

*George B. ...*

**ABSTRACTS OF PAPERS**

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Abstracts of Papers for the Fourteenth Annual Meeting of the  
Society of Toxicology, Williamsburg, Virginia  
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1. *Is Red Dye no. 2 Teratogenic? A Joint Government-Industry Approach to a Toxicological Problem.* J. F. HOLSON, JR., T. B. GAINES, H. J. SCHUMACHER and M. F. CRANMER, National Center for Toxicological Research, Jefferson, Arkansas.

Much controversy has centered around commercial usage of the red dye, amaranth (FD&C Red no. 2). This controversy arose from conflicting reports on this dye's embryo and/or fetotoxicity in rats. In an effort to provide a more substantial basis for evaluating Red no. 2 toxicity, a group of Ad Hoc advisors participated in the designing of a protocol which was subsequently implemented by three laboratories. The participating laboratories were Industrial Bio-Test Laboratories, The Division of Toxicology, Bureau of Foods, FDA, and the Teratology Division, National Center for Toxicological Research (NCTR). Each laboratory followed the same protocol with the exception that NCTR conducted the study on both Osborne-Mendel and CD rats, whereas Industrial Bio-Test and FDA used only CD and Osborne-Mendel rats, respectively. All of the following groups were randomly allotted 20 animals each and given 200 mg/kg Red no. 2 as follows: Red no. 2 gavage, 0-19 days, 6-15 days, and days 7, 8, and 9; Red no. 2 by drinking water (distilled, 0.2% Red no. 2), 0-20 days; control solution per gavage, 0-19 days, 6-15 days, and days 7, 8, and 9; sham gavage, 0-19 days; distilled water per gavage 0-19 days; untreated with distilled water ad lib., 0-20 days. At autopsy, particular attention was given to discerning early and late deaths. One-half of the fetuses from animals treated on days 7, 8, and 9 of pregnancy were examined further for internal anomalies and the remaining fetuses for skeletal defects. Results between strains, laboratories, routes, and days of administration were compared. Results from all three laboratories did not indicate fetotoxicity due to Red no. 2 administration under conditions of the protocol. However, there were striking differences between strains and laboratories in several reproductive parameters and in types and frequencies of observed terata. Analysis of this unique, joint Government-Industry study has revealed several problem areas worthy of consideration prior to implementation of such endeavors in the future.

2. *Teratologic Study in Dogs with FD & C Red no. 2.* K. MASTALSKI, D. H. JENKINS, J. B. PLANK, F. K. KINOSHITA, M. L. KEPLINGER and J. C. CALANDRA, Industrial Bio-Test Laboratories, Inc., Northbrook, Illinois.

A reproduction/teratology study was conducted in which purebred female beagle dogs in groups of 12 were fed FD&C Red no. 2 in the diet at levels of 0, 300, 900, or 3000 ppm. Six males were fed 3000 ppm of the diet. All dogs were fed their respective dietary level of FD&C Red no. 2 for at least 45 days prior to breeding for the first litter. Gestation day 0 was the first day of confirmed copulation. On gestation day 60, six females from each group were examined by caesarean section. The number of implantation sites, resorption sites, viable and dead fetuses, and corpora lutea were recorded. Live pups were examined, weighed, and placed in an incubator for 24 hr to determine viability. All pups were examined by X-ray and gross dissection. The remaining six females from each group were allowed to whelp their young and nurse them for 8 wk. Mortality and behavior, as well as physical and neurological signs, were recorded. All pups were X-rayed at 7 days of age. There were no effects on breeding performance, adult body weight, food consumption, progeny viability, survival, gross pathology, or skeletal development. The number of resorption sites was increased and the body weight of the pups was reduced in the caesarean sectioned dogs fed 900 ppm. These effects were not seen in the dogs fed

3000 ppm of FD&C Red no. 2. The weight gain of pups during 8 wk of nursing was reduced. After weaning of the first litter the adult dogs were rebred. All animals were maintained throughout the investigation on their respective dietary level of FD&C Red no. 2. All adult females were allowed to whelp their young and nurse them for 8 wk. No adverse effects were noted in either the adult dogs or the second litter progeny. The reduced body weight gain of the pups during the nursing period noted for the first litter was not seen in the second litter.

3. *Teratogenic Potential of Noise in Mice and Rats.* C. A. KIMMEL, R. O. COOK and R. E. STAPLES, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

The effect of noise stress on the developing embryo was studied in mice and rats. The first experiment was designed to determine whether background noise plays a role in producing spontaneous malformations. To exclude extraneous noise, mice (CF<sub>1</sub>S strain) were placed in an environmentally controlled IAC double-walled sound chamber from days 1 to 18 of gestation. Ambient noise levels in the chamber with animals present ranged between 30-45 dBA and were due almost entirely to self-generated noise of the animals. Control mice were housed in their regular quarters where noise levels measured 50-60 dBA. Animals were evaluated on day 18 of gestation. Mice housed in the chamber during pregnancy gained significantly less weight than controls but no other adverse effects were noted. Another experiment measured maternal and embryotoxic responses of CF<sub>1</sub>S strain mice exposed on days 7-10 or 11-14 and of CD strain rats exposed on days 6-15 to 100 dB (6 min of every half-hour on the exposure days) of white noise (20-20,000 cps) in the same chamber. Control and experimental groups were housed in regular animal quarters other than during the days of exposure to noise. Mice exposed on days 11-14 gained significantly less weight through pregnancy and percent resorptions were significantly increased. No change in incidence of malformed offspring was noted in either group of mice. Rats exposed to noise showed a significant decrease in maternal weight gain but developmental toxicity was not observed. These results appear contradictory to reports of other investigators who found increased malformations after combined audio-visual or varying types of audio stress. However, this study concerns the use of a single type of audio stress (white noise) which at the level of 100 dB had slight maternal and fetotoxic effects in mice and little effect in rats.

4. *Teratologic Evaluation of Zinc Omadine When Applied Dermally on Yorkshire Pigs.* J. H. WEDIG, G. L. KENNEDY, D. H. JENKINS, R. HENDERSON and M. L. KEPLINGER, Olin Research Center, New Haven, Connecticut; Industrial Bio-Test Laboratories, Inc., Northbrook, Illinois.

