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Abstracts of Papers for the Thirteenth Annual Meeting of the Society of Toxicology, Washington, D.C. March 10–14, 1974

1. *Narcosis in Rhesus Monkeys Following Oral Dosage with Glutethimide; Correlations with Serum Concentrations.* DAVID E. BAILEY, ALEX J. KUTCHES, KENNETH MORGAREIDGE and FRANCIS MEYER, Food and Drug Research Laboratories, Inc., Waverly Division, Waverly, New York, and Extracorporeal Medical Specialties, Inc., King of Prussia, Pennsylvania. (B. L. Oser.)

To test a device for removing glutethimide from blood in overdose cases, it was necessary to produce a severe narcosis in the Rhesus monkey. It was essential to determine the oral dosage that would produce clinically reproducible and predictable narcosis, and simultaneously to determine drug blood concentrations. A total of 21 monkeys (conditioned 3 months and weighing 3–5 kg) were given glutethimide at doses varying from 100 to 300 mg/kg as either compressed tablets or suspensions. Suspensions were prepared with vehicles of either corn oil, or 2% carboxymethylcellulose in water. Venous blood was drawn at hourly intervals and glutethimide concentration determined by gas chromatography. The first signs of incoordination were usually seen between 0.5 and 1.0 hr post dosing. However, onset of complete narcosis varied from 1 to 6 hr. There was extreme individual variation in relation to drug effect. Monkeys given 100 mg/kg showed only incoordination. At 150 mg/kg 3 monkeys were incoordinated, and 2 were completely unconscious. Of 4 monkeys given 300 mg/kg, 2 were incoordinated, 1 unconscious, and 1 died after 36 hr. Blood glutethimide concentrations and corresponding clinical signs were as follows: >5 μg/ml, ataxia and incoordination; 20–30 μg/ml, unconsciousness; 30–50 μg/ml, coma; and >50 μg/ml, death. Glutethimide blood concentrations correlated well with clinical signs of ataxia, incoordination and narcosis, however, clinical signs were not predictable based on the interval of time after dosing.

2. *The Development of Tolerance to Methadone Lethality After Morphine Pellet Implantation and Repeated Methadone Administration.* LAWRENCE W. MASTEN, CHARLES H. HINE and E. LEONG WAY, Department of Pharmacology, University of California, San Francisco, California.

The recent increase of methadone-related mortalities over the past few years has prompted an investigation by our laboratory into factors controlling methadone-induced lethality. An animal model was developed utilizing the mouse to study this problem in a framework similar to clinical methadone maintenance. Animals were rendered tolerant and dependent to morphine by the subcutaneous implantation of a specially formulated pellet containing 75 mg of morphine base. At the end of 3 days, the morphine pellet was removed and the resulting wound was clipped together. Three hr thereafter, 100 mg/kg methadone hydrochloride in aqueous solution was given by oral intubation. The administration was repeated at 24 hr-intervals. Mortalities associated with the 3-day morphine pellet implantation, the first 3 days of methadone administration and the last 3 days of oral methadone were 9.0, 20.8 and 0%, respectively. Substitution of water for methadone did not alter these percentages significantly. No deaths were noted in placebo pellet animals. The 3-day morphine implantation was found to increase the oral methadone LD50 by 3- to 4-fold when compared to placebo-implanted mice. Mice receiving methadone for 5 consecutive days following morphine pellet removal showed an additional 40% increase in the oral methadone LD50, whereas those receiving water exhibited a 16% decrease. These results indicate the presence of cross tolerance to lethality between morphine and methadone as well as a development of tolerance to methadone lethality following repeated, constant dosage of this compound. (This work was supported in part by USPHS Training Grant GM-00475 and NIH Grant GM-01839.)

3. *Effects of Subchronic Administration of Methadone on Reproduction in Rats.* LINDA K. MCDONALD, KENNETH BLUM, JAMES F. MADDUX and JACK E. WALLACE, Departments of Psychiatry, Pharmacology and Pathology, The University of Texas Health Science Center, San Antonio, Texas. (Jasbir M. Singh.)

Animal reproduction studies with follow-up observations into maturity could contribute significantly to understanding the protracted effects of chronic fetal methadone intoxication. This study was designed with the following specific objectives: (1) To identify in rats the signs of acute abstinence following physical dependence on methadone. (2) to investigate the consequences of methadone maintenance during gestation on birth weight, mortality, and growth of pups born to maternal methadone-dependent rats. Female rats, were made tolerant to and possibly dependent on methadone by subcutaneous injection. After 7 weeks of methadone administration "wet dog" shakes, a procedure used to quantitate morphine dependence in rats, was observed. The data further confirms the recent work of Singh and Singh (1973) who reported that when methadone is withdrawn from male rats chronically treated for 180 days signs of physical dependence are present. For the reproductive studies, methadone was administered sc in 2 equal doses a day at 12-hr intervals. The maximum dosage of 20 mg/kg was maintained for 30 days before males were introduced. A test of the number of deaths of experimental (methadone dependent) and control females and pups indicated a significant difference in each of the 2 groups. However, in the surviving siblings no difference was found in the rate of growth of the experimental and control pups. Fetal exposure to maternal methadone dependence throughout the period of gestation seems to create a higher perinatal death risk but there was no evidence of developmental difficulties up to 21 days of age. (Supported by NIMH Grant 626-1607-8150 and the Pharmaceutical Manufactures Association Starter Grant.)

4. *Studies of the Carcinogenicity of an Acetone Extract of Hashish.* B. G. PROCTER, P. DUSSAULT, G. RONA and C. I. CHAPPEL, Bio-Research Laboratories Limited, Pointe Claire, Quebec, Canada.

Four experiments were carried out in laboratory animals in an effort to obtain information which might indicate possible carcinogenicity associated with the use of hashish by man. All studies were conducted using an acetone extract of crude hashish which contained 2.4% Δ^9-tetrahydrocannabinol. The extract was investigated in a long-term skin painting study in mice and was also incorporated into cholesterol-based pellets whose carcinogenicity was studied in mice using a bladder implantation technique. The hashish extract was not carcinogenic in either of these studies. In further investigations, the extract did not prove to be a tumor "initiator", but as a tumor "promoter" it was at least as potent as croton oil. Thus, systematic *ante mortem* observation of developing skin tumors and *post mortem* microscopic study of representative tumors revealed that, following initiation with dimethylbenzanthracene, the hashish extract caused the development of a high incidence of multiple benign fibroepithelial and papillomatous neoplasms. This same effect was associated with croton oil but the tumors produced were noticeably fewer. On the other hand, malignancy was a relatively common attribute of the skin tumors that developed during croton oil administration, but very few malignant skin tumors developed in the animals treated with the hashish extract. (Supported by the Non-Medical Use of Drugs Directorate, Health & Welfare, Canada.)

5. *The Influence of Cannabinoid Mixtures on Drug-Induced Sleep in Mice.* D. J. MCCOY, D. J. BROWN and R. B. FORNEY, Department of Toxicology, Indiana University School of Medicine, Indianapolis, Indiana.

The purpose of this study was to determine whether cannabidiol (CBD) has any effect on ethanol and hexobarbital sleep time, and if the presence of CBD in a mixture with Δ-9-tetrahydrocannabinol (Δ-9-THC) exerted any effect on the actions of Δ-9-THC. The experimental design entailed ip injection of male albino mice with either CBD, THC, or a mixture of the two, followed, after a set time period by a hypnotic dose of either sodium hexobarbital (100 mg/kg) or ethanol (4 g/kg). The animals' loss-of-righting reflex (sleep time) was measured and the mean for each treatment group was compared to that of control. When hexobarbital was used as the

hypnotic agent, CBD was a much more potent enhancer of duration of sleep than was Δ-9-THC. A mixture of equal parts of CBD and Δ-9-THC gave an effect no greater than CBD alone. By increasing the time interval between injections, it was observed that CBD pretreatment (25 mg/kg) caused an increase in mean sleep time over control as much as 18 hr after administration. The mean sleep time of animals given CBD followed by ethanol, however, was just slightly increased over control. Δ-9-THC alone and Δ-9-THC with CBD increased ethanol sleep duration, but these pretreatments were statistically indistinguishable from one another. The magnitude and the duration of the CBD pretreatment on hexobarbital sleep time suggests not only the possible reinterpretation of some of the effects from crude marihuana extracts that have been attributed to Δ-9-THC, but also that other possible pharmacological properties of CBD be investigated.

6. *Effect of Cannabinoids on Pentobarbital Metabolism in the Rat.* B. B. COLDWELL, K. BAILEY, G. ANDERSON and C. PAUL, Drug Research Laboratories, Health Protection Branch, Health and Welfare Canada, Ottawa, Canada.

The widespread use of marihuana has focussed attention on the interaction of its components with drugs. This paper describes the effects of ip cannabidiol (CBD), cannabigerol (CBG), cannabinol (CBN), Δ^8-tetrahydrocannabinol (Δ^8-THC), and Δ^9-tetrahydrocannabinol (Δ^9-THC) on the in vivo metabolism of ^{14}C-pentobarbital (^{14}C-P) in rats. After oral ^{14}C-P, CBG initially depressed and later elevated ^{14}C blood concentrations; Δ^9-THC and CBD increased ^{14}C blood concentrations while CBN and Δ^8-THC had little effect. Following iv ^{14}C-P, blood ^{14}C values were elevated by CBD and unchanged by CBG, Δ^8-THC or Δ^9-THC. Urinary excretion of total ^{14}C and the major metabolite of pentobarbital (P) was decreased by CBD, CBG and Δ^8-THC during the first 6 hr following treatment. The effect of CBD on ^{14}C blood concentrations and on urinary excretion of ^{14}C was dose-related. In rats treated with CBD + P the liver concentrations of P metabolites were significantly lower and the liver and brain concentrations of unmetabolized P were significantly higher than in P-treated rats. Pentobarbital induction and sleeping times were potentiated by CBD and Δ^9-THC and antagonized by CBG. It is concluded that CBD and probably Δ^8-THC delayed P metabolism while CBG decreased the rate of P absorption and excretion, Δ^9-THC and CBN had little effect on P metabolism, and the CNS effects of CBD + P correlated with the brain P concentrations.

7. *Chronic Oral Toxicity of Cannabinoids in Monkeys.* GEORGE R. THOMPSON, ROBERT W. FLEISCHMAN, HARRIS ROSENKRANTZ and MONIQUE C. BRAUDE, Mason Research Institute, Worcester, Massachusetts, and NIMH, Rockville, Maryland.

Toxicity produced by oral administration of Δ^9-tetrahydrocannabinol (Δ^9-THC) or a crude marihuana extract (CME) was evaluated in rhesus monkeys. Both compounds were dissolved in sesame oil and administered by intubation for 90 days. Δ^9-THC was given in dosages of 50, 250 and 500 mg/kg/day while CME doses were 150, 750 and 1200 mg/kg/day. These high dosages were chosen specifically to produce toxicity and not to simulate human doses. Both compounds produced cumulative toxicity as indicated by delayed mortalities ($\geqslant$ day 10), but only moribund or deceased monkeys exhibited significant histopathology. Primary tissue changes in monkeys treated with Δ^9-THC included atrophy of the thymus and pancreas, and hemorrhagic colitis with associated myeloid hyperplasia of the bone marrow, vacuolar nephrosis and severe serum electrolyte derangement. Ulcerative colitis was also associated with CME administration in 1 moribund monkey, but tissue changes in other moribund animals included only lymphoid atrophy, aspiration pneumonia and bone marrow hypoplasia. Δ^9-THC and CME also produced adrenal hyperplasia in moribund monkeys, anorexia and weight loss. Hematological and hemochemical parameters were generally unaffected. Both compounds produced behavioral depression at all dose levels. Depression was most severe 8–24 hr after the first treatment, but the severity gradually subsided within 12–15 days. As tolerance developed, some monkeys exhibitied moderate hyperactivity. The general similarity of signs indicated that toxicity probably occurred by the same mechanism(s) in monkeys treated po with Δ^9-THC or CME.

8. *Distribution Studies of [^{14}C]-Delta-9-Tetrahydrocannabinol (THC) in Mice.* BERNARDO MANTILLA-PLATA and RAYMOND D. HARBISON, Department of Pharmacology and Center in Toxicology, Vanderbilt Medical Center, Nashville, Tennessee.

The purpose of this study was to determine the effect of vehicle, route of administration, and duration of treatment on absorption, distribution, and excretion of THC. ^{14}C-Labeled THC was prepared in 10% Tween 80 and saline (TW), 5% bovine serum albumin in water (BSA), 10% propylene glycol-1% Tween 80 and saline (PPG), or corn oil (COL) to deliver 5 mg/20 μCi/kg in a volume of 0.1 ml/10 g of body weight. ^{14}C concentration was measured in plasma, liver, brain, lung, and fat tissues at 1, 4, 8 and 16 hr after THC administration. ^{14}C concentration in all tissues was lowest after po or sc administration, higher after ip, and highest after iv injection. Liver contained and retained the largest amount of ^{14}C. Brain contained significantly less ^{14}C than other tissues at all time periods studied. Significantly higher ^{14}C-plasma concentration was seen at 1 hr after THC iv injection in PPG than that in TW and BSA. BSA produced significantly lower values at all time periods studied than that with PPG and TW. However, the ^{14}C-lung concentration was 4 times higher than that in PPG and TW. There was no significant difference in ^{14}C-brain concentrations 4 hr after iv administration of THC in TW, BSA and PPG. However, TW produced significantly higher ^{14}C-brain values 1 hr after iv injection of THC. Concentration of ^{14}C in all tissues studied was significantly lower after THC ip or po administration in COL when compared to those found with TW. Excretion was followed from 24 to 120 hr after injection of THC. There was significantly more ^{14}C excreted in the feces at all time periods studied when compared to the amount measured in urine. Chronic iv administration of ^{14}C-THC (5 days) produced high concentrations of ^{14}C in plasma, liver, lung and fat. Brain concentrations of ^{14}C were lower following chronic administration. In conclusion, the vehicle, route of administration, and the duration of treatment affect the distribution of THC in mice. (Supported by USPHS DA 00141 and ES 00267.)

9. *Absence of Tolerance to the Hypotensive Effects of Delta-9-Tetrahydrocannabinol in Hypertensive Rats.* S. C. LEWIS, D. J. BROWN and R. B. FORNEY, Department of Toxicology, Indiana University School of Medicine, Indianapolis, Indiana.

Other investigators have reported conflicting data concerning the development of tolerance to the hypotensive effects of delta-9-tetrahydrocannabinol (THC) upon repeated administration to spontaneously hypertensive rats (SHR). These experiments were undertaken to investigate the development of tolerance to the hypotensive effects of repeated low to moderate doses of THC. Randomly selected groups of SHR were administered 0.1, 1.0 or 10.0 mg THC/kg in a 0.1-ml corn oil vehicle, and a control group was administered the vehicle alone. The drug was administered ip. One hr prior to each of 9 successive daily injections of THC, systolic blood pressures were measured by means of a tail-cuff sphygmomanometer. On the tenth experimental day, 0, 6.6 or 66 mg THC/kg was administered to all pretreated animals by ip injection in a physiologic phosphate vehicle, and systolic pressures were measured prior to and 1 and 2 hr post injection. Age-matched naive animals were treated in a similar fashion. No change in systolic blood pressure significantly different from placebo could be observed during the 9-day treatment period with any of the 3 doses of THC. Nor could any difference be observed on the tenth day between the THC pre-treated groups and previously studied naive animals when challenged with a moderately potent hypotensive dose of THC. These experiments indicate that spontaneously hypertensive rats do not become tolerant to the hypotensive effects of delta-9-tetrahydrocannabinol when administered by the above protocol.

10. *The Effects of Delta-9-Tetrahydrocannabinol on Lung Resistance and Compliance in the Dog.* T. P. BRIGHT, M. O. FARBER, L. W. WINTER, D. J. BROWN and R. B. FORNEY, Pulmonary Research Laboratory, Veterans Administration Hospital, and Department of Toxicology, Indiana University Medical Center, Indianapolis Indiana.

Delta-9-tetrahydrocannibinol (THC) has been demonstrated to have many effects on experimental animals. Little is known, however, about its action on the lungs. The effects on lung resistance (R_L), compliance (C_L) and arterial blood oxygen tension of iv THC were

investigated in pentobarbital-anesthetized mongrel dogs artificially ventilated at constant rate and tidal volume. Six intact and 6 vagotomized dogs were given THC, 500 μg/kg and 6 dogs received 0.025 ml/kg (25 mg/kg) propylene glycol as carrier controls. Propylene glycol produced no effects on R_L or C_L. THC produced an increase in R_L within 1 min post-inection (mean 58% over control range 45–69%), and R_L remained elevated over control throughout the 20-min observation period. C_L changed significantly in only 1 dog which showed a decrease of 33% below control values at 1 min post-injection. The arterial blood oxygen tension did not decrease in any dog throughout the experiment, with a mean control value of 119 mm Hg (range 101–128); and mean 20 min post-THC of 116 mm Hg (range 104–130). One min following THC vagotomized dogs showed no significant change in R_L (mean 2% under control, range: 17% under, to 4% over control), and there was no change in C_L. This pattern persisted throughout the period of observation. The data presented suggest that THC produces a "bronchoconstriction" in anesthetized dogs through a central or reflex vagal mechanism. This alteration in bronchial mechanics does not, in itself, produce a profound change in overall ventilation since no change in arterial oxygen tension is seen.

11. *Effect of Apomorphine and Nalline on Methadone-induced Behavioral Changes.* JASBIR M. SINGH, Alcoholism Services Unit, Department of Psychiatry, Charity Hospital, New Orleans, Louisiana.

Many models have been developed to study behavioral changes such as clinical signs of aggression, produced by morphine. Methadone in mice produces circular or edging movements effect (CME) and the animals remain very close to the edges of the cage. Non-starved male white mice weighing 25–30 g were placed on an activity platform and the CME were recorded. The animals began to move in a circular manner with their tails up 2 to 3 min after ip administration of methadone (5–20 mg/kg). This effect of methadone was dose-dependent. The increase in CME was indicated by the number of impulses recorded. Tolerance is developed to the CME. Prior administration of apomorphine (1 mg/kg) blocked the CME induced by methadone. This effect is partially blocked (60–70%) by nalline (1 mg/kg). Some movements were recorded by apomorphine and nalline alone. These movements were not classified as CME because the animals were sitting in one corner on top of each other or they were moving in an unorganized manner.

12. *Comparative Deposition, Retention and Early Toxicity of Inhaled Insoluble ^{144}Ce Aerosols in Mice, Syrian Hamsters and Beagle Dogs.* C. H. HOBBS, R. O. MCCLELLAN, S. A. BENJAMIN, B. B. BOECKER, F. F. HAHN, D. L. LUNDGREN and R. L. THOMAS, Inhalation Toxicology Research Institute, Lovelace Foundation, Albuquerque, New Mexico.

As a part of a large program on the toxicity of inhaled radioactive aerosols that may be encountered in nuclear accidents, a series of studies has been initiated to provide interspecies comparisons on the toxicity of inhaled relatively insoluble forms of ^{144}Ce, a beta-emitting radionuclide. Mice, Syrian hamsters and beagle dogs have been exposed to aerosols of ^{144}Ce in fused clay or ^{144}Ce oxide, relatively insoluble forms of ^{144}Ce that are retained tenaciously by the lung. The various equipment used for acute, head-only exposure of the various species will be illustrated. The activity median aerodynamic diameters of the aerosols used ranged from 1 to 2 μm with geometric standard deviations of about 2. Of the total activity inhaled, the beagle dog deposited the largest percentage as the initial body burden, followed by the Syrian hamster and mouse. Also, of the total activity deposited as initial body burden, the dog deposited the largest percentage as the initial lung burden. The long-term retention of the material deposited in the pulmonary region varied widely with the species; effective half-lives in the lung were about 150–200 days, 90 days and 70 days for the dog, hamster and mouse, respectively. This resulted in substantially different radiation dose patterns to the lung of each species even for equivalent initial lung burdens in μCi/kg of body weight. Dose-response relationships will be discussed for each of the species. The highest doses in all 3 species have resulted in deaths at from 50 to 200 days post-exposure from radiation pneumonitis and/or pulmonary fibrosis. Primary pulmonary neoplasms have been observed in the dogs at later

times (2–3 years) but not in mice observed over their lifespan. These data illustrate the importance of species selection for these and other inhalation toxicology studies. (Supported by the Atomic Energy Commission under Contract AT(29-2)-1013.)

13. *Effects of NO_2 on Humoral Immunologic Defense Mechanisms.* W. C. FELSENSTEIN, J. CHRIS PRATHER, D. E. GARDNER and D. L. COFFIN, Experimental Biology Laboratory, National Environmental Research Center, Environmental Protection Agency, Research Triangle Park, North Carolina.

Increased susceptibility to pulmonary infections after exposure to NO_2 and other oxidant air pollutants has been well documented in animals. Nevertheless, the factors responsible are not well understood, expecially with respect to humoral immune defense mechanisms. One report (C. E. Buckley, personal communication) related significant changes in serum complement activity in dogs to brief exposure to NO_2, suggesting a possible sensitive biological indicator. Giant Flemish rabbits and groups of Balb C mice were exposed to 10 ppm NO_2 for 1 hr. Blood samples were obtained at 30, 60, 120 min and 18 hr after beginning of exposure. Complement, measured as the C3 fraction, and immunoglobulins were determined in rabbits, but immunoglobulins only were determined in mice. Electro-immunodiffusion was employed as an immunoassay. No significant differences in C3 levels between NO_2-exposed rabbits and controls were found, although considerable variations occurred between serial samples. Also, C3 levels were unchanged in rabbit serum through which NO_2 at 10,000 ppm had been bubbled for 1 hr. In contrast, there were moderate to 3-fold increases in IgA and IgG, respectively, in mice, with moderate to slight decreases for IgG_2 and IgM. Preliminary results in rabbits differed markedly, with slight drops in IgA and IgM and little change in IgG levels.

14. *Acute Toxicity of Cigarette Smoke Inhalation in Mice, in Relation to Nicotine and Tar Content.* PETER BERNFELD, Bio-Research Consultants, Cambridge, Massachusetts. (F. Homburger.)

The present work was undertaken to demonstrate the existence in cigarette smoke of substances with acute toxicity, other than nicotine and gas phase components (CO, HCN, etc.). Female mice (CAF1/J) were exposed to cigarette smoke from 10 commercial brands (undisclosed) and from 1R1 Kentucky reference cigarettes. The animals inhaled alternately 9.3% whole smoke (or gas phase) for 27 sec and fresh air for 33 sec. Acute toxicity was assumed to be inversely proportionate to the survival time of the animals under these conditions. There was an excellent correlation between tar and nicotine contents for all brands of cigarettes studied, independently of whether they were filter or nonfilter cigarettes. In contrast, only a superficial correlation existed between nicotine or tar contents of the cigarettes on the one hand, and the acute toxicity of their smoke under the test conditions, on the other hand. Four brands of cigarettes exhibited significantly higher, and 2 lower, acute toxicity than would be expected from their nicotine or tar contents. The gas phase did not have any toxic effects under the test conditions employed. These results suggest that the particulate phase of cigarette smoke contains components, other than nicotine, which contribute significantly to its acute toxic effects. Testing of commercial brands of cigarettes for this acutely toxic principle appears as meaningful as determinations of their tar and nicotine contents.

15. *Effect of Dibromotrifluoroethane Inhalation on Hepatic Drug Metabolism.* JAMES P. F. MURPHY, ETHARD W. VAN STEE and KENNETH C. BACK, 6570 Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio.

The fluoroalkane, dibromotrifluoroethane has been of interest to the U.S.A.F. as a potential fire control agent. Preliminary studies of the effect of exposure of mice to this compound on the duration of hexobarbital sleeping time suggests the possibility that it induced hepatic drug metabolizing enzymes. Inhalation of dibromotrifluoroethane, at concentrations ranging from 0.10 to 0.63% for 5 hr daily, for 3 days, reduced hexobarbital sleeping time almost 2-fold in mice. The increased rate of metabolism of hexobarbital and ethylmorphine by the hepatic 9000 *g* microsomal supernatant fraction prepared from mice exposed to dibromotrifluoroethane 0.1–0.63%, 5 hr daily for 1 through 4 days paralleled the effects observed in

vivo. Addition of 2,4-dichloro-6-phenyl-phenoxyethyldiethylamine (SKF525-A) 5×10^{-5} M/flask to the hepatic 9000 *g* microsomal supernatant fraction prevented the dibromotrifluoroethane-induced increase in hepatic drug metabolism. A structure-activity relationship was postulated from comparison of the effects of dibromotrifluoroethane and dibromotetrafluoroethane.

16. *Toxicity and Biochemical Changes in Rats After Inhalation Exposure to 1,1-Dichloroethylene, Bromobenzene, Styrene, Acrylonitrile or 2-Chlorobutadiene.* R. J. JAEGER, R. B. CONOLLY and S. D. MURPHY, Department of Physiology, Harvard School of Public Health, Boston, Massachusetts.

Previously, we showed that the inhalation toxicity of 1,1-dichloroethylene (DCE), a plastics monomer, was increased when male rats were fasted overnight prior to exposure. Toxicity was measured by serum transaminase (ST) elevation, by estimated 4 hr LC50, or by minimum lethal concentration (MLC). Enhancement of toxicity with fasting was correlated with a lower hepatic glutathione (GSH) concentration prior to exposure. After exposure to DCE both fed and fasted rats had a further decreased liver GSH concentration. It has been demonstrated by others (Reid and Krishna, *Exp. Molec. Pathol.* **18**, 80, 1973) that depletion of glutathione enhances hepatic injury produced by ip administration of bromobenzene (B). This suggested the possibility that DCE is similar in mechanism of toxic action to B. When B was administered by inhalation to fed and fasted phenobarbital-pretreated rats, fed rats were somewhat more sensitive to the toxic effects of B than were fasted rats. Without phenobarbital induction, both fed and fasted rats were resistant to the lethal effect of B, and hepatic injury was quite variable. Additional inhalation experiments with other plastics monomers known to react with GSH were undertaken. Acrylonitrile (ACN), styrene (S) and 2-chlorobutadiene (CBD), all depleted liver GSH. As anticipated, S and ACN caused no apparent liver injury. S caused death by pulmonary irritation and edema. The estimated LC50 for fed and fasted animals was 2700 ppm. ACN was lethal to all fasted rats at 275 ppm with no apparent effect on fed rats. The approximate LC50 values were 150 and 425 ppm for fasted and fed rats respectively. CBD caused liver injury and fasting enhanced its toxicity as measured by ST and MLC. Significant 24-hr elevation of ST occurred in fasted rats at 560 ppm while comparable values for fed rats were not measured until 4200 ppm. The MLC for fasted rats was below 560 ppm, the lowest concentration tested, while this value for fed rats was between 4200 and 8100 ppm. These data suggest that fed-fasted differences in rats are significant in inhalation toxicity testing. (Supported by Research Grants OH-00315 and ES-00002.)

17. *Comparative Toxicity of Aerosol Propellants.* D. M. AVIADO and M. BELEJ, Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania.

The inhalational toxicity of 15 aerosol propellants was examined in anesthetized monkeys. The usual pattern of bronchopulmonary response consisted of tachycardia, depression of myocardial contractility, fall in systemic arterial blood pressure, depression of respiratory minute volume and either bronchoconstriction or bronchodilation. A classification scheme based on the propellants' bronchopulmonary toxicity is proposed. Propellants classified as being of *high toxicity* induced these effects when inhaled in concentrations of 1–5%; those of *intermediate toxicity* required concentrations of 6–10%; and propellants of *low toxicity* elicited only minimal response at concentrations of 11–20%. A further distinction with respect to whether any particular substance is a high- or low-pressure propellant was made. Thus, the final classification of *low-pressure* propellants is as follows: *high* toxicity: trichlorofluoromethane (fluorocarbon 11), dichloromonofluoromethane (FC 21), trichlorotrifluoroethane (FC 113), trichloroethane and methylene chloride; and *intermediate* toxicity: dichlorotetrafluoroethane (FC 114), monochlorodifluoroethane (FC 142b), isobutane and octafluorocyclobutane (FC C-318). The *high-pressure* propellants were found to belong in one or the other of the following two groups: *intermediate* toxicity: dichlorodifluoromethane (FC 12), monochlorodifluoromethane (FC 22), propane and vinyl chloride; and *low* toxicity: chloropentafluoroethane (FC 115) and difluoroethane (FC 152a). The most toxic propellant among those

studied was FC 11 which induced cardiac arrhythmia when inhaled at a concentration of 2%; myocardial ischemia lowered the arrhythmic threshold to 1%. In the monkey with myocardial ischemia and infused with 0.5 μg/kg/min of epinephrine, inhalation of 0.5% of fluorocarbon 11 induced cardiac arrhythmia. The cardiotoxicity of this propellant is further enhanced in the infarcted heart. (Supported by the Food and Drug Administration under Contract No. FDA 71-310.)

18. *Continuous Exposure of Rats to Hexafluoroethane.* T. B. GRIFFIN, J. L. BYARD, F. COULSTON, L. GOLBERG and E. S. HARRIS, Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York, and NASA Manned Spacecraft Center, Houston, Texas.

Hexafluoroethane (Freon 116) is potentially useful as an atmospheric inerting agent for suppressing combustion in enclosed cabins of spacecraft, aircraft and submarines. The effects of virtually continuous exposure (23 hr/day) of rats to Freon 116 were studied in a chamber with a volume of about 0.32 m^3, modified to operate in the "closed dynamic" mode, involving recovery and reuse of the fluorocarbon. During the exposure the average concentrations of Freon 116, oxygen and carbon dioxide in the chamber were, respectively, 20.7, 20.1 and 0.39%. Growth of the animals in the exposure chamber was similar to that of the controls, although initially there was a period of growth retardation. Routine studies of serum chemistry and hematology revealed changes only in alkaline phosphatase and lactate dehydrogenase activities; similar enzyme changes had previously been observed in chambered control rats. Some evidence of hemoconcentration was observed in the exposed rats during early stages of the experiment. Possible cleavage of the carbon-fluorine bond of Freon 116 in the body of the rat was assessed through measurements of the rate of fluoride ion excretion in the urine and of the concentration of fluoride in bone. No evidence of such breakdown of Freon 116 was obtained. Exposure to the halocarbon did not induce N-demethylase or biphenyl hydroxylase in liver nor were levels of cytochromes *P*-450 or b_5 altered. There was no increase in lipid peroxidation. At autopsy, the lungs and other organs appeared normal. Histopathologic examination of the tissues is in progress. Anticipation of the inert character of hexafluoroethane appears to have been substantiated. (Supported in part by Contract 9-9964 from the National Aeronautics and Space Administration, by Research Grant 2P01-ES00226-07 from the National Institute of Environmental Health Sciences, NIH, and by National Institutes of Health Training Grant 2T01-ES00103-07.)

19. *Development of Biologic Standards for the Industrial Worker Exposed to Trichloroethylene.* R. D. STEWART, C. L. HAKE, A. J. LEBRUN, J. E. PETERSON, H. V. FORSTER and P. E. NEWTON, Department of Environmental Medicine, The Medical College of Wisconsin, Milwaukee, Wisconsin.

A controlled-environment chamber was used to expose human volunteers to the vapor levels of trichloroethylene (TCE) which might occur in an industrial setting. Ten male subjects were divided into 3 groups; 4 subjects spent 7½ hr/day, 3 subjects spent 3 hr/day and 3 subjects spent 1 hr/day, 5 days/week, in the chamber. Successive weekly average exposure concentrations were 20 ppm (steady), 100 ppm (steady), 100 ppm (fluctuating) and 200 ppm (steady). In addition, 10 females were divided into similar groups and exposed to a 100 ppm (steady) average concentration of TCE for 1 week. Extensive clinical testing was carried out to ensure the maintenance of good health in all subjects. Pulmonary function testing and cardiac monitoring revealed no deleterious effects of the repeated exposures. Electroencephalographic and visual evoked response studies revealed mild changes in 1 male and 3 females at the high exposure levels. Such changes may signal the beginning of deleterious changes in the functioning of the central nervous system. There were no consistent or dose-related decrements in the results of coordination, arithmetic, inspection or time estimation tests. Urine specimens were obtained from 24-hr collections and assayed for trichloroethanol (TCEt) and trichloroacetic acid (TCA). Values for 24-hr excretion of these metabolites were corrected to normal creatinine excretion. During the exposure of males to 20 ppm TCE, daily average TCEt excretion remained almost constant, while TCA excretion was below the measurable limits of the chromatograph. During the following weeks when daily exposures were to 100 and 200 ppm TCE,

daily average TCEt excretion increased for the first 4 days of each week, but seemed to level off on the fifth day. TCA excretion, while always lower than TCEt, increased each day of the week. There was a large variation in the excretion of TCA and TCEt among individuals identically exposed, and in some individuals on successive days of exposure. The measurement of urinary metabolites during and after TCE exposure has limited value as a biologic standard for the industrial worker. (Supported by Contract No. HSM-99-72-82, National Institute of Occupational Safety and Health.)

20. *Use of Breath Analysis to Monitor Trichloroethylene Exposures.* R. D. STEWART, C. L. HAKE and J. E. PETERSON, Department of Environmental Medicine, The Medical College of Wisconsin, Milwaukee, Wisconsin.

Trichloroethylene (TCE) post-exposure breath decay curves were obtained from 10 male and 10 female volunteers who were exposed daily in a controlled-environment chamber to TCE vapor, 20, 100 or 200 ppm for 1, 3 or 7½ hr. Alveolar breath samples were collected in glass pipettes for TCE analysis by gas chromatography. The series of TCE breath decay curves obtained was highly reproducible and the narrow range of TCE in the breath at a specific time in the early post-exposure period of persons identically exposed indicated that breath analysis could be used as a rapid method with which to estimate the magnitude of recent TCE exposure. The TCE breath concentration in the immediate post-exposure period accurately reflected the vapor concentration to which the subject had been most recently exposed. Breath samples collected 8–24 hr following exposure were accurate indicators of the time-weighted-average vapor exposure experienced by the subject on the previous day. (Supported by Contract No. HSM-99-72-82, National Institute of Occupational Safety and Health.)

21. *"Degreasers' Flush": Dermal Response to Trichloroethylene and Ethanol.* R. D. STEWART, C. L. HAKE and J. E. PETERSON, Department of Environmental Medicine, The Medical College of Wisconsin, Milwaukee, Wisconsin.

Trichloroethylene (TCE), one of the most widely used chlorinated aliphatic hydrocarbon solvents in America, has the reputation of being a reasonably safe industrial solvent. When properly used, few untoward responses have been reported. It can, however, elicit an unusual dermal response, a fascinating phenomenon referred to as the "degreasers' flush". Workmen drinking beer in their neighborhood tavern following an industrial exposure to TCE vapor have been observed to develop vivid red blotches on their faces. Unfortunately, this dermal phenomenon has not been adequately investigated and the mechanism of the response remains unknown. During a series of experimental human exposures to TCE vapor performed for the purpose of obtaining a series of diagnostic breath decay curves, the facial "flush" occurred in 1 of the subjects who violated the protocol and drank beer. This study was then initiated to investigate the factors responsible for this phenomenon. Ethanol was administered to 7 male volunteers being repeatedly exposed to TCE vapor to elicit the "degreasers' flush". In 6 exposed subjects transient, vasodilatation of superficial skin vessels occurred after the ingestion of small amounts of ethanol (<0.5 ml/kg). The dermal response reached maximum intensity 30 min after its onset, then faded completely within 60 min. The skin vasodilatation appeared in symmetrical patterns on the face, neck, shoulders and back. The mechanism responsible for the vasodilatation is unknown, but two factors appear necessary before the dermal response can be elicited: (1) repeated exposures to TCE, and (2) ingestion of a beverage containing alcohol. (Supported by Contract No. HSM-00-72-82, National Institute of Occupational Safety and Health).

22. *Methods for Evaluating Corrosive Effects of Ingesting Detergents.* G. G. CLOYD, N. M. BROWN and J. F. GRIFFITH, The Procter and Gamble Company Cincinnati, Ohio.

The common occurrence of accidental ingestion of detergents by young children each year necessitates an assessment of the potential corrosive effects of any new composition considered for detergent use. Experiments were conducted in New Zealand white rabbits, mongrel cats and beagle dogs to determine a model which could be used as a routine screening procedure to

test the corrosive nature of new products. Rabbits dosed with 300–500 mg of detergent material on the base of the tongue and necropsied 24 and 96 hr later showed little variation in the degree of corrosive injury produced when compounds with different caustic potentials were compared. Cats given 2 cc of dry product on the base of the tongue and necropsied at selected intervals post dosing showed a gradation in corrosive injury induced by different detergent materials, but had considerable variation within groups given the same material. Similar results were seen in dogs given 5 cc of dry material on the base of the tongue or 5 or 10 cc of detergent in the proximal esophagus after passage of a sigmoidoscope for administration of the product. Dogs were evaluated by endoscopy or necropsy examination at selected intervals post dosing. Dogs treated by gavage with 40% w/w solutions of detergents dosed at a specific volume of dry material/kg of bodyweight had a more uniform distribution and severity of lesions among animals dosed with the same product. They also showed a gradation in response with different detergents. The more uniform exposure of mucosal surfaces to test products following gastric intubation made this technique more reliable for assessing the corrosive nature of a detergent. Safety judgements can best be made by comparison of injury produced following dosing of test materials with results observed following dosing of compounds known to be safe or corrosive.

23. *Evaluation of Non-Phosphate Detergent Formulations for Ingestion Hazard.* NANCY M. BROWN and JOHN F. GRIFFITH, The Procter and Gamble Company, Cincinnati, Ohio.

One of the most important concerns in the development of new household detergent products is their safety in intended use as well as in accidental exposures such as ingestion by children. In contrast to a few years ago, marketed laundry products now include builder materials other than sodium tripolyphosphate. Some of these materials are more alkaline than tripolyphosphate, so that high levels of them increase the potential ingestion hazard of products. In evaluating such formulations for safety, conventional acute oral toxicity determinations in rats are not sufficient for measuring the effect of increased causticity. Two methods of oral dosing in beagle dogs have been used to assess the safety of new formulations relative to phosphate based products with which there is an abundance of safe experience. The results of these tests indicate a correlation between the causticity of a formulation, as measured by titration to pH 9.5, and gastric irritant and corrosive effects produced under experimental conditions. Serious damage was produced by gastric intubation or oral dosing of a highly alkaline product known to have produced gastric and esophageal damage in humans; mild effects were seen when a phosphate-based product to low causticity was tested by the same methods. Formulations containing moderate levels of alkaline ingredients produced intermediate effects corresponding to their causticity.

24. *The Oral Toxicity in Dogs of Several Commercial Detergent Products.* F. F. HAHN, B. A. MUGGENBURG and J. L. MAUDERLY, Lovelace Foundation for Medical Education and Research, Albuquerque, New Mexico. (R. O. McClellan.)

Dose-response relationships resulting from the ingestion of four commercial detergent products: (1) Tide, (2) Sears Heavy Duty Laundry Detergent, (3) Electrosol Dishwashing Detergent and (4) Tide with nitrilotriacetate (Canadian) were evaluated in 54 beagle dogs. Single oral doses of 2.5, 1.0, 0.5 and 0.1 g/kg were used for all the detergents plus an additional dose of 0.25 g/kg Electrosol. Fifteen additional dogs received 2.5 g/kg of wheat flour and served as controls. There were no deaths among dogs ingesting Tide and only a slight gastric irritation was observed at the higher doses. One dog died after ingesting Sears detergent and severe damage of the upper alimentary tract was found at the higher doses. All dogs (9) given the 3 highest doses of Electrosol died within 54 hr. Only mild gastric irritation resulted from the ingestion of Tide with nitrilotriacetate. The control dogs exhibited no signs of illness. Additional dogs were used in a serial sacrifice study to follow pathologic changes that develop from detergent ingestion. The single dose level used was based on the highest dose of the previous study that did not cause 100% mortality. Lesions of the upper alimentary tract occurred on contact with the detergents but varied in severity, with Electrosol and Sears

detergents causing the greatest tissue damage. Serious complications from aspiration pneumonia appeared between 3 and 24 hr after ingestion of Sears and Electrosol detergents. Proximal tubular nephrosis, a transient lesion that developed 24–72 hr after ingestion of Tide, Sears and Electrosol, began to heal by 144 hr. (Supported by interagency agreement between the National Institute of Environmental Health Sciences and the U.S. Atomic Energy Commission under AEC Contract AT (29-2)-1013.)

25. *Interspecies Study of Skin Irritation Produced by Detergents and Chemicals.* C. A. TYSON and G. A. NIXON, Professional and Regulatory Services Division, Miami Valley Laboratories, The Procter & Gamble Company, Cincinnati, Ohio. (J. F. Griffith.)

In a series of patch-testing experiments, 26 substances, including familiar household materials and common chemicals, were applied to intact and abraded sites on rabbits, guinea pigs and humans for 4 hr. The sites were graded after 4, 24 and 48 hr. A number of materials caused greatly different reactions on either intact or abraded sites of humans. Two irritants caused severe reactions on human skin after brief exposure under the patch but were only mildly to moderately irritating to the animals. From these results, it is concluded (a) that neither the rabbit nor guinea pig should be relied upon exclusively to predict potential hazard to human skin and (b) that use of abraded sites confuses interpretation of the results, making the animal tests less rather than more reliable for many household products and chemicals compared to the use of intact sites only. Although not perfectly reliable even if abraded site testing is omitted, the rabbit appears to be the better model and an acceptable one for screening detergents and chemicals prior to conducting human patch tests.

26. *Reproductive, Subacute and Chronic Safety Evaluation Studies of Amine Fluorides.* J. M. SMITH, W. R. RAPP, W. F. STRAUSS, M. M. DOLAN and S. L. YANKELL, Bio/dynamics Inc., East Millstone, New Jersey, and Menley & James Labs Ltd., Philadelphia, Pennsylvania.