A teratologic study in Yorkshire pigs was performed by applying zinc pyridine-2-thiol-1-oxide (zinc Omadine 30, 100, or 400 mg/kg doses prepared as a 50% (w/v) suspension in Aquaphor cream) in daily rotation to each of eight different 20 x 19-cm<sup>2</sup> treatment areas on the shaved dorsal surface from day 8 through day 32 of gestation. The materials remained on the skin for 8 hr/day. During the treatment period each animal was individually confined in such a manner as to prevent oral ingestion of the test materials. Each of the five experimental groups including a naive and treated control consisted of four animals. Piglets obtained by laparotomy on gestation day 100 were examined for gross abnormalities and for internal development. Skeletal development was assessed by X-ray. No signs of systemic toxicity or abnormal behavior were noted during the experimental period. Growth of maternal animals was unaffected by dermal treatment with zinc Omadine. A slight erythema which was observed on some of the zinc Omadine-treated animals during dosing was not apparent at sacrifice. The number of implantation sites, resorption sites, and viable fetuses among the test groups were not significantly different than the control groups. No evidence of any teratogenic effect was observed in the zinc Omadine-treated animals.

5. *Comparative Embryotoxicity and Teratogenicity of Various Tranquilizing Agents in Mice, Rats, Rabbits, and Rhesus Monkeys.* K. T. SZABO, M. E. DiFEBBO, Y. J. KANG, A. K. PALMER and R. L. BRENT, Pathology and Toxicology Department, Smith, Kline & French Laboratories, Philadelphia, Pennsylvania; Reproductive Toxicology Department, Huntingdon Research Centre, Huntingdon, England; and Stein Research Center, Thomas Jefferson University, Medical College, Philadelphia, Pennsylvania.

This is a report on the comparative embryotoxicity and teratogenicity of tranquilizers in mice, rats, rabbits, and rhesus monkeys. Six phenothiazine derivatives with a piperazine ring, one without this ring, and one nonphenothiazine were studied in pregnant animals during various stages of embryogenesis. Most of the doses ranged from sizable multiples to several hundred times the human therapeutic dose. In each species, the fetuses were removed near term by caesarean section and examined. The higher doses of the tranquilizing agents produced severe reductions in food and water consumption of the pregnant mice, resulting in markedly decreased weight gain. A number of fetuses were born with cleft palate; a majority of those were retarded in growth and weight. In mice, maternal nutritional deprivation during days 10-13 of pregnancy also resulted in a significant increase over the spontaneous incidence of cleft palate. A comparison of the various tranquilizing agents suggests that the mouse is a more sensitive species than the rat for the experimental induction of cleft palate. The rabbit and the rhesus monkey appear to be resistant.

6. *Teratogenicity of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin in CF-1 Mice.* F. A. SMITH, B. A. SCHWETZ and P. J. GEHRING, Toxicology Research Laboratory, Health and Environmental Research, Dow Chemical U.S.A., Midland, Michigan.

The effect of TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) on the developing embryo and fetus of CF-1 mice has been evaluated. An experimental no-adverse-effect level for TCDD in the developing embryo and fetus of mice is postulated. Pregnant CF-1 mice were given TCDD by oral gavage on days 6-15 of gestation at dose levels of 0, 0.001, 0.01, 0.1, 1.0 and 3.0  $\mu\text{g}/\text{kg}/\text{day}$ . Little or no maternal toxicity was observed at any dose level. Cleft palate and dilated renal pelvis were found at 3.0  $\mu\text{g}/\text{kg}/\text{day}$ . Cleft palate was found at 1.0  $\mu\text{g}/\text{kg}/\text{day}$ . No malformations were found at the intermediate dose levels of 0.1 or 0.01  $\mu\text{g}/\text{kg}/\text{day}$ . At the lowest or 0.001- $\mu\text{g}/\text{kg}/\text{day}$  dose level, exencephalies were found. Whether these latter observations were due to chance rather than treatment with TCDD is now under investigation. If this observation is not duplicated in further studies, the experimental nonteratogenic dose level of TCDD in CF-1 mice appears to be 0.1  $\mu\text{g}/\text{kg}/\text{day}$ .

7. *Reproduction and Teratologic Studies with Halothane and Forane.* S. SMITH, G. L. KENNEDY, M. L. KEPLINGER and J. C. CALANDRA, Industrial Bio-Test Laboratories, Inc., Northbrook, Illinois.

Two anesthetics, Halothane and Forane, were evaluated for their possible effects on the fertility, gestation, perinatal, and lactational performance of rats and for their teratologic activity in rats and rabbits. Exposures, in all cases, were for 1 hr and were conducted during the following time periods: Teratology, rats on gestation days 1-5, 6-10, or 11-15; rabbits on gestation days 6-9, 10-14, or 15-18; perinatal and lactation, on days 15-20 of gestation; and fertility and reproduction, on pre-mating days 11-15, 6-10, or 1-5. In reproduction and fertility phase, matings were conducted with untreated males and treated females and with treated males and untreated females to permit independent evaluation of effects on each sex. Exposure concentrations were approximately 1.4% or 2.2% Halothane, rats and rabbits, respectively, and 1.5% or 2.3% Forane, rats and rabbits, respectively. In all cases, control animals were similarly exposed to air flow only. The findings revealed no adverse effects of either Halothane or Forane with respect to fertility, gestation, fetal development, or progeny survival. Gravid rats treated on gestation days 6-10, and 11-15 with Halothane had increased numbers of resorption sites. Examination of fetuses obtained from female rats or rabbits which had been exposed to either of the anesthetics revealed no structural anomalies, and the two compounds are judged to be free of teratogenic properties under the conditions imposed.

8. *Teratogenicity Studies on Halogenated Benzenes (Pentachloro-, Pentachloronitro-, and Hexabromo-) in Rats.* K. S. KHERA and D. C. VILLENEUVE, Health Protection Branch, National Health and Welfare, Ottawa, Canada.

Fungicide, pentachloronitrobenzene (PCN), and fire retardants pentachloro- (PC) or hexabromobenzene (HB) were administered po by intubation at doses of 50, 100, and 200 mg/kg to rats on days 6-15 of gestation. The compounds were suspended in corn oil in 0.5, 1 and 2% concentrations and the suspensions were dosed at 10 ml/kg. Separate controls were included for each of the test compounds and were given equivalent amounts of the vehicle alone. The amounts administered were adjusted daily to maintain a constant dosage based on body weight. None of the test compound appeared to be toxic for the maternal organism or changed its body weight gain. On day 22, dams were killed and fetuses removed and examined for gross anomalies. Data on corpora lutea, deciduomata, dead fetuses and fetal weights were also collected. Two-third of fetuses were processed for determining skeleton defects and the remainder were fixed in Bouin's fluid for visceral examination. PCN and HB at all doses had fetal values including incidence of developmental anomalies comparable to the control values. PC reduced the mean fetal weight at 200 mg/kg. In addition, PC markedly increased the incidence of lumbar rib (uni- or bilateral) and retarded sternal ossification in a dose-related manner.

9. *Synthesis and Study of Some New Chelating Agents to Antagonize Methylmercury-Induced Toxicity.* M. M. JONES and R. D. HARBISON, Center in Toxicology, Vanderbilt Medical Center, Nashville, Tennessee.

Two new mercury chelating agents have been synthesized: phthalyltetrathioacetic acid (PTTA) and a novel chelating polymer (glutaraldehyde-butanedithiol polymer). Characterization of metal chelating properties were made using established titrimetric procedures. PTTA and the polymer form stable complexes with the mercuric ion in water and are capable of keeping the latter in solution at physiological pH. Adult male mice were treated with methylmercuric chloride (30 mg/kg) orally. Methylmercuric chloride (30 mg/kg) produced about 90% lethality in 7 to 10 days. PTTA was injected ip or orally. Binding polymer was administered orally. Both PTTA and binding polymer antagonized methylmercuric chloride-induced lethality. PTTA (250 mg/kg) administered simultaneously with methylmercuric chloride protected about 95% of methylmercuric chloride-treated animals from death at 7 days following treatment. PTTA (500 mg/kg) administered orally 24 hr following methylmercuric chloride treatment resulted in 50% survival. The protective effect induced by PTTA lasted only 1 wk following a single dose. Mercury binding polymer (500 mg/kg) administered 24 hr and 72 hr following methylmercuric chloride treatment resulted in 78% survival in the first week, 55% survival in the second week, and 55% survival at the end of 3 wk. A 10% survival rate was recorded in the corresponding control methylmercuric chloride-treated animals without antagonist. Both of these new mercury chelators have a selective specificity for mercury and do not chelate calcium and other essential ions to any significant extent. In conclusion, these two new mercury chelating agents produce significant protection against methylmercuric chloride-induced toxicity. (Supported by NIH Grants ES 00267 and ES 00782.)

10. *Toxic effects of Methylmercury in Infant Monkeys (Macaca irus).* J. F. TRUELOVE, R. F. WILLES and I. C. MUNRO, Health Protection Branch, Health and Welfare Canada, Ottawa, Canada.

The present study was undertaken to determine the effect of methylmercury on the developing infant monkey and to measure the dosing period required to produce signs of methylmercury intoxication and the blood concentrations of methylmercury acquired during this dosing period. Four infant monkeys were dosed with 500  $\mu$ g mercury as methylmercury/kg/day beginning at 2 days of age. Blood samples were taken weekly and analysed for methylmercury. Neurological examinations were conducted 5 days/wk on each infant. Body weights and caloric intakes were measured daily. Electroencephalograms and visual evoked responses

were recorded. When neurological signs of methylmercury intoxication were observed, dosing was stopped and the infant observed until advanced signs of intoxication were evident. The infants were then autopsied and tissues collected for histopathology and methylmercury analysis. Neurological signs of methylmercury intoxication were first observed after 28 to 29 days of dosing. The first signs noted were ataxia and a decrease in caloric intake. Even though dosing was terminated at the first signs of intoxication, the severity of the neurological signs observed, continued to increase. Blood mercury concentrations ranged from 8.0 to 9.4  $\mu\text{g/g}$  when the first neurological signs were observed. Brain mercury concentrations at autopsy ranged from 22 to 48  $\mu\text{g/g}$  and the blood/brain ratio ranged from 0.25 to 0.11. Preliminary qualitative assessment of the EEG indicated increased frequency and decreased amplitude in response to the methylmercury treatment. Visual evoked responses initially increased in amplitude then decreased to no response just prior to autopsy. Histopathological assessment indicated neuronal degeneration which was most pronounced in the visual cortex and cerebellum.

11. *Effect of Methylmercury on Electroencephalograms of Cats.* R. F. WILLES, J. F. TRUELOVE, D. KREWSKI and I. C. MUNRO, Health Protection Branch, Health and Welfare Canada, Ottawa, Canada.

The establishment of an acceptable daily intake for methylmercury in man relies on the assessment of early signs of methylmercury intoxication. Experiments were conducted to quantitatively assess the effects of methylmercury on EEG patterns in adult cats and to establish the correlation between EEG pattern changes, blood concentrations of methylmercury, and other signs of methylmercury intoxication. Six adult female cats were dosed orally with 250  $\mu\text{g}$  MeHg/kg/day. Six similar cats received 3  $\mu\text{g}$ /MeHg/kg/day and served as controls for the experiment. Recording electrodes were chronically implanted into several brain areas in all cats. EEG records were taken twice weekly from each cat using a physiological amplifier system interfaced to a minicomputer. The EEG data from the visual cortex was analysed via spectral analysis. Percentiles were calculated from the spectral density function and principle component analysis was used to compare the spectral percentiles between treated and control infants. Data for analysis was selected such that comparisons were made between data segments collected while the cats were in similar physical states (i.e., early sleep). Neurological examinations were conducted twice weekly until the first signs of methylmercury intoxication were observed, then daily until unequivocal signs were evident. Blood samples were collected weekly for methylmercury analysis. Only EEG data from the visual cortex has been analysed to date. The results have demonstrated that methylmercury produced a significant change in EEG patterns following 7 wk of treatment. The first neurological signs of methylmercury intoxication were observed at 9–10 wk of treatment and unequivocal signs of intoxication were evident between 11 and 13 wks of treatment. Blood mercury concentrations were 12.7  $\mu\text{g/g}$  at 7 wk, 15.8  $\mu\text{g/g}$  at 10 wk, and 16.3  $\mu\text{g/g}$  at 12 wk of treatment.