Toxicology studies were done on 2 combinations of 3 amine fluorides: I, hetaflur (hexadecylamine hydrofluoride); II, dectaflur (9-octadecenylamine hydrofluoride); and III, olaflur (2,2′-[[3-(2-hydroxyethyl) octadecylamino]propyl] iminodiethanol dihydrofluoride). A 1 : 1 ratio of II and I is intended for use in a mouthrinse and a 4 : 1 ratio of III and I is intended for use in a dentifrice. Both combinations have exhibited antiplaque activity in laboratory and clinical tests and clinical anticaries activity has been reported for the dentifrice. Oral dosing, following FDA guidelines, was planned to elucidate effects on reproductive processes and to examine overall effects with long-term administration. Dosage levels of the combinations ranged from 1 to 1.2, 5 to 6 and 25 to 30 mg/kg dosage day. In rats, data obtained after dosing for 6 months of a 2-year study indicate no alterations in appearance, mortality, hematology, chemistry and ratios of organ to body weight. In the high-dose group, some animals showed reduction of body weight gains, but the mid- and low-dose groups exhibited weight gains above control values. In dogs, the highest dose at the start of the study (30 mg/kg) was reduced to 10–12 mg/kg after 12 weeks because of excessive salivation, emesis and diarrhea. Thereafter, no toxicological signs were observed up to 1 year of dosing. Clinical laboratory values remained within normal limits. In reproductive studies in rats, no effects on mating, conception, organogenesis, fetal development, parturition, lactation, neonatal viability or newborn growth were noted. In rabbit teratologic studies, maternal weight gains were reduced at higher dose levels; however, no teratogenic effect was observed. These studies demonstrate the safety of these combinations of amine fluorides at exaggerated dose levels.

27. *Dermal Toxicity of Triphenyltin Chloride as Affected by Solvent and Solvent Temperature.* T. R. LEWIS, V. B. PERONE, H. D. SPECHT and W. SMALLWOOD, National Institute for Occupational Safety and Health, Cincinnati, Ohio. (W. D. Wagner.)

Triphenyltin chloride (TPTC) is a chemical precursor of triphenyltin hydroxide, an industrial bacteriostatic and fungistatic agent. In 1973, an industrial fatality occurred when a worker was drenched by a 1500-gallon slurry of TPTC spilled from an unbolted hatch of a mixing vessel.

The present study was designed to evaluate the systemic and local effects that would result from dermal contact with TPTC under conditions of different solvents, solvent temperatures and skin cleansing agents. Forty-eight healthy male and female rabbits, weighing 1.8–2.6 kg, were assigned to a 2 × 2 × 3 factorial design consisting of the 12 possible combinations of (1) two solvents, water or heptane, (2) two temperatures for each solvent 75° or 100°F, and (3) three skin cleansers, water, isopropyl alcohol or heptane. Acute primary skin irritation, tissue concentrations of tin in kidney, liver, heart, skin (2 layers) and body fat, hematological indices and histopathology of the kidney, liver, spleen, skin and bone marrow were the biological parameters investigated. Skin irritation did not differ with solvent. Significant differences occurred among the cleansers with all 3 showing significant differences from one another; skin cleansed with water had the highest degree of irritancy and that with heptane the least. Abraded animals had higher tin content in the heart, both layers of skin, kidney and liver than did unabraded animals. Muscle and body fat had essentially the same tin content. When heptane was the solvent, increased concentrations of tin were noted in both layers of the skin, but were not demonstrable in the kidney, liver or heart. Data on cleanser effects on tissue tin concentration are equivocal. No hematologic alterations were noted. TPTC causes primary skin irritation consisting of erythema and edema. Furthermore, significant skin absorption occurs, particularly from abraded areas.

28. *1,4-Dioxane Toxicity as Determined by a Two-Year Dose-Response Study in Rats.* R. J. KOCIBA, S. B. MCCOLLISTER, C. PARK, T. R. TORKELSON and P. J. GEHRING, Chemical Biology Research, The Dow Chemical Company, Midland, Michigan.

Four groups of rats, 60/sex/level, were maintained on drinking water containing 0, 1.0, 0.1 or 0.01 % 1,4-dioxane for up to 716 days. Male and female rats receiving 1 % dioxane (equivalent to approximately 1015 and 1599 mg/kg/day, respectively) showed decreases in body weight gains, survival rates and water consumption. Hepatocellular and renal tubular degenerative changes, accompanied by regenerative activity, were similar to those reported in previous studies following exposure to toxic levels of dioxane. Hepatocellular and nasal carcinomas, occurring at this dose level, were considered related to the lifetime exposure to these massive, toxic dosages of dioxane. In spite of the fact that male and female rats receiving 0.1 % dioxane (equivalent to approximately 94 and 148 mg/kg/day, respectively) in the drinking water had variable degrees of renal and hepatic degenerative changes, there was no indication of treatment-related tumor occurrence. Male and female rats receiving 0.01 % dioxane in the drinking water (equivalent to approximately 9.6 and 19.0 mg/kg/day, respectively) showed no evidence of tumor formation or other toxicological effects considered to be related to treatment. These data indicate a dose-reponse for the toxicity of dioxane. It is concluded that exposures to dioxane occurring under recommended conditions of use are not likely to be associated with untoward effects.

29. *Lack of Manifestation of Toxicity in Rats Inhaling 111 ppm 1,4-Dioxane for Two Years.* T. R. TORKELSON, B. K. J. LEONG, R. J. KOCIBA, W. A. RICHTER and P. J. GEHRING, The Dow Chemical Company, Midland, Michigan, and Industrial BIO-TEST Laboratories, Inc., Northbrook, Illinois.

Repeated 7-hr daily exposures to an average concentration of 111 ppm (0.4 mg/liter) 1,4-dioxane vapors were given 5 days/week for 2 years to groups of 288 male and 288 female Wistar strain rats. Groups of 192 male and 192 female rats similarly exposed to filtered room air served as controls. There were no observable compound-related effects with respect to demeanor, growth or mortality rate. Hematological studies during the 18th and 23rd months of exposure revealed no deviations from controls. Clinical chemical values determined at the termination of the experiment were within normal limits. Gross and microscopic examination of the major organs and tissues revealed no treatment-related lesions. In this study, no hepatic or nasal carcinomas were observed, although they have been reported to occur in rats maintained on water containing higher levels of dioxane. The incidence of all types of tumors in the control and exposed groups was not statistically different.

30. *Acute and Subacute Toxicity of 2,2,4-Trimethyl-1,3-Pentanediol (TMPD).* C. J. TERHAAR, W. J. KRASAVAGE and R. L. ROUDABUSH, Health and Safety Laboratory, Eastman Kodak Company, Rochester, New York.

2,2,4-Trimethyl-1,3-Pentanediol (TMPD) is a polyhydric alcohol approved as a component of polyester resins used in food packaging. Because of this potential use, its effect in animals was investigated. The oral LD50 of TMPD in rats, mice and guinea pigs is near 2000 mg/kg. The ip and iv LD50 in rats and mice is near 800 and 145 mg/kg respectively. Repeated skin applications to rabbits and guinea pigs caused only slight to moderate erythema. Moderate but transient irritation occurred after instillation of a 25% TMPD solution in 8% glycerine and 67% ethanol in rabbit eyes. The glycerine-alcohol solvent system reacted similarly. Fifteen male and 15 female rats were given TMPD in their diets at levels of 2.0, 1.0 and 0.5% for 60 days. Thirty of each sex served as controls. The females receiving 2.0% TMPD ate less and gained less weight than the controls and had a slight increase in average relative liver, adrenal, kidney, heart and brain weights. The relative liver, adrenal and kidney weights of the males receiving 2.0% TMPD were also increased. Relative organ weights of the 0.5% groups were similar to their controls. Hemograms, SGOT, SGPT and SAP values remained normal. The 1.0% group and one-half the controls (F_0) were placed on a 3-generation fertility study on day 30. There were 2 breedings in each generation. The only consistent finding was a decreased pup weight of the treated groups compared to their controls. Of the 6 groups of matings which delivered, 3 showed a higher mortality in the treated group than in the control, in 2 matings there was no difference and in 1, the control group had a higher mortality than the treated.

31. *Toxicological Properties of 2,3,5-Trichloro-4-(n-Propylsulfonyl)-Pyridine.* L. W. RAMPY, J. M. NORRIS, C. G. HUMISTON, R. J. KOCIBA and P. J. GEHRING, The Dow Chemical Company, Midland, Michigan.

Toxicological studies have been conducted on the subject film preservative for paint to elucidate handling hazards and its safety for the intended use. The single oral LD50 for male and female rats is 1700 and 1100 mg/kg, respectively. In rabbits, application of the dry powder produced slight skin and eye irritation while a 40% aqueous dispersion caused moderate irritation of both tissues; signs of untoward systemic effects were not observed. No signs of untoward effects occurred in rabbits upon repeated dermal application of 2 mg/kg/day in petroleum for 21 days. Skin sensitization was produced in guinea pigs by 0.1 and 0.25% solutions. The following untoward effects were seen in rats and beagle dogs maintained on diets providing the indicated daily doses (mg/kg/day) for 90 and 95 days, respectively: rats—depressed body weights, males (300); increased liver and kidney weights, males (300, 100 and 30) and females (300); dogs—depressed body weights and food consumption, females (65); increased liver-to-body weight ratios, males (65); increased kidney-to-body weight ratios, males and females (65). Dose-related depression of SGPT was seen in both species which was related to a direct effect of the compound on this serum enzyme. No toxic effects were seen at 6.5 and 10 mg/kg/day for dogs and rats, respectively.

32. *A Toxicological Investigation of the Acute, Subchronic, and Chronic Effects of Administering Di-2-Ethylhexyl Phthalate (DEHP) and Other Phthalate Esters.* W. H. LAWRENCE, M. MALIK, J. E. TURNER, A. R. SINGH and J. AUTIAN, The Materials Science Toxicology Laboratories, University of Tennessee Medical Units, Memphis, Tennessee.

Twelve esters of phthalic acid, including some of those most commonly used as plasticizers for biomedical devices, were subjected to one or more biological tests for acute, subchronic, and/or chronic toxicity. Certain aspects of subtle effects were investigated as well as overt toxic manifestations. The acute, ip LD50 of these compounds in mice ranged from 3.22 to more than 100 g/kg. A comparision of the acute to chronic LD50 values reveals most of these phthalates are 2–4 times more toxic chronically. However, di-n-octyl phthalate (DOP) was almost 22 times more toxic chronically, and the chronic toxicity of di-2-ethylhexyl phthalate (DEHP) was about 28 times greater. Pre-treatment of mice with these phthalate esters generally

resulted in an increase in pentobarbital sleeping time. A 12-week subchronic study of di-2-ethylhexyl phthalate in rats revealed very few abnormalities which could be attributed to the phthalate ester. Some of the new data are discussed in relation to previously published results of similar studies.

33. *Distribution and Kinetics of 2,4,5,2,'5'-Pentachlorobiphenyl (PCB) in the Male Rat.* H. B. MATTHEWS, J. R. BEND and M. W. ANDERSON, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. (J. R. Fouts.)

The distribution of [^{14}C]PCB in male rats was studied at times from 5 min to 7 days after iv doses of 0.06, 0.6 and 6.0 mg/kg. The PCB was rapidly cleared from blood with liver and lean tissues being major depots at early times (<1 hr). At 5 min postdose, hepatic uptake accounted for up to 35% of the total administered radioactivity. The compound is metabolized in the liver and excreted into the bile. At all times, only parent PCB was found in blood tissues. There was a biphasic decay with time for both blood and liver concentrations of PCB. Ratios of liver-to-blood concentrations of PCB decreased from 10 at early times to a steady-state value of 3 at 12 hr. Lean tissue-to-blood concentration ratios remained near unity during the study period; however, due to its mass, lean tissue served as a major storage site for PCB at early times. Fat-to-blood ratios rose steadily over the 7-day period and fat became the major depot at later times. Fat levels of PCB were as high as 46% of the total dose and were still 19% at 7 days. After 8 hr, about 80% of unexcreted PCB was in the fat. There was no evidence for saturation of storage or excretion sites as up to a 100-fold increase in dose had little effect on relative distribution of PCB. Pre-treatment of rats with an inducer (phenobarbital) or an inhibitor (SKF-525A) of hepatic microsomal enzymes had marked effects on the distribution pattern of PCB in all tissues 24 hr after dosing, particularly, in the fat. The results indicate that liver and lean tissue are the major sites of PCB clearance from blood at early times after dosing and storage in fat is the major determinant of PCB persistence in the body.

34. *Metabolism and Excretion of 2,4,5,2',5'-Pentachlorobiphenyl (PCB) in the Male Rat.* P. R. CHEN and H. B. MATTHEWS, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. (J. R. Fouts).

The metabolism and excretion of [^{14}C]PCB in male rats was followed as part of a pharmacokinetic study. After iv doses of 0.6 or 6.0 mg/kg, excretion of PCB occurred primarily in the feces of rats with about 50% excreted during the first 3 days after dosing. Another 12% was excreted in feces during the next 4 days. About 5% of the dose of PCB was excreted in the urine during the first 24 hr with only an additional 1% excreted thereafter. Analysis of radioactivity excreted in the feces revealed the presence of 2 metabolites as well as significant amounts of parent PCB. Both metabolites were isolated and analyzed by chemical ionization mass spectroscopy (MS). The data obtained from MS indicated the major metabolite was a monohydroxylated-PCB and the minor metabolite was a dihydroxy analog. NMR studies are in progress to determine the positions of the hydroxyl groups. Analysis of urinary radioactivity revealed the presence of only 1 metabolite, a glucuronide conjugate. That metabolism is closely coupled to excretion is suggested by the fact that no metabolites of PCB were found in liver and other tissues. The size of the dose apparently had no effect on the rate of excretion. During the first 24 hr, the rate of excretion of PCB and metabolites in the feces was markedly increased by animal pretreatment with an inducer, phenobarbital, and decreased by an inhibitor, SKF-525A, of the hepatic microsomal mixed-function oxidases. In conclusion, PCB is excreted primarily via the biliary route in rats. Excretion occurs both in the form of hydroxylated metabolites and as unchanged PCB, and the excretion rate is sensitive to the effects of an inducer or inhibitor of the hepatic microsomal hydroxylating enzymes.

35. *Studies of the Mixed Function Oxidase Catalyzed Metabolism of Carbon Disulfide.* R. DALVI, E. POORE and R. A. NEAL, Center in Environmental Toxicology, Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee.

Carbon disulfide administered in vivo to animals pretreated with phenobarbital produces a centrolobular hepatic necrosis with an accompanying marked decrease in the concentration of

cytochrome *P*-450 in the hepatic microsomes. Previous investigations in this laboratory had indicated that in the metabolism of parathion to paraoxon a reactive form of sulfur was released which became covalently bound to the microsomal membrane and decreased the concentration of cytochrome *P*-450 in the microsomes. In the present investigations ^{35}S-labeled carbon disulfide was incubated with rat liver microsomes and the microsomes examined for covalently bound sulfur. The results of these experiments have indicated that sulfur is bound to microsomes incubated with ^{35}S-labeled carbon disulfide. The amount of sulfur (nmol/mg protein) bound to microsomes isolated from rats pretreated with phenobarbital was 5–7 times greater than that bound to microsomes isolated from untreated animals. In addition, the amount of sulfur bound to microsomes from phenobarbital-treated rats was about 7 times greater in the presence as compared to the absence of NADPH. The amount of sulfur bound was also markedly decreased when the incubation was carried out in an atmosphere enriched with carbon monoxide. When microsomes isolated from phenobarbital-treated rats were incubated with ^{35}S-labeled carbon disulfide or ^{14}C-labeled carbon disulfide in the presence of NADPH, approximately 5 times more ^{35}S than ^{14}C was found to be bound to the microsomes. These results indicated the majority of the sulfur bound to the microsomes was in a form free of the carbon atom of carbon disulfide. The results of these studies suggested that carbon disulfide is metabolized by the mixed function oxidase enzyme system to carbonyl sulfide (COS) with the release of a reactive form of elemental sulfur. Mass spectrographic examination of an incubation which contained hepatic microsomes, carbon disulfide, and NADPH revealed the presence of carbonyl sulfide in the atmosphere over the incubation mixture. No carbonyl sulfide was detected in the atmosphere over an incubation which had contained microsomes, carbon disulfide but no NADPH.

36. *GC-MS Studies of the Metabolism of Safrole, an Hepatocarcinogen, in the Rat and Guinea Pig.* M. G. HORNING, L. BELL, M. J. CARMAN and W. G. STILLWELL, Baylor College of Medicine, Houston, Texas. (G. L. Plaa.)

The metabolism of safrole (4-allyl-l,2-methylenedioxybenzene), an hepatocarcinogen in the rat, was studied in the rat and guinea pig using GC-MS procedures. Two metabolites formed by the epoxide-diol pathway were identified in rat and guinea pig urine: a diol, (1,2-methylenedioxy-4-(2′,3′-dihydroxypropyl)-benzene), and a catechol diol, (1,2-dihydroxy-4-(2′,3′-dihydroxypropyl)-benzene). When synthetic safrole epoxide was administered to rats and guinea pigs, the major metabolites excreted in the urine of both species were the diol and the catechol diol; a small amount of a triol (1,2-methylenedioxy-4-(1′,2′,3′-trihydroxypropyl)-benzene), was also detected in rat urine. This supports the conclusion that these metabolities were formed from safrole by way of an epoxide. Unchanged safrole epoxide was also detected in the urine of both species. The presence of safrole epoxide in urine indicates that the epoxide is sufficiently stable in vivo to be circulated in the blood, and is also reasonably stable in urine. Epoxides are known to be cytotoxic, mutagenic, carcinogenic, or teratogenic substances. Their formation from drugs in common use, or from other materials which may be ingested by humans, should be regarded as a potentially hazardous circumstance. The hepatocarcinogen generated from safrole is probably an epoxide, and a possible structure for the active agent is 1,2-methylenedioxy-4-(1′-hydroxy-2′,3′-epoxypropyl)-benzene (the precursor of the triol isolated from rat urine after administration of safrole epoxide).

37. *Metabolism of Aflatoxin B_1 by an Intact and Reconstituted Rat Liver Microsomal Enzyme System.* LATA S. NERURKAR and T. COLIN CAMPBELL, Department of Biochemistry and Nutrition, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

The metabolism of aflatoxin B_1 (AFB_1), a potent hepatocarcinogen and hepatotoxin, was investigated using an intact and reconstituted microsomal system in the presence of an NADPH-regenerating system. The reconstituted system consisted of 3 partially purified microsomal fractions, i.e. cytochrome *P*-450, cytochrome *c* reductase and lipid which were isolated according to the methods described by Lu *et al.* (*JBC* **247**, 1727–1734, 1972; *Mol. Pharmacol.* **8**, 490–500, 1972; *JBC* **243**, 1331–1332, 1968), respectively. Approximately 40%

of the AFB_1 was consumed by the system, with 1% accounted for by AFM_1, AFP_1, and AFB_{2a}; 2.1% as an unidentified chloroform extractable material; and 36.6% as material unextractable with chloroform. Sixty-seven percent of the added label ($[^3H]AFB_1$) was found in the microsomal pellet when intact microsomes were used in the reaction. DEAE-cellulose chromatography of the solubilized $[^3H]AFB_1$-treated microsomal pellet indicated the presence of 3 radioactivity peaks, only 1 of which coincided with a combined peak for cytochrome *P*-450 and cytochrome c reductase activities. Additional fractions were prepared and independently contained cytochrome *P*-450 and cytochrome *c* reductase. The ^3H-radiolabel was essentially unextractable with chloroform and indicated rather light binding of AFB_1 or its metabolites to the microsomes.

38. *Cyclization and N-Dealkylation of Chlorguanide by Rabbit and Rat Hepatic Microsomes.* V. L. ARMSTRONG and CARL C. SMITH, The University of Cincinnati College of Pharmacy and College of Medicine, Department of Environmental Health, Cincinnati, Ohio.

Chlorguanide (CG) has been shown to be cyclized to chlorguanide triazine (CGT) and *p*-chlorophenylbiguanide (PBG) after administration to man, monkeys and rabbits. Rats, however, apparently N-dealkylate CG, but convert negligible amounts to CGT. The purposes of these studies were (1) to isolate the enzyme system(s) which metabolized CG, (2) to characterize the enzyme involved in the cyclization of CG, (3) to determine whether the same enzyme system accomplished the N-dealkylation, and (4) to elucidate the basis for species differences in the metabolism of CG. Initial studies involved slices, homogenates and subcellular fractions of the hepatic tissue of male, white, New Zealand rabbits. It was determined that CG was metabolized by the hepatic microsomal fraction and that maximal conversion to CGT and PBG required the presence of NADPH and oxygen. The biotransformation of CG was inhibited by the microsomal mixed function oxidase inhibitors SKF 525-A, Lilly DPEA and carbon monoxide. Pretreatment of rabbits with phenobarbital (PB) enhanced the formation of CGT and PBG. However, while the direction of effect of the inhibitors and inducer was the same for formation of CGT and PBG, the degree of effect differed. Also pretreatment with 3-methylcholanthrene (3-MC) enhanced the formation of CGT while the amount of PBG formed was unchanged or decreased. Using hepatic microsomes from male, Sprague-Dawley rats, it was shown that only minimal amounts of CG were converted to CGT, while considerable N-dealkylation occurred. Pretreatment of the rats with 3-MC resulted in a significant increase in cyclization without altering N-dealkylation. Our data suggest that the metabolism of CG was accomplished by the hepatic microsomal mixed function oxidase system. Also, N-dealkylation of CG probably involved cytochrome *P*-450, while cyclization to CGT required cytochrome *P*-448.

39. *Mechanism of Dose Threshold for Furosemide Hepatotoxicity.* M. WEIHE, W. Z. POTTER, W. L. NELSON, D. J. JOLLOW and J. R. MITCHELL, National Heart and Lung Institute, Bethesda, Maryland. (J. R. Gillette.)

We have recently reported that the commonly prescribed diuretic furosemide produces acute dose-dependent hepatic necrosis in mice. An active metabolite of furosemide appeared to be responsible for the necrosis. Studies on the *in vitro* covalent binding of furosemide to hepatic microsomal protein showed that the formation of an alkylating metabolite of furosemide to hepatic microsomal protein showed that the formation of an alkylating metabolite of furosemide was mediated by a cytochrome *P*-450 mixed function oxidase. In vivo covalent binding of furosemide to hepatic protein correlated with the severity of liver necrosis and preceded both the onset of necrosis and the onset of biochemical changes. We concluded that the covalent binding of a furosemide metabolite to liver macromolecules might be the cause of furosemide-induced liver necrosis. There was a dose threshold below which no covalent binding or liver necrosis could be detected. Unlike the previously described dose threshold for acetaminophen-induced hepatic necrosis, the furosemide threshold is apparently not due to a protective role of glutathione. We found instead that as one increases the dose, the concentration of furosemide in the liver increases in a strikingly disproportionate fashion. Both

covalent binding of the toxic metabolite and total metabolism of the drug increase directly as a function of the concentration of furosemide in the liver. Thus the threshold is an extrahepatic phenomenon. Greater than 90% of furosemide was bound reversibly to organic anion binding sites on plasma proteins at non-toxic doses. But, after toxic doses, furosemide plasma binding sites were saturated, as revealed by Scatchard plots. Therefore, the lack of toxicity of furosemide in patients after normal therapeutic doses presumably results from the tight binding of this organic acid to plasma proteins.

40. *Interaction of Nitrofurazone with Hepatic and Extrahepatic Tissues from Mice and Rats.* B. STRIPP, R. H. MENARD and J. R. GILLETTE, National Heart and Lung Institute, National Institutes of Health Bethesda, Maryland.

Nitrofurazone (NF), which is a widely used topical antibacterial agent can like other nitrofurans be reduced by xanthine oxidase, aldehyde oxidase and cytochrome *c* reductase. Reduction of the nitro group can lead to the formation of highly reactive N-hydroxylamines which have been implicated in carcinogenesis and cytotoxicity. Indeed, NF has been shown to induce mammary tumors in rats (Morris *et al.*, *Cancer Res.* **29**, 2145, 1969) and to cause spermatogenic arrest in rats and mice. After administration of [^{14}C]NF to mice we found covalently bound radioactivity in protein macromolecules of liver, kidney, mammary tissue, testes and blood. The NF-tissue interaction presumably involves labile SH groups since the amount of covalently bound radioactivity could be enhanced when the animals were pretreated with diethylmaleate. Moreover, administration of NF depleted the hepatic glutathion (GSH) content by 50% in 2 hr. Besides inhibiting spermatogenesis, NF administration also causes a loss of testicular cytochrome *P*-450. Furthermore, in vitro incubation with isolated testes microsomes and NF under conditions which were optimal for nitro reduction resulted in a loss of cytochrome *P*-450. The results suggest that a reactive metabolic intermediate may be formed extrahepatically at the site of toxicity as well as in the liver.

41. *The Influence of Maternally-Administered Phenobarbital on the Kinetic Parameters and Development of Hepatic Drug-Metabolizing Enzymes in the Perinatal Rat.* J. U. BELL and D. J. ECOBICHON, Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada.

The kinetic parameters K_m and V_{max} provide a means of characterizing the functional ability of an enzyme. The development of these parameters was studied in perinatal rats for 4 functionally-diverse hepatic enzymes (*O*-demethylase, nitro-reductase, carboxylesterase and bromosulfophthalein-glutathione conjugase), the period studied being from 3 days prepartum to 35 days postpartum. Significant changes were observed in both K_m and V_{max} for each enzyme during development. The transfer of phenobarbital (PB) across the placenta or in the milk and the influence on the kinetic parameters of the above enzymes in perinates was investigated by administering PB (75 mg/kg/day × 3 days, po) to pregnant and lactating dams, the pups being killed 24 hr after the last dose. Pups were killed at 3 days prepartum, at term and at 4, 7, 14, 21 and 28 days after birth. In an extended experiment, lactating rats were given PB (50 mg/kg/day, po) from parturition until the pups were 21 days old. Marked induction of enzymatic activity were observed postnatally, the increases appearing to be related to the maturity of the liver. Little effect was observed in animals 3 days before birth or at term, suggesting that the immature liver was not responding to the inducing agent.

42. *Hepatic and Extrahepatic Glucuronyltransferase and β-Glucuronidase.* B. R. SONAWANE and G. W. LUCIER, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. (R. L. Dixon.)

Comparative activities of microsomal glucuronyltransferase and β-glucuronidase were investigated in liver, lung, kidney and small intestine of guinea pigs, rats and rabbits. The glucuronyltransferase conjugating *p*-nitrophenol in guinea pigs was most active in liver followed by kidney, lung and small intestine. Female guinea pig liver glucuronyltransferase was 50% more active than the male enzyme. In male rats and rabbits a similar pattern of activity was observed except that enzyme from small intestines was more active than that from

kidneys and lungs of rabbits. Microsomal testosterone glucuronyltransferase was primarily detected in liver microsomes with only very slight activities detected in kidney, small intestine and lung microsomes. The formation of glucuronides of *p*-nitrophenol and testosterone was verified by co-chromatography on DEAE cellulose columns. Optimum concentrations of Triton X-100 added to guinea pig microsomal suspensions increased the conjugation of *p*-nitrophenol by approximately 6-fold in liver, 20-fold in kidney, 3-fold in lung and 10-fold in small intestine. Mg^{2+} enhanced the activity of glucuronyltransferase in all the tissues of guinea pigs with the highest increase observed in lungs. *β*-Glucuronidase hydrolysis of *p*-nitrophenyl *β*-D-glucuronide was also followed in microsomal fractions of liver, lung, kidney and small intestine of guinea pigs, rats and rabbits. *β*-Glucuronidase activity was greatest in liver microsomes of rats, followed by rabbits and guinea pigs. Kidney microsomes exhibited lowest activity in all the animals studied. Perinatal developmental patterns of glucuronyltransferase and *β*-glucuronidase were investigated in guinea pig liver, lung, intestine, kidney and placenta.

43. *Comparative Inductive Effects of Phenobarbital and Endrin on Liver Microsomal Activity in Endrin-Resistant and Susceptible Pine Voles.* R. W. HARTGROVE, JR., S. G. HUNDLEY and R. E. WEBB, Department of Human Nutrition and Foods, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Previous studies in this laboratory have shown a correlation between the relative toxicity of endrin and hepatic microsomal mixed function oxidase (MFO) activity in endrin-susceptible and resistant pine voles. Endrin-resistant voles have been shown to have a greater capacity to metabolize benzpyrene and this capacity is heritable at least through the first generation. The resistance mechanism must be viewed as a complex phenomena involving a genetic and an inductive aspect. This paper presents current findings on the relative inducibility of the hepatic mixed function oxidase system in the two strains. Voles were injected ip 3 consecutive days with (1) 50 mg/kg phenobarbital (PB), or (2) 1 mg/kg endrin in Tween 80 : saline (20: 80) carrier, or (3) a sham of Tween 80 : saline (20 : 80). Another group of resistant voles were treated with a dose that was equatoxic to susceptible voles. The voles were sacrificed 24 hr after the last treatment and MFO activity was determined by ethylmorphine (EM)-N-demethylation, aniline hydroxylation and *P*-450 content estimations. Induction with PB causes an increase in microsomal activity in both strains to an equal degree with both model substrate assays, as well as a marked increase in *P*-450 content above those values obtained for the sham injected controls. Endrin as it is administered in all these studies appears to cause no increase in microsomal MFO activity in either strain using the EM model substrate. *P*-450 content is decreased considerably, as is the proportion of the body weight represented by liver, as endrin is administered. The data for aniline, a type II substrate, hydroxylation does not in all cases follow the trends exhibited for EM N-demethylation. These results on first impression suggest that endrin does not induce microsomal activity.

44. *An Integrated Computer System for Large-Scale Chronic Carcinogenic Bioassays.* L. R. LAWRENCE, M. F. CRANMER and A. J. KONVICKA, National Center for Toxicological Research, Jefferson, Arkansas.

A computer system has been developed for the collection, storage, and retrieval of scientific data generated by large-scale chronic carcinogenic bioassays. Experimental data are collected via use of data collection terminals controlled by MODCOMP II/25 mini-computers. Data collected includes periodic feed consumption, animal weights, general health observations, mortality and animal disposition. These terminals, designed to NCTR specifications, consist of a photoelectric card reader for cage identification, a keyboard for animal identification and health observations, an electronic balance for feed consumption and animal weights, and a cathode-ray tube receiver for visual verification of all data collected. The resultant data base is transmitted daily via telecommunications from the mini-computers to an IBM 370/155 for storage on magnetic disk. All data can be accessed in native mode format (through NCTR I/O System) by most commonly-used, high-level languages including FORTRAN and PL/I to facilitate analysis of experimental data utilizing readily available statistical systems. The

system provides daily reports to managers and investigators summarizing current experiment status, thus facilitating more efficient conduct of such experiments. In addition, results of pathological examinations (gross and microscopic), hematological examination and genetic history data are collected.

45. *Induction of Tumors in Non-Human Primates with Various Chemical Carcinogens.* R. H. ADAMSON, P. CORREA and D. W. DALGARD, National Cancer Institute, Bethesda, Maryland, and Hazleton Laboratories, Vienna, Virginia.

Various chemicals which are carcinogenic in rodents have been evaluated for their carcinogenic potential in non-human primates, primarily rhesus and cynomolgus monkeys. Among carcinogens evaluated were various polycyclic hydrocarbons, fluorenylacetamides, azo dyes, urethane, various nitroso compounds, aflatoxin B_1, cycasin and its aglycone and procarbazine. Liver tumors have been induced by ip and/or oral N-nitrosodiethylamine (DENA), l-nitrosopiperidine, N-nitrosodipropylamine, aflatoxin B_1 and cycasin. Leukemias and lymphomas have been induced following administration of procarbazine and squamous cell carcinoma of the oral cavity and esophagus has been induced by oral administration of methylnitrosourea. DENA is the most potent liver carcinogen thus far tested. After ip administration of DENA every other week to newborn rhesus monkeys, 100% of the monkeys developed tumors within 10–15 months. All of the monkeys developed a significant elevation in alpha-fetoprotein levels 3–6 months in advance of gross or histologic evidence of tumors. Thus the non-human primates are good models for evaluating potential carcinogens and for developing biological markers for detecting preneoplastic changes as well as frank neoplasia.

46. *Mouse Dermal Study of Smoke Condensate from Chemosol-Treated Cigarettes.* J. L. GARGUS, J. B. SULLIVAN, R. T. HABERMANN, J. COPELAND and J. R. EVERLY, Hazleton Laboratories, Inc., Vienna, Virginia.

Canadian and German patent documents describe "Chemosol" (citric acid and deuterium oxide in distilled water) and its chemical and biological effects on cigarette tobacco. This study was designed to compare smoke condensates from Chemosol-treated and untreated cigarettes. Non-filter, 85-mm test cigarettes were fabricated from Chemosol-treated blend of flue cured Burley, Oriental and Maryland tobacco. Control cigarettes were made of the same blend. Test and control cigarettes were number code identified during the study. Borgwaldt smoking machines employing standard smoking procedures were utilized. Nicotine determinations averaged 77.6 mg/g of Chemosol-treated smoke condensate and 76.6 mg/g of control condensate. The benzo[a]pyrene value was 0.75 ng/mg in Chemosol-treated condensate and 0.76 ng/mg in control condensate. ICR mice received 3 weekly dermal applications of 0.1 ml acetone (control), 20 γ benzo[a]pyrene (positive control), and 25 mg of control or Chemosol-treated whole smoke condensate. No skin tumors were observed in 100 negative control or 200 vehicle control mice; 97 of 100 positive control mice developed tumors with a median latent period of 162 days; 105 of 200 control condensate mice developed 168 skin tumors (average of 1.6/tumor-bearing animal) with a median latent period 449 days. The 81 tumors were diagnosed as 54 carcinomas, 23 papillomas, 2 adenomas, 1 fibrosarcoma and 1 mast cell sarcoma. In Chemosol-treated condensate animals 106 of 200 developed 165 tumors (average 1.5/tumor-bearing animal) with a median latent period 351 days. The 88 tumors were diagnosed as 58 carcinomas, 18 papillomas, 1 adenoma, 10 fibrosarcomas and 1 hemangiosarcoma. Chemical and biological parameters investigated were comparable for untreated and Chemosol-treated tobacco smoke condensates.

47. *Distribution of Metabolites of Benzo[a]pyrene in the Isolated Perfused Rabbit Lung Preparation.* RICHARD W. NIEMEIER and EULA BINGHAM, University of Cincinnati, College of Medicine, Kettering Laboratory, Cincinnati, Ohio.

Epidemiological studies have shown a relationship between the levels of polycyclic aromatic hydrocarbons in the air and incidence of human bronchogenic carcinomas. However, few data are available to describe the ability of the lungs to cope with these compounds, i.e. absorption, excretion, binding, metabolic rates and patterns. An isolated perfused lung preparation

was developed for such studies. ^{14}C-Labeled benzo[a]pyrene (BaP) was administered to the isolated perfused rabbit lung preparation via the trachea. Estimations of metabolic pattern and amounts, as well as uptake of the parent compound and binding were made after solvent extraction, thin layer chromatography and liquid scintillation counting of various tissues. These tissues included blood, lung lavage fluid and the lung tissues. Studies suggested that 45% of the total metabolite produced by the lungs appeared in the blood after 3 hr of perfusion. Besides BaP, the metabolites recovered from the lung preparation included the 3,6-dione, the 3-hydroxy, two dihydrodiols, and epoxide and 2 or more very polar compounds. The 3-hydroxy, 3,6-dione combined represented only a fraction (25–30%) of the total metabolites appearing in the blood.

48. *The Effects of Benzo[a]pyrene and 3-Methylcholanthrene on Mouse Lungs.* WILLIAM HO and ARTHUR FURST, Institute of Chemical Biology, University of San Francisco, San Francisco, California.

The two carcinogenic hydrocarbons, benzo[a]pyrene (BP) and 3-methylcholanthrene (3-MCA), are often considered as alike in tumorogenic properties because of their similarity in chemical structure. While both are synthetically produced for commercial purposes, the former is usually associated with incomplete combustion of organic materials and some petroleum products; the latter is suggested to be a possible byproduct of abnormal cholic acid metabolism. We now report the differences in the physiologic responses, the clearance rates, and the carcinogenic potentials of these two aromatic compounds in NIH-Swiss mouse lungs. When BP and 3-MCA were administered to 2 respective groups of mice by the intratracheal instillation technique, 3-MCA was eliminated from the lungs at a slower rate. The higher mortalities and greater body weight loss in the 3-MCA animals suggested that this chemical is more toxic than BP. In addition, alveologenic squamous cell metaplasia and carcinoma were detected in 3-MCA-treated lungs, while BP induced only adenoma. The tumor incidence was higher and the neoplasm appeared sooner in the 3-MCA experimental animals. (Supported by the Council for Tobacco Research-USA, Inc.)

49. *Influence of a Polychlorinated Biphenol (PCB) on Biochemical Effects and Metabolism of Aflatoxin.* EDMUND L. PETERS, LEONARD FRIEDMAN and KATHERINE G. DAVIS, Food and Drug Administration, Washington, D.C.

PCB, a known stimulator of microsomal enzyme activity (*Toxicol. Appl. Pharmacol.* **23**, 112, 1972), was examined for its effects on the in vivo and in vitro inhibition of liver RNA synthesis by aflatoxin B_1 (B_1). The in vitro metabolism of B_1 by liver preparations from control and PCB-fed rats was also examined. Aroclor 1254 was fed in the diet at 25 and 100 ppm to male and female Osborne-Mendel adult rats for 60 days. This treatment produced liver enlargement and fatty liver but little or no increase in plasma SGOT or SGPT. In control rats, B_1 (0.50 mg/kg) inhibited incorporation of [^{14}C] orotic acid into liver RNA by 30.8 and 65.3% in females and males, respectively. However, inhibition was reduced to 0.0 and 27.3% in female and male rats fed 25 ppm PCB. Dietary PCB also abolished the 27.3% inhibition of protein synthesis produced by B_1 in male rats. Both levels of PCB stimulated the conversion of B_1 to aflatoxin M_1 by approximately 50-fold (while stimulating the metabolism of aminopyrine by only 2- to 3-fold). Inhibition of RNA synthesis in liver slices exposed to B_1 in vitro was also reduced by a similar PCB treatment but to a much smaller degree (65.6 vs 50.0%). Apparently, dietary PCB treatment of rats stimulated the conversion of B_1 to inactive or less active metabolites.

50. *Studies on the Excretion of Aflatoxin and Its Derivatives by Humans.* A. H. MERRILL, JR., L. SALAMAT and T. C. CAMPBELL, Department of Biochemistry and Nutrition, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, and Food and Nutrition Research Center, Manila, Philippines.

The human liver is capable of metabolizing aflatoxin (AF) B_1 in vitro to AFM_1 and AFP_1 (Merrill and Campbell, in press); however, only AFB_1 (Shank *et al.*, 1971) and AFM_1 (Campbell *et al.*, 1970) have been reported in human urine. To determine if humans excrete AFP_1 as

glucuronide and/or sulfate conjugates, urine from children known to have consumed AFB_1-contaminated peanut butter was treated with β-glucuronidase and sulfatase. Subsequent chloroform extracts failed to yield any fluorescent spots corresponding to standard AFB_1, AFM_1, AFP_1 or AFB_{2a} on thin layer chromatography. Four-hr hydrolysis of the samples with 0.2 N HCl also failed to yield either fluorescent spots corresponding to the standards or the acid-catalyzed water adducts of AFM_1 or AFP_1. The only fluorescence of interest was a bright blue spot with an R_f similar, but not equal to, AFB_2 which appeared in the urine both before and after consumption of the contaminated peanut butter; however, the fluorescent intensity after consumption was greater than before consumption. This spot is not aflatoxicol; however, it has not been ruled out as an aflatoxin derivative.

51. *Preclinical Toxicity of Cyclocytidine in Dogs and Monkeys.* JAIME L. SANYER and CHENG-CHUN LEE. Pharmacology and Toxicology, Midwest Research Institute, Kansas City, Missouri.

The toxicity of 2-2′-*o*-cyclocytidine (NSC-145668), a pyrimidine nucleoside reported having remarkable antitumor activity, low toxicity and resistant to serum pyrimidine deaminase in contrast with other compounds of this series, was studied in 28 beagle dogs and 12 rhesus monkeys. A single dose or 5 daily doses of 10 mg/kg (iv) were tolerated by dogs and 5 daily doses of 20 mg/kg were tolerated by monkeys. In dogs, a single dose of 640 mg/kg caused death in 4 days, and 5 daily doses of 160 mg/kg caused death in 12 days. In monkeys, 5 daily doses of 320 mg/kg caused death in 6 days. The toxicity of the compound was dose related. Toxic signs included severe emesis, anorexia, weight loss, miosis and bloody diarrhea (in dogs only). Severe leukopenia and elevation of transaminases occurred. Tissue lesions included necrosis and/or hyaline degeneration of the myocardium, inflammatory and destructive changes of the gastrointestinal tract, atrophy of lymphoid tissue and depression of the bone marrow, indicating that the target organs were the myocardium and those tissues with high rate of cell division. (Supported by NCI Contract No. NIH-71-2263.)

52. *Metabolism of 6-Aminochrysene in the Rat.* PRESTON H. GRANTHAM, GIAO NGUYEN BA, LETITIA C. MOHAN, ELIZABETH K. WEISBURGER, NG. PH. BUU-HOI and ROMEO RONCUCCI, National Cancer Institute, Bethesda, Maryland, and Continental Pharma, Brussels, Belgium.