12. *Effect of Spironolactone on the Distribution of Mercury.* C. D. KLAASSEN, University of Kansas Medical Center, Kansas City, Kansas.

The distribution and biliary excretion of  $^{203}\text{HgCl}_2$  (0.3 mg/kg, iv) was measured in rats treated with spironolactone (SP, 75 mg/kg, ip) for various time intervals. SP had no effect when it was administered once a day for 4 days, 24 hr prior to administration of  $\text{HgCl}_2$ . SP had its greatest effect when administered once, 15 min before  $\text{HgCl}_2$ . SP decreased the concentration of  $^{203}\text{Hg}$  in the plasma from 1.5 to 0.05  $\mu\text{g/ml}$ , while it increased the blood concentration from 1.5 to 5  $\mu\text{g/ml}$ . This treatment increased the concentration of mercury in the lung 12, heart 6, spleen 3, brain 3, muscle 2, stomach 1.7, and liver 1.5 times control, had no effect on the concentration of  $^{203}\text{Hg}$  in the intestine, bone, and testes, and markedly decreased the concentration in the kidney to 10% of controls. Biliary excretion of mercury was not increased. When SP was administered 30 min or 3 hr before administration of the  $^{203}\text{HgCl}_2$ , qualitatively similar but less dramatic effects on the distribution of mercury were obtained. SP administered 15 min after

HgCl<sub>2</sub> administration had a similar effect on the distribution of mercury as when administered 30 min before HgCl<sub>2</sub>, with the exception that the concentration of mercury in the kidney was not decreased. The two major metabolic products of SP, canrenone and thioacetic acid, were also given to determine their effect on mercury distribution. Canrenone had no effect while thioacetic acid produced an effect similar to that produced by SP. Thus it appears that the alteration in the distribution of mercury after SP treatment is due to thioacetate. It seems likely that the thioacetate complexes the mercury; this complex distributes in the body in a manner similar to organic mercurial compounds, which in comparison to inorganic mercurials, reach a lower concentration in the plasma and kidney and a higher concentration in the blood and other tissues. The decrease in the concentration of mercury in the kidney produced by SP is probably responsible for the decreased toxicity of mercury after SP treatment. (Supported by USPHS Grant GM 15956.)

13. *Hypersensitivity to Beryllium in Guinea Pigs*. M. J. PALAZZOLO and A. L. REEVES, Department of Occupational and Environmental Health, Wayne State University School of Medicine, Detroit, Michigan.

· Delayed hypersensitivity to beryllium in guinea pigs may be measured in vivo as dermal reactivity or in vitro as macrophage migration inhibition. Results of the two tests were in good agreement, with the latter test being the more accurate index of cell-mediated immunity. Inhalation exposure to BeSO<sub>4</sub> induced no hypersensitivity, but reduced substantially the hypersensitivity produced previously by intradermal treatments. Animals so treated responded to the inhalation of beryllium with a modified and alleviated reaction in the lungs, showing that the induction of dermal sensitivity to beryllium protected the guinea pigs from the severe lung lesions otherwise arising from beryllium inhalation. Both dermal sensitivity and macrophage migration inhibitory activity were passively transferable with sensitized lymphoid cells, with the transferred sensitivity persisting for about 7 days.

14. *Effect of Selenium on Cadmium-Induced Toxicity in Rats*. Z. MERALI and R. L. SINGHAL, Department of Pharmacology, University of Ottawa, Ottawa, Canada.

Cadmium seems to have a wide toxicological potential and has been implicated in the development of Itai-Itai disease, the signs of which include glycosuria and decreased pancreatic function. Selenium, on the other hand, has been reported to protect necrotic effects of cadmium on rat testes. The purpose of the present study was to investigate the influence of subacute cadmium (2.0 mg CdCl<sub>2</sub>/kg/day; ip, 7 days) on hepatic carbohydrate and cyclic AMP metabolism and to examine the effects of selenium (2.0 mg SeO<sub>2</sub>/kg/day; im, 7 days) on cadmium-induced metabolic alterations. Rats exposed to cadmium displayed suppressed pancreatic function as evidenced by intolerance to glucose (2.0 g/kg; ip), reduced serum immunoresponsive insulin (IRI) concentrations, as well as decreased insulinogenic indices. Selenium, on the other hand, by itself caused only slight alterations in the above parameters. However, when selenium was given 6 hr prior to the daily injection of cadmium, significant improvement was noted in glucose tolerance, IRI concentrations and insulinogenic indices when compared with the values observed for rats receiving cadmium alone. Cadmium treatment not only enhanced the activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase, and glucose 6-phosphatase but also elevated the endogenous levels of hepatic cyclic AMP. In contrast, exposure to selenium alone did not alter hepatic cyclic AMP concentration or the normal hepatic gluconeogenic potential. Although administration of selenium to rats exposed to cadmium failed to prevent the cadmium-induced elevation in hepatic cyclic AMP, it did protect against the cadmium-induced alterations in various gluconeogenic enzymes. Our data show that subacute exposure to cadmium suppresses pancreatic function, elevates hepatic cyclic AMP, and augments the gluconeogenic potential of rat liver. Although the administration of selenium prior to daily cadmium exposure prevented the effects of cadmium on hepatic gluconeogenic enzymes and pancreatic function, it failed to

exert any significant effect on the cadmium-induced increases in hepatic cyclic AMP concentrations. (Supported by Grants from the Medical Research Council and the Department of National Health and Welfare, Government of Canada).

15. *Pharmacological and Toxicological Effects of Alkali and Alkaline Earth Metal Ions as Measured by Deprivation-Induced Fluid Consumption.* J. E. ZABIK, B. R. FITSCH and R. P. MAICKEL, Department of Pharmacology, Medical Sciences Program, Indiana University, Bloomington, Indiana.

A variety of drugs have been shown to influence deprivation-induced fluid consumption in rats using a standardized test system developed in this laboratory (*Int. J. Neuropharmacol.* **8**, 337-346, 1969). Since it is well known that administration of aqueous solutions of Na<sup>+</sup> ions to animals can stimulate fluid consumption, it seemed of interest to test the effects of other metallic ions. Two series of ions were selected: alkali metals (Li<sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup>, Cs<sup>+</sup>) and alkaline earth metals (Mg<sup>2+</sup>, Ca<sup>2+</sup>, Sr<sup>2+</sup>, Ba<sup>2+</sup>). The anionic component was kept constant as the Cl<sup>-</sup> ion, and all dosages were by ip injection as solutions in distilled water. Dose-response data were collected over ranges of 0.02-0.06 mmol/kg (Ba<sup>2+</sup>) to 1-10 mmol/kg (Na<sup>+</sup>, K<sup>+</sup>) depending on the toxicity of each ion. Dose-related stimulation of deprivation-induced fluid consumption was evoked by Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, and Sr<sup>2+</sup>, while the other ions, except for Ba<sup>2+</sup>, had variable actions. Ba<sup>2+</sup> showed only a depressant action on deprivation-induced fluid consumption and was the most severely toxic. Differential effects of the ions on fluid consumption and overt behavior have been made. (Supported in part by NASA Grant NGL-15-003-117.)

16. *Acute Lethality of Selected Heavy Metals in Spontaneously Hypertensive Rats.* S. C. LEWIS, Department of Toxicology, Indiana University School of Medicine, Indianapolis, Indiana. (R. B. Forney)

Data from the literature on the acute lethality of most heavy metal elements in laboratory animals is frequently either contradictory or incomparable. No acute lethality studies could be found for animals bred specifically for their genetic predisposition to develop hypertension. Since strains of spontaneously hypertensive rats are widely used as an animal model in biomedical research of hypertension, determination of toxicologic data for these strains will allow more critical evaluation of their applicability to a specific research problem. These studies were undertaken to determine the acute median lethal dose of selected heavy metal and trace elements in young (4 mo) spontaneously hypertensive rats (SHR). All metal elements were administered as aqueous solutions of their common soluble salts and by the intraperitoneal route. When possible, the LD50 was calculated reflecting the mortality data from the first, third, and seventh day following injection. Seven-day LD50 values were as follows: 5.6 mg As/kg, 2.78 mg Cd/kg, 13.8 mg Co/kg, 27.0 mg Cu/kg, 279 mg Pb/kg, 1.71 mg Hg/kg, 4.16 mg Se/kg, 63.5 mg Sn/kg, 49.3 mg Zn/kg. A different result may well be realized in similar studies with older animals with more extensive and long-standing hypertensive changes. In 10 mo SHR, the LD50 of Cd and Zn were significantly reduced, being 1.73 mg Cd/kg and 28.7 mg Zn/kg. These results may be added to the presently limited store of toxicologic data available for SHR to further elucidate the characteristics of these animals for further biomedical research. (Supported by PHS T01 GM 1089-11.)

17. *Absorption and Tissue Distribution of Inorganic Lead in the Developing Infant Monkey (Macaca irus).* I. C. MUNRO, R. F. WILLES and J. F. TRUELOVE, Health Protection Branch, Health and Welfare Canada, Ottawa, Canada.

Excessive lead absorption has been implicated as a major factor in lead intoxication in young children. Experiments were undertaken to assess lead absorption from the gastrointestinal tract and subsequent tissue distribution of <sup>210</sup>PbNO<sub>3</sub> in infant monkeys. Infant monkeys were separated from their mothers within 12 hr after birth and reared in an infant nursery. Four infant monkeys at 10 days and 150 days of age were given a single oral dose of <sup>210</sup>PbNO<sub>3</sub> (10 μg Pb/kg, sp. act. 10 μCi <sup>210</sup>Pb/μg Pb). Feces and urine were collected over 8-hr time



periods for 96 hr postdosing. Blood samples were collected every 24 hr for 96 hr. At 96 hr postdosing the infants were anesthetized with sodium pentobarbital (40–60 mg/kg given ip). Following anesthesia the infants were exsanguinated via the abdominal vena cava and a variety of tissues were collected to assess  $^{210}\text{Pb}$  distribution within the infant. Lead-210 in all samples taken throughout the experiment was measured by gamma counting techniques. The apparent absorption of  $^{210}\text{PbNO}_3$ , as calculated from the  $^{210}\text{Pb}$  recovered in the feces, ranged from 65 to 85 % with no apparent difference between infants at 10 and 150 days of age. The ratio of  $^{210}\text{Pb}$  between erythrocytes and plasma was approximately 1 to 1. At 96 hr postdosing 98 % of the  $^{210}\text{Pb}$  contained in the erythrocytes was contained in the cytoplasmic constituents of the erythrocytes, presumably bound to hemoglobin. The remaining 2 % was associated with the erythrocyte membranes. Measurement of  $^{210}\text{Pb}$  in various tissues collected at autopsy indicated that the liver contained the highest concentration of lead followed by bile and bone. The lead concentration in bone was highest in the regions of bone growth, for example, the suture lines of the skull and diaphysis of long bones. Blood: brain ratios were between 4:1 and 6:1 and the  $^{210}\text{Pb}$  was distributed equally among the various brain areas. Blood:bone, blood:bile, and blood:liver ratios were 0.3, 0.06, and 0.03, respectively.

18. *In Vitro Enzymatic Defluorination of 2,2-Dichloro-1,1-Difluoroethyl Methyl Ether by Rat Liver Extracts.* F. D. BAKER, W. A. WARREN and J. BARRON, Division of Laboratories and Research, New York State Department of Health, Albany, New York. (L. Golberg)

2,2-Dichloro-1,1-difluoroethyl methyl ether (methoxyflurane; MOF) is a commonly used fluorinated anesthetic which causes abnormal biochemical and histological changes in both man and experimental animals following surgical anesthetization. The purposes of this work are to determine the role of hepatic tissue in the biotransformation of this compound and to study the requirements for enzymatic defluorination. Soluble fraction (cell sap) and microsome suspensions were prepared from the livers of adult male Wistar rats by conventional methods and were added to separate sealed glass bottles. An atmosphere for incubation was created by flushing the bottles with an appropriate gas for 3 min prior to adding MOF. All bottles were incubated at 37°C in a shaker bath, and samples were removed at intervals for determination of fluoride and MOF levels. All enzymatic activity was in the cell sap fraction; the rate was proportional to the protein concentration. The optimum pH was 8.5, and the maximum rate was obtained at MOF concentrations greater than 125 mM. Enzymatic activity was abolished by treatment of the cell sap at 60°C for 20 min. Washed microsomes, while retaining mixed function oxidase activity (aniline hydroxylase and *O*-demethylase), did not defluorinate MOF. The rate of defluorination (nmol F<sup>-</sup>/mg protein/ml/hr) was 2.1 in air, 2.3 in oxygen, 2.4 in nitrogen, 2.5 in argon, and 2.5 in carbon monoxide. The rates in argon and nitrogen were constant for at least 20 hr, whereas the rate in oxygen or air decreased after 4 hr. Large and small molecule fractions from the cell sap were prepared by dialysis and ultrafiltration. The large molecule fraction had 80 % of the activity of the unmodified cell sap, and addition of the small molecule fraction restored full activity. The results show that the reaction is enzymatic, nonmicrosomal, and possibly dependent upon a cofactor for complete activity. There is no apparent correlation of enzyme activity with sex or strain of rats.