6-Aminochrysene is an experimental carcinostatic agent for treatment of breast cancer or leukemia and for the splenomegaly of malaria. In view of no data on the metabolism of this drug, 6-aminochrysene-5,6-^{14}C was synthesized to use for such studies. After a single ip dose of the labeled 6-aminochrysene, only 3% appeared in the urine by 24 hr. At 10 days after injection 12% was in urine while 55% was in the feces. Of all organs surveyed, the liver and kidney had the highest radioactivity. Curiously, despite ip administration, the isotope level of the intestinal contents and especially the cecal contents was considerable. Separation of urinary metabolites showed that 9% of urine-^{14}C was present as free compounds, 23% as glucuronic acid conjugates, and 31% as sulfate conjugates while a considerable fraction 35%, was in the water-soluble fraction. In a 48-hr period, 42% of the dose was carried in the bile; the main biliary metabolite was the *N*-glucuronide of 6-aminochrysene. In contrast, the fraction of fecal material which was soluble in 80% ethanol yielded 44% of the fecal-^{14}C as free metabolites, 0.5% as glucuronides, 2.6% as sulfates and only 6% as water-soluble metabolites. 6-Aminochrysene was identified as a fecal metabolite. Although neither 12-hydroxy nor *N*-hydroxy-6-acetylaminochrysene could be identified, an unknown hydroxy-6-acetylaminochrysene metabolite was present in the urine and the feces. A parallel study with 6-aminochrysene-12-^{3}H showed substantial differences in distribution of isotope, indicating that exchange of the isotope or removal had occurred.

53. *Single- and Repeated-Dose Toxicity of 3-Tritylthio-L-Alanine Clinical Formulation (NSC 83265) Administered in Dogs and Monkeys by the Intravenous Route.* P. E. PALM, M. W. ROHOVSKY, E. P. ARNOLD, P. C. RACHWALL and M. S. NICK, Arthur D. Little, Inc., Cambridge, Massachusetts.

Twenty-two beagle dogs and 24 rhesus monkeys (1 male, 1 female/level) were administered the cancer chemotherapeutic agent 3-tritylthio-L-alanine, clinical formulation, by the iv

route at levels of 6.25, 12.5, 25, 50, 100 and 200 mg/kg, and 0.78, 1.56, 3.12, 6.25, 12.5, 25, 50 and 100 mg/kg respectively. Dosage regimens of ×1, ×5 and ×5(9)5(9)5, comparable to those to be used clinically, were employed with dogs, and ×1 and ×5 with monkeys. Death occurred in dogs after doses of 200 mg/kg (3.86 g/m^2) ×1, 50 mg/kg (0.98 g/m^2) ×4 and 25 mg/kg (0.50 g/m^2) × 5(9)5, and in monkeys after 100 mg/kg (1.20 g/m^2) ×1 and 50 mg/kg (0.63 g/m^2) ×3 and 4. The highest non-toxic doses were 25 mg/kg ×1, 6.25 mg/kg ×5 and ×5(9)5(9)5 in dogs, and 6.25 mg/kg ×1 and 0.78 mg/kg ×5 in monkeys. Clinical signs of marked toxicity in dogs included loss of consciousness, no response to pain stimuli 1 min after injection, incontinence, bradycardia, apnea, cardiac arrhythmias with ventricular fibrillation and death by cardiac arrest about 8 min after dosing. Liver involvement, indicated clinically by marked elevations in SGPT, SGOT and alkaline phosphatase, was noted in dogs receiving ≧ 50 mg/kg ×1 and ≧25 mg/kg ×5 or ×5(9)5(9)5. In monkeys, similar changes in enzyme activities were noted at lower levels (≧ 12.5 mg/kg ×1 and ≧ 3.12 mg/kg ×5). A nephrotoxic effect was also indicated in monkeys by marked increases in BUN and creatinine. At autopsy the high-dose monkeys were jaundiced and had hemorrhagic lesions in the mucosa of the urinary tract. Microscopic examination of tissues revealed liver damage, primarily centrilobular albuminous degeneration, at 100 mg/kg ×1 and ≧12.5 mg/kg × 5 in dogs and monkeys, and at 25 mg/kg ×5(9)5(9)5 in dogs. Bone marrow hypoplasia together with lymphocytic depletion of the splenic follicles was generally noted at the higher dosage levels in both species. While minimal kidney pathology was observed in dogs, monkeys which received 100 mg/kg ×1 and 50 mg/kg ×5 showed ulceration and necrosis of the transitional epithelium of the kidneys and ureters, and suppurative cystitis. (Supported by Contract NO1-CM-33727, Laboratory of Toxicology, Chemotherapy Program, National Cancer Institute.)

54. *Mutagenic Activity of Alkylating Agents as a Function of Number of Doses and Route of Administration.* D. W. ARNOLD, G. L. KENNEDY, JR., M. L. KEPLINGER and J. C. CALANDRA, Industrial BIO-TEST Laboratories, Inc., Northbrook, Illinois.

Previous studies have revealed the existence of a dose-response relationship and no-effect levels for methyl methanesulfonate (MMS) when administered as a single ip injection. Additional studies were conducted to investigate the effects of routes of administration and number of doses on the mutagenic activity of MMS. The criterion is the induction of dominant lethal mutations. Male albino mice were treated either via gavage or by ip injection with the compound administered as a single dose or as multiple doses. Mutagenic effects were monitored through early embryonic deaths found in utero among females having mated with treated males. Five consecutive doses of MMS at either 25 or 50 mg/kg gave a greater mutagenic response than did single doses of the same exposure levels. Mutation rates for the first 2 weeks following treatment for animals treated with a single ip injection of 25 mg MMS/kg were 8.9 and 8.3. At 50 mg/kg the mutation rates were 14.4 and 39.1. Animals treated with multiple doses had mutation rates of 24.8 and 21.5 (25 mg/kg) and 91.7 and 36.4 (50 mg/kg) during the first 2 weeks post-treatment. With a single ip injection of 100 mg/kg, mutation rates of about 30 and 35 have been obtained during the same time intervals. Animals given a single oral dose of 150 mg/kg showed mutation rates similar to those treated with a single injection of 50 mg/kg.

55. *Soft Contact Lens Studies in Rabbit Eyes.* FELIX A. DE LA IGLESIA, LORELIE MITCHELL and EDWARD SCHWARTZ, Warner-Lambert Research Institute of Canada, Sheridan Park, Mississauga, Ontario, Canada and Warner-Lambert Research Institute, Morris Plains, New Jersey.

The series of studies described here were aimed at analyzing the potential adverse effects of soft contact lenses (SOFTCON ®) when placed on the rabbit eye. SOFTCON contact lenses are composed of vifilcon A, which is a soft hydrophilic plastic co-polymer of 2-hydroxyethyl methacrylate and povidone, N.F. Lenses employed were obtained from routine inventory stock in their final packaged form and taken at random from different lots. Thus lens diameter base curvature and power differed. Lenses were fitted to each animal individually and the

contralateral eye served as control. In a 7-day study, lenses were kept in the eye for 24 hr and several solutions (anticholinergic, adrenergic, steroid, antibiotic and saline) were instilled daily. Lenses were kept for 8 hr in a second 7-day study and various OTC preparations were used. In 21-day studies, lenses were applied for 8 hr to groups of animals with and without induced eye irritation. Ophthalmoscopic examinations, including biomicroscopy, were carried out at the beginning and end of each treatment. Daily ocular reactions were graded according to Draize. The corneas were removed under anesthesia and studied for light microscopic and histochemical changes. Histochemistry was combined with densitometry to analyze some aspects of epithelial carbohydrate metabolism. The results showed that prolonged application of soft contact lenses failed to induce significant ophthalmologic changes, associated epithelial abnormalities or profound variations in corneal histochemical reactivity.

56. *Ototoxic Interaction Between Aminoglycoside Antibiotics and Diuretics.* Robert E. BRUMMETT, BUD WEST, JACK TRAYNOR and NEAL MANOR, Kresge Hearing Research Laboratory, Portland, Oregon.

Patients who have received both an aminoglycoside antibiotic and ethacrynic acid have been reported to experience a severe and permanent loss of hearing. This effect is produced by doses of both drugs that would ordinarily not produce a permanent hearing deficit. This clinical observation has been verified in the guinea pig for kanamycin and ethacrynic acid (West, Brummett, Himes, 1973).

This study was designed to determine if other aminoglycoside antibiotics as well as other diuretics were similarly capable of inducing this ototoxic interaction. Groups of guinea pigs were initially given a single sc dose of either kanamycin (400 mg/kg) or gentamicin (150 mg/kg). Two hr later they were given a subsequent single iv dose of either furosemide (50, 60, 70, 100 mg/kg); hydrodiuril (200 mg/kg); mercuhydrin (50 mg/kg); or mannitol (2500 mg/kg). All animals were maintained in the animal quarters for 30 days after drug administration. An evaluation of the drug effects on the ear was made by measuring the cochlea's ability to generate the a.c. cochlear potential in response to sound. Control sensitivity and intensity functions of the a.c. cochlear potential were obtained before the administration of either drug. They were again obtained 30 days later. Histological verification of cochlear damage was obtained by determining the number of missing hair cells in Corti's organ in all turns of the cochlea. The data indicate that an ototoxic interaction occurs with the aminoglycoside antibiotic gentamicin as well as kanamycin. Furthermore, it occurs with the diuretic furosemide but not with hydrodiuril, mercuhydrin or mannitol.

57. *Acute and Subacute Toxicity Evaluation of Intravenous Sodium Fluorescein in Mice, Rats and Dogs.* T. MCDONALD, K. KASTEN, R. HERVEY, S. GREGG, C. A. ROBB and A. R. BORGMANN, Alcon Laboratories Inc., Fort Worth, Texas. (O. G. Fitzhugh.)

Intravenous sodium fluorescein is used clinically for angiography. The purpose of this study was to evaluate the acute and subacute toxicity of iv sodium fluorescein. The iv LD50 of sodium fluorescein in mice, rats and dogs was approximately 1000 mg/kg. Albino rats were divided into 5 groups of 16 animals/group and administered at 72-hr intervals sodium fluorescein (400, 100, 20 and 0 mg/kg) with 1 group serving as untreated controls. Normal values were obtained for food and water consumption, hematology, clinical chemistry, urinalyses measurements. Significant changes were noticed on day 28 for increased hemoglobin and hematocrit at the 400-mg/kg dosage. Organ weights and histopathologic observations were normal. Beagle dogs were divided into 5 groups of 8 animals/group and given sodium fluorescein at 48-hr intervals (400, 100, 20 and 0 mg/kg) with 1 group as untreated controls. There were no treatment-related changes for food and water consumption, hematologic, clinical chemistry and urinalyses measurements; organ weights; gross appearance; histopathologic examination; and ocular (biomicroscopic) structures. Treatment-related changes were observed for incidence of salivation and emesis for dosages of 400 and 100 mg/kg with only emesis observed at 20 mg/kg. Increased activities of SGPT were observed on day 28 at 400 and 100 mg/kg, while increased activities of SGOT were noticed on days 14 and 28 for the

400 mg/kg dosage level. Three mortalities were observed at 400 mg/kg. Since the clinical dosage level is 10 mg/kg iv, administered at irregular intervals, sodium fluorescein represents little or no substantial clinical hazard.

58. *Nicotine Antagonists: Studies on ω-(N,N-Diethylamino)-n-Butyl 3,4,5-Trimethoxybenzoate (TMB-4) as an Antagonist of Nicotine.* B. V. RAMA SASTRY, C. P. ROBINSON and C. Y. CHIOU, Departments of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee and University of Florida College of Medicine, Gainesville, Florida.

During our search for specific antagonists of nicotine, we have investigated the antagonism between nicotine and TMB-4 on the isolated guinea pig ileal strip. TMB-4 (10 μM) was very effective for blocking (62%) the nicotine (10 μM) induced contraction of the ileal muscle. To clarify this antagonism we have investigated the potencies of TMB-4 for blocking contractions induced by 1,1-dimethyl-4- phenylpiperazinium (DMPP, 3.0 μM), 5-hydroxytryptamine (5-HT, 1.0 μM), bradykinin (0.03 μM), acetylcholine (ACh, 0.5 μM) and histamine (1.0 μM). ID50 values of TMB-4 for blocking the contractions induced by the above agonists were as follows (μM): DMPP 2.5, ACh 52, 5-HT 78, bradykinin 206, and histamine 253. These observations indicate that TMB-4 was more effective for antagonizing nicotine and DMPP than for antagonizing other agonists. TMB-4 (10^{-4} M) was not effective for blocking barium chloride induced contractions on the guinea pig ileum. In a concentration of 10^{-3} M, it blocked only 40% of the height of contractions induced by barium chloride. The above observations indicate that TMB-4 was more specific for blocking nicotinic agents at a membrane site than affecting calcium-availability for muscle contraction. TMB-4 was more effective for blocking DMPP induced contractions (I50, 2.5 μM) of the guinea pig longitudinal ileal muscle than for blocking the electrical stimulation of the potsganglionic nerves in the Auerback's plexus-longitudinal ileal muscle preparation (I50, 21.0 μM). These observations indicate that the major site of action for TMB-4 was a membrane site at the ganglia or the nerve terminal. (Supported by USPHS Grants NS-04699 and GM-00058.)

59. *The Treatment of Acute Ethylene Glycol Toxicosis with Pyrazole.* ETHARD W. VAN STEE, MICHAEL L. HORTON, ALAN M. HARRIS and KENNETH C. BACK, 6570 Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio.

Rats and dogs have been protected from the potentially lethal effects of the ingestion of large doses of ethylene glycol (EG) with pyrazole (P), a potent inhibitor of liver alcohol dehydrogenase. All rats given 1.35 ml/100 g of EG followed by 2.2 mM/kg ip of P, 6 and 30 hr after EG ingestion, survived. Control rats not treated with P died. The kidneys of the control rats contained large numbers of birefrigent oxalate crystals. The kidneys of the treated rats were free from oxalate crystals at 5 and 12 days post exposure. Dogs were given either 10.0 or 12.5 ml/kg of EG. Treatment was begun 5–6 hr post exposure. The control treatment consisted of $NaHCO_3$ administered iv according to calculated base deficit, B-complex vitamins with ascorbic acid, hydrocortisone, and 5% glucose in water. To this was added 0.9 μM/kg of P, 5–6 hr post exposure, and 0.5 mM/kg of P at 30 hr post exposure. Summary of survivals: 10 ml/kg EG, no P, 2/5; 12.5 ml/kg EG, no P, 1/1; 10 ml/kg EG + P, 9/11; 12.5 mg/kg EG + P, 10/19. The dogs that died had large numbers of oxalate crystals in the kidneys at necropsy. The dogs that survived had very few oxalate crystals in the kidneys by 7 days post exposure. The results suggest that pyrazole, despite its marked toxicity, may be of clinically significant value in the treatment of ethylene glycol poisoning when treatment is initiated within 5–6 hr of exposure.

60. *Five-Year Progress Report on Long-Term Oral Contraceptive Studies in Female Dogs and Monkeys.* F. X. WAZETER, R. G. GEIL, K. M. COOKSON, V. R. BERLINER and J. K. LAMAR, International Research and Development Corporation, Mattawan, Michigan, and Food and Drug Administration, Rockville, Maryland.

The study was designed to assess the long-term effects of certain steroids proposed or used in oral contraceptives in female beagle dogs and rhesus monkeys. The drugs are ethynerone

plus mestranol, Wy 4355 plus mestranol, anagestone acetate plus mestranol, ethynerone and mestranol. The dose levels are 2×, 10× and 25× the human dose in the dog study and 2×, 10× and 50× the human dose for monkeys. The animals, 16 per group, are dosed orally in a continuing cycle of 21 days on drug followed by 7 days off. In dogs, the 3 progestogens plus mestranol have produced the greatest dose-related tumorigenic effect in terms of total numbers of mammary nodules, numbers of affected dogs and nodule size. Ethynerone alone produced fewer but significant nodules, while dogs receiving mestranol alone had no more nodules than controls. In a number of dogs examined at a scheduled sacrifice at the end of 5 years, nodules were diagnosed histologically as lobular hyperplasia, mixed mammary tumor, adenoma and adenocarcinoma. These findings parallel results of examinations of nodules secured at biopsy or from dogs which have died. Alopecia, which was dose-related, occurred in dogs receiving the progestogen-mestranol combinations and mestranol or ethynerone alone. Diabetes mellitus developed in several dogs receiving anagestone acetate or Wy-4355 plus mestranol. Anemia also was produced by anagestone acetate and Wy-4355 plus mestranol in dogs. No significant mammary nodules or clinical signs have been noted in monkeys receiving these steroids. (Supported by Contract FDA 72-128.)

61. *Preclinical Safety Studies of a Synthetic Gonadotrophin Releasing Hormone (LH-RH).* R. D. HEMM, J. J. POLLOCK, L. ARSLANOGLOU and L. AUTHIER, Animal Health Division, Ayerst Laboratories, Chazy, New York.

Since the structural characterization of the natural LH releasing hormone, this polypeptide has been synthesized and is being developed for human use. Although the biological activity of LH-RH has been investigated in great detail in a variety of species, there are few reports of its toxicological potential in animals. To define the toxicologic potential, subacute toxicity studies were conducted in rats and monkeys and teratologic studies were performed using mice, rats and rabbits. Synthetic LH-RH (AY-24,031) was administered iv to male and female Long Evans rats for 28 consecutive days at doses of 5, 25 and 50 μg/kg and was administered sc for 28 days to male and female rhesus monkeys at a daily dose of 500 μg/kg per monkey. In a separate study, AY-24,031 was administered sc at doses of 4, 20 and 100 mg/kg to adult female rhesus monkeys from day 8 through 15 of 3 consecutive menstrual cycles. Conventional physical, clinical laboratory and post-mortem examinations were performed. No alterations occurred in monkeys receiving continuous or intermittent administration of AY-24,031. Slight reduction of prostate and seminal vesicle weight occurred in male rats at all doses, but AY-24,031-treated rats were not otherwise affected. AY-24,031 was administered sc at doses of 4, 50, and 100 μg/kg to pregnant female Long Evans rats and New Zealand white rabbits from gestation day 6 through 15 and 6 through 18 respectively, and at doses of 4, 40 and 100 μg/kg to CRCD rats and CD-1 mice. AY-24,031 administration produced no toxic alterations in the pregnant dam or fetus. It was concluded that in the species tested, AY-24,031 was not toxic for the adult or for the developing fetus.

62. *Toxicology of W-2587 (5-Dimethylaminoacetyl-1,2,3,4,4a,5,6,11a-octahydromorphanthridine Hydrochloride)—A New Anti-hypertensive Agent.* B. N. BANERJEE and N. IVINS, Wallace Laboratories, Cranbury, New Jersey.

W-2587 is a novel tricyclic compound with an octahydromorphanthridine nucleus. It effectively lowers blood pressure in normotensive and hypertensive animals, probably through a central action. Diuresis with saluresis but not kaluresis is an additional property of this compound. The preclinical toxicity of this compound was evaluated in rats, mice, rabbit, dog and monkeys. The oral, ip and iv LD50 values in rats were 1400, 235 and 31 mg/kg respectively. In mice, oral and ip LD50 values were 460 and 172 mg/kg. In the dog the LD50 was 166 mg/kg, and the maximum tolerated dose (MTD) without any toxic signs was 24 mg/kg. The MTD in rhesus monkey was 80 mg/kg. Two-week iv irritation and toxicity studies were conducted in rabbits, dogs and rats in doses up to 10 mg/kg. The compound was administered as a 1% solution in sterile water. No vascular irritation was noted at any dose evaluated. At the highest dose (10 mg/kg), however, 3 of 4 rabbits showed signs of ataxia and convulsions. Thirty-day

oral toxicity studies were conducted in rats and monkeys with does levels of 200, 100 and 50 mg/kg/day for rats and 50, 25 and 10 mg/kg/day for rhesus monkeys. No toxicity was noted in either species except for possible changes in the liver. A significant, dose-dependent, liver weight increase was noted in rats. This was not associated with any gross, histopathological or chemical abnormalities even in the highest dose group (200 mg/kg). Monkeys treated with the high dose of 50 mg/kg showed significant increases in liver weights and SGPT values. However, no histopathological changes were noted. No behavioral or toxicological effects were observed in monkeys that received either 25 or 10 mg/kg daily for 30 days.

63. *The Effect of Propranolol on Myocardial Necrosis Induced by Minoxidil in Dogs.* T. BALAZS, F. EARL and W. BUSEY, Food and Drug Administration, Washington, D.C.

Minoxidil (6-amino-1,2-dihydro-1-hydroxy-2-imino-4-piperidinopyrimidine) is a vasodilator antihypertensive agent, pharmacologically related to hydralazine, which produces reflex tachycardia. Its tendency to cause myocardial hypoxia was detected in clinical studies and prevented by propranolol (Pettinger and Mitchell, *New Engl. J. Med.* **289**, 167–171, 1973). We investigated these effects in dogs. Two beagle dogs were each administered 1, 3, 10 or 30 mg/kg minoxidil orally on 3 consecutive days and were killed 24 hr thereafter. Left ventricular papillary muscle necroses of dose-related severity were found, which were histologically similar to that described for related agents (*Tox. Appl. Pharm.* **20**, 442–445, 1971). This lesion is considered to be the consequence of myocardial hypoxia due to the pharmacologic effects. In a second experiment, 16 dogs were dosed with minoxidil (10 mg/kg) on 2 consecutive days; 8 of these were also dosed with propranolol (3 mg/kg tid orally), concurrently. In a third experiment, 12 dogs were dosed with minoxidil as above; 6 of these received propranolol (3 mg/kg bid) for 16 days prior to but not during the days of dosing with minoxidil. The incidence and/or severity of the lesions was less in the dogs treated with propranolol than in minoxidil controls in both experiments. The difference reached a statistical significance ($p < 0.1$) only in the second experiment. Though propranolol increases the hypotensive effect of minoxidil in dogs as well as in man (Pettinger and Weeton, *Clin Res.* **21**, 472, 1973) it appears to decrease its adverse cardiac effect.

64. *Myocardial Sensitization to Epinephrine by Halothane and Two Halogenated Cyclobutanes.* HAROLD H. BORGSTEDT, Departments of Pharmacology and Anesthesiology, University of Rochester School of Medicine and Dentistry, Rochester, New York.

Myocardial sensitization to epinephrine (Epi) is an important factor in the consideration of halogenated hydrocarbons as potential inhalation anesthetics. Purebred beagle dogs were exposed to 1.25% halothane, 1.5% 1-bromo-1,2,2-trifluorocyclobutane (42M9), and 2.5% 1-chloro-1,2,2-trifluorocyclobutane (22M13) for 1 hr after induction with 15 mg/kg of thiopental sodium and 0.1 mg/kg of atropine given iv. Graded doses of Epi, beginning with 0.25 μg/kg and doubling the dose every 5 min for each successive step, were administered while the ECG was monitored continually. The incidence of premature ventricular contractions, ventricular tachycardias and ventricular fibrillation was scored according to the system of Thompson and Galysh (*Fed. Proc.* **27**, 705, 1968). The mean scores were found to be 21 for halothane, 26 for 42M9, and 22 for 22M13, the differences are not statistically significant; 42M9 and 22M13 apparently have the same sensitizing potential as halothane. (Supported by a grant from W. E. Grace and Co., Dewey and Almy Chemical Div.)

65. *Cardiovascular Actions of Intravenous Methaqualone in the Cat.* P. P. MATHUR, J. A. QUEST, G. S. ROWLES, G. N. MIR and L. T. MULLIGAN, Research Division, William H. Rorer, Inc., Fort Washington, Pennsylvania. (Jerry Smith.)

The cardiovascular actions of methaqualone (MTQ), a non-barbiturate hypnotic agent, were studied in 54 chloralose-anesthetized cats. Femoral arterial blood pressure (BP) and heart rate (HR) were continuously monitored. Isovolumic ventricular contractile force (CF) was measured by a Walton-Brodie strain gauge arch. MTQ (0.5 to 10.0 mg/kg iv) produced dose-dependent decreases in BP ranging from 14 ± 3 to 73 ± 6 mm Hg. The hypotensive

response was associated with decreases in HR and CF at doses of 1.0 mg/kg or higher. Pretreatment with atropine (1 mg/kg iv) or bilateral vagotomy did not influence the measured responses to the drug. In vagotomized cats: (1) spinal section markedly reduced the depressant effects of MTQ on BP, HR and CF, and (2) removal of the stellate ganglia reduced the negative chronotropic and inotropic effects of the drug. These observations suggested MTQ was acting centrally to reduce sympathetic outflow. This possibility was tested by administering MTQ into the vertebral artery of vagotomized cats. Dose-dependent falls in BP, HR and CF were observed with doses ranging from 31.2 to 500 μg/kg. Vascular smooth muscle effects of MTQ were examined in the isolated hindquarters preparation perfused at constant blood flow. Intra-arterially administered drug (31.2–1000 μg) produced decreases in perfusion pressure ranging from 7 ± 2 to 41 ± 3%. The decreases in perfusion pressure were not altered by iv pretreatment with phentolamine (5 mg/kg); propranolol (1 mg/kg); atropine (1 mg/kg) or tripelennamine (2.5 mg/kg). The results indicate that the cardiovascular effects of MTQ result primarily from a centrally-mediated action to reduce sympathetic neural outflow to the heart and blood vessels. The results suggest that this agent can directly relax vascular smooth muscle as well.

66. *Effects of Hexachlorobenzene on Liver Porphyrin Levels and Microsomal Enzymes in the Rat.* D. L. GRANT, F. IVERSON, G. V. HATINA and D. C. VILLENEUVE, Food Research Laboratories, Health Protection Branch, Ottawa, Canada. (K. S. Khera.)

The present study was conducted to provide additional toxicological information on hexachlorobenzene (HCB), a very persistent fungicide. Male and female Wistar rats were fed diets containing HCB (0, 10, 20, 40, 80 or 160 ppm) for 274 days. A number of biochemical parameters and HCB residues in serum and liver were measured. The effect of sex on acquired porphyria was evident, as female and male rats receiving the 80 ppm HCB diet had 202 and 0.12 nmol porphyrin (calculated as coproporphyrin)/g liver. The HCB residues in liver were similar in males and females and dose-related. The liver:body weight ratio was increased in both sexes fed the 80 and 160 ppm diets. These 2 diets also caused decreased body weight gain of females. In vitro hepatic aniline hydroxylase and N-demethylase activities and cytochrome *P*-450 content were increased in males fed 40 ppm or more HCB but were unaltered in the females at all levels. However, pentobarbital sleeping time was decreased in female rats fed dietary levels of at least 20 ppm HCB.

67. *Toxic Effects of Hexachlorobenzene in the Rat: Correlations of Electron Microscopy with Other Toxic Parameters.* T. KUIPER-GOODMAN, D. GRANT, G. KORSRUD, C. A. MOODIE and I. C. MUNRO, Health and Welfare Canada, Food Research Laboratories, Health Protection Branch, Ottawa, Canada. (H. C. Grice.)

This study was designed to investigate the effect of subacute feeding of diets containing the fungicide hexachlorobenzene (HCB) in the rat. The 700 male and female rats of the Charles River (COBS) strain were fed a diet consisting of Master Fox cubes with 4% corn oil to which was added HCB to give 0, 0.5, 2.0, 8.0 and 32.0 mg/kg body weight. Groups of 4 rats of both sexes were killed at 3, 6, 9 and 12 weeks of feeding and at various times after feeding with HCB was discontinued. Livers were excised for electron microscopy and for measurement of drug metabolizing enzymes, HCB concentrations and porphyrin concentrations. Males showed a significant increase in liver weight which after morphometric analysis of electron micrographs was found to be mainly due to an increase in smooth endoplasmic reticulum. This was correlated to increased amounts of drug metabolizing enzymes at the 2 highest dose levels. The enzymes were at a maximum at 6 weeks after feeding of HCB and were still elevated 8 weeks after feeding of HCB had been discontinued. Sorbitol dehydrogenase, an indicator of liver damage, was maximal at 6 weeks in the serum of males. Histochemically, this was correlated to a depletion of this enzyme in the liver and a change in distribution of other enzymes was also noted. These effects were seen to a lesser extent in the females. Females were more sensitive than males to the lethal effects of HCB; in the highest dose group 26% of the females died and no males died. Females developed a more severe porphyria with very high porphyrin concentrations in the liver. These results indicate a sex difference in the responses to HCB.

68. *Microsomal Enzyme Induction by Hexabromobiphenyl.* T. M. FARBER and A. BAKER, Food and Drug Administration, Washington, D.C.

Male Osborne-Mendel rats were fed for 30 days ad libitum dietary levels of 500, 50, 5, 0.5 and 0 ppm of hexabromobiphenyl (HBB) to determine the effect of HBB on microsomal drug metabolism and to compare it to Arochlor 1254 (PCB) fed at the level of 5 ppm. Significant reductions in food consumption and growth were observed only in the 500-ppm group. Liver weights and liver-to body weight ratios were increased over controls at the 500, 50 and 5 ppm levels of HBB, with increases in liver weights of greater than 50% in the 500 and 50 ppm rats. Dose-related increases in the amounts of microsomal protein and cytochrome *P*-450/g of liver were seen. Significant increases in the microsomal demethylation of aminopyrine, the nitroreduction of p-nitrobenzoic acid and the hydroxylation of pentobarbital were seen at all levels of HBB, which were not related to dose. Although the 500 ppm group demonstrated the greatest increases in liver weight, microsomal protein/g of liver and cytochrome *P*-450/g of liver, the greatest degree of microsomal activity was seen at the 50 ppm rather than at the 500 ppm. However, the highest amount of cytochrome *P*-450/mg of microsomal protein and the greatest increase in the activity of NADPH cytochrome *c* reductase, an enzyme believed by many workers to be the rate-limiting step in the microsomal electron-transport system, was observed in the 50-ppm group. Changes in cytochrome b_5 content related to the increase in microsomal drug metabolizing activity were not observed. HBB at 5 ppm appears to be a more potent microsomal inducer than PCB at the same level of administration. PCB caused a 30% increase in the demethylation of aminopyrine while HBB produced a 92% increase. If compared on a mole per mole basis HBB appears to be at least 5 times as potent as PCB as a microsomal inducer.

69. *Potentiation of Carbon Tetrachloride Hepatotoxicity by Polychlorinated Biphenyls.* GARY P. CARLSON, Department of Pharmacology and Toxicology, College of Pharmacy, Univ. of Rhode Island, Kingston, Rhode Island.

Polychlorinated biphenyls (PCB's) have been recognized as widespread pollutants of the environment. Since PCB's have been shown to be inducers of drug metabolism, it was of interest to ascertain if they increase the toxicity of carbon tetrachloride (CCl_4) as phenobarbital (PB) does since the cytochrome *P*-450 species induced by PCB treatment differs from that induced by PB and also from that induced by 3-methylcholanthrene, an agent which protects against CCl_4 hepatotoxicity. Adult male Charles River rats were injected ip daily with either corn oil or 25 mg/kg of Arochlor 1254 for 6 days. On the seventh day they were exposed to CCl_4 (3600–4200 ppm) for 2 hr in a dynamic inhalation chamber and were sacrificed 24 hr later. Administration of Arochlor 1254 resulted in an increase in the liver-to-body weight ratio which was further increased by exposure to CCl_4. Pretreatment with this PCB resulted in a significantly greater decrease in glucose-6-phosphatase activity following CCl_4 exposure than in the controls. No elevations in serum glutamic pyruvic transaminase (SGPT) or serum glutamic oxalacetic transaminase (SGOT) were found with the Arochlor alone but this compound potentiated the rises due to CCl_4 5-fold. Arochlor 1254 significantly increased p-nitroanisole demethylation, cytochrome *c* reductase activity and cytochrome *P*-450 content. Subsequent exposure to CCl_4 resulted in a 72% decrease in p-nitroanisole demethylation and a 88% decrease in cytochrome *P*-450. In the controls these values were decreased by 43 and 41% respectively. In neither group was cytochrome *c* reductase altered by the CCl_4 exposure. The potentiation of CCl hepatotoxicity was further evidenced by histological observations which revealed slight to moderate damage in the livers of the controls but massive necrosis in the Arochlor group. (Supported by NIEHS Grant No. 00596.)

70. *The Effect of Isomerically Pure Chlorobiphenyls on the Structure of Kidney Tubules in the Rat.* M. M. HANSELL and D. J. ECOBICHON, Departments of Anatomy and Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada.

Commercial polychlorinated biphenyls are mixtures of various compounds with only the total chlorination determined. Studies of the effects of isomerically-pure chlorobiphenyls on

liver carried out in these laboratories showed that the effect of the compound was related to the position and degree of chlorination. In a parallel study, an attempt was made to determine if there was a similar relationship in kidney. The agents used were Aroclors 1254 and 1260, biphenyl and a series of isomerically-pure mono-, di-, tetra-, hexa-, and octochloro-biphenyls. Groups of 50–60 g male Woodlyn Wistar rats were injected (ip) with agent dissolved in peanut oil at a dosage of 50 mg/kg/day for 3 days, and killed with chloroform 4 days after the final injection. Samples of kidney were immediately immersion fixed in 1% OsO_4 for electron microscopy. In a second experiment, groups of rats were injected with 3 compounds (4-mono-, 4,4′-di- and 2,5,2′,5′-tetrachlorobiphenyl) at a rate of 100 mg/kg/day for 7 days and killed 1 day later. The glomerulus appeared to be unaffected by treatment, but alterations could be found in renal tubule cells. These included increases in the number of vacuoles, lipid droplets, microbodies, lysosomes and smooth endoplasmic reticulum. Some tubule cells contained mitochondria having altered morphology and disruption of the ordered appearance of the basal infoldings of the plasma membrane. In both experiments, biphenyl and mono- and dichlorobiphenyls caused more marked changes than did the more highly chlorinated compounds.

71. *Effect of Chronic Ingestion of Polychlorinated Biphenyls on the Rat.* J. V. BRUCKNER, K. L. KHANNA and H. H. CORNISH, School of Public Health, The University of Michigan, Ann Arbor, Michigan.

Widespread industrial use of polychlorinated biphenyls (PCB's) has apparently resulted in their contamination of the environment and accumulation in tissues of many biological species, including man. As little information is available on the effect of prolonged, low-level exposure of mammals to PCB's, a chronic feeding study was initiated. A commercial PCB mixture (Aroclor 1242) was mixed with ground chow and fed to male Sprague-Dawley rats at levels of 0, 5 and 25 ppm. After intervals of 2, 4 and 6 months on this regimen, groups of rats were sacrificed and examined for PCB-induced bioeffects. Significant elevations of urinary porphyrin excretion and liver microsomal hydroxylase activity were initially observed in rats consuming 25 ppm for a 2-month period. Both the 5 and the 25 ppm regimens markedly induced porphyrin excretion and hydroxylase activity in the 4- and 6-month groups. Significant increases in liver weight and total liver lipid were also noted after feeding 5 or 25 ppm for 4 and 6 months. Histopathological examination revealed lipid vacuolation of the renal and hepatic parenchyma in the animals. Such findings indicate that long-term, low-level exposure to PCB's may result in alteration of a variety of biologic parameters.

72. *Dominant Lethal Studies in Rats of the Polychlorinated Biphenyls, Aroclor1242 and 1254.* SIDNEY GREEN, FRANCES M. SAURO and KENNETH A. PALMER. Food and Drug Administration, Washington, D.C. (Leonard Friedman.)

An investigation of the possible mutagenicity of the polychlorinated biphenyls, Aroclors 1242 and 1254, as measured by the induction of dominant lethality, was undertaken. Random-bred male and female Osborne-Mendel rats weighing 275–350 and 200–250 g, respectively, were used. The protocol followed the usual dominant lethal procedures. Male animals were dosed (po) acutely or subacutely (5 days) and subsequently mated to females over the entire spermatogenic cycle. Aroclor 1242 was administered as a solution in corn oil at acute dosages of 625, 1250 and 2500 mg/kg and subacutely at 125 and 250 mg/kg. Aroclor 1254 was administered subacutely as a solution in corn oil at 75, 150 and 300 mg/kg. Additionally, rats of one group were administered Aroclor 1254 at 150 mg/kg subacutely and were starved overnight after the last dosage, prior to the admittance of females. The negative control group received corn oil. The positive control compound triethylenemelamine was administered acutely at 0.5 mg/kg and subacutely at 0.2 mg/kg. The results, except in the case of the positive control, showed random, unrepeatable positive effects that were not related to dose or stage sensitivity. These responses were considered to be due to normal variation. Consequently Aroclors 1242 and 1254 do not appear to be mutagenic as measured by the induction of dominant lethality in the rat.

73. *Toxicodynamics of PCB 1254 in the Beagle Dog and Miniature Swine.* J. L. COUVILLION, F. L. EARL and E. J. VAN LOON, Special Pharmacological Animal Laboratory, Division of Toxicology, Food and Drug Administration, Department of Health, Education and Welfare, Washington, D.C.

Reproductive effects of Aroclor 1254 were studied in the beagle dog and miniature swine at daily oral doses of 0.25, 1 and 5 mg/kg in the dog and 1, 10 and 30 mg/kg in the pig. An anorectic response was induced in the dog at the 5 mg/kg dose while a higher dose of 30 mg/kg was required for the pig. In both species fetal liver concentrations and fetal abnormalities showed that PCB penetrated the placental barrier. The rate of decay of PCB concentration in blood was measured in the adult beagle dog. After 3 weeks of conditioning at 10 mg/kg, po, dogs were infused iv with 50 mg/kg Aroclor 1254 as a 10% emulsion. A half-life of 35 hr was obtained with a first-order decay rate. Tissue analysis for PCB in treated animals showed that adipose tissue and liver were major storage sites.

74. *The Reproductive Effects of PCB 1254 in Beagle Dogs and Miniature Swine.* F. L. EARL, J. L. COUVILLION and E. J. VAN LOON, Special Pharmacological Animal Laboratory, Division of Toxicology, Food and Drug Administration, Department of Health, Education and Welfare, Washington, D.C.

Forty-six bred bitches were given levels of 0.25 (10 dogs), 1.0 (16 dogs) and 5.0 (20 dogs) mg/kg/day polychlorinated biphenyl (Aroclor 1254) from the day of breeding to day of necropsy. Sixteen dogs were maintained as untreated controls. A significant decrease in the number of bitches pregnant, the number of live pups/litter at birth and the number of live pups after 2 weeks was observed in those bitches given PCB at a level of 5.0 mg/kg/day. There was a significant increase in the percentage of resorptions. Teratogenic abnormalities consisted of enlarged fontanelles, cleft palates and superfluous phalanges. The lower doses were essentially no-effect levels on reproductive performance. Eighteen bred sows were given levels of 1.0 (5 sows), 10.0 (6 sows) and 30.0 (7 sows) mg/kg/day of PCB 1254, from 21 days prior to breeding to day of necropsy. Five sows served as non-treated controls. Dose-related effects were seen in all treatment levels as decreases in number of pregnancies, number of live pigs farrowed per litter and percentage of live pigs after 2 weeks of age. Only the number of resorptions in the 10.0 mg/kg group did not show a dose-related decrease over the 1.0 mg/kg group. Syndactyly and cleft palates were observed in the 10 mg/kg group with patent fontanelles and cleft palates being observed in the 30 mg/kg group.

75. *Persistence of PCB Homologs and Isomers in the Domestic Hen and the Rat.* FREDERICK D. BAKER, BRIAN BUSH and CASIMIR TUMASONIS, Division of Laboratories and Research, New York State Department of Health, Albany. (L. Golberg.)

This study was undertaken to (1) separate, identify, and quantitate the 18 major components of Aroclor 1254 in adult hens and pregnant rats, in the embryos and fetuses, and in the progeny of both species during and after exposure; (2) compare the metabolic patterns of Aroclor 1254 for both species; (3) correlate toxicity and abnormalities with specific isomers; and (4) examine the possible relationship between induced mixed-function oxidase activity and hydroxylation of isomers. Aroclor 1254, emulsified in drinking water, was administered at a level of 6 mg/kg body weight (50 ppm) to hens and to rats over periods of 6 and 9 weeks respectively. After the intake was terminated, the populations were observed for 20 and 13 weeks respectively as the level of PCB declined. Egg yolks, tissues of viable 18-day-old embryos, and surviving 5-day-old chicks were analyzed by thin-layer chromatography and by gas chromatography during the course of the experiment. Data suggest that 4-4′-chlorination is more important than degree of chlorination in determining persistence of chlorinated biphenyl in the hen, chick embryo and hatched chick. Results with both hens and rats indicate that fertility was not affected at the level of PCB employed and that the degree, and termination, of embryonic and/or fetal development was related directly to length of exposure. Mixed-function oxidase activity (MFO) on liver microsomal preparations from rat mothers, fetuses and pups was

measured by determination of liver microsomal cytochrome *P*-450 and aniline hydroxylase levels. Preliminary results indicate sex-related MFO response.

76. *Computer-assisted Reduction and Display of Gas Chromatographic Data from Long-term Toxicity Studies of Commercial Mixtures of Polychlorinated Biphenyls in Chickens and in Rats.* BRIAN BUSH, FA-CHUN LO and FREDERICK D. BAKER. Division of Laboratories and Research, New York State Department of Health, Albany. (L. Golberg.)

Homologs and isomers of PCB represented by 18 gas chlomatographic zones have been traced through the tissues of adult animals to their young in long-term studies of the chicken and the rat. Three or more tissue types from each animal sacrificed have been analyzed and in order to facilitate the chemical analysis and to reduce the mass of data produced, several novel techniques have been developed and will be described. The Hewlett-Packard 7600A system was employed. In the chicken experiment, integrator output was converted into logarithmic bar charts to give a visual display of relative changes in concentration during residence in the animals, using a Wang Programming Calculator and Data Plotter. Conclusions were drawn from the data, in spite of the large disparities observed between individuals, by determining frequencies of occurrence above and below datum lines drawn across the bar charts. In the rat experiment, Fortran programs were written to display the data as linear or logarithmic bar charts, tabulate nominal concentrations of each chromatographic zone, plot the variation of each zone concentration with time for each tissue type in the adult, fetus and pup throughout the experiment. Detailed observations and conclusions will be described in an accompanying presentation.

77. *Subacute 2-AAF Toxicity in C57BL/6 and BALB/c Strain Mice.* G. SCHIEFERSTEIN and T. J. HALEY, National Center for Toxicological Research, Jefferson, Arkansas.

Ninety-day subacute toxicity of 2-Acetylaminofluorene (2-AAF) has been determined in 1200 mice each of the C57BL/6 and BALB/c strains equally divided between the sexes. The concentrations of 2-AAF were 0.05, 0.025, 0.01, 0.005 and 0.001 % of the diet. No signs of toxicity were seen. Slight weight reduction was seen in the C57BL/6 males and BALB/c females at the 0.05 % AFF level. A dose-dependent urinary bladder epithelial hyperplasia was seen in both strains and the males were more susceptible than the females. Squamous metaplasia of the uroepithelium was observed in the BALB/c mice with this effect greater in the males. One BALB/c male at the 0.025 and 0.05 % levels had bladder carcinoma. Adrenal gland spindle cell hyperplasia was greater in the females of both strains. Bladder concretions were observed in males of both strains. No other significant histopathological changes were observed. The data obtained will be utilized in the design of the large chronic studies to be conducted at NCTR.

78. *Histologic Response of Mouse Urinary Bladder to 2-AAF in a Large Subacute Experiment.* WILLIAM E. JAQUES and CHARLES H. FRITH, National Center of Toxicological Research, Jefferson, Arkansas. (T. J. Haley.)

These studies are concerned with the premalignant and pathogenetic factors in chemically induced injury to the mouse uroepithelium. One thousand, two hundred mice equally divided between the C57BL/6j and BALB/cSt/BR strain equally sexed and weighing from 17 to 31 g were fed dietary 2-AAF for 90 days. The mice were divided and fed 2-AAF concentrations of 0.05, 0.25, 0.01, 0.005 and 0.001 %, respectively. There were 240 control animals. Complete necropsies were performed at sacrifice but significant change were observed only in the urinary bladder. The transitional cell epithelium displayed progressive thickening from the usual 3–4 cell layer. This degree of hyperplasia was quantitated into 3 grades. Squamous metaplasia was a significant feature in many of the mice. Two examples of overt neoplasia of the urinary bladder occurred with one being in situ and the other demonstrating early invasiveness. Dietary levels of 0.01–0.05 % gave a 100 % response. The studies support the concept of hyperplasia which may precede neoplasia and squamous metaplasia being a prerequisite for squamous carcinoma of the urinary bladder.

79. *Chronic Dose Response Studies in Mice fed 2-AAF.* N. A. LITTLEFIELD, C. CUETO, JR., A. K. DAVIS, and K. MEDLOCK, National Center for Toxicological Research, Jefferson, Arkansas.

Large numbers of mice have been exposed to 2-acetylaminofluorene (2-AAF) in the diet in order to provide quantitative information on carcinogenic dose-response relationships. Weanling BALB/c and C57BL/6 mice of both sexes were fed 2-AAF ad libitum in their diet for 18 months at concentrations of 0, 100, 150 and 500 ppm. Occurrence of bladder tumors was considered as the endpoint for determination of dose response curves. Data were accumulated from mice that died during the 18-month period, from moribund mice that were necropsied, and from mice necropsied at the terminal sacrifice after 18 months of exposure to the carcinogen. The results from an interval sacrifice after 1 year of exposure to the high dose showed 38 and 88% incidence of bladder tumors in the BALB/c males and females, respectively, while the C57BL/6 mice exhibited a bladder tumor incidence of 29% in the males and 11% in the females. The results exhibited both a sex-related and a strain-related difference in response to 2-AAF. The BALB/c mice appeared to be more susceptible to early development of bladder tumors than the C57BL/6 mice.

80. *Histopathologic Changes in Mice Fed 2-Acetylaminofluorene in a Chronic Study.* CHARLES H. FRITH and WILLIAM E. JAQUES, National Center for Toxicological Research, Jefferson, Arkansas. (C. Cueto.)

This investigation concerns the histopathologic changes produced by feeding 2-acetylaminofluorene (2-AAF) to large numbers of mice in a chronic study. Beginning at weanling age, BALB/c and C57BL/6 mice of both sexes were fed 2-AAF in their diet for up to 18 months at concentrations of 0, 100, 250 and 500 ppm. Complete necropsies were performed on over 2000 animals that died or became ill and were sacrificed, and approximately 50 tissues from each mouse were studied histologically. Significant changes were observed in the urinary bladder and the liver. The changes in the bladder ranged from hyperplasia and squamous metaplasia of the transitional epithelium to carcinoma with extensive metastases. Bladder neoplasms included transitional cell carcinomas, transitional cell carcinomas with squamous metaplasia, squamous cell carcinomas and anaplastic carcinomas. Bladder tumors developed much earlier in the BALB/c mice than in the C57BL/6 mice and were more common in the female animals. A definite sex difference in the C57BL/6 mice was not established. Histopathologic changes in the liver included single or multiple hepatomas, some of which metastasized to the lungs. There was also an apparent increase in reticulum cell sarcoma. Bladder tumors, hepatomas, and reticulum cell sarcomas were noted in both sexes of both strains at all three concentrations of 2-AAF. A small number of hepatomas and reticulum cell sarcomas were noted in the controls, but bladder tumors were not found in untreated animals.

81. *Determination of 2-Acetylaminofluorene and its Metabolites by Liquid and Gas Chromatography.* FLOYD R. FULLERTON and C. D. JACKSON, The National Center for Toxicological Research, Jefferson, Arkansas. (T. E. Shellenberger.)

A quantitative method was developed to separate 2-acetylaminofluorene (2-AAF) and its hydroxy metabolites utilizing a combination of high speed liquid chromatography and gas chromatography. Trimethylsilyl derivatives of 2-AAF, N-OH-AAF, 1-OH-AAF, 3-OH-AAF, 5-OH-AAF and 7-OH-AAF were formed with N,N-bis (trimethylsilyl) trifluoroacetamide (BSTFA) and good separation of the compounds was achieved using gas chromatography. The GC was equipped with a 6 ft × ¼ in. glass column packed with 3% OV-1 on Chromosorb G and a dual flame ionization detector. It was found, however, that interfering peaks are present in biological extracts when injected directly into the GC without prior cleanup. A liquid chromotographic method was utilized as a primary cleanup of biological extracts which also allowed direct quantitation of N-OH-AAF, 5-OH-AAF and 7-OH-AAF. The liquid chromatograph was equipped with a 280 nm UV detector. Another single peak consisting of 2-AAF, 1-OH-AAF and 3-OH-AAF was collected from the liquid chromatograph and TMS derivatives formed prior to injection into the GC. This combination has been successfully applied

to urine analysis and should be equally applicable to other substrates. The lower limits of detection ranged from approximately 25 ng of 2-AAF and 3-OH-AAF using gas chromatography to 150 ng of 5-OH-AAF and 7-OH-AAF using liquid chromatography.

82. *Tissue binding of [9-^{14}C] 2-Acetylaminofluorene in Mice During Oral Administration.* C. D. JACKSON, CONSTANCE WEIS and T. E. SHELLENBERGER, The National Center for Toxicological Research, Jefferson, Arkansas.

This study was made to determine the extent of tissue binding of 2-acetylaminofluorene (AAF) during oral administration of the carcinogen at different dose levels. Weanling, female BALB/cJ and C57BL/6J mice were maintained on diets containing 5 or 500 ppm [9-^{14}C] AAF for 8 weeks. The amount of ^{14}C bound to liver and bladder tissue, as well as blood concentrations, were determined at various intervals. Results obtained with the two dose levels were essentially parallel with amounts of ^{14}C being approximately 100-fold higher in mice on the 500 ppm diet. Blood values of ^{14}C in both strains of mice increased for 2 weeks and then remained constant with the concentrations in BALB/cJ mice being twice that of C57BL/6J mice on the same diet. Amounts of bound ^{14}C in liver tissue also reached a plateau at 2 weeks and were approximately 40% higher in C57BL/6J mice. In contrast, the amount of ^{14}C bound to bladder tissue continued to increase over the entire 8-week period. In a second experiment, weanling, female BALB/cJ and C57BL/6J mice were maintained on diets containing AAF at various levels over the range of 0.5–500 ppm and sacrificed at the end of 2 weeks. Blood and tissue concentrations were determined as above. The results, plotted as log ^{14}C bound vs log concentration of AAF in the diet, indicate that the amount of AAF bound to both liver and bladder tissue is linear over the dose range studied.

83. *The In Vitro Metabolism of 2-Acetylaminofluorine in Mouse Liver.* B. J. GOUGH, K. P. BAETCKE and T. E. SHELLENBERGER, National Center for Toxicological Research, Jefferson, Arkansas.

A modified in vitro system for the metabolism of 2-acetylaminofluorine (2-AAF) was developed. The system depends upon utilization of a NADPH-generating system, low amounts of microsomal protein (0.5–1.0 mg) and substrate (660 ng), and utilization of HEPES buffer. With these modifications, the in vitro metabolism of 2-AAF was found to be linear with time and after 30 min incubation, formation of N-hydroxy-2-acetylaminofluorine (N-OH-2-AAF) represented 30% or greater of the 2-AAF added originally. Using this modified in vitro system in a 14-day preliminary study, mice fed a purina diet were found to metabolized 30% more of the added 2-AAF to N-OH-2-AAF than did mice receiving a synthetic diet. In another study, 2-AAF was added to diets of mice (100 ppm in Purina or synthetic) for periods of up to 18 weeks. Determinations of in vitro 2-AAF metabolism were made at periodic intervals and it was found that feeding of 2-AAF in the diet exerted little, if any influence on the conversion of 2-AAF to N-OH-2-AAF or to the 5- or 7-OH metabolites when compared to control animals.

84. *Toxicity of 2-Acetylaminofluorene and its Metabolites in Liver Slices.* C. D. JACKSON and C. C. IRVING, Veterans Administration Hospital and Department of Biochemistry, University of Tennessee Medical Units, Memphis, Tennessee. (T. E. Shellenberger.)

Rat liver slices were investigated as a possible in vitro system for studying the initial effects of a hepatocarcinogen on liver cells. Binding of N-hydroxy-2-acetylaminofluorene (N-HO-AAF) to RNA and DNA of liver slices from male Holtzmann rats was 5- to 6-fold greater than that of females. Inhibition of RNA and protein synthesis by N-HO-AAF was 2–3 times greater in liver slices from male rats compared to females. The glucuronide conjugate of N-HO-AAF (N-G10-AAF) was half as effective in inhibiting RNA synthesis as N-HO-AAF. 2-Acetylaminofluorene (AAF) had little or no effect on RNA synthesis in liver slices. Although 0.2 mM N-HO-AAF reduced both RNA and protein synthesis in slices 80%, there was no inhibition of DNA synthesis with the concentrations studied (0.05–0.8 mM). Inhibition of RNA synthesis in liver slices by several N-hydroxy-N-arylacetamides (0.2 mM) was found to be as follows:

N-hydroxy-2-acetylaminofluorene, 80.3%; N-hydroxy-2- acetylaminophenanthrene, 54.4%; N-hydroxy-4-acetylaminostilbene, 37.9%; N-hydroxy-4-acetylaminobiphenyl, 35.3%. These results indicate that the effect of AAF and its metabolites on rat liver slices in vitro is similar to that observed in vivo and that the glucuronide conjugate may contribute significantly to the hepatotoxicity observed in vivo during oral administration of AAF. The results also indicate that the metabolism and/or reactivity of the N-hydroxy-N-arylacetamides is highly dependent on the structure of the aryl stubstituent. (Supported by Veterans Administration Research Funds.)

85. *Percutaneous Absorption of Hexachlorophene Following Daily Whole Body Washings.* B. CALESNICK, C. H. COSTELLO, J. P. RYAN and J. DIGREGORIO, Hahnemann Medical College and Hospital, Philadelphia, Pennsylvania, and Colgate-Palmolive Company, Piscataway, New Jersey.

The purpose of this study was to study the absorption of hexachlorophene (HCP) through normal skin while washing with detergent-based skin cleansing products containing 3% HCP. A group of human volunteers were initially screened to rule out any skin or systemic disease. No soap or cosmetics containing HCP were used for at least 2 weeks before the start or during the course of this study. Subjects were divided into 2 subgroups which were balanced with respect to age, sex, weight, color and baseline HCP values. One group was assigned HyperpHaze and the other pHisohex. Each subject washed his or her body (excluding scalp) daily for 3 min with 30 ml (containing 900 mg HCP) of the respective detergent-based skin cleansing product and rinsed in the shower in their normal manner. Blood samples for gas chromatographic analyses were obtained prior to this study, weekly during the study and then weekly after treatment was discontinued until blood concentrations of HCP returned to control values. Final statistical analysis of data was conducted on 36 subjects (18 for HyperpHaze and 18 for pHixohex). During the 8-week usage test, blood concentrations of hexachlorophene (ng/ml) reached a plateau in weeks 3, 4 and 5 (pHisohex means = 543.8, 638.7 and 616.3 respectively; HyperpHaze means = 631.3, 655.3 and 592.6 respectively) and dropped to a secondary lower plateau in weeks 6, 7 and 8 (pHisohex means = 508.7, 417.2 and 395.4 respectively; HyperpHaze means = 398.8, 332.5 and 330.8 respectively). At weeks 6, 7 and 8 there were differences in the hexachlorophene blood concentrations which were significant ($P < 0.01$). HyperpHaze reached a lower plateau then pHisohex. Throughout the study, there were no adverse systemic effects noted directly to the product. In summary, HCP was absorbed through intact healthy skin after daily bathings with detergent-based skin cleansers containing 3% HCP. HCP blood values reach an initial plateau between 3 and 5 weeks; fell to a secondary lower plateau at 6–8 weeks and returned to normal values within 2–3 weeks after ending product usage. (We wish to thank the following for their invaluable assistance: Ken Hensen, William Gregory, Robert Steltenkamp and Annette Dinan.)

86. *Placental Transfer of Hexachlorobenzene in the Rabbit.* D. C. VILLENEUVE, L. G. PANOPIO and D. L. GRANT, Food Research Laboratories, Health Protection Branch, Ottawa, Ontario. (K. S. Khera.)

Hexachlorobenzene (HCB) has gained significance as an environmental contaminant through its use as a fungicide and an industrial chemical. While information is available on the metabolism and distribution of this compound no data have been presented on its placental transfer. White, New Zealand rabbits were mated (day 0) and then orally administered HCB from day 1 to day 27 with subtoxic doses of 0, 0.1, 1.0 or 10 mg/kg. On day 28 the dams were killed, fetuses excised and tissues removed. HCB content of maternal and fetal tissue was determined by GLC. HCB was found to cross the placenta and accumulate in the fetus in a dose-dependent manner. In the dams, the tissue with the highest HCB concentration was fat followed by liver, heart, kidney, brain, lung, spleen and plasma. In the fetus, liver concentrations were higher but brain concentrations lower than the corresponding maternal organs. No fetotoxic effects were observed at any of the dose levels.

87. *Hexachlorobenzene: Teratogenicity and Dominant Lethal Studies in Rats.* K. S. KHERA, Food Research Laboratories, Health Protection Branch, Health and Welfare Canada, Ottawa, Canada.

Hexachlorobenzene (HCB) was associated with large scale human poisoning in Turkey during the years 1955–1959. It has been detected in birds, fish, mussels and animal tissues (*Toxicol. Appl. Pharmacol.* **18**, 994, 1971) and finds wide use as a fungicide on cereals. Its teratogenic and mutagenic potential in mammals is at present, not known. For teratogenicity evaluation rats were given single daily po doses of 0, 10, 20, 40, 60, 80 or 120 mg HCB/kg during 6–9, 10–13, 6–16 or 6–21 days of gestation; 80 and 120 mg/kg doses manifested maternal neurotoxicity and reduction in fetal weight. The incidence of uni- and bilateral 14th rib among fetuses was significantly increased above control values when HCB was administered from days 10–13, 6–16 and 6–21 days of gestation. The incidence was related to the duration of treatment and the dose. Measures on live and dead fetuses, resorption sites, fetal weight and visceral and other skeleton anomalies were within control limits. For the dominant lethal test male rats distributed in 4 groups each of 15 males were dosed orally with 0, 20, 40 or 60 mg/kg for 10 consecutive days. Fourteen sequential mating trials (at each trial a male was caged with 2 virgin females for 5 days) were conducted. No significant differences in the incidence of pregnancies, corpora lutea, live implants and deciduomas in the test and control groups were seen.

88. *A Computer System for the Collection and Statistical Analysis of Teratology Data.* JAMES W. NOVEROSKE, ROBERT T. DREW, KAREN WOLFORD, WARREN P. GREENBERG, JOSEPH L. CIMINERA and NEETI R. BOHIDAR, Merck Institute for Therapeutic Research West Point, Pennsylvania. (Paul A. Mattis.)

In 1972, the Department of Safety Assessment of the Merck Institute initiated a formal project involving the Teratology Section, Systems and Programming, and Biometrics Research personnel to develop a computer-oriented teratology data analysis system. This system (utilizing an IBM 370 series computer) was designed to handle the collection, storage, retrieval, and statistical analysis of all teratologic data. To date, this effort includes the collection and storage on magnetic tape of more than 100,000 parameter values routinely measured or observed from more than 5000 female mice, rats and rabbits. Parameters printed out and summarized in a tabular form are maternal weights, clinical signs and pregnancy status; number and location of implants, resorptions, live and dead fetuses per female; and the sex, weight and anatomical alterations of each fetus. At the present time, a computerized statistical package has been implemented to analyze maternal body weight gains, fetal weights and number of resorptions, dead and live fetuses per litter. The computer, as programmed, first examines the data with respect to the validity assumptions of normality and homogeneity of variances. It then selects the appropriate statistical methodology to be used depending upon the response parameter and whether or not a parametric or nonparametric analysis is required. A retrieval capability for cumulative control data is now being developed.

89. *The effect of Indomethacin on Parturition in the Rat when Administered in Combination with Prostaglandin $F_2\alpha$.* J. IULIUCCI, W. WILLIAMS, A. CERNISKI and E. SCHWARTZ, Warner-Lambert Research Institute, Morris Plains, New Jersey.

Recent studies have indicated that non-steroidal anti-inflammatory agents are capable of delaying the onset of parturition in rats (Chester, *Nature* **240**, 37–38, 1972). It was suggested that the mechanism for the delay may be related to the ability of these agents to inhibit the synthesis of prostaglandins. A study was undertaken to determine whether the administration of prostaglandin $F_2\alpha$ ($PGF_2\alpha$) could alter the delayed rat parturition which results from the oral administration of indomethacin. Carworth Farms (CFN) pregnant rats were administered indomethacin (1 mg/animal, bid) by gastric intubation from day 18 of gestation until parturition. One-half of the treated animals also received $PGF_2\alpha$ (1 mg/animal, bid) sc from day 20 of gestation until parturition. Animals dosed with 1% gum tragacanth by gastric intubation from day 18 served as a vehicle control group. Females that were given the combination

of indomethacin and $PGF_2\alpha$ delivered their pups earlier than the vehicle control or indomethacin treated animals. However, no significant delay in onset of parturition was noted between the indomethacin-treated group and the vehicle control group. In an attempt to ascertain an oral dose of indomethacin which could cause a delayed onset of parturition, 2 strains of gravid rats (CFN and CRCD) were administered the anti-inflammatory agent at doses of 0.5–2.0 mg/kg, bid, from day 18 of gestation until parturition. Only 1 CFN animal and 2 CRCD animals treated with 1.0 and 1.5 mg/kg, respectively, had a delayed onset of parturition; all other parturition times were not different from those of the vehicle controls.

90. *Effect of Single and Daily Injections of Phenobarbital on Ovulation in Hamsters.* JOHN J. ALLEVA, MARY V. WALESKI, FREDERIC R. ALLEVA and TIBOR BALAZS, Food and Drug Administration, Washington, D.C.

Ovulation in hamsters normally occurs every fourth day between 1:00 AM and 3:00 AM and can be blocked by hypophysectomy or injection of certain centrally acting drugs at 2:00 PM but not 4:00 PM of the preceding day (day 4 of the 4-day cycle). This indicates that pituitary gonadotropins (PG) responsible for ovulation are released into the circulation between 2:00 PM and 4:00 PM on day 4 and that a central mechanism controls this release. Phenobarbital is an effective blocking agent. In the present study we observed effects of acute and chronic injections of phenobarbital on the central mechanism that controls PG release. Effects of the drug on PG release were monitored by daily examination of vaginal washings. Hamsters were exposed daily throughout life to light from 4:00 AM to 8:00 PM (LD 16:8). The drug was dissolved in saline and injected sc (0.5 ml/100 g). In the acute study, a single injection was given at 180–182 days of age on day 4 of the cycle. A dose of 140 mg/kg at 5:00 AM, 9:00 AM, 2:00 PM, 2:30 PM, or 3:00 PM blocked PG release in 0/5, 10/10, 10/10, 6/10 and 1/10 hamsters, respectively; 70 or 35 mg/kg at 2:00 PM blocked 9/10 and 4/8 hamsters; 70 mg/kg at 9:00 AM blocked 5/12 hamsters, Blocks lasted 1–5 days, after which 4-day cyclicity resumed. In the chronic study, the first of 50–53 consecutive daily injections was given at 80–82 days of age. In 1 group, injections were given at 1:30 PM. These caused an initial series of blocks lasting 1–8 days. Eventually, however, the injections were ineffective, as evidenced by the return of normal 4-day vaginal cyclicity. Two weeks after the last daily injection, a single injection at 1:30 PM on day 3 was effective again. In another group, the daily injections were given at 3:30 PM except for the last injection which was given at 1:30 PM on day 4 of the cycle. Only this last injection blocked PG release. This suggests that the eventual failure of the daily 1:30 PM injections to block PG release resulted from a change in the central mechanism.

91. *Influence of Environmental Contaminants on Biochemical Adaptation to Stress in Birds.* MICHAEL P. DIETER, Patuxent Wildlife Research Center, Laurel, Maryland. (L. Stickel.)

Adult Coturnix males (*Coturnix coturnix japonica*) were stressed by motion for 0.5–3 hr on a laboratory shaker operating at low or high speed. The extent of the stress was measured by elevations of plasma enzymes; the stress was not physically harmful, but was capable of evincing major biochemical changes that were repeatable from one test to another. In 2–3 hr plasma creatine kinase and lactate dehydrogenase activities had increased 10-fold in birds shaken at high speed. Moreover, these plasma enzymes were increased proportionally to the amount of time the birds were stressed. There was also a doubling of aspartate aminotransferase activity in birds similarly stressed. but this was not proportional to time on the shaker. Other groups of birds were fed diets for 12 weeks containing 100 ppm Aroclor 1254, 4 ppm Morsodren or 165 ppm malathion, concentrations previously found to significantly elevate plasma enzyme activities. Form the 9th through the 12th week, half of the birds on the contaminated feed and equal numbers on clean feed were shaken at low speed 1 hr/day for 5 days a week, in an attempt to adapt the birds to the stress. Plasma enzyme responses in these 2 groups of birds were compared after 1 hr of shaking at high speed. Training almost entirely prevented the rise in plasma enzyme activities previously found in untrained birds shaken at high speed. For example, there was only 16.6% of the creatine kinase response to stress remaining in trained birds fed the clean diets. However, in trained birds fed Aroclor 1254, 49.3%

remained, in those fed Morsodren 48.8% remained, and in those fed malathion 31.5% of the response to stress remained. Aspartate aminotransferase and lactate dehydrogenase responses to stress remaining were also 2–3 times greater in trained birds fed contaminants than in those on clean feed, suggesting that biochemical adaptation to stress was severely inhibited by ingestion of polychlorinated biphenyl, organophosphate or mercury.

92. *Biological Availability of Arsenic from Fish.* I. C. MUNRO, S. M. CHARBONNEAU, E. SANDI, K. SPENCER, F. BRYCE and H. C. GRICE, Food Research Laboratories, Health Protection Branch, Health and Welfare Canada, Ottawa, Canada.

Although it has been well established that certain species of marine life contain high amounts of arsenic, little experimental data exists concerning the bioavailability of this arsenic to mammals. In order to gain some information on the potential public hazard, studies were undertaken to compare the excretion of arsenic from fish and arsenic trioxide. Adult female Yorkshire pigs with urinary catheters were given a series of 4 single meals; low arsenic fish (control); high arsenic fish (0.3 mg As/kg); arsenic trioxide (0.3 mg As/kg) and arsenic trioxide with low arsenic fish (0.3 mg As/kg). Urine samples were collected daily for 3 days prior to dosing, every 12 hr for 4 days after dosing and then daily for 6 days. Fecal collections were done daily for 3 days prior to dosing and for 10 days thereafter. Following administration of the high arsenic fish, 80% of the arsenic was excreted in the urine and the remaining 20% appeared in the feces. Excretion was virtually complete in 4 days. Following administration of arsenic trioxide, 80% of the arsenic was excreted in the urine in the 1st day, while the remaining 20% appeared to be retained in the body.

93. *The Fish as a Toxicological Tool.* LIONEL E. MAWDESLEY-THOMAS, Huntingdon Research Centre, Huntingdon, England

So often with traditional toxicological methods the possible hazards associated with the logarithmic increase in foreign compounds which are entering our environment, may take months or years to be fully appreciated. Many of the foreign compounds which are produced are included under the general heading of insecticides and herbicides. As well as studying the efficacy and toxicity of these compounds on the pest species, it is possible to study their effects *directly* on wild-life. This is in contradistinction to pharmaceuticals, where in the majority of cases the animal data has to be extrapolated to man, and it is here that the problem arises. In wild-life studies one is able to study the effect of a compound on a species, and in this paper the fish is being considered, not only at the LD50 levels but also at the actual levels found in the environment. Fish toxicity testing has advanced considerably over the past 5 years and standard tests are made using the gold-fish, cat-fish, blue-gill, trout and harlequin fish routinely. New methodologies in the field of dosimetry have been developed in these laboratories together with analytical techniques related to fish, the waters in which they are found together with some advances in histological and histochemical principles.

94. *The Effects of Inducers on Acute p-Xylene Toxicity.* R. T. DREW and J. R. FOUTS, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

One aspect of the program in aerotoxicology at NIEHS is to determine whether known modifiers of hepatic microsomal mixed-function oxidase activity will alter the acute toxicity of common solvents. This paper describes the effects of animal pretreatment with either phenobarbital (PB), 3-methylcholanthrene (3-MC), or chlorpromazine (CPZ) on the acute toxicity of both inhaled and injected *p*-xylene. Female rats were injected ip daily for 3 days with either PB (75 mg/kg in saline), CPZ (15 mg/kg in saline), or 3-MC (20 mg/kg in corn oil). On the fourth morning the animals were either exposed to *p*-xylene vapors in an inhalation chamber for 4 hr or given an ip injection of 50% (v/v) *p*-xylene in mineral oil. Injected animals were given doses of 1, 2, 3, 4 or 5 ml *p*-xylene/kg. Animals exposed via inhalation received a single exposure to *p*-xylene at concentrations ranging from 3630 to 7530 ppm. The LD50 for animals injected with *p*-xylene and the LC50 for animals inhaling *p*-xylene were calculated for control groups and for groups pretreated with each of the enzyme modifiers. All 3 compounds

raise the LC50 of inhaled *p*-xylene with the greatest effect being from PB. Only 3-MC increases the LD50 of injected *p*-xylene. Methods have been developed for measuring the in vitro metabolism of *p*-xylene in microsomes prepared from rat liver. These methods have been used to measure the effects of animal pretreatment with PB, 3-MC or CPZ on the in vitro metabolism of *p*-xylene by microsomes prepared from rat liver.

95. *The Effect of Phenobarbital on Chronic Benzene Toxicity in Rats.* R. T. DREW, C. HARPER, J. G. ZINKL, B. N. GUPTA and M. D. HOGAN, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. (J. R. Fouts.)

Previous studies in our laboratory have shown that pretreatment of rats with phenobarbital increases the rate of benzene metabolism, as measured in vitro, without altering the acute toxicity of benzene. The present studies were designed to determine (1) whether previous inhalation exposure to benzene vapors will increase the rate of benzene metabolism in rats, and (2) if phenobarbital ingestion will alter any effects of chronically inhaled benzene. Rats were exposed to benzene vapors 4 hr/day for 3 successive days and sacrificed on the fourth morning. Exposure to an average concentration of 4000 ppm benzene doubles the rate of hepatic microsomal metabolism of benzene to phenol while exposure to 2000 ppm increases the rate by about 10%. Groups of 44 rats were either exposed to air (control); exposed to 1650 ppm benzene vapor 6 hr/day, 5 days/week; or exposed to 1650 ppm benzene vapor 6 hr/day, 5 days/week and also given 1 mg/ml phenobarbital in their drinking water. Subgroups of 11 animals were sacrificed after 2, 4, 8 or 12 weeks of exposure. The parameters investigated include growth rate, both red and white blood cells, benzene metabolism in vitro by lung and liver microsomes and histological changes in lung, liver, heart and kidney. Exposure to benzene causes a decrease in weight gain, a decrease in the number of lymphocytes in blood, and a slight increase in rate of hepatic microsomal benzene metabolism. Phenobarbital partially reverses the decreases in weight gain and lymphopenia and increases the rate of hepatic microsomal benzene metabolism 10-fold.

96. *Modification of Benzene Metabolism and Myelotoxicity in Male Albino Rats by Pretreatment with Phenobarbital, SKF-525A or 3-Methylcholanthrene (3-MC).* DAVID P. GILL, JOE B. NASH and SYDNEY ELLIS, University of Texas Medical Branch, Galveston, Texas.

The present studies were undertaken to evaluate the importance of the oxidation of benzene to phenol and other products in the production of leucopenia by benzene. Male rats of Sprague-Dawley descent were pretreated with phenobarbital, 40 mg/kg ip, twice daily for 3 days, or 3-MC 30 mg/kg ip for 2 days, or SKF-525A 80 mg/kg ip, or diluent and then were given 10 ml/kg sc of an equal mixture of benzene and corn oil. The urinary excretion of phenol and the peripheral white blood cell count were monitored. Phenobarbital was found to increase the conversion of benzene to phenol, while decreasing the fraction of phenol excreted as conjugates. Phenobarbital pretreatment also protected animals from the leucopenic action of benzene. SKF-525A caused a significant reduction in the conversion of benzene to phenol, but had no effect on benzene-induced leucopenia, whereas 3-MC significantly increased phenol production only early in the experimental period, aad had no effect on the leucopenia produced by benzene. These findings indicate that the metabolism of benzene is subject to modification by specific drug pretreatments, but there does not appear to be a simple relationship between the myelotoxicity of benzene and its early phases of oxidation.

97. *Benzene Toxicity and Its Metabolism.* E. W. LEE, L. S. ANDREWS, J. KOCSIS and R. SNYDER, Thomas Jefferson University, Philadelphia, Pennsylvania.

The study of the relationship between benzene metabolism and benzene-induced depression of erythropoiesis, was facilitated by a recently developed method that measures the hematopoietic effects of single doses of benzene (Lee *et al.*, *Res. Commun. Chem. Pathol. Pharm.* **5**, 547, 1973). While urinary excretion of radioactivity after a single dose of [^{3}H]benzene (a relatively simple measure of overall benzene metabolism) is essentially complete (90%) in 24 hr, hematopoietic effects are best measured 48 hr after benzene administration, i.e. ^{59}Fe is given

48 hr after [^{3}H]benzene and ^{59}Fe incorporation into circulating erythrocytes is measured 24 hr later. Thus, 1 dose [1 ml (880 mg/kg)] of [^{3}H]benzene provides simultaneously both a means of measuring benzene metabolism and a challenge to the hematopoietic system. Among benzene-treated mice 2 sub-groups could be distinguished with respect to ^{59}Fe incorporation into erythrocytes. In sub-group A ^{59}Fe incorporation was 84% of controls and in sub-group B, 30% of controls. Mice in sub-group A, which tolerated this dose of benzene without showing much bone marrow depression, also excreted significantly less radioactivity ($p < 0.05$) than mice in sub-group B, which were sensitive to the hematopoietic toxicity of this dose of benzene. Toluene, which showed no hematopoietic toxicity, inhibited benzene metabolism and decreased the hematopoietic toxicity of benzene. Kinetic studies in vitro showed toluene to be a competitive inhibitor of benzene metabolism. These data show that animals which metabolize less benzene are more resistant to the toxic activity of benzene. (Supported by USPHS ES-00322.)

98. *Methemoglobinemia and Metabolism of Nitro Compounds.* A. M. KAPLAN, K. L. KHANNA and H. H. CORNISH, University of Michigan, Ann Arbor, Michigan.

Nitrobenzene has been thought to be an active methemoglobin-generating agent only in vivo; however, a study has shown that small amounts of methemoglobin were actually formed in vitro. The metabolism of nitrobenzene and other aromatic nitro compounds is presumed to occur via the microsomal nitroreductase enzyme system. Studies were conducted to determine the effect of microsomal enzyme induction on nitrobenzene methemoglobin formation. Male Sprague-Dawley rats were dosed with phenobarbital (50 mg/kg/day for 3 days, ip) and with caffeine (100 mg/kg/day for 4 days, ip) prior to receiving nitrobenzene (75 mg/kg in corn oil, ip). With each animal serving as its own control, blood samples were collected at various time intervals after administration of nitrobenzene and the percent methemoglobin determined. When compared with controls there was no effect of microsomal enzyme induction on the rates of formation or the amount of methemoglobin formed. However, rather than the plateauing of the methemoglobin concentration, seen in nitrobenzene-treated controls between 120–240 min, the methemoglobin values rapidly decreased in the induced animals. The in vitro microsomal nitroreduction of p-nitrobenzoic acid and hydroxylation of acetanilide were both increased 2-fold in the induced groups. Since nitrobenzene is largely excreted as the hydroxylated product the present data would suggest that increased microsomal enzyme activity has increased the rate of hydroxylation and excretion of nitrobenzene in the induced animals. Thus the more rapid excretion of nitrobenzene could account for the noticeable decrease of methemoglobin concentrations seen in the induced animals. (Supported by Grant No. 5TO1 ES 00138, E.H.S.)

99. *Initial Events Involving Nuclear-Cytoplasmic Relationships in Rat Liver After Phenobarbital.* J. D. YOUNG and D. COURI, The Ohio State University College of Medicine, Columbus, Ohio.

Events which occur in rat liver cytoplasm following treatment with phenobarbital suggest the involvement of hepatocyte nuclei in the induction process. In studies designed to examine the mechanism by which phenobarbital may activate transcription of RNA in nuclei, we have investigated the relationship of drug-protein binding in rat liver cytosol and nucleoplasm to activation of RNA polymerase as a function of time. Male albino Wistar rats were administered radiolabeled phenobarbital, and protein binding was measured in liver cytosol and nucleoplasm by gel filtration chromatography. The activities of RNA polymerase I (nucleolar) and II (nuclear) were assayed in intact isolated liver nuclei. Although phenobarbital bound to several fractions of protein in the cytosol in vivo, only binding to the high molecular weight fraction changed in magnitude with time. This binding was greater in magnitude at 5 min than at 20 min and did not occur when the drug was added in vitro. The protein-drug complex involved less than 0.5% of the total drug present in the cytosol and was easily dissociated by high ionic strength buffers or rechromatography. Drug binding to protein in the nucleoplasm indicated a reciprocal relationship in time with binding in the cytosol. In support of this relationship, cytosol was found to enhance the uptake of drug into isolated nuclei. In animals

treated with phenobarbital (80 mg/kg ip) or saline, the activity of RNA polymerase I was 220% of control at 5 min and 180% of control at 30 min, while RNA polymerase II activity did not change significantly within 60 min. The results of these experiments suggest that phenobarbital causes an early stimulation of RNA polymerase I activity in rat liver nuclei at a time which is related to binding changes occurring in the cytoplasm and nucleus.

100. *Influence of Barbiturate Analogues on Liver Microsomal RNA Degradation During Development of Metabolic Tolerance.* R. T. LOUIS-FERDINAND, Department of Pharmacology, School of Pharmacy, University of Maryland, Baltimore, Maryland. (Eugene Miller.)

The extent of hepatic mixed function oxidase (HMFO) stimulation by inducers such as phenobarbital may be inversely related to the degree of ribonuclease (RNAase) inhibition produced by these agents (Louis-Ferdinand and Fuller, *Toxicol. Appl. Pharmacol.* **23**, 492, 1972). In order to compare the influence of barbiturate analogues on microsomal RNA degradation and HMFO activity, barbiturate analogues dimethoxyphenobarbital (DMMP), monomethoxymethyl phenobarbital (MMMP), 1,3,bis-acetoxymethyl phenobarbital (BAP), mephobarbital (MEPH), Barbital (B) or barbituric acid (BA) (100 mg/kg, ip) were administered to male Sprague-Dawley rats (100–125 g). Twenty-four hr later the animals were sacrificed. The livers were perfused and homogenized with 4 volumes (w/v) ice-cold 0.25 M sucrose. Liver fractions were prepared by differential centrifugation. Liver microsomal demethylation of aminopyrine (AP) p-chloro-N-methylaniline (PCMA) or RNAase activity was determined spectrophotometrically. Relative percents RNAase inhibition observed following administration of the barbiturate analogues were: BA (19%) < DMMP (23%) < B (28%) < BAMP (28%) < MEPH (33%) < MMMP (38%). Conversely, the relative percents stimulation of AP demethylation were BA (110%) < B (130%) < DMMP (133%) < BAMP (163%) < MEPH (213%). No statistically significant differences from respective control PCMA demethylation activity were found at 24 h. A statistically significant ($p < 0.05$). positive correlation was found between the effect of the barbiturates on AP demethylation and RNAase activity ($r = 0.886$). These results suggest that the degree of microsomal RNAase inhibition and the stimulation of HMFO activity by barbiturates are inversely related. (Supported by PHS Grant RO1-GM19084.)

101. *Dearylation of Dialkyl p-Nitrophenyl Phosphates: A Case of Substrate Specificity of Mixed-Function Oxygenases.* PAUL A. CAMMER and ROBERT M. HOLLINGWORTH, Department of Entomology, Purdue University, West Lafayette, Indiana. (T. S. Miya.)

The mixed-function oxygenase system (MFO) of mammalian liver microsomes is known to metabolize a large number of xenobiotics with a wide variety of structures. A high degree of structural specificity within a series of homologous compounds is not often observed. In this work the MFO of liver microsomes from mice is shown to exhibit such selectivity in the dearylation of certain dialkyl *p*-nitrophenyl phosphates (analogs of paraoxon). Microsomal pellets were prepared from phenobarbital-treated mice. Release of *p*-nitrophenol from a series of dialkyl *p*-nitrophenyl esters with alkyl groups ranging from 1 to 5 carbon atoms in length was measured spectrophotometrically. K_m and V_{max} values were determined for each substrate. Methyl and ethyl paraoxon were not readily metabolized by MFO. Though considerable dearylation of *n*-propyl paraoxon was observed, this activity was again lost with the *n*-butyl and *n*-amyl analogs. When the 2 alkyl substituents were not identical, only the compounds with a *n*-propyl group were metabolized in this manner. Dearylation of analogs with branched chains was generally successful with only the di-*s*-butyl and di-*i*-butyl forms. Certain modifications of the alkyl groups on ethyl paraoxon elicited dearylation. For instance, both di-(2-chloroethyl) paraoxon and di-(2-methoxyethyl) paraoxon proved to be good substrates, whereas di-(2,2,2-trichloroethyl) paraoxon was not metabolized. The high degree of substrate specificity in this series appears to be associated with the presence of a 3-carbon length or equivalent.

102. *Glucuronide Formation in Rainbow Trout: Effect of Salicylamide on the Acute Toxicity, Conjugation and Excretion of 3-Trifluoromethyl-4-Nitrophenol.* J. J. LECH, Medical College of Wisconsin, Milwaukee, Wisconsin.

In rainbow trout, the selective sea lamprey larvicide, 3-trifluoromethyl-4-nitrophenol (TFM) was previously found to be conjugated with glucuronic acid and excreted to a great extent in bile. In order to determine the degree and significance of conjugation of TFM in trout, studies utilizing salicylamide, an inhibitor of glucuronide formation, were carried out. Pre-exposure of rainbow trout to 25 mg/liter salicylamide decreased the LC50 of TFM from 5.05 to 2.67 mg/liter. Coincidental with the increase in toxicity were elevated blood concentrations of unconjugated TFM and a depression of TFM glucuronide in blood and bile. An increase in the concentration of unconjugated TFM was also found in brain, muscle and heart from salicylamide-treated trout. Salicylamide increased the half-life of ip injected TFM from 1.59 to 4.13 hr and inhibited the glucuronidation of TFM by trout liver extracts in vitro. These studies indicate that glucuronide formation appears to be an important mechanism in the protection of trout from the toxic effects of TFM and possibly other water-borne phenols.

103. *Metabolism of Intravenously or Orally Administered Butylene Glycol Adipic Acid Polyester.* P. L. WRIGHT, G. J. LEVINSKAS, M. L. KEPLINGER and J. C. CALANDRA, Monsanto Company, St. Louis, Missouri and Industrial BIO-TEST Laboratories, Inc., Northbrook, Illinois.