19. *Effect of Acetaminophen-Induced Hepatotoxicity on the Metabolism and Biliary Excretion of [<sup>35</sup>S]Bromosulfophthalein.* H. S. BUTTAR, Drug Research Laboratories, Health Protection Branch, Health and Welfare Canada, Ottawa, Canada. (S. B. B. Coldwell)

Hepatic damage is a well-recognized consequence of acetaminophen (A) overdosage in humans and animals. The purpose of the present study was to evaluate the overall functional capacity of liver using [<sup>35</sup>S]bromosulfophthalein (BSP) in biliary-fistulated adult male Wistar rats pretreated orally with different doses of A for varying time intervals. Bile and tissue levels of <sup>35</sup>S-radioactivity were determined by liquid scintillation spectrometry. The maximal hepatic damage seemed to occur between 12–18 hr after single doses of A (0.5 and 1 g/kg), hepatic excretory function returned to control values by 48–72 hr. Administration of BSP (100 mg/kg, iv) to rats treated 18 hr previously with 0.5 or 1 g/kg of A caused a dose-dependent retardation

in biliary flow and in 60-min cumulative excretion of the dye, but conversely, produced a significant increase in the liver and plasma concentrations of  $^{35}\text{S}$ . Following acute (0.25 g/kg), or subacute (0.5 g/kg, twice daily for 7 days) treatment with A, the total excretion of  $^{35}\text{S}$  in bile and the retention of  $^{35}\text{S}$  in liver or plasma remained essentially the same as that for the controls. The hepatic clearance of BSP was more rapid in the rats treated subacutely with A, than in those given equal doses on an acute basis. This suggested that the intensity of A-induced hepatotoxicity became less severe after the repeated administration of this drug. TLC of bile showed two major and one minor metabolite of BSP. Acute administration of 1 g/kg of A caused a marked increase in the biliary excretion of unchanged BSP and a corresponding decrease in BSP-GSH conjugate at 6, 12, and 18 hr, returning to control values after 24 hr. The results suggest that the hepatic uptake, metabolism, and excretion of BSP are reversibly impaired following A-induced liver injury.

20. *Early Liver Changes Produced by Mirex and Their Reversibility.* J. L. BYARD and K. A. PITTMAN, Department of Environmental Toxicology, University of California, Davis, California, and Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York.

The reversibility of early liver changes produced by a brief exposure to Mirex was measured to determine if any irreversible changes might occur which could be related to the later development of liver nodules. Male Charles River CD-1 mice were fed 60 ppm Mirex in the diet for 2 wk and then continued on the same diet containing no Mirex. Mice were sacrificed at intervals and body weight, liver weight, and the concentrations of RNA, DNA, and cytochrome P-450 in the liver were measured. Samples of liver, fat, and plasma were taken for analysis of Mirex. The body weights of treated mice were not different from that of controls throughout the experiment. Gross examination revealed that, up to 4 mo, livers of treated mice were enlarged but otherwise were not different from control mice. The liver weight of treated animals doubled after 2 wk of feeding Mirex, declined gradually thereafter, and was still somewhat elevated at 4 mo. The total amount and concentration of cytochrome P-450 in the liver was elevated in the treated mice. The amount of cytochrome P-450 was elevated within 2 days after feeding Mirex, reached a maximum increase of six times the control amount at 2 wk, and was still three times the control amount at 4 mo. The concentration of RNA and DNA in the liver declined to a minimum as liver weight increased to a maximum. However, at the same time, total RNA and DNA increased to about 150% of the control values. By 2 mo, the increase in total RNA had partially regressed but still remained higher than control values. The increase in DNA did not appear to have regressed. Analysis of Mirex in the plasma indicated a rapid rise to a maximum of 5.5  $\mu\text{g}/\text{ml}$  at 2 wk, then a rapid fall to 1.6  $\mu\text{g}/\text{ml}$  at 3 wk, followed by a gradual decline to 0.4  $\mu\text{g}/\text{ml}$  at 2 mo. Analyses of Mirex in liver and fat are in progress. The data available at this time indicate that the measured parameters of liver growth, except for DNA, are partially reversible and are directly related to the concentration of Mirex in the plasma. Additional studies are in progress to test the relationship between the apparent irreversible increase in DNA and the later development of liver nodules. (Supported by NIH Grant 2P01-ES00226-07 and by NIH Training Grant 2T01-ES00103-07.)

21. *Comparative Ultrastructural and Cytochemical Studies in Livers of Mice, Rats, and Monkeys Fed Various Levels of Mirex.* J. C. FULFS, R. ABRAHAM and F. COULSTON, Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York.