Santicizer® 334F is a linear polyester (1700–2200 average molecular weight) composed of alternating units of adipic acid and 1,3-butylene glycol, terminated by a mixture of myristic, palmitic and stearic acids. The metabolism of Santicizer 334F containing 1 ^{14}C-labeled adipate was determined following iv or oral administration. The dose was 3 mg/kg or 2.3 μCi/rat. Partition of excreted ^{14}C between carbon dioxide, urine and feces was nearly identical for iv and orally dosed rats. Of the excreted activity, 50.1% appeared as CO_2, 28.6% was in urine and 11.2% was in feces. Tissues, excluding the site of injection and liver, contained 2.6% of the oral dose and 4.8% of the iv dose 96 h post-dosing. Livers from the orally dosed animals contained 0.3% and livers of the iv dosed animals contained 4.6% of the respective doses. Extraction and characterization of the ^{14}C-containing residues in the livers demonstrated that the material was not parent Santicizer 334F. Solvent partition and chromatographic behavior suggested that the residual ^{14}C had been incorporated into naturally occurring cellular compounds. These studies demonstrate that this butylene glycol adipic acid polyester underwent hydrolysis when administered either orally or iv. The subsequent metabolic fate was similar following either route of administration.

104. *The Metabolism of Hexafluoropropene by Washed Rat Red Blood Cells.* JAMES V. DILLEY and ELLIOTT S. HARRIS, Northrop Services, Inc., and the National Aeronautics and Space Administration, Lyndon B. Johnson Space Center, Houston, Texas.

Previous studies in this laboratory have shown that hexafluoropropene is metabolized by rats as evidenced by an increase in fluoride ion excretion following an acute inhalation exposure. These studies have been extended in an attempt to identify the primary tissue responsible for hexafluoropropene metabolism and to obtain some information concerning the enzyme system involved in the metabolism of this compound. Washed red blood cells were found to be at least 10 times more active in hexafluoropropene metabolism than any of the other tissues examined. The reaction favored an alkaline medium, was heat-sensitive above 40°C and was sensitive to sulfhydryl inhibitors to some extent. The K_m was about 6.44×10^{-4} M. Spectrophotometric evidence indicated that hemoglobin might be involved in at least part of the metabolic reaction. This study presents an example of another compound that is metabolized by an extrahepatic detoxication mechanism.

105. *Phenothiazine Tranquilizer PD-50's Exerted Against Amphetamine Effect in Aggregated Mice.* C. D. PROCTOR and J. B. CHO, Meharry Medical College, Nashville, Tennessee.

Prior reports from our laboratory have indicated that phenothiazine tranquilizers can reverse the excitatory, lethal response produced in aggregated mice by amphetamine (Proctor

et al., *Toxicol. Appl. Pharmacol.* **6**, 1, 1964; *Arch. int. Pharmacodyn.* **163**, 87, 1966). Other workers have ascertained that while the phenothiazine tranquilizers exert this protective effect, barbiturates and meprobamate do not afford such protection without producing marked neurological deficit (Lasagna and McCann, *Science* **125**, 1241, 1956). We have extended the latter observation ascertaining that diazepam, chlordiazepoxide, benactyzine, hydroxyzine and oxazepam do not protect against the amphetamine effect in non-neurological deficit dosage. Considering these findings collectively we have become interested in the possibility that the median protective doses (PD-50's) exerted by phenothiazine tranquilizers against a 100% lethal, 20 mg/kg ip dose of *dl*-amphetamine in aggregated mice might afford a screening model useful in predicting the approximate rank order potency of these agents. Adult albino ICR Swiss mice were used, in groups of 10–20 mice/dose level of tranquilizer, all of the latter being given 15 min prior to the 20 mg/kg amphetamine. After ip injection of the amphetamine the mice were aggregated according to the method cited in Proctor *et al.* and observed over 4 hr for lethality. PD-50's for the tranquilizers, obtained by the method of Litchfield and Wilcoxon (*J. Pharmacol. Exp. Ther.* **96**, 99, 1949), has revealed a rank order of tranquilizer protective potency exerted against the *dl*-amphetamine lethality in aggregated mice such that: fluphenazine > perphenazine > thiopropazate > chlorpromazine > thioridazine > promazine. The phenothiazine protection afforded was effected without causing marked neurological deficit in the mice.

106. *An Effect of Amphetamine on Mouse Brain Polyribosomes.* M. A. BLACKSHEAR, M. L. HARRIS, R. BENNETT and C. D. PROCTOR, Meharry Medical College, Nashville, Tennessee.

Aoki and Siegel (*Science* **168**, 129, 1970) have shown that hyperphenylalaninemia can cause disaggregation of brain polyribosomes in 4-day-old rats. Loo and Whitaker (*J. Neurochem.* **14**, 813, 1967) have shown that in experimental phenylketonuria, such as hyperphenylalaninemia, a Schiff base formed in vivo from phenylethylamine and pyridoxal may be involved with the disaggregation of polyribosomes. Considering these reports together has caused us to wonder whether or not amphetamine, an agent with chemical structure similar in Schiff base formation potential to that of phenylethylamine, could form such a base and/or influence brain polyribosome aggregation. Utilizing the methods of Campagnoni and Mahler (*Biochem.* **6**, 956, 1967) for harvesting and characterizing brain polyribosomes and the in vivo methods of Proctor *et al.* (*Arch. int. Pharmacodyn.* **163**, 79, 1966) for drug administration, we have studied the effect of *dl*-amphetamine at 20 mg/kg ip on brain polyribosome aggregation in Swiss ICR adult albino mice at 30 min post-injection times. Animals injected with physiological saline served as controls. Some experiments were run on aggregated mice (25 cm^2 floor space/mouse), while others were run in mice solitarily confined (522 cm^2 floor space/mouse). Our preliminary results indicate that *dl*-amphetamine is capable of causing disaggregation of heavy polyribosomes into lighter polyribosomes in mouse brain. This effect was more marked in crowded mice given *dl*-amphetamine than it was in mice kept in solitary state after amphetamine administration.

107. *An Effect of Amphetamine on Incorporation of Leucine into Mouse Brain Protein.* M. L. HARRIS, M. A. BLACKSHEAR, R. BENNETT and C. D. PROCTOR, Meharry Medical College, Nashville, Tennessee.

Preliminary findings made in our laboratory utilizing the methods of Campagnoni and Mahler (*Biochem.* **6**, 956, 1967) have shown that 20 mg/kg of *dl*-amphetamine, given ip to adult mice, is capable of causing disaggregation of heavy polyribosomes into lighter polyribosomes in mouse brain in vivo. This finding has engendered interest on our part as to whether or not brain protein synthesis would be affected by this *dl*-amphetamine effect. Experiments were run in Swiss ICR adult albino mice, one group being given 20 mg/kg of *dl*-amphetamine, another group (controls) being injected with a physiological saline. Thirty min post-injection the mice were sacrificed and the brain polyribosomes harvested, characterized and fractionated according to the methods of Campagnoni and Mahler (*Biochem.* **6**, 956, 1967). Employing [UL-^{14}C]*l*-leucine, incorporation of leucine into protein was studied in various fractions of the

fractionated polyribosomes according to the method of Mans and Novelli (*Arch. Biochem. Biophys.* **94**, 48, 1961). The results obtained indicate a decreased incorporation of leucine into brain protein caused by the *dl*-amphetamine, an effect which paralleled the decrease in heavy polyribosomes induced by the amphetamine. This action of the amphetamine was more marked in crowded mice (25 cm^2 floor space/mouse) than in mice solitarily confined (522 cm^2 floor space/mouse).

108. *Mechanism of Action of Phencyclidine on the Peripheral Sympathetic Nervous System.* H. HITNER and G. J. DIGREGORIO, Department of Pharmacology, Hahnemann Medical College, Philadelphia, Pennsylvania. (B. Calesnick.)

Phencyclidine (PCP) is an anesthetic agent which in low doses produces psychotomimetic effects similar to LSD. PCP has previously been shown to produce pressor responses in rats and cats and also to potentiate the pressor responses to norepinephrine (NE). In the isolated guinea pig vas deferens preparation, PCP (5×10^{-5} M) was found to potentiate NE-induced contractions while completely blocking Ach-induced contractions. In vivo on the cat nictitating membrane preparation, PCP potentiated both electrical and epinephrine (EP)-induced contractions. After surgical denervation of the superior cervical ganglion the potentiation of EP by PCP was significantly reduced. Preliminary studies on the compound action potentials of the frog sciatic nerve indicates that PCP has a local anesthetic action at 10^{-2} M while at 10^{-3} M PCP facilitates nerve conduction causing a rise in the height of the action potential. It appears that the mechanism of action of PCP may involve an ion effect related to either Na^+ or Ca^{2+}.

109. *Antianesthetic Effects of Cyclic AMP and Analeptic Drugs as Determined by Reversal of Amobarbital-Induced Narcosis.* M. L. COHN, B. J. KRAYNACK and M. COHN, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania. (M. Eisler.)

Dibutyryl cyclic AMP (an analog of adenosine 3′:5′-cyclic monophosphoric acid) has a remarkable regulatory effect on the duration of narcosis induced by amobarbital (Cohn *et al.*, *Neuropharm.* **12**, 1973). Usefulness of analeptic drugs to lessen duration of narcosis has never been demonstrated. Antianesthetic effects of dibutyryl cyclic AMP and analeptic drugs, picrotoxin, d-amphetamine, pentylenetetrazal, caffeine, theophylline, strychnine, methylphenidate and doxapram were compared. Sprague-Dawley male rats weighing 85–125 g were anesthetized in the morning with a 1% solution of sodium amobarbital (80 mg/kg ip). Immediately after loss of the righting reflex, groups of rats were injected either ip or intracerebroventricularly into the right lateral ventricle with the appropriate agents. Only dibutyryl cyclic AMP and picrotoxin administered intracerebroventricularly shortened duration of amobarbital-induced narcosis. Whereas dibutyryl cyclic AMP, without any toxic manifestations, shortened narcosis in a dose-related manner, picrotoxin at all doses tested induced the Straub-tail phenomenon, shivering, piloerection, moderate-to-severe convulsions, micturation, defecation and phonation. Despite marked hyperventilation, doxapram in a dose-related manner, prolonged amobarbital-induced narcosis. None of the other analeptic drugs administered intracerebroventricularly demonstrated any antianesthetic properties. The lack of antianesthetic properties of the analeptic drugs tested may explain their failure to reverse barbiturate poisoning in man. Thus, pateints suffering from drug overdosage should not be treated with these analeptic drugs because such therapy may add to the toxicity already present. In contrast, it has been shown in rats and squirrel monkeys that due in part to its effective antianesthetic action, dibutyryl cyclic AMP is an effective antidote to barbiturate overdosage (Cohn *et al.*, *Res. Comm. Chem. Path. Pharm.* **6**, 1973).

110. *Recovery of Infant Monkeys from the Effects of Repeated Bathing with an Antibacterial Scrub Formulation Containing Hexachlorophene.* E. R. HART, K. PITTMAN, H. P. DROBECK and J. F. KURTZKE, Litton Bionetics, Inc., Falls Church, Virginia, Sterling-Winthrop Research Institute, Rensselear, New York, and Georgetown University, Washington, D.C.

Two studies were conducted to examine the systemic effects of repeated washing of baby monkeys with a scrub formulation containing hexachlorophene. In the first study, 5 newborn

rhesus monkeys were washed daily for approximately 5 min, thoroughly rinsed in flowing lukewarm tap water and gently dried. Five control monkeys were washed with a non-hexachlorophene-containing preparation. In the second study 8 cynomolgus and 8 rhesus monkeys were treated for 90 days. Four were killed at the end of this period. The others were allowed post-treatment recovery periods ranging from 6 to 18 weeks. Seven additional monkeys (3 cynomolgus and 4 rhesus) served as controls. Two animals in the first group showed papilloedema upon ophthalmoscopic examination. Deficits of CNS function were not detected by neurological examination including electroencephalography. The 9 monkeys killed immediately after 90 days treatment (5 from the first study and 4 from the second) showed the same vacuolation of the white matter, particularly, of the cerebellum, brain stem and spinal cord as has been attributed by others to hexachlorophene toxicity. No control animals showed this. In the other 12 monkeys, the longest recovery time was associated with disappearance of the vacuolation. The hexachlorophene content of the blood at various times will be presented.

111. *Lead-Induced Hyperactivity.* E. K. SILBERGELD and A. M. GOLDBERG, The Johns Hopkins University, School of Hygiene and Public Health, Baltimore, Maryland. (D. Blake.)

To establish an animal model of lead toxicity, mice were exposed to lead from birth. Lead was administered to their mothers by substituting solutions of lead acetate for the drinking water. The offspring were thus exposed to lead indirectly through their mothers' milk and after weaning directly through their drinking water. In addition to retardation of growth and development, the lead-treated offspring were more aggressive and hyperactive than controls. At 30–150 days of age, lead-treated mice were at least 3 times as active as controls. Previously reported results have demonstrated pharmacological parallels between lead-induced hyperactivity in this model and the childhood behavior disorder, minimal brain dysfunction hyperactivity. That is, the hyperactive animals are quieted by several central nervous system stimulants and exacerbated by phenobarbital. The mechanism by which lead produces hyperactivity is unknown. In the neuromuscular preparation it has been shown that lead decreases function by acting on prejunctional cholinergic mechanisms. To test whether central cholinergic pathways are affected by lead, drugs with known cholinergic effects were administered to both control and lead-treated hyperactive mice. These included: physostigmine, atropine, and benztropine. Drugs known to increase the availability of acetylcholine, such as physostigmine, suppress hyperactivity. These results support the hypothesis that lead acts, at least in part, on central cholinergic pathways and that this effect of lead may be involved in the induction of the hyperactivity. (Supported by NIEHS 00034 and 00454.)

112. *Comparative Neurotoxicity and the Distribution of Methyl Mercury in the CNS of the Beagle Dog and Miniature Swine.* E. MILLER, P. P. SAPIENZA, F. L. EARL, T. C. MICHEL, V. L. OLIVITO and E. J. VAN LOON, Special Pharmacological Animal Laboratory, Division of Toxicology, Food and Drug Administration, Department of Health, Education and Welfare, Washington, D.C.

The purpose of the present investigation was to determine the feasibility of using the dog or miniature swine as a credible model for the study of methyl mercury (MeHg) poisoning of the CNS. MeHg was administered simultaneously at the levels of 2000, 1000, 500 and 250 μg/kg/day to 1 group of minipigs and 2 groups of beagle dogs at each dose level. Whenever a pig became moribund and was sacrificed, a dog fed the corresponding dose and the same cumulative dose of MeHg was sacrified, even though the dog did not exhibit any overt signs of neurotoxicity. The other group of dogs was fed the same doses until they became moribund. The cumulative dose of MeHg at which the minipig reached the final stages of the Minamata syndrome (e.g. paresis, convulsion, prostration) did not produce any overt signs of neurotoxicity in the dog. The concentrations of Hg in the CNS of these dogs as compared to the pig were significantly higher. However, when dogs were administered MeHg until they reached

the final stages of the Minamata syndrome, the contents of Hg found in the CNS of a dog exceeded those found in the CNS of the pig by 50%. The cumulative dose at which the dog becomes moribund following the administration of MeHg is 70% higher at the levels of 2000, 1000 and 500 μg/kg/day and 250% higher at the level of 250 μg/kg/day than for the pig. At 100 μg/kg/day, dogs exhibit no overt signs of MeHg poisoning after 3 years, yet the pig becomes moribund in 1 year. The results support the conclusion that of the two species discussed the miniature swine provides a much more sensitive model for the study of MeHg poisoning.

113. *Changes in Brain Biogenic Amines and Body Temperature After Cyclodiene Insecticides.* P. D. HRDINA, R. L. SINGHAL and D. A. V. PETERS, Department of Pharmacology, University of Ottawa, Ottawa, Canada. (J. A. Thomas.)

The purpose of this study was to investigate whether exposure to organochlorine pesticides belonging to the cyclodiene group is capable of producing neurotoxic effects (tremor, convulsions and hyperthermia) and alterations in brain biogenic amines similar to those reported earlier for p,p'-DDT (*Toxicol. Appl. Pharmacol.* **25**, 276, 1973). Following an acute dose of α-chlordane (200 mg/kg), endrin (50 mg/kg), heptachlor (200 mg/kg) or heptachlor epoxide (100 mg/kg) in rats, slight tremor, paralysis of hind legs and convulsive episodes were seen which were most pronounced during the 3rd and the 4th hr. In addition, treatment with these pesticides was found to produce hypothermia which was in sharp contrast to the marked increase in body temperature noted with p,p'-DDT. The α-chlordane-induced hypothermia was dose-dependent and the decrease in body temperature coincided with a fall in the concentration of brain-stem NE. While no changes were seen in brain-stem 5-HT, the concentration of ACh in cortex and striatum dropped to 72 and 73% of the control values respectively, 4 hr after pesticide administration. Treatment with α-methyl-p-tyrosine (100 mg/kg) while partially antagonizing the hypothermic response, failed to alter the other neurotoxic signs produced by α-chlordane. Chronic treatment (45 days) with small daily doses of endrin (0.5 and 2.0 mg/kg), heptachlor (3 and 15 mg/kg) or heptachlor epoxide (1 and 5 mg/kg) resulted in significant decreases in cerebro-cortical ACh, but failed to alter the concentrations of brain-stem NE. In contrast, significant increases in brain-stem 5-HT were noted with both doses of endrin and heptachlor. There were no apparent signs of neurotoxicity or any changes in body temperature in rats which received chronic treatment with either of these insecticides. It is suggested that while increased release and utilization of NE may be involved in the acute hypothermic effect of α-chlordane, the observed decrease in brain ACh is related to the convulsive episodes seen after acute treatment with this cyclodiene insecticide. (Supported by the Department of Health and Welfare, Canada.)

114. *Effect of Acute and Chronic Dieldrin Exposure on Brain Biogenic Amines of Male and Female Rats.* STEVEN R. WAGNER and FRANK E. GREENE, Department of Pharmacology, Milton S. Hershey Medical Center, Pennsylvania State University College of Medicine, Hershey, Pennsylvania.

There have been numerous attempts to elucidate the etiology of neurotoxic manifestations of dieldrin in mammals (e.g., hyperexcitability, muscle fasciculations and clonic-tonic convulsions) by observing associated changes in brain neurochemistry. However, information regarding the possible effects of dieldrin on brain concentrations of norepinephrine (NE), dopamine (DA), and serotonin (5-HT) appears to be lacking. We have performed acute and chronic intoxication studies using adult male and female rats to determine not only if the brain concentrations of these biogenic amines were affected, but also whether sex differences were apparent. Five hr after an oral dose of dieldrin (50 mg/kg), severe neurotoxic signs were observed, especially in the male rat. This was accompanied by a 25% decrease of the whole brain NE content from the control value. NE contents were not significantly decreased in the female, and DA and 5-HT contents were not altered in either sex. Two days after a sublethal oral dose of dieldrin (40 mg/kg), no changes were observed in the whole brain contents of NE, DA and

5-HT. Whole brain values for NE, DA, and 5-HT were also measured after rats were maintained for 1, 2, 4, 6, 8 and 10 weeks on 50 ppm dieldrin in semi-synthetic diet. Although the brain content of NE, DA and 5-HT in dieldrin-fed rats were frequently below control values after 4 weeks, these differences were seldom significant. Since regional fluctuations of these biogenic amines may have been masked in the whole brain estimations, a separate study was performed in which regions designated as midbrain (M), striatum (S), hippocampus (Hc), medulla-pons (MP), cortex and pooled cerebellum samples were analyzed after 1, 2, 4 and 8 weeks of feeding 50 ppm dieldrin. Significant differences were observed in NE contents in M, S, Hc and MP in both males and females which indicated that partial depletion occurred initially, followed by a return to normal values in subsequent weeks. Changes in 5-HT followed a similar pattern in M, S and MP, but were less significantly affected than NE in these regions. DA contents were unaffected. No obvious sex differences were noted in either of these chronic studies. It is concluded that dieldrin is capable of altering brain tissue concentrations of NE, DA and 5-HT under certain conditions and that sex differences are minimal. (Supported by NIEHS Research Grant 9-RO1-ES00638-03.)

115. *Choline Acetylase (ChA) Inhibitors: Studies on the Mechanism of the Inhibition of ChA by Iodoacetylcholine (IACh).* B. V. RAMA SASTRY and G. I. HENDERSON, Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee.

Rat brain and human placental ChAs (choline acetyltransferase) were assayed by the formation of [^{14}C]acetylcholine (ACh) from choline (Ch) and [^{14}C]acetyl-CoA(ACoA). IACh was a potent inhibitor of the formation of ACh by both enzymes (I50: 2.4×10^{-7} to 1.9×10^{-6} M). The mechanism of this inhibition was studied by the following experiments: (1) variation of initial velocity as a function of Ch concentration (5×10^{-5} to 2×10^{-4} M, ACoA 10^{-5} M) at different concentrations of IACh (5×10^{-7} to 2.5×10^{-6} M); (2) variation of initial velocity as a function of ACoA concentration (6×10^{-6} to 2.5×10^{-5} M, Ch 10^{-4} M) at different concentrations of IACh; (3) dialysis of inhibited ChAs; and (4) inhibition of ChAs by iodo-acetate (IA). According to the primary plots (1/s vs 1/v) in experiments 1 and 2, IACh was a non-competitive inhibitor of both enzymes. The secondary plots (IACh concentration vs intercepts or slopes of the primary plots) were linear and gave the following inhibitory constants (M): Rat brain ChA, K_{ii} 3.3×10^{-7} and K_{is} 2.2×10^{-7}; Human placental ChA, K_{ii} 5.4×10^{-7} and K_{is} 2.1×10^{-6}. In experiment 3 only 60–70% of the inhibition was reversible. In experiment 4 high concentrations of IA (10^{-2} M) inhibited both ChAs by about 30–40%. These observations support the statements that (1) the inhibition of ChAs by IACh was of linear-noncompetitive type, (2) a ternary intermediate was formed during the enzyme-reaction which supports a Theorell-Chance mechanism for both ChAs (Sastry and Henderson, *Biochem. Pharmacol.* **21**, 787, 1972) and (3) the carboxymethylated-ChAs from both enzymes were about 60–70% as active as the original ChAs. (Supported by USPHS Grants NS-04699 and GM-00058.)

116. *Reproduction and Teratology Studies with the Fungicide Ferbam.* JAN L. MINOR, JOHN Q. RUSSELL and CHENG-CHUN LEE. Pharmacology and Toxicology, Midwest Research Institute, Kansas City, Missouri.

The reproductive and teratogenic effects of the fungicide, ferric dimethyldithiocarbamate (ferbam), were studied in rodents. Daily feeding, equivalent to 23, 66 or 109 mg/kg/day, to male rats for 13 weeks caused deaths and weight loss at the highest dosage without any effect on reproduction. Daily feeding, equivalent to 15 or 51 mg/kg/day, to females for 2 weeks caused a severe weight loss at the high dose and a slight, but not significant, decrease in neonatal survival. Ferbam, administered to rats on days 6–15 of gestation at dosage of 150 mg/kg/day resulted in some deaths, increased resorption, decreased fetal weight, and caused a slight increase in soft and skeletal tissue anomalies. Ferbam, administered to mice on days 6–14 of gestation at 30 or 300 mg/kg/day, failed to show any teratologic affect. These studies suggest that high doses of ferbam caused mild teratologic effects in rats. (Supported by the National Institute of Environmental Health Sciences, Contract No. NIH-NIEHS-72-2084.)

117. *Routine Teratogenicity Screening Procedures: Experiences and Recommendations.* KENNETH MORGAREIDGE, DAVID E. BAILEY, ALEX J. KUTCHES, JOSEPH MCLAUGHLIN and T. F. X. COLLINS, Food and Drug Research Laboratories, Inc., Waverly Division, Waverly, New York, and U.S. Food and Drug Administration, Washington, D.C.

Experience gained in the course of submitting some 74 substances to a rigidly defined teratologic screening procedure now permits certain tentative conclusions to be drawn. Groups of 20 pregnant rats, mice or hamsters or 10 rabbits were dosed ig with 1 of 4 different levels of each agent during the second trimester of gestation, yielding data representing over 20,000 litters in rodents and 3000 litters in rabbits. Concurrent groups of controls were sham-dosed or treated with a positive teratogen. All test substances were selected from the list of so-called GRAS materials (21 CFR 121.101), and included gums, cellulosics, metal salts, acid salts, organic acids and salts thereof, antioxidants, essential oils, 5- and 6-carbon sugars and miscellaneous items. No substance tested proved to possess unequivocal teratogenic properties in any of the 4 species. However, embryotoxicity and maternal morbidity were not uncommon at the highest dose levels (one-tenth of the oral LD50). The limitations of the method include problems related to size and route of dosage; animal variability related to strain and, probably, season of the year; as well as the subjective nature of the data. Preliminary recommendations include: (1) replication of groups within tests; (2) reduction in number of species to 2 (rats and mice), since hamsters add nothing in sensitivity and rabbits are the most variable in quality and response; (3) reconsideration of the utility of positive controls, since a single dose level provides insufficient information; and (4) adoption of statistical standards defining the ranges of response parameters both within and between assays. It is concluded that the present protocol, while adequate to detect strongly positive teratogens, lacks sensitivity to distinguish weakly active materials from wholly negative ones.

118. *Teratogenic Studies with Certified Colors in Rats and Rabbits.* C. M. BURNETT, H. P. K. AGERSBORG, JR., J. F. BORZELLECA, E. EAGLE, A. G. EBERT, E. C. PIERCE, J. C. KIRSCHMAN and R. A. SCALA, Inter-Industry Color Committee Task Force, Cosmetic, Toiletry and Fragrance Association, Inc., Washington, D.C.

To supplement the spectrum of toxicological information on certified synthetic organic color additives subject to human ingestion, 25 colors which have been permitted in foods, drugs and cosmetics in the United States have been tested for teratogenic effects in rats and rabbits. The studies were done in 4 commercial contract laboratories using a standard protocol fortified with additional control groups. Administration was by gavage during organogenesis at doses based on the highest no-effect level in rats and dogs in prior 2-year feeding studies. None of the colors produced evidence of skeletal or soft tissue abnormalities in the fetuses. The higher doses of 7 colors, notably xanthenes, caused maternal and fetal toxicity in rabbits. Only 1 of these colors showed effects in the rat. The data emphasized the importance of multiple discrete control groups in the evaluation of fetal toxicity in these studies. The following colours were studied: FD & C Green No. 3, Yellow No. 5, Yellow No. 6, Red No. 2, Red No. 3, Red No. 4, Blue No. 1, Blue No. 2, Violet No. 1, D & C Green No. 5, Green No. 6, Yellow No. 10, Red No. 7, Red No. 9, Red No. 10, Red No. 19, Red No. 21, Red No. 27, Red No. 30, Red No. 33, Red No. 36, Orange No. 5, Orange No. 10, Orange No. 17 and Blue No. 6.

119. *Multi-generation Reproduction Studies with Certified Colors in Rats.* E. C. PIERCE, H. P. K. AGERSBORG, JR., J. F. BORZELLECA, C. M. BURNETT, E. EAGLE, A. G. EBERT, J. C. KIRSCHMAN and R. A. SCALA, Inter-Industry Color Committee Task Force, Cosmetic, Toiletry & Fragrance Association, Inc., Washington, D.C.

To further supplement the spectrum of toxicological information on certified synthetic organic colors subject to human ingestion, 25 colors which have been permitted in foods, drugs and cosmetics in the United States are under test in multi-generation reproduction studies in rats. The studies are almost completed at 4 contract laboratories. Doses were administered in the diet, and were based on multiples (1×, 10×, 30×, and 100×) of the A.D.I., or of the projected safe dose determined from data of previous long-term feeding studies in rats

and dogs. However, no doses in excess of 1000 mg/kg/day were used. The human usage level of the color was also a factor in determining the levels used in the diet. Data through the F2b litter give no indication of adverse effects on reproductive performance. The following colors were studied: FD & C Green No. 3, Yellow No. 5, Yellow No. 6, Red No. 2, Red No. 3, Red No. 4, Blue No. 1, Blue No. 2, Violet No. 1, D & C Green No. 5, Green No. 6, Yellow No. 10, Red No. 7, Red No. 9, Red No. 10, Red No. 19, Red No. 21, Red No. 27, Red No. 30, Red No. 33, Red No. 36, Orange No. 5, Orange No. 10, Orange No. 17 and Blue No. 6.

120. *Enhancement of Dinoseb-induced Teratogenicity by Maternal Food Deprivation in Mice.* MAURLINE PREACHE and JAMES E. GIBSON, Department of Pharmacology, Michigan State University, East Lansing, Michigan.

Dinoseb (2-*sec*-butyl-4,6-dinitrophenol), a commonly used herbicide, has teratogenic potential in mice at or near the dose levels required for maternal toxicity (Gibson, *Fd. Cosmet. Toxicol.* **11**, 31, 1973). Food deprivation was combined with dinoseb treatment to determine whether the teratogenicity would be altered. Pregnant mice were assigned to 1 of 4 groups that were either maintained on normal diet and treated with dinoseb (15.8 mg/kg/day, ip) or given a control injection of the vehicle on days 10–12, or were deprived of food but not water for 24 hr on the ninth day and given the dinoseb (15.8 mg/kg/day, ip) or control vehicle injections on the succeeding 3 days. The fetuses were removed by cesarean section on day 19 and were weighed, measured (crown–rump distance), and examined for external abnormalities. Mothers that received control injections, whether or not they were food deprived, produced litters in which greater than 95% of the fetuses were normal. Fetal weight and the proportion of normal fetuses/litter were decreased by dinoseb alone or in combination with food deprivation. Litters from dinoseb-treated mothers that had been food deprived had a higher incidence of anomalies than those of nondeprived mothers, but fetal weight was not further reduced by the deprivation. External anomalies included club feet, a reduction in the size of the fore limbs and the tail and absence of digits on both fore and hind limbs. Skeletal examinations indicated that the club foot anomaly was related to a reduction in size or absence of the tibia and also revealed a high incidence of fused ribs and vertebrae as well as the absence of digital bones. Soft tissue examinations most frequently revealed hydronephrosis sometimes accompanied by hydroureter. In conclusion, maternal food deprivation for 24 hr preceding dinoseb treatment altered dinoseb-induced teratogenicity by increasing the incidence of anomalies that were qualitatively the same as those observed with dinoseb alone.

121. *Distribution, Placental Transfer and Perinatal Toxicity of Paraquat in Mice.* JAMES S. BUS, MAURLINE M. PREACHE and JAMES E. GIBSON. Department of Pharmacology, Michigan State University, East Lansing, Michigan.

The purpose of this study was to examine the teratogenicity, placental transfer, maternal distribution and postnatal toxicity of paraquat in Swiss-Webster mice. Paraquat, 1.67, 3.35 and 6.7 mg/kg ip or 20.0, 40.0 and 100.0 mg/kg po, was administered daily to pregnant mice on days 8–16 of gestation. Fetuses were examined for terata on day 19. Maternal distribution and placental transfer of paraquat was determined following administation of [^{14}C]paraquat, 20.0 mg/kg po, to pregnant mice on day 10 or 11 of gestation. Samples of maternal plasma, liver, lung and kidney plus placenta and embryo were collected at 0.25, 0.5, 1.0, 2.0 and 4.0 hr later and radioactivity was determined. In other experiments, paraquat was added at 50, 100 and 150 ppm in the water supply of pregnant mice starting at day 8 of gestation and continuing until the litters reached 42 days of age. Total litter body weights and mortality were recorded at 1-week intervals. A 100% maternal mortality was induced at 6.7 mg/kg ip and 40.0 and 100.0 mg/kg po before day 16 of gestation was reached. Paraquat, 3.35 mg/kg ip, significantly increased ($p < 0.05$) fetal resorptions ($22.5 \pm 3.5\%$) and was associated with high maternal mortality. Paraquat, 1.67 and 3.35 mg/kg ip and 20.0 mg/kg po, slightly increased gross, soft tissue and skeletal anomalies. Radioactivity after [^{14}C]paraquat was highest in liver and kidney, followed by lung, placenta and plasma. Radioactivity in the embryos reached maternal plasma values 2 hr after [^{14}C]paraquat but was low at all other times. Paraquat at 50 and

100 ppm did not alter neonatal growth rate compared to controls; however, 100 ppm paraquat increased neonatal mortality to 67%. The level of 150 ppm was lethal to the dams within 20 days after initiation of treatment. Despite low paraquat teratogenicity which may be due to low levels of paraquat reaching the embryo, postnatal mortality of mice receiving paraquat throughout perinatal development was high.

122. *Embryo- and Fetotoxicity of Inhaled Carbon Tetrachloride, 1,1-Dichloroethane and Methyl Ethyl Ketone in Rats.* B. K. J. LEONG, B. A. SCHWETZ and P. J. GEHRING, Chemical Biology Research, The Dow Chemical Company, Midland, Michigan.

The effects of subanesthetic concentrations of carbon tetrachloride (300 and 1000 ppm), 1,1-dichloroethane (3800 and 6000 ppm) and methyl ethyl ketone (1000 and 3000 ppm) on rat embryonal and fetal development have been studied. Groups of pregnant Sprague-Dawley rats were exposed to each solvent 7 hr/day on days 6 through 15 of gestation. All 3 solvents caused some degree of retarded fetal development, such as delayed ossification of sternebrae. Methyl ethyl ketone caused, in addition to the aforementioned embryo toxicity, a low incidence of true terata (acaudia, imperforate anus and brachygnathia) at 3000 ppm. These studies did not reveal a correlation between the toxicity incurred by the mother exposed to these solvents and that incurred by the embryos or fetuses.

123. *Embryo- and Fetotoxicity of Inhaled Chloroform in Rats.* B. A. SCHWETZ, B. K. J. LEONG and P. J. GEHRING, Chemical Biology Research, The Dow Chemical Company, Midland, Michigan.

This study evaluated the effects of subanesthetic concentrations of chloroform on rat embryonal and fetal development. Pregnant Sprague-Dawley rats were exposed to 30, 100 or 300 ppm chloroform for 7 hr/day on days 6 through 15 of gestation. Exposure to chloroform caused an apparent decrease in the conception rate (300 ppm) a high incidence of fetal resorption (300 ppm), retarded fetal development (30, 100, 300 ppm), decreased fetal body measurements (300 ppm) and a low incidence of acaudate fetuses with imperforate anus (100 ppm). Chloroform was not highly teratogenic but was highly embryotoxic at 100 and 300 ppm. At 30 ppm, only minor toxicity was observed. The results of this study do not reveal a correlation between maternal toxicity and embryo- or fetotoxicity as the result of exposure to chloroform.

124. *Teratology Studies on Orally Administered Chloroform in the Rat and Rabbit.* D. J. THOMPSON, S. D. WARNER and V. B. ROBINSON, Life Sciences Research and Development Laboratories, The Dow Chemical Company, Zionsville, Indiana.

Subanesthetic concentrations of chloroform have been shown to be embryocidal and teratogenic when administered by inhalation to pregnant rats during organogenesis (B. A. Schwetz *et al.*, personal communication). Since chloroform is found in numerous preparations intended for ingestion by man, the present studies were done to evaluate the teratogenicity of chloroform when administered orally to the rat and rabbit. Total daily doses of 0, 20, 50 or 126 mg/kg/day were given bid by esophageal intubation to pregnant rats on days 6–15 of gestation. Rabbits were given single daily doses of 0, 25, 35 or 50 mg/kg/day by stomach tube on days 6–18 of gestation. Dose levels for both studies were based upon preliminary tests in which doses up to 501 and 398 mg/kg/day were administered to pregnant rats and rabbits, respectively. Fetuses were removed by cesarean section 1 or 2 days prior to expected parturition and were examined for external, skeletal, and/or soft tissue abnormalities. In the preliminary study in rats, anorexia and maternal weight gain suppression occurred at levels of 126 mg/kg/day. Doses of 316 or 501 mg/kg/day were hepato- and nephrotoxic and resulted in the death of some animals. Similar evidence of toxicity occurred in the rabbit at dose levels of 63 and 100 mg/kg/day, respectively. In the main studies, anorexia and weight gain suppression in dams of both species, and subclinical nephrosis in the rat and hepatotoxicity in the rabbit occurred at the highest dose levels. There was no evidence of teratogenicity in either species at any dose level tested.

125. *Teratologic and Reproduction Studies with Cyclazocine.* S. SMITH, G. L. KENNEDY, M. L. KEPLINGER, J. C. CALANDRA and J. A. NUITE, Industrial BIO-TEST Laboratories, Inc., Northbrook, Illinois, and National Institute of Mental Health, Rockville, Maryland.

Studies were conducted to determine the effects of cyclazocine on embryo and fetal development, fertility, and reproduction. For fertility and reproduction, Charles River strain albino rats were gavaged with 3, 10 or 30 mg/kg/day. Treatment was initiated 63 and 14 days prior to mating for males and females respectively. Dosing continued until sacrifice at the end of mating for males, on day 14 of gestation for one-half of the females or after weaning 21-day-old pups. Food consumption, behavior and mating and fertility indices were similar for control and treated groups. Females treated with 30 mg/kg of cyclazocine gained less weight than controls during the pre-mating period. Females sacrificed on gestation day 14 had similar numbers of implantation sites, resorption sites, corpora lutea and viable fetuses for all groups. Parturition indices, pup survival and pup body weight were also similar to controls. Initiation of treatment on day 15 of gestation led to no untoward effects. The teratogenic potential was studied in rats and New Zealand rabbits. Rats were dosed from days 6 to 15 of gestation and rabbits from days 6 to 18 with 3, 10 or 30 mg/kg/day. Rats were sacrificed on day 20 and rabbits on day 29 to determine the number of implantation sites, resorption sites, corpora lutea, and viable fetuses as well as any external, internal and skeletal abnormalities. Cyclazocine had no effect on the body weight gained by bred rats. Body weights of all treated rabbits decreased during treatment, but the overall gain during pregnancy was similar to controls. The number of viable fetuses/100 implantation sites and fetal body weights for rats and rabbits were similar to the respective controls. External fetal abnormalities occurred with an incidence of 1.4% in rats after treatment of adults with 10 mg/kg cyclazocine. No other external, internal or skeletal abnormalities were noted in offspring of either species. The findings of these studies indicate that cyclazocine had no effect on fertility and reproduction in rats and was not teratogenic to rats or rabbits. (Supported by NIMH Contract HSM-42-72-171.)

126. *Teratogenic and Biochemical Aspects of the Interaction of 5-Diazouracil and 5-Fluorouracil in the Mouse Embryo.* R. G. SKALKO and R. D. SAX, Birth Defects Institute, New York State Health Department and Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York. (L. Golberg.)

The teratogenic activity of 5-fluorouracil (5-FU) in laboratory rodents is potentiated by the simultaneous administration of the natural pyrimidines thymidine, thymine and uracil. The site of interaction appears to be the maternal liver, as the natural pyrimidines inhibit the catabolism of FU by the soluble enzyme, pyrimidine dehydrogenase (*Teratology* **2**, 99, 1969). The present study was performed to further clarify the role of drug interaction in teratogenesis utilizing 5-diazouracil (5-DU), a non-competitive inhibitor of the same enzyme, in conjunction with FU on day 10 (Witschi stage 18) of mouse development. The ip administration of FU to pregnant ICR mice on day 10 produces the following results: 10 mg/kg—non-teratogenic; 20 mg/kg—teratogenic but not embryolethal; 40 mg/kg—highly embryolethal. When 10 mg/kg DU is administered 3 hr before, 1 hr before or simultaneously with 10 mg/kg FU, the combination is embryolethal (96–100% of implantation sites). If DU is administered 1 or 3 hr after FU, no embryotoxic effects are observed. The tissue distribution of 10 mg/kg [^{3}H]FU was analyzed in homogenates of selected maternal organs, placentas and embryos between 15 min and 8 hr after administration. In all tissues, the disappearance rates are biphasic, having a rapid exponential phase (k_1) with a $t_{\frac{1}{2}}$ of 23–50 min, and a slow exponential phase (k_2) with a $t_{\frac{1}{2}}$ of 100–600 min. The effect of simultaneous administration of 10 mg/kg DU is the loss of k_1 and a prolongation of tissue availability times ($t_{\frac{1}{2}}$ = 130–390 min). Thus, DU administration prolongs the availability time of FU for placental transport and results in elevated concentration within the embryo between 15 min and 8 hr after injection. Histological analysis indicates that these altered kinetic parameters coincide with increased levels of cell death between 12 and 48 hr in primitive ependymal cells and areas of condensed mesenchyme. Patterns of necrosis following 10DU-10FU treatment are identical to those following 40FU alone.

127. *An Examination of the In Vitro Metabolism of Parathion in Rat Lung and Brain.* B. J. NORMAN and R. A. NEAL, Center in Environmental Toxicology, Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee.

Although in a quantitative sense the liver is the major organ involved in the metabolism of parathion to its toxic oxygen analog, paraoxon, extrahepatic metabolism of parathion to paraoxon may play a more important role in the toxicity of this pesticide. We have therefore undertaken studies examining the lung and brain of the rat for its ability to metabolize parathion to paraoxon. These studies have indicated that on the basis of the protein content of the "microsomal fractions" the rat lung metabolizes parathion to paraoxon at a rate that is approximately 10% of rat liver. These studies have also revealed that the "microsomal fraction" from rat brain can metabolize parathion to paraoxon at a rate that is about 3% that seen with microsomes isolated from the liver. The metabolism of parathion to paraoxon using "microsomes" isolated from rat lung and brain has been shown to require the presence of NADPH and is inhibited by carbon monoxide. The metabolism of parathion to paraoxon by rat lung and brain does not appear to be increased by prior treatment of the animals with phenobarbital or 3-methylcholanthrene. The apparent K_m for the metabolism of parathion to paraoxon by "microsomes" isolated from the lung appeared to be significantly lower than the apparent K_m for this reaction catalyzed by rat liver microsomes. The apparent K_m for metabolism of parathion to paraoxon by "microsomes" isolated from the brain appears to be very similar to that for liver microsomes. The results of these studies indicate there is a low but significant rate of metabolism of parathion to paraoxon in the rat lung and brain.

128. *The Influence of Age and Sex on the Toxicity and Multiple Pathways of Metabolism of Methyl Parathion and Parathion in Rats.* G. M. BENKE and S. D. MURPHY, Department of Physiology, Harvard School of Public Health, Boston, Massachusetts.

Methyl parathion (MPS) and parathion (PS) are 2 widely used organophosphorus insecticides. The literature reveals that there are marked age and sex differences in LD50 values for rats for MPS and PS. The present investigation of multiple pathways of metabolism of these compounds in vitro was undertaken in an attempt to explain these differences. The following pathways were studied: oxidative cleavage to *p*-nitrophenol and the corresponding dialkylthiophosphate; activation to the oxygen analogue; glutathione-dependent degradation of MPS and PS; and hydrolysis and binding of methyl paraoxon (MPO) and paraoxon (PO). Livers of male and female Holtzman rats 1, 12, 24, 37 and 60 days old were used to determine changes in enzyme activity occurring during this period, and LD50 values (ip) were also determined for both sexes at each age. For both compounds there was a direct correlation of LD50 with age, i.e., older animals were less susceptible. In adult females the LD50 for MPS and PS was 8 and 3 respectively, while in males the LD50 was 5.8 for both compounds. The calculated ratio of rates of oxidative cleavage/activation in adult male livers for both insecticides was equal (1.34) while in females for MPS it was 1.75 and for PS 0.94. The ratio was lower with the younger animals, indicating a lesser tendency for the liver to cleave than activate the parent insecticide. Liver and plasma of younger animals were also less able to hydrolyze MPO and PO by A-esterases. Glutathione-dependent detoxification was lower in 1- and 12-day-old male and female rat livers, but there was little difference between weanling and adults. Liver and plasma of younger rats, especially 1- and 12-day-olds, also had much less capacity to bind MPO and PO. These results indicate that several alternate pathways of metabolism must be considered in attempted correlations with age and sex differences in toxicity. (Supported by Training and Research Grants ES 00045, ES 00084, and ES 00002 from the National Institute of Environmental Health Sciences, USDHEW.)

129. *Propanil-Induced Methemoglobin Formation in Relation to its Metabolism In Vitro.* A. Y. K. CHOW and S. D. MURPHY, Department of Physiology, Harvard School of Public Health, Boston, Massachusetts.

Previous studies in this laboratory showed that ip administration of the herbicide, propanil (3,4-dichloropropionanilide) produced methemoglobin in mice. In this study, we compared

species and sex differences in the metabolism of propanil in relation to its methemoglobin production in vivo. Adult male and female mice, rats and guinea pigs were given single ip doses (1.83 mmol/kg) of propanil and methemoglobin concentrations (as percent total hemoglobin; % MHb) were determined at intervals of 0.5–3 hr. Three hr after propanil, the mean % MHb values in male mice, rats and guinea pigs were 53.9, 50.1 and 5.1 respectively. The corresponding values for females were 28.3, 65.1 and 5.0. Parallel studies using equimolar doses of 3,4-dichloroaniline (DCA), a hydrolytic metabolite of propanil, showed no sex differences in methemoglobinemic response in any species; the % MHb values 3 hr after DCA in male mice, rats and guinea pigs were 33.4, 62.3 and 15.2. Of 9 tissues assayed for acylamidase activity only liver and kidney hydrolyzed propanil. Male mice had significantly higher liver and kidney acylamidase activity than females which may explain the sex difference in propanil-induced methemoglobinemia in this species. The resistance of guinea pigs to propanil methemoglobinemia was not due to low acylamidase activity. The relative capacities of NADP-glucose 6-PO_4-fortified liver homogenates from both sexes of each species to activate DCA to methemoglobin-producing metabolites were compared using an in vitro system. Livers from mice and guinea pigs were about equally active in producing methemoglobin forming metabolites from DCA, while rat livers were less effective. Sex differences in liver capacity to activate DCA were not observed in mice and guinea pigs, but male rat livers were more active than female rat livers. These results are in contrast to those expected from the in vivo studies with DCA suggesting that other factors such as species differences in methemoglobin reduction may be involved. (Supported by Research Grants ES 00084 and ES 00002 from the National Institute of Environmental Health Sciences, USDHEW.)

130. *Interaction of Endrin and Dieldrin with Hepatic Microsomal Cytochrome P-450.* J. R. HAYES, R. W. HARTGROVE, S. G. HUNDLEY, T. C. CAMPBELL and R. E. WEBB, Departments of Biochemistry and Nutrition and Human Nutrition and Foods, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

The kinetic parameters for binding of endrin to hepatic microsomes were determined for two strains of the vole (*Pitymys penetorum*) resistant (R) and susceptible (S) to the toxicity of endrin, respectively. The LD50 values were 18.9 and 2.6 mg/kg for the R and S strains, respectively (Petrella and Webb, *Fed. Proc.* 32, 320Abs., 1973). Endrin produces type I binding when added to microsomes of either strain. No significant differences were observed between the binding kinetics for these two strains. The K_s (spectral dissociation constant) values were 5.6 ± 0.7 μM (R) and 4.4 ± 0.4 μM (S); the ΔA_{max}/mg protein values were 0.045 ± 0.007 (R) and 0.051 ± 0.001 (S); and the ΔA_{max}/nmol cytochrome *P*-450 measurements were 0.029 ± 0.004 (R) and 0.035 ± 0.002 (S). Therefore, the capacity of microsomes to bind endrin was not related to the endrin resistance exhibited by these 2 strains. Since metabolism of endrin by these 2 strains also differs significantly (Petrella and Webb, *Fed. Proc.* 32, 320Abs., 1973), the absence of a difference in binding may indicate that binding and catalytic kinetic parameters are not related in the microsomes of this species. Dieldrin, the stereoisomer of endrin, known to differ in its toxicity betwen these 2 strains only approximately 2-fold, also showed no difference in its binding to microsomes.

131. *Biochemical Changes Produced in the Liver by Mirex.* J. L. BYARD, U. CH. KOEPKE, R. ABRAHAM, L. GOLBERG and F. COULSTON, Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York.

In a study of the hepatic effects of Mirex, Charles River CD-1 mice were fed 1–90 ppm Mirex in the diet for periods ranging from 5 days to 13 months, Swiss Webster, BALB/c and C57 mice were fed 15 ppm Mirex for 4 months, and Sprague Dawley rats received 5 and 30 ppm Mirex for 13 and 9 months respectively. At regular intervals, heavy mitochondrial, light mitochondrial, microsomal and soluble fractions were prepared from livers of 2–6 animals/group. Measurements of DNA, protein, respiration, oxidative phosphorylation, mixed function oxidase, glucose-6-phosphatase and diene conjugates were carried out. Mirex stimulated an increase in relative liver weight which was slight at 1 ppm and 230% of the control value at

30 ppm. The protein in each liver fraction increased in approximate proportion to the increase in relative liver weight. Mixed function oxidase activity and cytochrome *P*-450 were stimulated several-fold in all livers. The total DNA content of the liver was elevated 120–360%, and tended to increase as the level of Mirex and the duration of feeding increased. While the amount of DNA in the liver increased, the DNA concentration decreased, indicating that new protein resulted from stimulation of RNA and/or protein synthesis as well as DNA synthesis. Respiration in the light mitochondrial fraction was stimulated 2- to 3-fold by Mirex. In comparison with controls, the mitochondria in this fraction has a more intact inner membrane, as measured by permeability to NADH, and a tighter coupling of oxidative phosphorylation to respiration. Decreased glucose-6-phosphatase activity was not associated with lipid peroxidation since the concentration of diene conjugates was unchanged. No marked sex differences were observed in the biochemical parameters in Charles River CD-1 mice. Strain differences were not evident in male Swiss Webster, BALB/c, C57 or CD-1 mice fed 15 ppm Mirex for 2 and 4 months. At comparable doses and lengths of exposure, the biochemical parameters were much less changed in rats than in mice. (Supported by Research Grant 2P01-ES00226-07 from the National Institute of Environmental Health Sciences, NIH, and by National Institutes of Health Training Grant 2T01-ES00103-07.)

132. *Comparative Lindane Metabolism and Enzyme Induction in Rats Pretreated with Eight Different Chlorinated Pesticides.* R. W. CHADWICK and J. J. FREAL, Environmental Protection Agency, National Environmental Research Center, Primate and Pesticides Effects Laboratory, Research Triangle Park, North Carolina. (R. S. McCutcheon.)

Barbiturates and miscellaneous drugs, including organochlorine pesticides, are believed to bring about a generalized nonspecific induction of microsomal enzymes through similar mechanisms. However, previous work indicates that the metabolism of toxicants may be selectively altered by pretreatment with different pesticides and drugs. This study compares the effect of pretreatment with 1 of 8 organochlorine pesticides on the metabolism of lindane and on the activity of various hepatic microsomal enzymes in the rat. Forty-eight weanling, female rats were randomly assigned to 1 of 8 treatment groups and received daily oral injections of 1 mg of either chlordane, DDT, hexachlorobenzene, Mirex, Penphene, Pentac, Toxaphene or just peanut oil. On the eighth day all animals received 1.7 mg of lindane (containing 1.49 μCi of [^{14}C]lindane) in place of their previous treatment and they were sacrificed at 24 hr later. In general, DDT, Mirex, chlordane and hexachlorobenzene appear to be the most effective inducing agents followed by Toxaphene, Pentac and Penphene. However, while in the vitro activity of various microsomal enzymes was increased to the greatest extent by pretreatment with Mirex, the metabolism of lindane was significantly highest in the animals receiving DDT. DDT and hexachlorobenzene pretreatment resulted in the excretion of more free chlorophenols and neutral metabolites, while Mirex and chlordane stimulated the excretion of more conjugated chlorophenols and highly polar metabolites. DDT was unique in stimulating the excretion of the metabolite 2,3,4,5-tetrachlorophenol. The results of this study indicate the possibility of rather selective enzyme responses to certain organochlorine pesticides and suggest that these compounds may induce microsomal enzymes through dissimilar mechanisms.

133. *Transfer of Endrin via the Milk in Endrin-Susceptible and Resistant Pine Mice and the Resultant Effects on Liver Microsomal Activity in the Neonate.* S. G. HUNDLEY, R. W. HARTGROVE, JR., and R. E. WEBB, Departments of Biochemistry and Nutrition, and Nutrition and Foods, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Many lipophilic pesticides are known to be transferred to offspring via mother's milk. The present study was conducted to determine the transfer of endrin from dam to pup in endrin-susceptible and resistant pine mice and to compare the activity of the hepatic mixed function oxidase (MFO) system in pups from dams treated with or without endrin. Dosing of the dams with endrin began 1 day after birth with either (1) oral doses of endrin in corn oil or (2) a mixture of endrin in ground lab feed. The total amount of endrin present in the whole pup was determined by gas chromatography. MFO activity was determined in 2.5-week-old pups on the

basis of maximal activity of ethyl morphine N-demethylase and aniline hydroxylase. No difference was observed in the amount of endrin present in pups from endrin-resistant and susceptible dams maintained on an identical endrin dosing schedule. Though a decrease in MFO activity was seen in pups from either endrin-resistant or susceptible dams treated with endrin as compared to the activity in pups from dams maintained on a normal diet, the magnitude of decrease was greater in pups from endrin-susceptible dams.

134. *Effects of Perinatal Dieldrin Exposure on Hepatic Microsomal Enzymes of Immature and Adult Rats.* F. E. GREENE, J. T. STEVENS, M. R. I. SOLIMAN and K. A. OBERHOLSER, Department of Pharmacology, The M. S. Hershey Medical Center, The Pennsylvania State University, Hershey, Pennsylvania.

There have been numerous studies concerning the effects of intrauterine exposure to insecticides on the activity of hepatic drug metabolizing enzymes in the neonate. However, little data is available on the long-term effects of such exposure. We have examined the effects produced by feeding a semi-synthetic diet containing 50 μg/gm of dieldrin to SD rats during the last week of pregnancy on certain hepatic microsomal cytochrome *P*-450-dependent pathways at 1, 5, 14, 21 and 42 days of age. Metabolism of [^{14}C]dieldrin was also examined in adult animals. As expected, microsomal *P*-450 concentrations were higher in offspring of dieldrin-exposed mothers. Differences appear to reach maximum at 5 days when control values were 0.21 ± 0.03 nmol *P*-450/mg and values in exposed rats 0.60 ± 0.06 nmol *P*-450/mg. At 14 days these values were 0.60 ± 0.01 nmol/mg and 1.40 ± 0.16 nmol/mg in perinatally exposed rats. Significant differences in *P*-450 values were also seen at 21 but not at 42 days. Ethylmorphine-N-demethylase (ETM) activity paralleled differences in *P*-450 concentrations at 5 and 14 days, but differences between perinatally exposed and control rats were no longer significantly different at 21 days of age. The sustained induction is probably the result of continuous ingestion of small amounts of dieldrin during nursing. At 42 days of age the *P*-450-dependent system, as measured by ETM and aniline metabolism, NADPH oxidase and NADPH cytochrome *c* reductase activity, was identical in the perinatally exposed and control groups. There were, however, small but significant differences in fatty acid composition of the microsomal membranes. Perhaps the most significant effect produced by perinatal dieldrin exposure is permanent impairment of dieldrin metabolism. In a typical experiment the rate of [^{14}C]dieldrin metabolism was 35.5 ± 3.8 pmol/mg microsomal protein/min in control and 24.1 ± 2.4 pmol/mg microsomal protein/min in 6-week-old perinatally exposed male rats. These results demonstrate that perinatal exposure to common environmental contaminants such as dieldrin may produce both reversible and irreversible changes in hepatic microsomal enzymes. (Supported by NIEHS Research Grant 9-R01-ES00638-04.)

135. *Individual and Combined Effects of Mirex and Polychlorinated Biphenyls on Mouse Liver Cells.* R. ABRAHAM, U. CH. KOEPKE, L. GOLBERG and F. COULSTON, Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York.

Liver enlargement, proliferation of smooth endoplasmic reticulum and increases in microsomal enzymes are common hepatic responses of rodents to the administration of single polychlorinated compounds, but little is known regarding the combined effects of 2 such compounds. A study was therefore undertaken of a mixture of polychlorinated biphenyls (PCB) (Aroclor 1254) and Mirex in Charles River CD-1 mice. The compounds were administered in the diet at the following levels for 7 days: group I, chow; group II, PCB 500 ppm; group III, Mirex 90 ppm; and group IV, Mirex 90 ppm and PCB 500 ppm. Loss of body weight, increases in liver weight and demethylase activity were recorded in all 3 experimental groups, being most pronounced in group IV. Glucose 6-phosphatase activity was decreased in groups II and III and remained unchanged in group IV. In mice of group IV there were striking structural changes in hepatic lysosomes, with formation of large myeloid bodies (lysosomes containing cellular debris) and numerous autophagic vacuoles that were PAS-positive and stained for acid phosphatase. These changes were reflected in the elevated activities of both total and sedimentable acid phosphatase. The lysosomes were normal in all other groups. Further changes included loss of glycogen in groups III and IV and hepatic cell necrosis in group IV.

The capacity of the combination of toxicants to elicit unique lysosomal responses poses questions regarding the biological interaction between environmental chemicals. (Supported by Research Grant 2P01-ES00226-07 from the National Institute of Environmental Health Sciences, NIH, and by National Institutes of Health Training Grant 2T01-ES00103-07. Dr. Abraham is the recipient of a Research Career Award No. 1 K04 ES70607-01.)

136. *Toxicity Studies of an Insect Juvenile Hormone Analogue in Domestic Animals.* H. E. SMALLEY, J. E. WRIGHT, H. R. CROOKSHANK and R. L. YOUNGER, U.S. Department of Agriculture, College Station, Texas.

Insect juvenile hormone analogues (JHA) are being studied and developed for use as specific insecticides. Field tests in cattle-feed lots have shown the efficacy of 1 of these compounds in control of the stable fly, *Stomoxys calcitrans* (L), and its promise has led us to study the toxicity. Studies on a Stauffer JHA, R-20458, were done on cattle, sheep and swine on the proposed commercial compound of 74% purity. The cattle were sprayed with the recommended dose (1% solution) and the sheep and swine were given the compound orally, by dose gun for single doses and mixed in their feed on longer studies. Biochemical, hematological and histopathological studies were done on all species. There were no differences seen in treated cattle in any parameter tested; there was, however, an exacerbation of the reaction of the animals to parasitic grubs (*Hypoderma* spp.) in the skin. Large, single oral doses of the JHA to sheep caused a dose-related leukopenia which lasted for 2–3 weeks. There were no changes seen in sheep on long-term feeding tests in the biochemical or hematological studies, but there was a marked testicular atrophy seen in rams at all levels, none in the controls. No toxicity was noted in any of the swine studies. Further studies were done in sheep on the same JHA but of 96.2% purity. We did not find the leukopenia in the acute dose nor was there the testicular atrophy seen in the rams. All other parameters remained within normal ranges. The toxicity of the Stauffer JHA, R-20458, appears to be negligible when used in a state of purity of 96.2%.

137. *The Toxicological Properties of 4-((4,8-Dimethyldecyl)oxy)1,2-(Methylenedioxy)-benzene and 1-((7-Ethoxy-3,7-Dimethyloctyl)oxy)-4-ethyl Benzene, two Insect Juvenile Hormone Mimics.* J. M. NORRIS, C. G. HUMISTON, B. A. SCHWETZ, R. J. KOCIBA, G. C. JERSEY and C. E. WADE, Chemical Biology Research, The Dow Chemical Company, Midland, Michigan. (P. J. Gehring.)

The object of this study was to evaluate the toxicity of 4-((4,8-dimethyldecyl)oxy)-1,2-(methylenedioxy)benzene (DMB) and 1-((7-ethoxy-3,7-dimethyloctyl)oxy)-4-ethyl benzene (EDB) which are juvenile hormone mimics that cause gigantism and increased life span of the larvae of the yellow mealworm *Tenebrio molitor* when incorporated in their feed. The single oral doses of 4000 mg DMB/kg and 8000 mg EDB/kg were not lethal. Higher doses were not given. Decreased food consumption and body weight gains occurred in rats receiving 100 mg DMB/kg/day for 98 days. Increased liver weights, histopathological changes consisting of minimal focal lipid vacuolar degeneration and necrosis of individual hepatocytes, elevated alkaline phosphatase activity and depressed packed cell volume, hemoglobin concentrations and red blood cell counts were observed at 100 and 50 mg/kg/day; 1 and 10 mg/kg/day had no toxicological effect. A 90-day dietary feeding study with EDB showed that 100, 50, 10 or 1 mg/kg/day caused no toxicological effect. The reproductive capacity of rats maintained on 100, 50, 10 or 1 mg DMB/kg/day for 62 days before mating was not effected at any of the dose levels as demonstrated by the duration of the estrus cycles, fertility and reproductivity. Neonate survival and growth, and the litter size from dams, was decreased at 100 mg DMB/kg/day but not at lower levels.

138. *Enhancement of Effect of Exposure to O_3 and NO_2 by Exercise.* D. E. GARDNER, J. W. ILLING and D. L. COFFIN, Experimental Biology Laboratory, National Environmental Research Center, Environmental Protection Agency, Research Triangle Park, North Carolina.

Small laboratory animal models have been widely used by environmental toxicologists to investigate the potential health hazard of air pollutants. With few exceptions, during the

exposure to the pollutants the animals were at rest. In the natural environment the population at risk is not always at rest, but in contrast, is frequently active. This study was undertaken to determine if exercise during exposure to a gaseous air pollutant would increase the risk resulting from the inhalation of viable organisms. Two groups of mice were exposed to either 0.3, 0.2 or 0.1 ppm of ozone or 3.0, 2.0 or 1.0 ppm of nitrogen dioxide for 3 hr. One group of animals were at rest while the second was on a newly designed mechanized exercise wheel. After the exposure to the gas the groups were combined and together they were then exposed, while at rest, to an aerosol of viable microorganisms. A significant increase in mortality was noted in the exercise animals as compared to those at rest.

139. *Time/Dose Response for NO_2 Exposure in an Infectivity Model.* D. L. COFFIN, D. E. GARDNER and E. J. BLOMMER, Experimental Biology Laboratory, National Environmental Research Center, Environmental Protection Agency, Research Triangle Park, North Carolina.

The concentrations of NO_2 in polluted atmosphere is subject to wide variation according to peak traffic load, industrial productivity, intensity of sunlight and meteorological conditions. Normally, NO_2 has a very low basal concentration with superimposed spike or pulse when the above conditions are optimal for its production. Thus it is important to determine the relative importance of a short-term relatively high-dose vs exposure for long periods at minimal dose levels. In other words, which is more important, time or concentrations in the toxic response to NO_2? Utilizing the most sensitive animal biological system for NO_2 effect, the infectivity model, experiments were carried out to answer this question. Parameters employed were increases in mortality, indicators of pulmonary inflammation, changes in pulmonary macrophages and alterations in immunological factors. Data indicate that when employing exposure times ranging from 0.5 to 28 hr utilizing a constant CT the effect is skewed, with higher mortality occurring from short-term high concentration abruptly tapering off at concentrations lower than the 2-hr threshold (3.5 ppm $\times$ 2 hr).

140. *Histopathologic Effects of Nitrogen Dioxide Exposure and Heat Stress in Cynomolgus Monkeys.* W. M. BUSEY, W. B. COATE and D. W. BADGER, Experimental Pathology Laboratories, Inc., Hazleton Laboratories, Inc., and the National Institute of Occupational Safety and Health, Cincinnati, Ohio.

Histopathologic studies were conducted on the lungs from male cynomolgus monkeys exposed continuously for 90 days, with and without heat stress, to 5 and 10 ppm of nitrogen dioxide. Similar studies were conducted on the lungs from air exposed monkeys with and without heat stress. Nitrogen dioxide-related microscopic alterations were seen in the respiratory bronchioles, alveolar ducts and adjacent interalveolar septa. These alterations were characterized by an interstitial infiltration of mononuclear macrophages, lymphocytes and occasionally polymorphonuclear leucocytes. Bronchiolar epithelial hyperplasia was also frequently seen in the monkeys exposed to both 5 and 10 ppm of nitrogen dioxide. Granular pneumonocyte hyperplasia was occasionally seen in alveoli adjacent to affected bronchioles. Focal pulmonary edema was present in the lungs from some monkeys exposed to 10 ppm of nitrogen dioxide. No microscopic morphological effect of heat stress was seen in the lungs from either the nitrogen dioxide-exposed or control monkeys.

141. *Physiological Effects of Nitrogen Dioxide Exposure and Heat Stress in Cynomolgus Monkeys.* W. B. COATE and D. W. BADGER, Hazleton Laboratories Inc., and the National Institute of Occupational Safety and Health, Cincinnati, Ohio. (W. M. Busey.)

In order to assess the effects of hot environments on nitrogen dioxide toxicity, groups of 10 male cynomolgus monkeys were exposed continuously for 90 days to 5 and 10 ppm of NO_2 at 72° E.T. and at 88° E.T. Air control groups were maintained at the respective effective temperatures. Hematological studies included CBC, methemoglobin, plasma volume and red cell survival. Serum chemistry, blood gases, chest X-rays and body weights were monitored.

Pulmonary function tests were conducted. The results indicated that NO_2 at 10 ppm impaired distribution of ventilation of the lungs, increased respiratory rate, and decreased tidal volume. The addition of heat-stress to this level of NO_2 exposure did not further impair distribution of ventilation but it decreased dynamic compliance of the lungs whereas NO_2 alone did not. The combination of 10 ppm NO_2 and heat-stress resulted in a mean loss in body weight over the 90-day exposure whereas all other groups gained weight. No effects were found on hematological or serum chemistry attributable to NO_2 or heat-stress. No deleterious effects were found on blood gases from either factor. No effects on airway resistance were obtained. General observation indicated that the animals were hypoactive in the chambers at 88° E.T. with and without NO_2. No synergistic effects of heat-stress were found at 5 ppm NO_2.

142. *Peripheral Airway Obstruction in Primates following Long-Term Inhalation Exposure to Coal Dust.* W. J. MOORMAN, T. R. LEWIS and W. D. WAGNER, National Institute for Occupational Safety and Health, Cincinnati, Ohio. (H. Stokinger.)

One of the earliest physiologic responses to the inhalation of coal dust is pulmonary obstruction, primarily of the small, peripheral airways. Therefore, techniques were developed to assess peripheral airflow in cynomolgus monkeys exposed to coal dust at the Threshold Limit Value and Federal Coal Mine Dust Standard (2 mg/m^3 respirable dust). Two types of coal were studied: Pennsylvania coal from an area with a high incidence of lung disease, and Utah coal from an area of low incidence. Both treatment groups were maintained for 2 years at 2 mg/m^3, 6.5 hr daily. Twelve monkeys were assigned to each treatment group and control group. The following pulmonary function tests were employed to detect small airway obstruction: forced expiratory volume in 1 sec, maximum midexpiratory flowrate, and maximum expiratory flow at small lung volumes. All coal exposure groups demonstrated marked reduction in flowrates for the above parameters; however, no differences were demonstrated between Pennsylvania and Utah coal exposures. This combination of technique, species and results presents a useful animal model for studying environmental and occupational human respiratory disease.

143. *Influence of Hypoxia or Hypercapnia during Sensory Irritation by Airborne Chemicals.* Y. ALARIE and J. KOMINSKY, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania.

During sensory irritation of the upper respiratory tract by airborne chemicals a decrease in respiratory rate occurs which is not compensated by a proportional increase in tidal volume. In order to evaluate the state of the peripheral and central chemoreceptors during this reflex inhibition mice were exposed to various sensory irritants at concentrations capable of inducing 80% decrease in respiratory rate and hypoxic or hypercapnic gas mixtures were introduced. The results indicate that with low oxygen (12–14%) a rise in respiratory rate can be observed while high concentrations of CO_2 (2–5%) did not modify the respiratory rate. Conversely during low oxygen tidal volume remained unchanged while it increased significantly with high concentrations of CO_2. It is concluded that hypoxic or hypercapnic stimuli are still effective during respiratory inhibition due to stimulation of trigeminal nerve endings of the upper respiratory tract by airborne chemical irritants and that in mice low oxygen increased respiratory rate primarily while CO_2 increased tidal volume. (Supported in part by Research Grant 5 R01-OH-00367, D.H.E.W.)

144. *Red Cell 2,3-Diphosphoglycerate and Oxygen Toxicity in Rats.* J. B. WEISSBERG and J. D. CRAPO, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. (R. L. Dixon.)

Studies were undertaken to investigate red cell 2,3-diphosphoglycerate (2,3-DPG) metabolism in rats exposed to high oxygen tensions. Previous investigators have shown that 2,3-DPG decreases the affinity of hemoglobin for oxygen by specifically binding to deoxyhemoglobin. Elevations in red cell 2,3-DPG with resultant enhanced availability of oxygen to tissues have been reported in instances of lowered arterial oxygen tension. If under high oxygen tensions

2,3-DPG concentrations decline, less oxygen would be available to tissues, and the toxic effects of oxygen may be reduced. Large male rats (greater than 9 weeks of age) were exposed to 100% O_2. No change in red cell 2,3-DPG concentrations occurred after 24, 48 and 60 hr of exposure. A 31% increase in 2,3-DPG was observed after 24 hr of exposure to 10% O_2. However, younger (3- to 4-week-old) male rats showed a 22% decline in red cell 2,3-DPG after 24 hr in 100% O_2. This effect persisted throughout a 7-day exposure period. The younger animals were also shown to be less responsive to the toxic effects of oxygen. While older rats died after 60–72 hr of exposure to 100% O_2, the young rats survived for prolonged periods. A differential response between young and older rats exposed to toxic levels of oxygen has been observed, and it is postulated that 2,3-DPG may have a role in protection against the toxic effects of oxygen.

145. *Metal Cytotoxicity: Comparative Studies with Rabbit Alveolar Macrophages and Human Lung Fibroblasts (Strain WI-38).* M. D. WATERS, D. E. GARDNER and D. L. COFFIN, Experimental Biology Laboratory, National Environmental Research Center, Environmental Protection Agency, Research Triangle Park, North Carolina.

Two model systems in vitro were employed to study the relative cytotoxic properties of soluble salts of several metals that occur as environmental pollutants. Rabbit alveolar macrophages and human lung fibroblasts (Strain WI-38) were exposed to Cd^{2+}, Ni^{2+}, Mn^{2+} and Cr^{3+} as chlorides and to VO_3^- as ammonium vanadate. Trypan blue exclusion tests for cell viability after 20 hr indicated that the relative toxicity of the metals in both test systems was Cd > V > Ni > Mn > Cr. Metal concentrations that caused a reduction in cell viability to 50% in 20 hr ranged from approximately $1–3 \times 10^{-4}$ M with Cd^{2+} and VO_4^- to $5–7 \times 10^{-3}$ M with Mn^{2+} and Cr^{3+}. In the macrophage test system, changes in cell viability could be correlated with morphological alterations observed by scanning electron microscopy and with changes in hydrolase specific activity (acid phosphatase and lysozyme). The uptake of precursors for DNA, RNA and protein biosynthesis by WI-38 fibroblasts was depressed at somewhat lower concentrations of metals than those which caused reduction in cell viability. The preliminary data suggest the potential usefulness of these tissue culture systems in screening for relative cytotoxicity of pollutant materials, particularly in cases where small sample size is a consideration.

146. *Distribution and Excretion of Radioactivity in the Rat after Intraperitoneal Administration of the Lung-Edemagenic Toxin, [^{14}C] 4-Ipomeanol.* M. R. BOYD, L. T. BURKA, B. J. WILSON and B. V. R. SASTRY, Center in Environmental Toxicology and Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee.

4-Ipomeanol [1-(3-furyl)-4-hydroxypentanone] is a poisonous metabolite produced by the mold-damaged sweet potato (*Ipomoea batatas*). In experimental animals the compound characteristically exhibits lung toxicity, with prominent pulmonary edema and congestion. Recent studies in our laboratory have centered on the question of why the lung is the primary target organ. We have initially studied the distribution and excretion of radioactivity in rats after administration of [^{14}C] 4-ipomeanol. The radiolabeled toxin was prepared from the reaction of ethyl-3-furoylacetate with either [2-^{14}C]bromoacetone or [1,3-^{14}C]bromoacetone, followed by partial reduction with sodium borohydride. Toxin was given ip at doses of 2, 10 and 30 mg/kg. Approximately half of the administered radioactivity appeared in the urine within 2 hr, with only traces occurring in the feces and expired air. The greatest tissue concentration of radioactivity occurred in the lungs. Other organs showing significant concentrations were liver, kidney and GI tract. The maximal accumulation of activity occurred in the tissues within 0.5–1 hr. The values thereafter declined over the next 1–2 hr, then leveled off to a plateau representing residual activity. Subsequent studies have shown that this residual activity is particularly high in lung, liver and kidney, and probably represents toxin or its metabolite(s) which has become tightly bound to tissue macromolecules, primarily protein. Binding occurs maximally in lung, and therefore may indicate that the binding phenomenon is involved in the toxic mechanism of 4-ipomeanol.

147. *The Effects of Freon 11 on Isolated Auricles.* J. H. WILLS and D. SILBER, Center of Experimental Pathology and Toxicology, Albany Medical College, Albany, New York.

Although exposure of spontaneously beating auricles from the heart of the rat to vapors of Freon 11 stops the contractions, the Freon 11 seems to produce no permanent damage. Upon pumping gas (95% O_2, 5% CO_2) containing vaporized Freon 11 (1% v/v) through a bath of Locke's solution in which the auricles were suspended, the contractions of the auricles initially increased in force but decreased in frequency. Later, or with higher concentrations of Freon 11, the contractions decreased in force also and might stop. Aeration of the bath thereafter with gas free of Freon 11 resulted in eventual resumption of spontaneous, rhythmic contractions. Decreasing the concentration of Ca in the Locke's solution lowered both the force and rate of beating of the auricles and their responsiveness to epinephrine. Verapamil, a drug thought to inhibit the coupling by Ca of excitation to contraction in cardiac muscle, increased the force of contraction of the auricles but did decrease their rate of beating.

148. *Statistical Analyses of Clinical Biochemistry Data Obtained from the Pesticide Community Studies.* K. P. BAETCKE, ANITA PEOPLES, M. F. CRANMER and D. L. GAYLOR, National Center for Toxicological Research, Jefferson, Arkansas.

A quality control program (intra- and interlaboratory) was instituted in conjunction with the EPA Pesticide Community Studies to ensure standardization of methodologies from laboratory to laboratory and to permit accurate determinations of biological variation in the human population based on clinical biochemistries. The clinical tests conducted by the 13 participating laboratories included determinations of cholesterol, total protein, albumin, uric acid, glucose, BUN, serum creatinine and phosphorus. Means, SD and coefficients of variation were determined for each test based on data collected during 13 test periods in 1972. The average coefficient of variation for all test periods exceeded 10% for normal serum creatinine only; variation of all other tests averaged 6–7%. Similar statistical data was collected on clinical biochemistries obtained on human participants in the long-term studies of the Community Studies program. It was found that biological variation exceeded analytical variation by as much as a factor of 11:1. A combination of analytical variation, based on quality control data, and biological variation, based on chemistries of human subjects, was utilized successfully to predict the chemical differences required between human populations for the differences to be statistically significant. Such information can be utilized to ascertain which clinical tests are the most appropriate for detecting effects of chronic exposure to chemical insult by the human population and to determine when analytical methodology R & D is desirable.

149. *Toxicology of USB 3584.* J. B. PLANK, D. C. LINDBERG, G. L. KENNEDY, M. L. KEPLINGER, J. C. CALANDRA and J. D. STONE, Industrial BIO-TEST Laboratories, Inc., Northbrook, Illinois and U.S. Borax Research Corporation, Anaheim, California.

Studies have been conducted to define the general toxicologic properties of USB 3584 (N^3, N^3-diethyl-2,4-dinitro-6-trifluoromethyl-m-phenylenediamine) and of its 25% emulsifiable concentrate formulation. Investigations included a battery of acute and subacute studies in rats, rabbits, fish, wild birds and chicks. Subacute feeding studies in mice and rats, a dominant lethal mutagenic study in mice and a teratologic study in rabbits. The USB was relatively non-toxic in acute studies, but the formulation was severely irritating to rabbit eyes upon unlimited contact and to rabbit skin upon repeated contact under occlusive conditions. Eye irritation was greatly diminished by washing after a 4-sec contact. In 90-day feeding studies, 600 ppm was a no-effect level in both rats and dogs. At 2000 ppm, both rats and dogs experienced body weight depressions, rats showed liver weight elevations and dogs showed a moderate anemia. No evidence was obtained to indicate cataractogenic, mutagenic, teratologic or carcinogenic activity for USB 3584.

150. *Metabolic Studies with USB 3584.* G. L. KENNEDY, M. L. KEPLINGER, O. E. FANCHER, J. C. CALANDRA and J. D. STONE, Industrial BIO-TEST Laboratories, Inc., Northbrook, Illinois and U.S. Borax Research Corporation, Anaheim, California.

A metabolic study with [^{14}C]USB 3584 (N^3, N^3-diethyl-2,4-dinitro-6-trifluoromethyl-m-phenylenediamine) in albino rats showed that the compound is rapidly and extensively metabolized. Unaltered USB 3584 was detected only in the feces. Chromatographic analysis suggested the occurrence of multiple metabolites in both feces and urine. None of these were identified. Tissue concentrations of USB 3584 and/or its tagged metabolites were low, estimated total recoveries from all tissues being 1.5–1.7% caluclated as unaltered USB 3584. Treatment of a dairy cow with a single dose of [^{14}C]USB 3584 after preconditioning with the unlabeled compound, at levels corresponding to 0.3 ppm in the total diet in each case, gave no detectable concentrations of radioactivity in whole milk and barely detectable levels in the concentrated lipid fraction. The ^{14}C activity of all tissues was below the detection limit of 0.36 ppb for 150-mg samples. Only liver samples had counts of 1–3 above background. Exposure of bluegill sunfish to [^{14}C]USB 3584 revealed no accumulation of residues in fish tissue and the retained residues were rapidly cleared upon termination of exposure.

151. *Toxicity and Reproduction Studies in Quail and Ducks with Dieldrin and USB 3584.* D. H. JENKINS, J. B. PLANK, M. L. KEPLINGER and J. C. CALANDRA, Industrial BIO-TEST Laboratories, Inc., Northbrook, Illinois.

Subacute dietary LC50 values of USB 3584 (N^3, N^3-diethyl-2,4-dinitro-6-trifluoromethyl-m-phenylenediamine) in Mallard ducks and bobwhite quail were determined to be >1000 and >5000 ppm respectively. Quail showed decreased food consumption and weight depression at dietary levels of 5000 ppm and higher over the 5-day period of treatment. The 5-day LC50 for dieldrin in quail was found to be 54 ppm. Reproduction studies were conducted in young adult ducks and quail at dietary levels of 10 and 30 ppm with USB 3584 and at 3 ppm with dieldrin. Treatment began 6 and 12 weeks prior to collecting eggs for hatching for ducks and quail respectively. There was no evidence of toxic effects upon parental animals from any treatment. The USB 3584 caused no adverse effects with respect to egg production or quality, shell thickness, hatchability, weight and viability of progeny and incidence of progeny abnormalities. Dieldrin slightly decreased the hatchability of eggs from ducks and appeared to slightly decrease the 14-day viability of quail chicks.

152. *Pathologic Effects of a Preparation of 2,4,5-Trichlorophenoxyacetic Acid on Maternal Mice.* BENJAMIN HIGHMAN and HERBERT J. SCHUMACHER, National Center for Toxicological Research, Jefferson, Arkansas. (T. J. Haley.)

To study the possible teratologic and pathologic effects of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), several strains of maternal mice were sacrificed for pathologic study at intervals during peroral treatment with this compound. Beginning 6 days after mating, the mice received on 9 successive days 30–120 mg/kg of 2,4,5-T dissolved in 0.2 ml of a 1:9 mixture of acetone and corn oil. This report concerns the effect of this preparation on C57BL/6 and CD-1 maternal mice. Pathologic findings were found in all treated groups of maternal mice but were most frequent and severe in mice that died or were sacrificed after 4–8 doses of 120 mg/kg of 2,4,5-T. Over 50% of these maternal mice showed myocardial lesions, and a great majority showed depletion of lymphocytes in the thymus, spleen or lymph nodes and hypocellularity of the bone marrow. Many developed bronchopneumonia and other severe secondary infections. The possibility that some of these lesions may be due to dioxins or other contaminants in the current preparation cannot be excluded and is under study.

153. *Failure of Parathion to Alter Male Mouse Reproductive Organ Activity.* JOHN A. THOMAS and MICHAEL G. MAWHINNEY, Department of Pharmacology, West Virginia University Medical Center, Morgantown, West Virginia.

Recent reports from this laboratory revealed that certain of the organochloride pesticides

(e.g. DDT or dieldrin), but not carbaryl, can reduce the metabolism of androgens in sex accessory organs of the male mouse. Parathion (1.3, 2.6 and 5.2 mg/kg daily × 5-po), like carbaryl, failed to interfere with the assimilation of [1-^{3}H] testosterone (T-^{3}H) by the prostate gland. These dose regimens had no effect upon testicular weights or upon prostate gland weights. The metabolism of T-^{3}H to its major radiometabolites by either the prostate gland or by hepatic microsomes revealed few parathion-induced changes in these tissues. Parathion-induced alterations in the formation of testosterone polar radiometabolites (e.g. 6-β, 7-α or 16-α hydroxytesterone-^{3}H), unlike DDT or dieldrin, were not particularly evident following the 5-day treatment period with this organophosphate. In general, these studies reveal that despite the relative high acute toxicity of the organophosphate-type pesticide, they are not as likely as the organochlorides to produce alterations in male reproductive organs. (Supported in part by Grant No. EP00871 of the U.S. EPA.)

154. *Toxicological Studies on the Avicide 3-Chloro-p-Toluidine.* W. C. FELSENSTEIN, R. P. SMITH and R. E. GOSSELIN, Dartmouth Medical School, Hanover, New Hampshire.

3-Chloro-p-toluidine (3-CPT) induced methemoglobinemia when given in lethal doses to mice and rats, but not when given to chickens. In mice the ip LD50 for 3-CPT (Hydrochloride salt) was 1.9 mmol/kg with 95% confidence limits of 1.7 and 2.1. Death in laboratory rodents, however, appeared to be unrelated to the methemoglobinemia. Peak values were moderate and had declined to low but persistent levels at time of death, which occurred in hours or several days in rats and mice, respectively. Heinz bodies appeared at 30 hr but there was no evidence of hemolysis. Blood concentrations of 3-CPT in fatally poisoned mice appeared to decrease linearly with time. The estimated half-life was 6.6 hr. In mice methemoglobinemia was attenuated, but mortality increased by methylene blue. Hyperbaric oxygen increased and ANIT decreased methemoglobinemia without affecting mortality; hypoxia had no effect on the latter. Cyanosis persisted after return of methemoglobinemia to low values and was not relieved by hyperbaric oxygen. No significant amounts of inactive pigments other than methemoglobin were found. Mortality in mice after 3-CPT was increased by cold stress or contemporaneous administration of Dial-Urethane. A profound and persistent hypothermia in mice and rats occurred after 3 CPT and mice showed a dramatic, transient decrease in pulmonary ventilation. Elevated ambient temperatures partially blocked the hypothermic response but, at least in mice, warming did not prevent death. In species resistant to 3-CPT methemoglobinemia death appears to be due to an uncharacterized block of energy metabolism. (Supported by USPHS Grant HL 14127 from the National Heart and Lung Institute and a Research Training Program, Primate and Pesticides Effects Laboratory, EPA.)

155. *Quantitative Aspects of Immunosuppression by Selected Pesticides.* J. C. STREET and R. P. SHARMA, Departments of Animal and Veterinary Science, Utah State University, Logan, Utah.