Mirex was fed at levels of 1 and 5 ppm to mice, 5 ppm to rats, while monkeys received 20 ppm in their diets. Mice and rats were killed, and their livers obtained at 2, 6, and 12 mo. Monkey livers were obtained by surgical biopsies at 3, 6, 12, and 18 mo. Cytochemical techniques were employed to detect activities of lysosomal  $\beta$ -glycerol-phosphatase (ACpase) and glucose 6-phosphatase (G-6-pase). ACpase and G-6-pase remained unchanged and comparable to controls in livers of mice receiving 1 ppm. G-6-pase decreased in centrilobular areas while ACpase increased in time in the 5 ppm group. At 12 mo, liver cells that lost their G-6-pase activity were surrounded by Kupffer cells that contained strong ACpase. In contrast, rat livers

demonstrated no increases in ACpase and only a marginal loss in G-6-pase. Surprisingly, and in spite of high levels of Mirex ingested, monkey livers showed no loss of G-6-pase or activation of ACpase. Ultrastructurally, the underlying feature in all livers was proliferation of smooth endoplasmic reticulum (SER), displaying species variation. Thus, in mice, intense proliferation of SER that was both time and dose dependent, was localized in specific regions of the cytosol. Hepatic cells, damaged and necrotic in mice fed 5 ppm, were phagocytosed by activated Kupffer cells. SER proliferation in rat liver cells was less conspicuous than in mice and in monkeys; the nature of this change was diversified, occupying the entire hepatic cell. Except for this change, rat and monkey liver cells were normal. These studies emphasize species and enzyme variations in response to Mirex. An interesting aspect observed was the lack of lysosomal catabolism in liver enlargement. (Supported in part by Research Grant 2P01-ES00226-08 from the National Institute of Environmental Health Sciences, NIH, and by National Institutes of Health Training Grant 2P01-ES00103-08, and by a Grant from Gesellschaft für Strahlen und Umweltforschung, Neuherber, Germany. Dr. Abraham is the recipient of a Research Career Award No. 1 K04 ES70607-02.)

22. *The Role of Microsomal Metabolism of Nitrobenzene in Methemoglobin Formation.* A. M. KAPLAN and K. L. KHANNA, University of Michigan, Ann Arbor, Michigan. (R. Hartung)

The metabolism of nitrobenzene and other aromatic nitro compounds can occur via the soluble and/or microsomal nitroreductase enzyme systems. Since the reduction intermediates, namely the nitroso and hydroxylamino derivatives are presumed responsible for methemoglobin formation, studies were conducted to evaluate the role of the soluble and the microsomal fractions of nitroreductase in the metabolism of nitrobenzene. Male Sprague-Dawley rats were dosed with 3-amino-1,2,4-triazole (2.5 g/kg ip), SKF-525A (80 mg/kg ip), or CCl<sub>4</sub> (1.0 or 2.0 ml/kg ip) prior to receiving nitrobenzene (75 mg/kg in corn oil, ip). In rats pretreated with large doses of aminotriazole, SKF-525A, or CCl<sub>4</sub>, the circulating concentrations of nitrobenzene methemoglobinemia were significantly depressed to approximately the same level, namely 15%. Microsomal nitroreductase activity was markedly decreased by pretreatment with all three compounds. Cytochrome P-450 concentrations in liver microsomes were decreased in rats pretreated with aminotriazole or CCl<sub>4</sub>. The content of cytochrome P-450 closely paralleled liver microsomal nitroreductase activity; however, in rats pretreated with SKF-525A, cytochrome P-450 values were unaffected. These inhibition studies show that the primary effect of aminotriazole and CCl<sub>4</sub> was on the microsomal rather than the soluble nitroreductase activity. Since this inhibition was accompanied by a marked reduction in methemoglobin values, these results are consistent with a major metabolic role for microsomal nitroreductase in the metabolism of nitrobenzene. (Supported by Grant No. 5T01 ES 00138, E.H.S.)

23. *The Effect of Technical and 99% Pure Pentachlorophenol on the Rat Liver. Light Microscopy and Ultrastructure.* R. D. KIMBROUGH and R. E. LINDER, Center for Disease Control, Atlanta, Georgia; Environmental Protection Agency, Research Triangle Park, North Carolina.

Technical pentachlorophenol may be contaminated with a number of impurities (Villanueva *et al.*, *J. Agr. Food Chem.*, **21**, 739, 1973), namely, hexa-, hepta-, and octachlorodibenzo-*p*-dioxin, polychlorodibenzofurans, and polydichlorodiphenyl ethers. The effect of a technical pentachlorophenol containing a relatively high concentration of these contaminants on the rat liver was compared to the effect of a relatively pure pentachlorophenol. Groups of ten male rats were fed 1000 ppm pure or technical pentachlorophenol for 3 mo and compared to two groups of ten controls each. All of the exposed rats had statistically significant ( $p < 0.001$ ) enlargement of the liver at the end of the 90-day feeding period; on light microscopic examination, the livers of rats fed technical pentachlorophenol showed foamy cytoplasm or pronounced vacuolation of the hepatocytes, inclusions, single hepatocellular necrosis, slight interstitial fibrosis, and a prominent brown pigment in macrophages and Kupffer cells. Ultrastructural observations consisted of an increase in smooth endoplasmic reticulum, many lipid vacuoles, and atypical mitochondria. The livers of rats fed the relatively pure pentachlorophenol showed

enlarged hepatocytes. Many cells contained inclusions in their cytoplasm. Electron microscopic studies showed a slight increase in smooth endoplasmic reticulum, atypical mitochondria and some lipid vacuoles. The livers of all control rats were normal. Groups of ten male and female rats were exposed to 500, 100, 20, and 0 ppm of technical pentachlorophenol for 8 mo. The livers of the rats fed 500 ppm were also enlarged at autopsy.

24. *Bioactivation of 1-Nitronaphthalene*. D. JOHNSON and K. KHANNA. (R. Hartung)

Certain carcinogenic metabolites can be formed by oxidation of aromatic amines. The possibility exists that nitroreduction of analogous aromatic nitro compounds may produce the same active intermediates. To study the potential bioactivation of aromatic nitro compounds, 1-nitronaphthalene (1-NN) was chosen as a prototype since two possible intermediates in the metabolism of both 1-NN and 1-naphthylamine, 1-nitrosonephthalene and N-hydroxy-1-naphthylamine, have been shown to be liver carcinogens in rats. It was thought that the occurrence of liver toxicity in acute studies, suggesting binding to liver macromolecules, correlated with urinary metabolite excretion patterns, might serve as an indicator of potential liver carcinogenesis and suggest the tentative identity of an active intermediate. Single ip injections of 1-NN (100 mg/kg) were given to male Sprague-Dawley rats maintained in individual metabolism cages. A majority of the rats developed severe pulmonary edema which was abolished by phenobarbital pretreatment. Histopathological studies showed that extensive liver necrosis occurred in both groups within 48 hr. The urinary excretion pattern indicates that nitroreduction and/or hydroxylation are the primary metabolic pathways. These findings suggest that certain aromatic nitro compounds may have carcinogenic potential if metabolized to active intermediates and binding to liver macromolecules occurs.