Dose-response evaluations of immune suppression by 4 insecticides were made using rabbits. Test compounds (p,p′-DDT, methyl parathion, carbaryl and carbofuran), were given orally, by means of treated feed, during a 28-day pretreatment period. Antigens were administered on day 29 into the foot pad (sheep erythrocytes plus Freund's complete adjuvant) and the status of the immune systems were evaluated over the following 28 days while continuing chemical treatments. The most sensitive indicator of immune suppression was a histological test of fluorescent anti-rabbit globulin-binding by sections of popliteal lymph node. By that test, significant suppression was observed with doses as low as 0.92 mg/kg DDT, 0.04 mg/kg methyl parathion and 0.49 mg/kg carbofuran. Supporting evidence of suppression was observed as reductions in germinal centers in spleen as well as thymus cell cortical atrophy. Methylparathion and DDT apparently suppressed cell-mediated sensitivity to tuberculin although higher dosages were required. In contrast, hemolysin and hemagglutinin titers were not systematically affected by dosages of any chemical up to 40-fold over those producing histological effects. Serum globulin values were also unaffected. Carbaryl, at dosages up to 8.38 mg/kg, gave no consistent indications of suppression in these studies. These observations

confirm previous reports of immune suppression by insecticides such as DDT, an effect which can now be extended to additional pesticidal chemicals as indicated by the results obtained with the organophosphate and carbamate compounds studied.

156. *Delayed Neurotoxic Potential of a Series of Alkyl Esters of 2,2-Dichlorovinyl Phosphoric Acid in the Chicken.* J. R. ALBERT and S. M. STEARNS, Shell Development Company, Modesto, California.

The absence of any delayed neurotoxic effect in the chicken has been reported for dimethyl 2,2-dichlorovinyl phosphate (dichlorvos). Replacement of 1 methyl group with an ethyl, isopropyl, pentyl or chloroethyl substituent results in chemicals with reported neurotoxic potential (Aldridge and Johnson, 1971; Aldridge and Barnes, 1966). In this study *bis* and C_1,C_n n-alkyl and C_1, chloroethyl esters of 2,2-dichlorovinyl phosphoric acid were tested in the 14- to 17-month-old hen for their delayed neurotoxic potential. The oral LD50 values in mg/kg determined for mixed C_1,C_n n-alkyl esters were C_1,C_1 (dichlorvos): 11.4 ± 1.4; C_1,C_2: 3.4 ± 1.0; C_1,C_3: 3.4 ± 0.6; C_1,C_4: 2.6 ± 0.6; C_1,C_5: 6.9 ± 1.1; C_1C_6: 21.1 ± 4; C_1,C_7: 50 ± 2; C_1,C_8: 78 ± 10; C_1,C_9: 134 ± 35; C_1,C_{10}: 339 ± 50; and $C_1,ClCH_2CH_2$: 11.8 ± 0.7. All induced qualitatively similar cholinergic effects. No delayed effects occurred except for the C_1,C_{10} ester (1 of 13). Delayed effects were found following supralethal doses (2 × LD50 plus atropine) with impure preparations of C_1,C_3, C_1,C_4, C_1,C_8, C_1,C_{10} esters and the $C_1,ClCH_2CH_2$ ester. The *bis* content of these ranged from 0.6 to 4.0%; a highly purified C_1,C_8 preparation, *bis* < 0.1%, did not cause delayed effects, *bis* n-alkyl substituted compounds had LD50 values of C_2: 3.0 ± 0.6; C_3: 9.4 ± 3.2; C_4: 13.7 ± 2.3; C_5: 25.7 ± 1.7; C_6: 82 ± 19; C_7: 677 ± 129; C_8: >3200; C_{10}: >1800; and $ClCH_2CH_2$: 41.5 ± 5.4 mg/kg. Cholinergic effects were prominent in the C_3 through C_7 esters and the chloroethyl compound. Delayed paralytic effects occurred in all of the *bis* C_3-C_{10} and *bis* $ClCH_2CH_2$ chemicals at less than lethal levels. The C_2 analog induced the effect at lethal levels. The contribution of *bis* impurities (that can occur in the synthesis of the mixed esters) was tested with the C_1,C_8 and *bis* C_8 pair. Using similar (po) and dissimilar routes (po, im) of varying amounts of the esters indicated that the *bis* esters may be responsible for the effects found in the hen with impure preparations of the mixed esters.

157. *Studies on Dichlorvos-Induced Intestinal Enteritis in the Dog.* V. L. KIRKLAND, J. R. ALBERT and K. VAN KAMPEN, Shell Development Company, Modesto, California, and Intermountain Laboratory, Salt Lake City, Utah.

During a study of the acute oral lethality of technical dichlorvos in the dog, a consistent finding in succumbing animals was a marked inflammation of the mucosal surface of the intestine. The severity and extent of involvement ranged from a moderate to severe hyperemia over the proximal third of the duodenum, the principal area of involvement, to a severe hyperemia throughout the intestinal tract and colon. Consistent histopathology was found in affected animals. A cholinergic mechanism was postulated for the intestinal hyperemic effects of dichlorvos, and studies to substantiate this are the subject of this report. Anesthetized dogs were prepared for respiratory and vascular monitoring and cholinesterase determinations. Drugs were administered po except as noted. Necropsy centered on the digestive tract. With technical dichlorvos, the hyperemic effect was marked and consistent at 25 mg/kg, less marked and variable at 10 mg/kg (the minimal lethal dose in intact dogs) and absent at 3.2 mg/kg. Using 20% formulated dichlorvos (TASK® Dog Anthelmintic) the effect was mild and variable at 99 mg a.i./kg and absent at 33 mg a.i./kg, the therapeutic dose. Physostigmine, 40 mg/kg, elicited effects comparable to 25 mg/kg of technical dichlorvos. Cardiorespiratory responses following either technical or formulated dichlorvos or physostigmine were typical. The effects, except ChE inhibition, of both drugs were blocked by atropine (IV) but not by hexamethonium (IV). The dose-effect relationships (incidence and severity) of the hyperemic response indicate a close relationship to the lethality curve established in the intact animal. Histopathologically, the appearance of the affected portions of intestine was comparable to that in intact dogs. The intestinal lesion in dogs may be the result of local ischemia, as mesenteric arterial blood flow, measured in some dogs, was reduced in dichlorvos-treated animals showing the lesion.

158. *Needs of the WHO Expert Committee on Pesticide Residues for Toxicological Data.* FRANK C. LU, Chief, Food Additives, World Health Organization, Geneva, Switzerland.

In 1961, the World Health Organization convened a joint meeting of its Expert Committee on Pesticide Residues with the FAO (Food and Agriculture Organization of the United Nations) Panel of Experts on the Use of Pesticides in Agriculture. The experts recommended that studies be undertaken to evaluate the hazards to the consumer on the basis of toxicological and other pertinent data on those pesticides which are known to leave residues in food. The WHO Expert Committee has met jointly with the FAO Working Party of Experts on Pesticide Residues annually since 1966. Their tasks are to review the relevant data and establish, where possible, acceptable daily intakes of the pesticide residues for man, and to propose pesticide residue limits (e.g. tolerances) and methods of analysis for their determination. The recommendations are summarized in their reports, which are transmitted to governmental authorities as well as the Joint FAO/WHO Codex Alimentarius Commission as a basis for elaborating international limits of residues of pesticides. The Commission is an intergovernmental body and has at present a membership of 100 countries. Before each meeting, WHO collects data from all sources including the manufacturers. This procedure has in general proved satisfactory in acquiring the needed information. However, where no single manufacturer has an exclusive commercial interest in a product, there is then no economic incentive to generate the required data. Some pesticides in this category are effective in the control of pests in agriculture and inexpensive. They merit due consideration. It is hoped that members of the Society of Toxicology might be interested in providing the required information on such pesticides.

159. *The Effect of Glutathione Depletion on the Renal Deposition of Methylmercury in Rats.* RUDY J. RICHARDSON and SHELDON D. MURPHY, Department of Physiology, Harvard School of Public Health, Boston, Massachusetts.

Glutathione (GSH) complexes of mercury (Hg) may play a significant role in the tissue accumulation and excretion of this metal. As a test of this hypothesis, the effect of GSH depletion on Hg deposition in tissues was measured in rats. Tissues were depleted of GSH by ip administration of compounds that form mercapturic acid derivatives. GSH was determined colorimetrically. Hg was administered as [^{203}Hg]methylmercuric chloride, 1.0 mg/kg ip, and total Hg content of tissues measured by gamma scintillation spectrometry. When methylmercury was administered 0.5 hr after diethylmaleate (DEM) or propylene glycol (PG), significantly lower Hg concentrations in kidney and brain of DEM-treated animals were observed at 2.5 hr. While liver, lung, kidney and brain followed a dose-response relationship between DEM administered and GSH depletions, only kidney consistently displayed a corresponding dose-dependent effect of DEM on Hg concentration. Kidneys of rats given 0.31, 0.62, 1.55 and 3.10 mmol/kg of DEM in PG solution had GSH concentrations at 2.5 hr that were 102, 96, 72 and 47% of control values, respectively; and 76, 74, 38 and 28% of the Hg concentrations of kidneys from corresponding methylmercury-treated PG controls. When methylmercury was given at 0.5, 1.0, 2.0, 3.0 and 4.0 hr after 2.48 mmol/kg of DEM, kidney GSH concentrations at 2 hr after Hg administration were 57, 75, 80, 83 and 107%; and Hg concentrations were 20, 44, 53, 73 and 106% of controls for each respective time point. In addition to DEM, sodium maleate, acrylamide and benzyl chloride each lowered renal GSH concentrations and resulted in decreased deposition of Hg in kidney. Oxidation of renal GSH by sodium tetrathionate produced the same effect as the depleting agents on kidney Hg levels. These results suggest that renal GSH is a determinant in the deposition of Hg in kidney. (Supported by Research Grant OH-00315 from NIOSH and Training and Research Grants ES-00084 and ES-00002 from NIEHS, USDHEW.)

160. *Whole Body Half-Life of Methylmercury in Adult Cats.* R. F. WILLES, J. G. HOLLINS, F. R. BRYCE, S. M. CHARBONNEAU, I. C. MUNRO and H. C. GRICE, Food Research Laboratories, Health Protection Branch, Health and Welfare Canada, Ottawa and Biological Sciences, National Research Council, Ottawa, Canada.

Six adult female cats were given a single oral dose of mercury-203 labelled methylmercury (55 μCi total dose, specific activity 2.0 μCi/mg). Whole body counts were taken daily for 4 days,

3 times weekly until 3 weeks, weekly until 3 months and bi-weekly until 5 months. Twenty-four hr feces and urine collections and hair samples were counted at each of the above time intervals. Five months after dosing, each cat was anesthetized and the total hair was clipped off the body. A whole body count minus hair was taken. The cats were then exsanguinated by cardiac puncture, following which the skin of the cat was removed. A total carcass count was then taken after which the major organs were removed and the mercury-203 in each tissue measured with a crystal scintillation counter. All radioactive counts were corrected for mercury-203 half-life and machine efficiency to allow comparisons between cats and various organs. The average whole body half-life for the 6 cats was calculated as 143.7 ± 2.2 days. This value has not been corrected for excretion into the hair.

161. *Chronic Toxicity of Methylmercury in the Adult Cat.* S. M. CHARBONNEAU, I. C. MUNRO, E. A. NERA, F. A. J. ARMSTRONG, R. F. WILLES and H. C. GRICE, Food Research Laboratories, Health Protection Branch, Health and Welfare Canada, Ottawa, Canada.

To obtain data on the effects of long-term consumption of fish containing methylmercury, a chronic toxicity study was undertaken in which adult cats were fed methylmercury-contaminated fish. Doses of 3, 8.4, 20, 46, 74 or 176 μg Hg/kg/day were fed to groups of cats either as methylmercuric chloride or as methylmercury-contaminated fish. Food consumption, weight change, blood mercury concentrations, hematology, urinalysis, serum blood urea nitrogen values and neurological assessment were conducted on all cats. Clinical signs of methylmercury toxicity consisting of ataxia, hypalgesia and loss of balance occurred in cats receiving 176 μg Hg/kg/day after 14 weeks of treatment and in those consuming 74 μg Hg/kg/day after 36 weeks of treatment, at which time the animals were sacrificed. Total mercury and methylmercury concentrations in tissues were correlated with toxic signs, clinical investigations and histopathological findings.

162. *Toxicity of Methylmercury in Pregnant Rhesus Monkeys.* W. J. DOUGHERTY, F. COULSTON and L. GOLBERG, International Center of Environmental Safety, Holloman AFB, New Mexico, and Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York.

An evaluation of the toxic and possible teratogenic effects of methylmercury chloride (MMC) was carried out in 40 mature pregnant rhesus monkeys (*M. mulatta*). Following diagnosis of pregnancy, the monkeys were assigned either to the control group of to 1 of the test groups receiving MMC in doses of 0.05, 0.16 or 0.5 mg Hg/kg. The MMC (K & K Laboratories, Plainview, N.J., Lot No. 98443), dissolved in 5.0 mM Na_2CO_3, was administered by stomach tube daily from day 20 through day 38 of pregnancy; the controls received the Na_2CO_3 solution. All animals were observed daily for indications of toxicity, predominantly those ascribable to methylmercury poisoning, i.e., loss of weight and reduced spontaneous activity followed by signs of progressive ataxia. Monkeys developing these signs became moribund and were killed. All animals that died or were killed, including fetuses, as well as two infants from each group were autopsied and examined histopathologically, total Hg being measured in blood and selected organs. Of the 10 control monkeys, 9 delivered live offspring and 1 aborted. (The spontaneous abortion rate among untreated females in our colony is approximately 12%). Of the 9 monkeys receiving 0.05 mg/kg MMC, there were 7 live births and 2 abortions, no adult deaths and no apparent effect of MMC. In 10 monkeys given 0.16 mg/kg, there were 7 live births and 1 abortion in a female exhibiting no sign of toxicity; 2 additional monkeys aborted after the appearance of MMC intoxication. Nine of 11 monkeys given 0.5 mg/kg MMC aborted subsequent to the development of MMC toxicity. One monkey aborted and died without showing evidence of MMC poisoning. Four fetuses were recoved from this group. In affected groups, the time of first appearance of toxic manifestations varied from day 35 to day 95 of gestation. Malformations were not observed in any infant or fetus. Infants still living appear clinically normal and are being evaluated for possible behavioral decrement. (Supported by Research Grant 2P01-ES00226-07 from the National Institute of Environmental Health Sciences, NIH, by National Institutes of Health Training Grant 2T01-ES00103-07, and by the New York State Department of Health.)

163. *The Absorption, Distribution, and Excretion of Orally Administered $^{133}BaCL_2$ in Weanling Male Rats.* J. J. CLARY and R. G. TARDIFF, National Institute for Occupation Safety and Health and U.S. Environmental Protection Agency, Cincinnati, Ohio.

The absorption, distribution and excretion of $^{133}BaCl_2$, administered po or ip, were studied in weanling male rats to develop criteria on which to establish a drinking water standard and an industrial air limit for soluble barium (Ba). In the first metabolic study, groups of rats received a single oral dose (1, 5, 25 and 125 mg/kg) of $^{133}BaCl_2$ and were sacrificed at 0.5, 2, 4, 8, 16 or 24 hr after exposure. ^{133}Ba concentrations were measured in blood, heart, brain, liver, kidneys, adrenals, femur and muscle of all animals and in urine and feces of animals sacrificed at 24 hr. In the second metabolic experiment, groups of animals received a single oral dose of $^{133}BaCl_2$ (cf., 3 or 6 mg/kg), and the animals were sacrificed at 0.5, 1, 2 or 4 hr post administration. In addition to the tissues listed above, the gastro-intestinal (g-i) tract and its contents were assayed for ^{133}Ba. In the third metabolic study, a single dose of $^{133}BaCl_2$ (cf. or 15 mg/kg) was administered ip. Total urine and fecal excretion of ^{133}Ba was determined for 7 days after injection. The animals were sacrificed on the 7th day, and the same tissues listed in the 2nd metabolic study were counted for ^{133}Ba activity. Results of the first metabolic study indicate that ^{133}Ba is rapidly absorbed from the g-i tract with the peak concentration in the blood and soft tissues occurring 30 min after administration. However, ^{133}Ba exhibited a peak concentration in the femur at 2 hr post treatment. Total uptake of Ba increased with increasing dosage; however, relative uptake was inversely related to the dosage. Results of the second metabolic study delineated the saturation point for the oral absorption of ^{133}Ba and revealed a relatively high percentage of ^{133}Ba in the combined g-i tract and contents. Results of the third metabolic investigation demonstrated that ^{133}Ba is excreted in both the urine and feces, but with the majority of the excretion occurring via the fecal route. These studies indicate that soluble Ba (as $BaCl_2$) is rapidly taken up by the soft tissues, more slowly taken up by the skeleton, and excreted primarily in the feces.

164. *Decreased Antibody Formation in Lead-Exposed Mice.* LOREN D. KOLLER and SHARLOTTE A. KOVACIC, Environmental Health Science Center and the Department of Veterinary Medicine, Oregon State University, Corvallis, Oregon. (Virgil Freed.)

The number of plaque-forming cells and the serum hemolytic activity was determined for control and lead-exposed mice responding to injections of sheep rod blood cells (SRBC). Swiss-Webster mice were exposed to various doses of lead acetate (2500, 250, 25 or 0.25 ppm) in their drinking water for 8 weeks. Control and lead exposed mice were then injected with SRBC and the primary immune response was determined on days 3 through 7. Additional mice were given a second injection of SRBC, and the secondary immune response was determined on days 8 through 14. A decrease in the immune response was observed in the lead-exposed mice. A more pronounced effect was observed in the secondary response. This effect was correlated with the occurrence of lead inclusion bodies in the kidneys of the mice that were examined microscopically. Kidneys were also analyzed for an estimation of the amount of lead each mouse accumulated. It is concluded that prolonged exposure to subclinical doses of lead reduced antibody formation (primarily IgG) in mice.

165. *Toxicity Produced in Rats Fed Dry Paint Chips for 13 Weeks which Contained Different Concentrations and Forms of Lead.* T. R. CASTLES, J. L. SANYER, J. A. HOCH, H. M. MILLER and J. L. SPIGARELLI, Midwest Research Institute, Kansas City, Missouri.

This study evaluated in rats the toxicity and body lead-burden produced by a modern interior paint film containing different concentrations and forms of lead. Female and male rats were fed a diet containing 0.1% paint chips (0.5–1 mm size) in rat chow mash for 13 weeks. These chips contained no added lead (control); 0.08, 0.53 or 2.05% lead as lead octoate; 0.42, 1.95 or 12.43% lead as lead chromate; or 66.05% lead as basic lead carbonate. Paint chips (0.06–0.08 mm size) from old dwellings similar to those implicated in lead poisoning were

obtained from the National Bureau of Standards (NBS) for comparison. No gross changes in appearance, feed consumption, body weight, hematology or urinalysis were observed during the experiment. Porphyrin metabolism was normal in rats fed lead octoate and lead chromate. Body lead-burden was normal in the octoate and chromate groups, except for an elevated blood lead in the rats fed chips containing 12.43% lead as lead chromate. Erythrocyte δ-aminolevulinic acid dehydrase activity was decreased 50% in rats fed lead carbonate or NBS powdered lead paint, without concomitant changes in protoporphyrin, δ-aminolevulinic acid or coporporphyrin concentrations. The lead concentrations in bone, blood and kidneys were increased in both groups. In addition, lead accumulated in the livers and brains of rats in the NBS lead paint group. Histological examination of tissues yielded none of the classic pathology seen in lead poisoning. In conclusion, we found that the lead in these modern paint formulations was not absorbed readily by the rat, as evidenced by the lack of toxicity and a change in lead-body burden. (Supported under Contract No. 62-W-62 GL & NPC from the National Paint and Coatings Association.)

166. *Alterations in Rat Liver and Kidney Cortex Metabolism After Acute and Chronic Exposure to Cadmium.* Z. MERALI, S. KACEW and R. L. SINGHAL. Department of Pharmacology, University of Ottawa, Ottawa, Canada (J. A. Thomas.)

Exposure to cadmium has been shown ot produce itai-itai disease, hypertension and testicular atrophy, as well as an increase in the excretion of urinary protein and glucose. The purpose of this study was to investigate the acute and chronic effects of cadmium on serum urea, blood glucose and hepatic glycogen, as well as on the activities of the 4 key, rate-limiting enzymes involved in kidney cortex and liver gluconeogenesis. Rats injected ip with an acute dose of cadmium chloride (60 mg/kg) displayed metabolic changes as early as 1 hr which were reflected in increased blood glucose (4-fold) and serum urea (2-fold) and a decrease in hepatic glycogen content (2-fold). The activities of the 4 gluconeogenic enzymes, pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase, showed small but consistent increases in both liver and kidney cortex. Chronic exposure to a smaller dose of cadmium (1 mg/kg/day) for 45 days also enhanced blood glucose and serum urea and reduced hepatic glycogen concentrations. Following chronic cadmium treatment, the activities of the 4 hepatic and renal gluconeogenic enzymes also were significantly elevated when compared to the control values. In rats given cadmium (1 mg/kg) for 45 days and then maintained for 28 days without any additional treatment (withdrawal group), the observed increases in blood glucose and serum urea still persisted, as did the drop in liver glycogen. Similarly, enzyme activities in livers and kidney cortices of rats in the "withdrawal" group remained significantly above those seen in the control animals. The results show that exposure to cadmium produces hyperglycemia, hyperuremia and glycogenolysis, as well as stimulating the process of renal and hepatic gluconeogenesis, and that the various biochemical changes persist for a prolonged period even after the cessation of heavy metal treatment. (Supported by a grant from the Department of National Health and Welfare, Canada.)

167. *Some Effects of Cadmium-Induced Hypertension on Vascular Smooth Muscle.* ROBERT GOLDEN and ROLF HARTUNG, University of Michigan, Ann Arbor, Michigan.

Long-Evans hooded rats were maintained on 100 ppm cadmium chloride in their drinking water until they were hypertensive. The hooded rat proved to be more susceptible than 2 other strains to the hypertensive effects of cadmium. Animals conceived and born to mothers maintained on 100 ppm cadmium in their drinking water, developed hypertension faster and to a higher level than animals placed on cadmium after birth. The contractility of helically cut strips of thoracic aorta from hypertensive and control animals was measured in a muscle bath. The vascular smooth muscle (VSM) from cadmium hypertensive animals was found to be less responsive to norepinephrine, KCl and serotonin on a dose-response basis. When Cd^{2+} (10^{-4} M) is added to the bathing medium it produces a contraction in the control tissue but

not in the VSM from the hypertensive animal. Once a normal tissue has been exposed to Cd^{2+} in vitro repeated washing will not cause the strip to return to the same resting tension (baseline). This response may be due to a cadmium-induced defect in the relaxing mechanism of VSM. Length-tension relationships were investigated to rule out any possible differences in "Stiffness" between the tissues from control and hypertensive rats. There was no change between the ratios of the maximum responses over the entire range of resting tensions (0.75–4.0 g). The VSM tissue from a cadmium hypertensive animal does not respond to the addition of CD^{2+} in vitro, which suggests that any response may have already occurred in vivo. If this same response does occur in vivo it may help to explain the cadmium induced hypertension as there would be an increase in the total peripheral resistance.

168. *On the Role of Cadmium-Binding Protein in the Transport and Excretion of Cadmium in the Rat.* M. G. CHERIAN and J. J. VOSTAL, Department of Pharmacology and Toxicology, University of Rochester School of Medicine and Dentistry, Rochester, New York.

It has been shown that the repetitive administration of small doses of cadmium chloride (0.25 mg/kg) can induce the formation of high concentrations of metallothionein—a protein with high affinity for cadmium—in the liver and kidney. In the present study, radioactive $^{109}CdCl_2$ was administered iv in a dose of 1 mg/kg to control rats and to rats pretreated with 0.25 mg Cd/kg sc 24 hr earlier; a significant increase in the retention of cadmium in the liver and decreased biliary excretion of cadmium were observed in pretreated animals as compared with the controls. No differences in cadmium concentrations in the kidney and in urinary excretion were recorded between the two groups. Although nearly all tissue radioactivity was recovered in the high-molecular-weight protein fractions of liver and kidney supernatants in control animals, more than 90% of liver cadmium and 70% of kidney cadmium was bound to a protein of molecular weight approximately 11,000 in kidney and liver supernatants from pretreated rats. Remarkably, most of cadmium excreted in bile was not in metallothionein complex but in the form of a low-molecular-weight peptide identified by gel filtration and thin-layer chromatography as cadmium-glutathione complex; only traces of cadmium were excreted into the urine. In contrast, when purified rat metallothionein labeled with ^{109}Cd was administered to control animals most of the injected radioactivity accumulated in the kidney instead of liver. Moreover, significant amounts of cadmium appeared in urine; however, most of the urinary radioactivity was excreted in the form of a cysteine adduct with cadmium. The results indicate that the induced synthesis or administration of a specific cadmium-binding protein have profound, although completely different, effects on the distribution and disposition of cadmium in the organism. (Supported in part by the U.S. Public Health Service Grant No. GM 15190.)

169. *Biliary Excretion of Cadmium in the Rat.* J. J. VOSTAL and M. G. CHERIAN, Department of Pharmacology and Toxicology, University of Rochester School of Medicine and Dentistry, Rochester, New York.

Only a small fraction of the parenterally administered cadmium is excreted into the urine, whereas amounts up to several percent of the administered dose may be excreted via feces after a single sc administration of cadmium in the rat. The role of liver retention and biliary secretion of cadmium was therefore studied in rats with cannulated biliary ducts after iv administration of various doses of cadmium chloride labeled with radioactive isotope ^{109}Cd. In contrast with a relatively constant rate of biliary excretion of cadmium after ip administration (Caujolle *et al.*, *Europ. J. Toxicol.* **4**, 310, 1971) higher concentrations of cadmium were found in the bile during the first 3 hr after iv injection of cadmium chloride; only minimum amounts of cadmium were excreted with bile after this period. The total amount of cadmium excreted into the bile within the 5-hr experimental period was dependent on the dose. When low doses of cadmium were administered (0.1 mg Cd/kg) only a fraction of 1% of administered dose appeared in the bile; cumulative 5-hr biliary excretion of cadmium represented, however, more than 15% of the administered dose after injection of 2 mg Cd/kg. Cadmium concentration in the liver increased

linearly with the administered amount up to the dose of 1 mg Cd/kg; relative increments of liver concentrations were smaller when the dose exceeded 1.5 mg/kg. In contrast, biliary concentrations of cadmium increased more rapidly when more than 1 mg Cd/kg was administered. The possibility was explored that the changes might be related to the presence of different forms of cadmium in bile and different excretory mechanisms might be involved. Gel filtration of biliary and tissue complexes of cadmium on Sephadex G 75 indicated that, independent of the dose, approximately 70% of the radioactive cadmium in kidney and liver supernatants is bound to high-molecular-weight proteins, whereas more than 98% of biliary cadmium is associated with a peptide with molecular weight lower than 5000 and mobility on the thin-layer chromatography systems identical with cadmium-glutathione complex. None of the biliary cadmium was found in fractions corresponding to metallothionein and also the amounts of cadmium bound to this cadmium binding-protein in liver and kidney supernatants were lower than 10% of total radioactivity. (Supported in part by the U.S. Public Health Service Grant No. GM 15190.)

170. *Are Fluorocarbon Propellants Metabolized? Studies on ^{14}C-labeled Trichlorofluoromethane (F-11) and Dichlorodifluoromethane (F-12) in Beagles and Humans.* D. A. BLAKE and G. W. MERGNER, University of Maryland, School of Pharmacy, Baltimore, Maryland.

The possible biotransformation of F-11 and F-12 was investigated in male and female beagles ($n = 15$) and humans ($n = 4$) after a 7–20 min inhalation. Dogs were anesthetized with ketamine and succinylcholine, intubated and respired artificially. Unanesthetized humans were exposed through a face mask. F-11 (1000–5000 ppm) or F-12 (1000–10,000 ppm) containing up to 180 μCi of ^{14}C-fluorocarbon was delivered from 100-liter Teflon bags. Exhaled air was collected via a unidirectional valve in similar bags for 1 hr. Venous blood samples were withdrawn at appropriate times and assayed for fluorocarbon content. Exhalation bags were assayed for ^{14}C-fluorocarbon and $^{14}CO_2$. Urine was collected for up to 3 days and assayed for non-volatile radioactivity. In some experiments, dogs were euthanized 24 hr after exposure and tissues removed for determination of non-volatile radioactivity. Essentially all of the administered fluorocarbon was recovered in the exhaled air within 1 hr. Mean results: F-11: dogs—101.6%, humans—99.2%; F-12: dogs—103.0%, humans—101.9%. Only traces of radioactivity were found in urine (F-11: dogs—0.01%, humans—0.09%; F-12: dogs—0.04%, humans—0.03%) or exhaled carbon dioxide (F-11: dogs—0.3%, humans—0.25%; F-12: dogs—0.14%, humans—0.12%). All tissues contained low levels of non-volatile radioactivity 24 hr after exposure but together represented less than 1% of the administered dose. All of these trace levels can be attributed to the radiolabeled impurities present in the administered gas mixture. Neither phenobarbital pretreatment (60 mg, 3 times a day for 3 days) nor prolonged exposure (50–90 min) produced any alteration of these results in dogs. Thus, it can be concluded that F-11 and F-12 are refractory to biotransformation after a short inhalation exposure and that they are rapidly exhaled in their unaltered chemical form. (Supported by a contract with the Cosmetic, Toiletries and Fragrance Association.)

171. *Kinetics of Uptake and Elimination of Trichlorofluoromethane (F-11) and Dichlorodifluoromethane (F-12) in Beagles and Humans.* JOSEPH ADIR, DAVID A. BLAKE and GERTRUDE W. MERGNER, University of Maryland School of Pharmacy, Baltimore, Maryland.

Blood concentrations of 6 beagles and 2 volunteers who inhaled F-11 and F-12 on different occasions were analyzed by a digital computer program to determine the kinetics of uptake and elimination of these two substances. The data fitted a model in which the body was conceived as consisting of a highly perfused central compartment and a slowly accessible peripheral compartment. The transfer between the 2 compartments as well as the uptake and elimination via the lung were assumed to follow first-order kinetics. Mathematically, the model implies that the blood concentration-time curve can be described by a bi-exponential function with an initial rapid distribution (α-phase) and a slower elimination (β-phase). The rate constant

for the transfer of F-11 from the alveolar space to the blood is larger than that of F-12, indicating that pulmonary blood flow may not be the rate-determining step. Simulation of the model using the averaged pharmacokinetic parameters shows that at the same inspired concentration, the F-11 levels in the central and peripheral compartments are substantially higher and persist longer than those of F-12. The two-compartment model also adequately described the pharmacokinetics of F-11 and F-12 in humans. The kinetics of the fluorocarbons are consistent with their blood solubilities, oil/water partition characteristics and other physiologic considerations. (Supported in part by a grant from the University of Maryland, Computer Science Center, at College Park.)

172. *Influence of the Physical State of the Plasticizer, Di(2-ethylhexyl)phthalate (DEHP) on its Biological Disposition and Action.* R. J. RUBIN and C. O. SCHULZ, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland. (D. Blake.)

There has been considerable recent interest in the toxicology of phthalate ester plasticizers (*Env. Health Perspectives* 3, 1973). Our laboratory had previously shown that in rats iv DEHP (500 mg/kg) significantly altered reticuloendothelial (RE) clearance. Since DEHP is a highly insoluble oil, administration of large doses required the use of sonicated aqueous emulsions. We have now sought to develop detergent-containing vehicles for the administration of solubilized DEHP. The sonicated aqueous emulsion (DEHP 50 mg/ml) was milky white and completely opaque; it had 19×10^6 droplets/ml with a diam. of 1.9–2.6 μ. DEHP (100 mg/ml) in Vehicle E (50% DMSO, 5% Tween 80, and 45% saline) was opalescent and contained 5.4×10^6 droplets/ml, 10.5–42 μ in diam. DEHP (50 mg/ml) in Vehicle A (25% DMSO, 10% Tween 80, and 65% saline) was completely translucent and had no visible droplets. Addition of rat plasma to these preparations resulted in the settling out of additional oil droplets with the first two preparations, but had no effect on the solubility of DEHP in Vehicle A. DEHP (250 mg/kg iv) as a sonicated emulsion resulted in a 75% increase in the half-time for RE clearance in rats. In Vehicle E this same dose caused a 200% increase while in Vehicle A there was no significant effect. These results clearly implicate the particle size and number in the RE effect and indicate no direct inhibitory effect of DEHP. The solubilized DEHP disappeared from blood logarithmically with a half-time of 21 min. In contrast, the emulsion form displays two distinct decay rates having a half-time of 9 and 22 min, respectively. These results suggest that the emulsified DEHP is rapidly taken up by RE clearance followed by a slower rate of metabolism of the solubilized molecules. With the solubilized dosage form of DEHP, only the latter rate of clearance pertains. In support of this, whereas 50% of the emulsified dose is in the liver after 1 hr and 10% after 24 hr, only 10% of the solubilized dose is found in the liver after 1 hr and 0.2% after 24 hr. The solubilized DEHP is found primarily in the eviscerated carcass, equally distributed among the muscles and bony skeleton. The solubilized dosage form of DEHP produced a marked pulmonary edema at 200 mg/kg. At 300 mg/kg 50% of the rats died of asphyxiation. The vehicle alone or the sonicated emulsion of DEHP did not produce edema. DEHP solubilized in 15% Tween 80 only was equally edematous. These results emphasize the necessity for knowledge of the physical state of DEHP in evaluating its toxicologic potential.

173. *Distribution of Dieldrin Following a Single Oral Dose.* W. J. HAYES, JR., Center in Toxicology, Department of Biochemistry, Vanderbilt University, Nashville, Tennessee.

Two groups of investigators have used the classical model for the distribution of thiopental following iv injection to predict the distribution of dieldrin following accidental ingestion. They concluded that the concentration of dieldrin in the brain is reduced first by redistribution to muscle and only later reduced by redistribution to fat. The model as originally designed for thiopental had been evaluated by analysis of samples from persons undergoing surgery under thiopental anesthesia. Since it is not appropriate to give large doses of dieldrin to people or to collect samples of muscle and various vital organs from them, the distribution of dieldrin was studied in rats. Dieldrin dissolved in corn oil was administered to these animals by stomach tube. The highest concentration of dieldrin in the brain was reached in 4 hr, and the concentration decreased gradually thereafter. The concentration in muscle remained essentially

steady during the interval from 4 to 48 hr. There was no peak for muscle that could be interpreted as replacing a peak for brain. The concentration of dieldrin in fat was already slightly higher than that in the brain at 1 hr, very much higher at 4 hr when the concentration in the brain was maximal, and the concentration in the fat continued to increase during the first 24 hr. Either on the basis of concentration or on the basis of the total amount in each organ, no reason was found to assign any special importance to muscle as a sink into which dieldrin is redistributed from the brain. The fat appears to be far more important in this regard. The results indicate the danger of applying a mathematical model to a new situation without checking the results experimentally.

174. *Comparative Effect of Stress and Ethanol on Hexobarbital in the Rat. I: In vitro Metabolism.* HO CHUNG and DAVID R. BROWN, University of Maryland School of Pharmacy, Baltimore, Maryland. (David A. Blake.)

Ethanol and stress have each been shown to alter drug metabolism. The overall objective of this study is to determine the combined effect of stress and ethanol on drug metabolism. Adult male Sprague-Dawley rats were stressed by hind-leg ligature (HLL) for 0.5, 1 or 2.5 hr, sacrificed, livers perfused with cold 1.15% KCL and liver subcellular fractions prepared by differential centrifugation. In the stress-ethanol experiments, 1 or 3 g/kg ethanol was administered po immediately before application of HLL. The hexobarbital oxidase (Hb oxidase) activity of $9000 \times g$ supernatant was inhibited 31 and 59% by 1 and 2.5 hr stressing respectively. Administration of 3 g/kg ethanol po (15% solution w/v) inhibited Hb oxidase activity 45%. Combination of 1 hr of stress with 3 g/kg ethanol produced 81% inhibition of Hb oxidase. Plasma ethanol concentrations were 105 and 20 mg/100 ml 15 and 240 min after removal of stress respectively. A dose-response relationship was found for ethanol inhibition of Hb oxidase and a time-response relationship was found for stress induced inhibition. The increases in Hb oxidase inhibition were paralleled by increases in Hb-induced sleep-time in the ethanol alone and stress-ethanol treated rats. In contrast, the sleep-time, decreased in the stress-alone rats, although Hb oxidase activity in vitro was inhibited and plasma Hb concentrations at awakening were the same as controls. The results of this study indicate that the combination of stress with ethanol decreases the rate of Hb metabolism more than either alone. (Supported by a Pharmaceutical Manufacturers Association Foundation Research Starter Grant.)

175. *Comparative Effect of Stress Alone and in Combination with Ethanol on Hexobarbital (HB) in the Rat. II: Pharmacokinetics Studies.* JOSEPH ADIR, HO CHUNG and DAVID R. BROWN, University of Maryland, School of Pharmacy, Baltimore, Maryland. (David A. Blake.)

The pharmacokinetics of HB was determined in untreated rats (control), in hind-leg ligated animals (stress) and in rats given 3 g/kg ethanol simultaneously with applying the ligature (stress-ethanol). After the ip administration of 100 mg/kg HB the rats were sacrificed at pre-determined time intervals for a total of 3 hr, and the unchanged HB concentrations in the plasma determined spectrophotometrically. Analysis of the terminal points of the log HB concentration-time curve indicates that the half-life of elimination ($t_{\frac{1}{2}}$) was increased by almost 2-fold in the stress groups and 3-fold in the stress-ethanol group, as compared with $t_{\frac{1}{2}}$ of the control group. Since biotransformation is the major route of elimination of this drug, it appears that stress has affected the metabolism of HB and this impairment is augmented in combination with ethanol. The increase in $t_{\frac{1}{2}}$ in the stress-ethanol group parallels the observed increase in sleep-time. In contrast, a shorter sleep-time of the stress group was observed, in spite of the increase in $t_{\frac{1}{2}}$ of HB compared to the control group. The area under the plasma concentration-time curve (AUC) is usually taken to represent the amount of drug reaching the systemic circulation. In the stress-ethanol group the AUC was found to be approximately 120% that of the control group, an increase which correlates rather well with the corresponding increase in sleep-time (130%). On the other hand, the AUC of the stress group is about 80% that of the control animals, and this decrease is in excellent agreement with the observed reduction (76%) of sleep-time of the stress group compared to the untreated rats. It seems,

therefore, that stress alone and in combination with ethanol altered the absorption and/or distribution of HB in the rat, in addition to its elimination. (Supported by Pharmaceutical Manufacturers Association Foundation, Starter Grant.)

176. *Alteration of Hexobarbital Disposition by Dietary Glucose in Mice.* R. B. FORNEY, JR., J. ASHMORE and R. B. FORNEY, Indiana University School of Medicine, Indianapolis, Indiana.

Increasing the amount of glucose in the diet can potentiate the toxicity of some drugs. When mice are given a 30% glucose solution to drink ad libitum for 2 days, the duration of hexobarbital-induced sleep is prolonged. This effect has been associated with a decrease in the disappearance of the drug in vivo. These studies were performed to determine if dietary glucose affects the tissue distribution or action of hexobarbital. The total body concentration of hexobarbital in mice awakening from sleep is less ($p < 0.05$) in glucose pre-fed (3.08 ± 0.16 mg/100 ml) than in control mice (3.65 ± 0.18 mg/100 ml). The high sugar diet reduced the rate of disappearance of hexobarbital from blood, brain and liver. Unusually high liver concentrations indicate that the drug is being sequestered there. The glucose effect was observed in sodium barbital (368 mg/kg, iv) induced narcosis, suggesting that the action is not restricted to an alteration in biotransformation. The ip ED50 of hexobarbital for inducing hypnosis was 46.3 mg/kg in glucose pre-fed mice compared with 55.0 mg/kg in control animals, a significant increase in potency. Distribution of the barbiturate to the brain appears to be dose-dependent and unaltered by glucose consumption. The initial brain concentrations of hexobarbital in the 2 groups of sleeping mice were found to be similar when the ED50 dose for each group was administered.

177. *Sex Variations in the Biliary and Urinary Excretion of [^{14}C]Pentobarbital and its Metabolites in Rat.* H. S. BUTTAR, B. B. COLDWELL and B. H. THOMAS, Drug Research Laboratories, Health Protection Branch, Health and Welfare Canada, Ottawa, Canada.

Sex variations can modify the metabolism and excretion and thus alter the toxicity of many drugs. The influence of sex on the rate of disappearance of [^{14}C]pentobarbital (PB, 35 mg/kg; iv) from the blood and on the appearance of PB-derived ^{14}C in the bile and urine was investigated in biliary-fistulated Wistar rats. The half-life ($t_{\frac{1}{2}}$) for disappearance of ^{14}C from blood was significantly longer ($p < 0.005$) in the females than in the males (7.1 ± 0.6 vs 3.9 ± 0.4 hr). The cumulative excretion of ^{14}C over 6 hr was 1.5-fold greater in the bile and 1.4-fold greater in the urine of males compared with the females. The bile/blood concentration ratios of ^{14}C were 4- to 16-fold higher in the males and 2.8- to 6-fold higher in the females, indicating an active transfer of PB and/or its metabolites from the hepatocytes into the bile. The PB-induced choleresis was correlated with the bile concentration of ^{14}C in both sexes. Paper chromatography of bile and urine from male rats showed 4 metabolites of PB; only 3 metabolites were detected in the bile and urine from female rats. The results suggest that the reduced biliary and urinary excretion of PB-derived ^{14}C in female rats is due to the slower metabolism of PB by these animals.

178. *Metabolic Disposition of Tilorone Hydrochloride in Mice, Rats and Dogs.* R. H. HOOK, J. M. WILLIAMS, G. F. BRUNZIE and G. J. WRIGHT, Merrell-National Laboratories, Division of Richardson-Merrell Inc., Cincinnati, Ohio.

Tilorone hydrochloride, 2,7-bis[2-(diethylamino)ethoxy]fluoren-9-one dihydrochloride, is an orally active antiviral agent (Krueger and Mayer, *Science* **169**, 1213, 1970). Metabolic studies in mice, rats and dogs have been performed with [^{14}C]tilorone hydrochloride. Single oral doses of [^{14}C]tilorone hydrochloride were administered to dogs (20 mg/kg), rats (100 mg/kg) and mice (250 mg/kg). Tilorone was readily absorbed following oral administration. Elimination of 50% of the dose required 3 days in mice and 6–7 days in rats and dogs. Tissue ^{14}C concentrations in spleen, liver, lymph nodes, lung, eyes and adrenals were found to be 10–20 times higher than the ^{14}C concentrations in fat or muscle of rats and dogs. Also, low

plasma ^{14}C concentrations in comparison with tissue ^{14}C concentrations resulted in high tissue-plasma ratios. Extraction studies have demonstrated the presence of unchanged drug as well as metabolites in rat tissues; the relative percentage of unchanged drug in tissues decreased with time. Metabolic transformations of tilorone involving the side chains (N-deethylation and N-oxide formation) and the ring system (reduction of the carbonyl group) have been found. Thus, tilorone hydrochloride administered orally to experimental animals is eliminated slowly, exhibits strong, selective tissue affinity, and undergoes several metabolic transformations.

179. *Effect of Acetylsalicylic Acid on the Tissue Distribution and Metabolism of Acetaminophen.* B. H. THOMAS, W. ZEITZ and B. B. COLDWELL, Drug Research Laboratories, Health Protection Branch, Health and Welfare Canada, Ottawa, Canada.

Hepatic necrosis due to large doses of acetaminophen has caused several human fatalities. A metabolite of acetaminophen is believed to be responsible. This study was designed to determine if acetylsalicylic acid (ASA) changes the tissue concentrations of acetaminophen and its metabolites when it is given in combination. Male Wistar rats were divided into 2 groups and dosed orally with either ASA (210 mg/kg) and [^{14}C]acetaminophen (150 mg/kg) (AA′) or [^{14}C]acetaminophen alone (150 mg/kg) (A′). Half the rats in each treatment group were killed 30 min after dosing and the remainder at 4 hr. Radioactivity in samples from various tissues was determined by digestion and liquid scintillation counting. At 30 min the concentration of radioactivity in blood and all major organs was lower in the AA′ group than the controls (A′). These data correlate with the high level of radioactivity retained in the stomach contents of rats from the AA′ group. By 4 hr the relative concentrations have reversed, with higher concentrations in the blood, liver, muscle and brain of the AA′ group. Paper chromatography of deproteinized extracts of plasma, liver and kidney showed that at 30 min both unchanged acetaminophen and its metabolites were lower in the AA′ group. At 4 hr the unchanged drug concentration was higher in all tissues from the AA′ group compared to the controls. Over 90% of the radioactivity in brain was unchanged acetaminophen at both times. We conclude that ASA reduced the rate of acetaminophen absorption and probably its metabolism.

180. *Stimulation of the Development of the Hepatic Excretory Mechanism for Ouabain in Newborn Rats with Microsomal Enzyme Inducers.* CURTIS D. KLAASSEN, University of Kansas Medical Center, Kansas City, Kansas.

The LD50 for ouabain in a 3-day-old rat is about 1/40 that in the adult rat. This difference is largely due to the inability of the liver to remove the ouabain from the plasma and to excrete it into the bile. We treated newborn rats with known microsomal enzyme inducers to determine if they would enhance the development of the hepatic excretory system and thus decrease the toxicity of ouabain. 1-, 6-, 11-, 16-, 21-, 26-, 31-, 36- or 41-day-old rats were pretreated for 4 days with phenobarbital (PB, 30 and 50 mg/kg), pregnenolone-16α-carbonitrile (PCN, 75 mg/kg), spironolactone (S, 75 mg/kg) or 3-methylcholanthrene (3-MC, 20 mg/kg), and on the fifth day ouabain was administered (4 mg/kg iv, 2 ml/kg). Fifteen min later the amount of ouabain in the plasma, the liver and the amount excreted into the intestine was determined. PB, PCN and S enhanced the hepatic clearance of ouabain in the neonates but 3-MC did not. PCN and S were more effective than PB. In the 10-day-old-rats, 3–4 times more ouabain was in the liver and bile of the PCN and S rats than in control rats. When 15 mg/kg of ouabain was administered (ip, 10 ml/kg) to 10-day-old rats, 92% died within 24 hr, after 3-MC pretreatment 90% died. However, the rats were protected when they were pretreated with the other inducers: after PCN none died, after S 33% died and after PB 68% died. In summary, some microsomal enzyme inducers will markedly enhance the development of the hepatic excretory mechanisms that remove ouabain from the plasma, thus decreasing its toxicity. (Supported by USPHS Grant AM14513.)

181. *Experiences with Isolated Canine Livers Perfused with a Heart-Lung Machine.* H. REINERT, Centre de Recherche Pfizer, 37400 Amboise, France.

The heart-lung machine of Cuypers *et al.* (*Arch. Internat. Physiol. Bioch.* **72**, 245, 1964) was modified for isolated canine liver perfusion with whole blood. The purpose of the study was to

evaluate the suitability of the method for the rapid assessment of hepatotoxicity of drugs. The notorious difficulties with canine liver perfusion can be reduced by use of hypothermia and minimal quantities of blood and low arterial perfusion pressure at start of perfusion, avoidance of any interruption of circulation and rapid preparation. In control experiments perfusion pressures, flows and clinical chemical results remained normal for up to 5 hr. A fall in hepatic artery perfusion pressure, induced artificially or by drugs, resulted in a rise in hepatic venous pressure, reduced portal venous flow, increased SGOT, LDH, ICD and urea, but not SGPT and alkaline phosphatase. This led to the conclusion that the primary function of the hepatic artery is to provide a constant high perfusion pressure in an otherwise labile low-pressure system, the high-pressure gradient maintaining sinusoidal blood flow. Norepinephrine and dopamine are vasodilators and this effect is enhanced by alpha-blockers. The adrenergic receptors in the smooth muscle of the hepatic vascular system, like those in the coronary arteries, are thus predominantly of the beta type. Some members of a series of pyribenzamine derivatives were hepatotoxic in vivo in dogs and in this system. Structure/activity studies, using the isolated perfused liver technique, showed that a hepatotoxic effect is found only in those derivatives which have a trimethylenediamine side chain. The method appears useful for the rapid evaluation of the hepatotoxic potential of compounds.

182. *The Application of Image Analysis in Pathology and Toxicology.* A. J. NEWMAN, Huntingdon Research Centre, Huntingdon, England. (Paul M. Newherne.)

Automated methods of image analysis are being applied in several diverse fields of pathology. The present paper illustrates some techniques which are particularly relevant to toxicology. Fatty change in the liver caused by the administration of carbon tetrachloride to rats is evaluated by staining with osmium and area-sizing the osmiophilic material present in sections. Similarly, bile duct hyperplasia can be well demonstrated with sirius red, the contrast being heightened by the use of a green filter. Variations of the size of cells in both the liver and adrenals are illustrated by change in the numbers of nuclei present in a field of standard area, and changes in linear intercept can be used to study emphysema in the lung and the dimensions of muscle fibres. The methods described provide the pathologist with rapid and objective means of obtaining numerical data which express morphological changes in a form which lends itself to statistical analysis. The results clearly reflect treatment-related effects and could be used for ranking various analogues in terms of a specific pathological feature. The author is convinced that in the coming years techniques of this kind will be used widely in toxicology and considers that further investigations should be initiated along the present lines.

183. *Application of Radiorespirometry as a Fast Screening Method for Toxic Substances. I. Effect of Methylmercurychloride.* S. D. LEE, R. M. DANNER, L. MCMILLAN and R. ILTIS, E.P.A. National Environmental Research Center, Cincinnati, Ohio. (Wesley Clayton.)

The primary objective of this investigation is to develop new, or apply existing techniques that are capable of detecting early biochemical changes before the appearance of overt toxic signs in the process of toxicologic assessment of low concentrations of environmental pollutants. Various reports have been published on biologic effects of pollutants; yet, there is a paucity of information concerning the effects of pollutants on biochemical interactions at low, relevant concentrations of environmental pollutants using in vivo nonterminal experiments. Rats exposed to SO_2 showed decreased metabolic activity, as determined by suppressed CO_2 output following injection of [U-^{14}C]glucose; the degree of suppression was related to SO_2 concentration between 27.7 and 200 ppm. On the other hand, $^{14}CO_2$ release from rats following [1-^{14}C]palmitate injection was enhanced. These phenomena indicated an effect similar to "Pasteur effect." Radiorespirometric investigation on metabolic effect of CH_3HgCl provided an extremely sensitive measure. Intragastric administration of CH_3HgCl (0.05 and 0.10 mg/kg) caused suppression in $^{14}CO_2$ output following an iv injection of [1-^{14}C]glucose. This effect was cumulative when treatment was repeated 1 week later. To date the alterations reported herein are the earliest effects observed after exposure to low concentrations of CH_3HgCl. These metabolic alterations were demonstrable in non-terminal in vivo experiments.

Furthermore, a mathematical model has been developed from these results which enables one to predict the effect over a longer period of time by examining the $^{14}CO_2$ excretion pattern over a 30- to 60-min period (initial slope). In addition, one can predict dose-response over a wide range of toxicant concentrations by simulation on analog computer and interpolation following a small number of strategic experiments.

184. *Application of Radiorespirometry as a Fast Screening Method for Toxic Substances. II. Mathematical Modelling and Computer Simulation for Prediction of Effects.* R. ILTIS and S. D. LEE, E.P.A. National Environmental Research Center, Cincinnati, Ohio. (Wesley Clayton.)

A mathematical model has been developed for the rate of excretion of ^{14}C from rats during radiorespirometric investigations. The derivation of the model was based on biological processes that occur in the course of $^{14}CO_2$ elimination from rats in non-terminal in vivo experiments. The purpose was to prove that radiorespirometric tests could be used as a fast screening method for environmental pollutants. With this method $^{14}CO_2$ output pattern, following administration of ^{14}C-labeled substrate, over a 4- to 6-hr period can be predicted by examining the initial slope (30–60 min). The model has a form $Y = \alpha.\tau.\ \exp\ [-\beta(\tau^2/2)]$ and its integral is used to measure the severity of toxicity. The model is tested on an analog computer and curve fitting of the data is done on a digital computer. Since the model has 2 unknown coefficients α and β, 2 equations, i.e., 2 points, will provide a solution and thus permit prediction. Another advantage of the method is the possibility of predicting effects for a given concentration of administered toxic agent, provided the control and some intermediate curves for the particular animal are available. This prediction requires simulation on an analog computer and interpolation between curves.

185. *Structure-Activity Relationships for Diabetogenic Activity of Nitrosourea Compounds in Mice.* TOM ANDERSON, MARY MCMENAMIN and PHILIP S. SCHEIN, Section of Clinical Pharmacology, Medicine Branch, National Cancer Institute, Bethesda, Maryland.

Nitrosamine and nitrosourea compounds containing a methyl or ethyl end group rapidly depress hepatic pyridine nucleotide (NAD) concentrations. Until the present time only 1 member of this group, streptozotocin, has been demonstrated to be diabetogenic. In this compound the cytotoxic moiety, 1-methyl-1-nitrosourea (MNU), is attached to the carbon-2 position of the glucopyranose carrier, which facilitates its uptake into the pancreatic islet. In addition to lowering hepatic pyridine nucleotide concentrations, streptozotocin depresses pancreatic islet NAD with resultant beta cell necrosis. The failure to produce diabetes with MNU attached to the carbon-1 position of glucopyranose and galactopyranose, or the carbon-3 position of glycopyranose suggests specificity for the carbon-2 position of the glucopyranose carrier for diabetogenic activity. Based upon these results, the pharmacologic properties of 2-deoxy-(3-ethyl-3-nitrosoureido)-glucopyranose, were examined. This compound, like streptozotocin, was found to depress hepatic and pancreatic NAD concentrations and was diabetogenic in the mouse as documented by elevated blood glucose, decreased serum insulin, and histological evidence of pancreatic islet beta cell necrosis. Compared to streptozotocin, a 10-fold increase in drug dosage was required for diabetogenicity. This was predicted by prior structure-activity studies demonstrating the relative inefficiency of the ethyl, in comparison to the methyl end groups of nitrosamine and nitrosourea compounds in reducing NAD concentrations.

186. *Effects of CCl_4 Liver Damage on Biliary Absorption and Reexcretion of Compounds Administered to Rats by Retrograde Intrabiliary Injection (RII).* R. E. PETERSON and J. M. FUJIMOTO, Medical College of Wisconsin, Milwaukee, Wisconsin.

The effect of CCl_4 liver damage on absorption and reexcretion of compounds administered by RII (*J. Pharmacol. Exp. Ther.* **185**, 150, 1973) was assessed. Rats were pretreated 24 hr with CCl_4 (1 ml/kg, ip) or corn oil. Compounds were given in 32 μl by RII and the bile duct cannula was occluded for 0.5, 3 or 6 min. After occlusion 30 drops of bile were collected and analyzed

for the compounds. After 0.5 min the concentration of compounds in individual drops of bile from 1 to 30 were similar in both treatment groups, but after 3 and 6 min the concentration of inulin, urea, ouabain, morphine and morphine-3-glucuronide in drops Nos. 2, 3 and 4 were significantly lower in the CCl_4 group. Since these drops were derived from the duct and ductule portion of the biliary tree, CCl_4 may alter function at this site. The RII caused a rise in intrabiliary pressure to 175 mm Hg in both groups which dropped to 12 mm Hg after 0.5 min occlusion. Pressure remained at this level for longer occlusion periods in both groups. Thus, a change in intrabiliary pressure in CCl_4-treated rats is not responsible for the low concentration of compounds in drops Nos. 2–4. Processes depressed by CCl_4 pretreatment were bile flow and excretion into the biliary tree during occlusion of ouabain, morphine and morphine glucuronide given iv. Also, reexcretion of the same compounds after RII tended to be less in the CCl_4 group. Thus, CCl_4 liver damage causes a decrease in the concentration of compounds (given by RII) in drops of bile obtained from the distal portion of the biliary tree. This effect of CCl_4 could be due to alteration in function at a duct or ductular site or a deficit at the hepatocyte level. (Supported by USPHS Grant GM 16503.)

187. *Effect of Isopropanol Pretreatment on the Hepatotoxic Response to Several Chemicals.* G. L. PLAA, G. K. HANASONO, G. J. TRAIGER and H. P. WITSCHI. Département de Pharmacologie, Université de Montréal, Montréal, Canada.

CCl_4-induced liver damage in mice and rats can be markedly potentiated by isopropanol (I), or acetone (A) pretreatment (Traiger and Plaa, *J. Pharmacol. Exp. Ther.* **183**, 481, 1972). The objective of the present study was to determine if an 18-hr pretreatment with I (2.5 ml/kg, po) or A (1.0 ml/kg, po) could enhance the 24-hr hepatotoxic response produced by single doses of other necrogenic agents. In addition, the effect of I or A on the 24-hr hyperbilirubinemic response to alpha-naphthylisothiocyanate (ANIT) was examined at po doses of 100 or 300 mg/kg. SGPT activity after single ip doses of chloroform (0.5 ml/kg) or trichloroethylene (1.5 ml/kg) were approximately 41- and 8-fold higher, respectively, in I-pretreated mice. I produced little or no potentiation of SGPT activity in mice given ip 1,1,2-trichloroethane (0.15 ml/kg) or 1,1,1-trichloroethane (2.5 ml/kg); A also enhanced the response to chloroform, but had relatively little effect on the other compounds. I did not affect beryllium-induced changes in serum isocitric dehydrogenase (ICD) activity when rats were given single iv injections of Be and sacrificed 4 hr (39 μmol/kg) or 24 hr (7 or 17 μmol/kg) later. Similarly, I produced no increases in either serum ICD activity of rats given allyl alcohol (10, 20 or 40 ml/kg, po), or in SGPT activity of rats given galactosamine HCl (200 or 400 mg/kg, ip). Thus, only necrogenic agents producing predominantly centrilobular damage were potentiated by I. A or I pretreatment enhanced the hyperbilirubinemic response (2-fold) of rats to ANIT at the higher dose only, suggesting tha cholestatic responses may also be affected by I or A. (Supported by the Medical Research Council.)

188. *Effect of Carbon Tetrachloride on the Membranous and Polysomal Components of the Liver Endoplasmic Reticulum.* J. A. CASTRO, M. I. DIAZ GOMEZ, E. C. DE FERREYRA, M. C. VILLARRUEL, N. D'ACOSTA, C. R. DE CASTRO and O. M. DE FENOS, CITEFA. Zufriategui y Varela. V. Martelli. Pcia de Bs As., Argentina.

Carbon tetrachloride (CCl_4) activating ability to produce $\cdot CCl_3$ is higher in the smooth endoplasmic reticulum (SER) than in the rough endoplasmic reticulum (RER), while CCl_4-induced lipid peroxidation is as intense in SER as in RER. $^{14}CCl_4$ gets irreversibly bound to lipids and proteins from either SER or RER not only to the membranous portion of RER but also to the polysomal component. More than 95% of the label from $^{14}CCl_4$ in lipid is associated with phospholipids. Half of the label is bound in the phosphatidylcholine fraction, the other half is distributed similarly among lysophosphatidylcholine, sphingomyeline and phosphatidylethanolamine, while only a very minor fraction is associated with diphosphatidylglycerol. Cystamine administration decreased the extent of the labeling but not its pattern which is similar in SER and RER. The ability to produce polysome breakdown is CCl_4 > chloroform > methylene chloride = control. Promethazine and cystamine partially prevented CCl_4-induced

polysome breakdown, while N,N′-diphenyl-p-phenylenediamine did not have any effect. Other hepatocarcinogens like dimethylnitrosamine, thioacetamide, tannic acid or dimethylaminoazobenzene also cause polysome breakdown but do not induce a lipid peroxidation process. Results are discussed in relation to the relative contribution of $\cdot CCl_3$ free radicals and lipid peroxidation to CCl_4-induced damage to SER and RER.

189. *On the Glucose Protection Against Alloxan-Induced Insulin Release from Isolated Mouse Pancreatic Islets.* WING-PUN FUNG and JOHN H. MENNEAR, Purdue University, West Lafayette, Indiana.

Glucose has been shown to protect mice and rats against the development of alloxan-induced diabetes. However, it is difficult to prove, through the use of in vivo experiments, that the protective effect of glucose is mediated by a direct action on the pancreas. An in vitro technique has been developed to facilitate the study of the direct effects of alloxan on isolated pancreatic islets. Isolated islets were exposed to alloxan (10^{-2}–10^{-3} M) at 0° C. When islets were exposed to alloxon at 0°C throughout the incubation period (3.5 hr) minimal insulin release was observed. Islets which had been exposed to alloxan (10^{-3} M) at 0°C for 30 min then transferred to fresh incubation medium and incubated at 37°C for up to 6.5 hr released significantly more insulin than did control islets. When islets were exposed to alloxan (10^{-3} M) in the presence of 60 mg/ml of D-glucose, increased insulin release was not observed. In the presence of 40 mg/ml of D-glucose, alloxan produced a significant release of insulin; however, the onset of effect was delayed. Exposure to 10^{-2} M alloxan was not antagonized by D-glucose at concentrations as high as 200 mg/ml. This high concentration of glucose did, however, delay the onset of insulin release. The results of these experiments suggest that the ability of glucose to protect against alloxan-induced diabetes is mediated at the level of pancreatic *beta* cell. Also, the protective effect of glucose in vitro is dose related.

190. *Interspecies Variability in Metabolism of Ingested Sulfite.* A. F. GUNNISON, J. T. GUNNISON and E. D. PALMES, New York University Medical Center, Institute of Environmental Medicine, New York, New York.

Although the toxicity of sulfites in laboratory mammals has been investigated for many years, little consideration has been given to their gut absorptivity and no awareness shown of the persistence of the sulfite once absorbed into the plasma of the test species. The purpose of our study was to compare the absorption and metabolism of a standard dose of sulfite administered via gastric intubation in several mammalian species, i.e. the mouse, rat, rabbit, rhesus monkey and baboon. The key measurements made were the plasma sulfite level and total absorbed sulfur, the latter determined simultaneously in specific instances using ^{35}S tracer. The data obtained enabled us to determine for each species the rate of sulfite absorption from the gut and the efficiency of oxidation of plasma sulfite. We found that although the rate of sulfite absorption was roughly similar in all species tested it was heavily dependent upon the rate of gastric emptying. Conversely, the metabolism of plasma sulfite as reflected by plasma sulfite concentrations varied by a factor of approximately 10 between some species. Following gavage with 2 mmol sulfite/kg, the rabbits and primates produced peak plasma sulfite concentrations of approximately 1000 nmol/ml as compared with nearly 100 nmol/ml in rats and mice. This interspecies variation is interpreted as a result of variation in the activity of the enzyme, sulfite oxidase, which catalyzes the in vivo oxidation of sulfite to sulfate. Since rats have been by far the most frequently used species for chronic sulfite toxicity experiments, the difference observed here between primate species and rats with respect to the systemic potential of ingested sulfite is cause, we believe, for reevaluation of the sulfite toxicity literature.

191. *An In Vitro Method to Determine Binding of* [1-^{14}C]*Halothane in the Liver.* LEONARD C. HOWARD, DAVID A. BLAKE and DAVID R. BROWN, University of Maryland School of Pharmacy, Baltimore, Maryland.

In vivo binding of [1-^{14}C]halothane or a metabolite to subcellular fractions of rat, mouse and guinea pig liver had been demonstrated. (Cohn and Hood, *Anes.* **31**, 55, 1969; Howard

et al., J. Pharm. Sci. **62**, 1021, 1973; *Fed. Proc.* **32**, 702, 1973). The bound label was not released by dialysis, precipitation with trichloroacetic acid, or by extraction with hot methanol-benzene (1:1). A radiolabeled macromolecule of >100,000 was separated on Sephadex gel. Incubation with proteases released the label but RNAase and DNAase did not. A similarly bound moiety is produced by incubation at 37°C of [1-^{14}C]halothane with 9000 × *g* fractions of rat, mouse, guinea pig and monkey liver in a system containing 4 μM NADP, 40 μM glucose-6-PO_4, 5 units glucose-4-PO_4 dehydrogenase, 20 μM $MgCl_2$, 40 μM nicotinamide, 0.2 M PO_4 buffer pH 7.4 and oxygen. The relative ratios of binding produced by subcellular fractions are: whole homogenates (0.4), 9000 × *g* supernatant (1.0), nuclear fractions (0.1), mitochondrial fraction (0.7), microsomes (1.0) and cytosol (0.1) calculated per mg protein. Omission of oxygen decreased binding 42%, of cofactors 76% and incubation at 4°C decreased binding 90%. Pretreatment with SKF 525A or 3 methyl cholanthrene did not alter in vitro binding. Phenobarbital increased binding 2-fold; piperonyl butoxide or CCl_4 decreased binding 22 and 93% respectively. Exposure to anesthetic concentrations of halothane on 3 successive weeks significantly increased in vitro binding (1.3-fold). In vitro binding by the liver was 7 times higher than the next most active tissues, the kidney, lung and heart. The results of this study show that the binding of [1-^{14}C]halothane is relatively specific for the liver and primarily associated with the metabolic activity of the microsomal fraction. (Supported by NSF Traineeship to Leonard Howard.)

192. *Absorption and Disposition of Carrageenan Fractions.* R. ABRAHAM, L. GOLBERG and F. COULSTON, Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York.

The lack of adverse effect of an undegraded kappa-lambda carrageenan (HMR) contrasts with the capacity of degraded iota carrageenan (C16) to induce ulceration of the cecum in guinea pigs, ulceration of the colon in monkeys and squamous metaplasia of the rectum in rats. In an attempt to clarify further the biological activities of carrageenans, fractions of kappa, lambda, or iota carrageenan were given either to rats, or guinea pigs, or both. Six fractions of iota carrageenan having intrinsic viscosities (dl/g) of 0.28, 0.68, 1.62, 4.19, 5.34 and 7.51 were given daily by stomach tube at a level of 500 mg/kg to Charles River rats for 6–9 months. Staining with alcoholic toluidine blue and electronmicroscopy of the liver, kidney, lymph nodes and the intestinal tract did not reveal the presence of carrageenan; there was no evidence of histopathological change in these organs. When these fractions were fed for 12 weeks at a concentration of 2% in the diet of guinea pigs, metachromatic material was seen in lysosomes of Kupffer cells and cecal macrophages of animals receiving fractions whose intrinsic viscosities ranged from 0.28 to 4.19, but a seventh fraction of carrageenan with intrinsic viscosity 0.11 was not retained. Fractions of kappa carrageenan (intrinsic viscosities 0.17, 1.45 and 11.95) and lambda carrageenan (intrinsic viscosities 0.50, 2.24 and 10.25) were given to guinea pigs as 1% solutions in drinking water for 3 weeks. With carrageenans having the lowest intrinsic viscosities metachromatic material was observed in the lysosomes of Kupffer cells and cecal macrophages. Tissue retention of κ- and λ-fractions was not accompanied by histopathological changes. Chemical analyses of carrageenans in tissues and urine, now in progress, may clarify the basis of the species differences observed with iota carrageenan, as well as the striking disparities in the behavior of different types of carrageenan chains in the guinea pig. (Supported by FDA Contract 69-7, by Research Grant 2P01-ES00226-07 from the National Institute of Environmental Health Sciences, NIH, and by National Institutes of Health Training Grant 2T01-ES00103-07. Dr. Abraham is the recipient of a Research Career Award No. 1 K04 ES70607-01.)

193. *The Fate of 2,2,4-Trimethyl-1,3-pentanediol (TMPD) Orally in Rats and Percutaneously in Rabbits, Guinea Pigs and Man.* B. D. ASTILL and D. W. FASSETT, Health and Safety Laboratory, Eastman Kodak Company, Rochester, New York.

TMPD, 2,2,4-trimethyl-1,3-pentanediol is an approved polyhydric alcohol for polyester resins used in food packaging, with potential use as an insect repellant. Safety evaluation

included its oral fate in rats, and its fate in rabbits, guinea pigs and humans after skin application. Single oral 0.4 g/kg doses to rats yielded *ca.* 55% in the urine, principally as increased glucuronide output. Single oral 0.2 g/kg doses of [3-^{14}C]TMPD were eliminated in 3–4 days in urine (94–99%) and feces (2%); $^{14}CO_2$ was <0.1%. Urinary metabolites (48 hr) were: unchanged TMPD (0.8–1.7%) and its O-glucuronide (72–73%), 2,2,4-trimethyl-3-hydroxyvaleric acid (HTMV) (2.9–3%) and its glucuronide (4.3–4.4%). Tissue and organ concentrations were <0.02%. Single 2.0 g/kg applications of TMPD-ethanol-glycerol (2:1:2) to clipped rabbit skin increased urinary glucuronide output (*ca.* 30% of the dose). Single 0.18 g/kg skin applications using [3-^{14}C]TMPD were mostly absorbed, and eliminated via the urine (75–82%), with <0.5% as $^{14}CO_2$, and 2% in feces. Volatilization from the site was *ca.* 14%. There was <0.02% at 72 hr in tissues and organs. Absorption from depilated guinea pig skin was less extensive, 0.09 mg/kg applications yielding 26–33% in urine, 4–5% in feces, <0.5% as $^{14}CO_2$, <0.01% at 48 hr in tissues and organs; the remainder was volatilized. Metabolites (24 hr) were: TMPD (rabbit 5–47%, guinea pig 2–5%), TMPD glucuronide (rabbit 43–2%, guinea pig 11–12%), HTMV (rabbit 9–17%, guinea pig 2–4%), HTMV glucuronide (rabbit <1%, guinea pig 7–9%). Application of 3.7–4.9 g TMPD in ethanol-glycerol (2:2:1) to the exposed torso of 11 male human subjects did not increase urinary glucuronides. When TMPD was maintained in the same medium in contact with covered and sealed areas of the forearm (5 subjects, 8 experiments) for 2.5–3.5 hr at a level of 0.4–2 mg TMPD/cm^2, 82–93% was recovered from the application area. The fate of TMPD after oral intake or skin absorption is typical of similar glycols, although the extent of skin absorption is species dependent.

194. *Progress of Chronic Toxicity Studies with Brominated Soybean Oil.* D. E. GORDON, J. B. PLANK, G. L. KENNEDY, M. L. KEPLINGER and J. C. CALANDRA, Industrial BIO-TEST Laboratories, Inc. Northbrook, Illinois.

Previous studies have suggested that heart lesions and enzyme alterations could be caused by the ingestion of high levels of brominated vegetable oil. Studies are now in progress to assess the long-term toxicological effects of brominated soybean oil. These experiments include 2-year chronic studies in rats, dogs and mini-pigs; a multigeneration rat study; a 3-phase study utilizing rats and rabbits; an 18-month mouse study; and a dominant lethal study in rats. The brominated soybean oil is mixed in the diet at levels of up to 3600 ppm. In addition to standard toxicologic parameters, ECG's lipid profiles, LDH isozymes and multiple sections of cardiac tissue with a variety of special stains are being examined carefully. Data from interim sacrifices of all chronic studies in all species have been evaluated, and to date no deleterious effects have been noted. Through 1 generation there have been no effects on reproduction.

195. *The Oral Toxicology of Dichlorodifluoromethane.* H. SHERMAN, J. R. BARNES and E. F. STULA, Haskell Laboratory for Toxicology and Industrial Medicine, E. I. du Pont de Nemours and Company, Newark, Delaware.

Dichlorodifluoromethane has been in use since 1967 as an immersion freezing agent for foods. Male and female rats have been administered dichlorodifluoromethane for 2 years by intragastric intubation at dose levels of 25–11 and 250–130 mg/kg, starting with offspring that had been exposed to the compound in utero. Except for a very slight decrease in the rate of body weight gain by animals that received the higher dose level, no clinical signs of toxicity were observed. The oral administration of the test compound did not affect mortality, nor did it alter any of the clinical laboratory measurements (hematology, urine analysis, liver function test). After 16 months, there was no histologic evidence of toxicity attributable to the administration of the dichlorodifluoromethane. The compound was not teratogenic to rats; it did not interfere with reproduction and lactation, and it did not alter the dominant lethal mutation index in rats. Dichlorodifluoromethane has also been administered in the diet to dogs for 2 years at levels of 300 ppm (*ca.* 10 mg/kg) and 3000 ppm (*ca.* 100 mg/kg) without any evidence of toxicity.

196. *Preliminary Assay for the Teratogenicity of Mycotoxins.* A. WALLACE HAYES, RONALD D. HOOD and KAREN SNOWDEN, Departments of Microbiology and Biology, The University of Alabama, University, Alabama.

The mycotoxins are metabolites produced by storage molds that are toxic either by contact or by inadvertent ingestion of the toxin when present in foodstuffs or feeds. Since information on embryocidal and teratogenic effects of mycotoxins is limited, a preliminary screening was undertaken to determine if such metabolites were teratogens in a mammalian species. Albino mice were used as the test species. Animals were examined on gestation day 18 for evidence of embryonic mortality and fetal malformation. The following mycotoxins have been screened: aflatoxin B_1, citrinin, ochratoxin A, pentatremorgen, rubratoxin B and sterigmatocystin. Data indicating that both ochratoxin A and rubratoxin B are potent embryocidal and teratogenic agents has been reported previously. Citrinin, pentatremorgen and sterigmatocystin were administered ip on day 8 or 10 of gestation in successively higher doses until maternal mortality became limiting. Rubratoxin B was administered orally in doses equal to or in excess of ip doses (on the basis of comparable lethality) previously reported to be teratogenic for the mouse. Treatment with citrinin (5–50 mg/kg, ip), pentatremorgen (1.0–2.5 mg/kg, ip) or sterigmatocystin (0.25–1.5 mg/kg, ip) failed to significantly effect fetal mortality, gross malformation rate or fetal weight at gestation day 18. Rubratoxin B (50 mg/kg, po, day 8; 100 mg/kg, po, day 9 or 10; 125 mg/kg, po, day 6, 7, 8 or 9) caused increased levels (27–61 %) of prenatal mortality, except for the 100 mg/kg, day 9 group. Prenatal-growth rate was unaffected and only a few gross anomalies were noted. (Supported in part by Grant No. ES-00464.)

197. *Some Effects of Graded Levels of Dietary Copper Sulfate Supplementation in Broiler Chickens.* M. J. NORVELL, C. C. CALVERT, M. C. THOMAS and W. D. GOATCHER, Division of Nutritional Sciences, Bureau of Veterinary Medicine, Food and Drug Administration, Rockville, Maryland, and Agricultural Environmental Quality Institute, U.S. Department of Agriculture, Beltsville, Maryland. (D. J. Wagstaff.)

Very little information is available on the effects of dietary supplementation of excess trace elements in broiler chickens. The purpose of this study was to determine the effects of excess levels of dietary copper sulfate supplementation in broiler chickens on mortality, weight gain, relative liver weight, tissue and feces copper residues and histopathological effects on the liver, pancreas, duodenum, ileum and crop. Day-old broiler female chickens were randomly assigned to individual floor pens and fed commercial broiler diets containing graded levels of copper sulfate (up to 720 ppm copper) for 8 weeks. At 2, 4, 6 and 8 weeks of age birds were individually weighed and sacrificed. Selected tissues were then taken for analysis and histological observation. Samples of feces, feed, liver and breast muscle were analyzed for copper and iron by atomic absorption spectrophotometry. The results of this study indicated that growth was inversely related to dietary copper supplementation and that liver, muscle and feces copper was directly related to dietary copper supplementation. All reported effects were statistically significant at the higher levels. Parameters not significantly affected by dietary copper supplementation were mortality, relative liver weight and histological observations on the crop, duodenum, ileum, pancreas and liver. The results of this study indicate that excessive dietary copper supplementation is detrimental to growth and significantly elevates the copper levels of liver and muscle tissues in broiler chickens.

198. *Studies on Impurities in Commercial Saccharin.* R. LACOMBE, B. STAVRIC, C. A. MOODIE, D. R. STOLTZ, R. F. WILLES and I. C. MUNRO, Food Research Laboratories, Health Protection Branch, Health and Welfare Canada, Ottawa, Canada. (H. C. Grice.)

Extensive testing of saccharin for carcinogenic effects has failed to produce unequivocal results. While preliminary reports from 2 laboratories suggest that chronic administration of saccharin to rats results in the development of bladder tumors, this finding is not supported by similar studies conducted in several other laboratories. One of the reasons considered for the discrepancies in the cancer studies was the possibility of differences in the nature of chemical impurities in the samples used. Preliminary investigations showed that saccharin

is produced by different synthetic processes. Analysis of the saccharin samples used in the different cancer studies revealed that the samples differed qualitatively and quantitatively in their content of impurities. *o*-Toluenesulfonamide, *o*-sulfamoylbenzoic acid, *p*-sulfamoylbenzoic acid and ferrous sulphate were identified in some of the samples tested. *o*-Toluenesulfonamide was found to be a major impurity in most of the samples used in the cancer studies. The concentration of *o*-toluenesulfonamide in these samples varied from 2.5 to 5050 ppm.

199. *Assessment of the Carcinogenicity of Commercial Saccharin.* I. C. MUNRO, C. A. MOODIE and H. C. GRICE, Food Research Laboratories, Health Protection Branch, Health and Welfare Canada, Ottawa, Canada.

Groups of 60 male and 60 female Charles River rats were fed diets containing sodium saccharin to provide daily doses of 0, 90, 270, 810 or 2430 mg saccharin/kg of body weight/day. The animals were treated for a period of 26 months. Food consumption, body weight and clinical examinations were conducted weekly on all rats. The animals were free of the bladder parasite *T. Crassicauda*. Four bladder tumors were found in treated animals, 1 in a male and female from the lowest dose group and 2 in males given 810 mg/kg, the second highest dose. The tumors were transitional cell papillomata, none of which were invasive. Three bladder calculi were observed grossly and several others were noted in urine samples examined microscopically. The presence of bladder calculi was not associated with saccharin or with the presence of bladder tumors. Saccharin administration was not accompanied by an increase in tumor incidence, although high doses were associated with reduced body weight in both sexes and increased mortality in male rats.

200. *Combined Chronic Feeding and Three-Generation Reproduction Study of Sodium Saccharin in the Rat.* JEAN M. TAYLOR and LEO FRIEDMAN, Food and Drug Administration, Washington, D.C.

The carcinogenic and reproductive effects of sodium saccharin were studied in the Charles River CD rat in a combined chronic and 3-generation reproduction study. For the chronic study, 48 male and 48 female F_{1a} weanling offspring per test group were selected and continued on the same test regimen as their parents. The dietary levels were 0, 0.01, 0.1, 1.0, 5.0 and 7.5% sodium saccharin. Calcium cyclamate was fed as a reference compound at 5.0%. The rats were mated to produce 2 litters. Serial sacrifices were performed at 14 and 18 months. The study was continued until the number of survivors in a group fell to 20% of the starting number. The last rats were killed approximately 28 months after the first weanlings were selected for the chronic study. There were no significant differences between test and control groups in hematological values, organ weights or survival. Average weaning weights were decreased in litters from parents receiving 5 and 7.5% sodium saccharin and 5% calcium cyclamate; other reproductive indices showed scattered variations but these were not consistent for all generations. During the chronic study some of the initial weaning weight depression was overcome, but rats on 5 and 7.5% sodium saccharin and 5% calcium cyclamate had lower average body weights throughout the study. Histopathological examination of the urinary bladders from rats on the chronic study longer than 18 months revealed an increased incidence of transitional cell carcinomas in the 7.5% sodium saccharin group.

201. *Further Observations on the Metabolism of Saccharin in Man.* J. L. BYARD, E. W. GOLBERG and F. COULSTON, Institute of Comparative and Human Toxicology, Albany, New York.

As a continuation of studies previously reported from this laboratory (*Food Cosmet. Toxicol.* **11**, 391 and 403, 1973) each of 4 men took 500 mg of [^{14}C]saccharin (9.8 μCi), and their excreta were collected at intervals up to 96 hr. More than 98% of the ^{14}C was recovered within 48 hr (92.1% in urine, 5.8% in feces), and only a further amount of 0.3% was excreted over 48–72 hr (none over 72–96 hr). The mean 24-hr excretion of 92.4 $\pm$ 1.4% of the dose indicates a body half-life of about 6.5 hr. In most of the urine samples GLC analysis gave results for saccharin (SA) corresponding very closely to those calculated from the ^{14}C counts, but in several there

were discrepancies exceeding considerably those expected from the precision (± 3%) of the GLC method. Very high values in 2 samples made it likely that the subject had inadvertently ingested extraneous SA, while 2 low values (both in 0–8 hr samples) suggested that some alteration of the administered SA had occurred. However, chromatographic analysis (high-pressure, liquid–liquid and thin layer) failed to reveal the presence in these samples of labeled compounds other than SA in more than trace amounts. Hydrolysis of the aberrantly low samples with strong alkali brought the GLC and ^{14}C analyses into satisfactory agreement. The fecal ^{14}C, within the limits of precision of the HPLLC analysis, proved to be in the form of unchanged SA. Subsequently, these same subjects, together with 2 others, took 500 mg of unlabeled SA; urine only was collected for the ensuing 72 hr. Analysis of these samples by GLC gave rates of excretion quite similar to those described above: the mean overall 72-hr excretion as SA was 90.3% of the dose, but alkaline hydrolysis brought this figure up to 94.3%. These results substantiate the earlier findings in animals. (Supported by FDA Contract 69–7, by Research Grant 2P01-ES00226-07 from the National Institute of Environmental Health Sciences, NIH, and by the National Institutes of Health Training Grant 2T01-ES00103-07.)

202. *Evaluation of the Toxicologic Potential of a Nutritive Sweetener (Aspartame) in the Dog.*
K. S. Rao and R. G. McConnell, Searle Laboratories, Chicago, Illinois.

Aspartame [SC-18862, 3-amino-N-(a-carboxyphenethyl) succinamic acid, methyl ester; the methyl ester of aspartyl phenylalanine; APM] is a dipeptide sweetening agent which organoleptically has about 180 times the sweetness of sugar. It is hydrolyzed in the gut to its constituent amino acids, aspartic acid and phenylalanine, and these are metabolized as natural constituents in the diet. In the present study, chronic toxicity of Aspartame was evaluated in the beagle dog. The compound admixed with the basal diet was administered daily to 5-month-old male and female pups for 106 consecutive weeks. Aspartame was administered at daily intake levels of 1, 2 and 4 g/kg. This amounts to a maximum of 11% APM in the diet of high dose dogs. Control animals received the compound-free basal diet only. Five animals of each sex per group were employed. Conventional physical, hematology, clinical chemistry, urinalysis and postmortem examinations were performed. Survival was 100% in all groups. No compound related variations in food consumption, body weight gain, hematology and clinical chemistry parameters were observed. A significant increase in excretion of urinary phenylketones was observed in some high dose dogs at weeks 2, 4 and 26 of treatment. However, at subsequent intervals all high dose dogs were negative in this regard. Postmortem gross and microscopic findings were unremarkable. It is concluded that daily oral administration of Aspartame up to 4 g/kg for 106 consecutive weeks to 5-month-old beagle dogs of both sexes, causes no biologically meaningful detrimental effects.

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