25. *Effect of 2-Butanol and 2-Butanone on Rat Hepatic Ultrastructure and Microsomal Drug Metabolizing Enzyme Activity*. G. J. TRAIGER, J. V. BRUCKNER and P. H. COOKE. Department of Pharmacology and Toxicology, School of Pharmacy, University of Kansas, Lawrence, Kansas. (D. Wenzel)

In the rat, CCl<sub>4</sub>-induced hepatotoxicity can be markedly enhanced by a 16-hr pretreatment with either 2-butanol or 2-butanone (Traiger and Robert, *Pharmacologist* 15, 585, 1973). Since the hepatotoxic action of CCl<sub>4</sub> is known to be associated with the microsomal drug oxidase system, the present study was undertaken to determine the effect of 2-butanol (2.2 ml/kg po) and 2-butanone (1.87 ml/kg po) on hepatic ultrastructure and microsomal drug metabolizing activity. Rats were sacrificed 16, 28, and 40 hr after dosing for the in vitro determination of microsomal acetanilide hydroxylase and aminopyrine N-demethylase activity. A 50-97% increase in acetanilide hydroxylase activity was found in animals sacrificed 16, 28, and 40 hr after administration of 2-butanol or 2-butanone. Microsomal aminopyrine N-demethylase activity was significantly elevated only in those animals given a 40 hr pretreatment with 2-butanone. Less pronounced increases in N-demethylase activity were noted in rats sacrificed 16 or 28 hr after dosing with either agent. Electron microscopic examination of hepatocytes 16 hr after either 2-butanol or 2-butanone revealed a marginal increase in the amount of smooth endoplasmic reticulum. However, hepatocytes examined from animals that had been sacrificed 40 hr after receiving 2-butanol or 2-butanone exhibited a marked proliferation of the smooth endoplasmic reticulum. These results would indicate that the potentiation of CCl<sub>4</sub> hepatotoxicity by 2-butanol or 2-butanone may be related in part to their stimulatory effect on the drug metabolizing system of the endoplasmic reticulum. (Supported by a Pharmaceutical Manufacturers Association Foundation Research Starter Grant.)

26. *Hepatotoxicity Produced by the Lung-Toxic Furanoterpenoid, 4-Ipomeanol, after mixed-Function Oxidase Induction: Reversal of Target Organ for Toxicity and Covalent Binding in Rats Pretreated with 3-Methylcholanthrene*. M. R. BOYD, Center in Environmental Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee. (B. J. Wilson)

Intraperitoneal administration of toxic doses (LD<sub>50</sub>, 24 mg/kg) of 4-ipomeanol (1-[β-furyl]-4-hydroxypentanone) to normal rats produces predominantly lung pathology, characterized by pulmonary edema, vascular congestion, and hemorrhage. No gross hepatotoxicity is seen.

When  $^{14}\text{C}$ -labeled toxin is given to normal rats, radioactivity becomes selectively bound to lung. The binding is covalent in nature and requires metabolism by an enzyme system needing oxygen and NADPH and inhibited by carbon monoxide. In rats pretreated with 3-methylcholanthrene (3MC), toxic doses of 4-ipomeanol (LD50, 64 mg/kg) produce gross hepatic necrosis, and minimal pulmonary pathology. In vivo covalent binding of  $^{14}\text{C}$ -labeled toxin is greater in liver than in lung in 3MC-pretreated rats. In vitro this is reflected by an increase in the covalent binding to liver microsomes, but relatively little change in covalent binding to lung microsomes from 3MC-pretreated animals. These results provide further support for our conclusion that the mechanism of toxicity of 4-ipomeanol involves mixed-function oxidase catalyzed covalent binding of the toxin to its target tissue. The data further illustrate that the relative rates of toxication and detoxication pathways available to 4-ipomeanol in both liver and lung may determine which is the target organ.

27. *Inhalation Toxicity Studies of Vinyl Chloride Monomer in Phenobarbital or Aroclor 1254-Pretreated Rats.* R. J. JAEGER, E. S. REYNOLDS, S. SZABO, M. T. MOSLEN and S. D. MURPHY, Department of Physiology, Kresge Center for Environmental Health, Harvard School of Public Health and Departments of Pathology, Peter Bent Brigham Hospital, and Harvard Medical School, Boston, Massachusetts.

Recent evidence suggests a causal relationship between long-term vinyl chloride monomer (VCM) exposure and angiosarcoma of the liver in rats, mice, and man. Short-term exposure has not been associated with acute liver injury, and we have confirmed this observation using normal male rats exposed to 100,000 ppm of VCM for 6 hr and killed 24 hr later. The index of liver injury used was serum alanine- $\alpha$ -ketoglutarate transaminase activity (SAKT). Under similar conditions of exposure, 7-day phenobarbital (PB)-treated rats (400  $\mu\text{mol/kg/day}$  in the drinking water) had elevated SAKT after 40,000, 50,000, and 60,000 ppm of VCM. Exposure to 60,000 ppm resulted in an approximate 50-fold elevation of SAKT in PB-treated, fasted rats while PB-treated, fed rats only increased approximately 7-fold. Although SAKT was elevated after exposure of PB-treated rats to 40,000 ppm, there was no fed-fasted difference. SAKT in PB-treated rats exposed to 20,000 and 5000 PPM was not elevated. Repeated daily exposure of PB-treated, fed rats for 5 days (50,000 ppm) did not result in SAKT elevation when the rats were killed immediately after the last exposure. Rats, treated for 7 days with Aroclor 1254 (1254 at 400  $\mu\text{mol/kg/day}$ ) and then fasted had elevated SAKT 24 hr after exposure to 25,000 ppm. Since both PB and 1254 are associated with increased microsomal enzyme activity, and it appeared that VCM hepatotoxicity might be associated with the production of a toxic metabolite, shorter induction intervals were tested. PB for 3 or 5 days or 1254 for 3 days caused increased SAKT after 50,000 ppm exposure. Zoxazolamine paralysis time (ZPT) was decreased by these treatments. A single treatment with 1254 did not enhance SAKT following 50,000 ppm of VCM even though ZPT in nonexposed animals was decreased by more than 90% at this time. The role of enzyme induction in VCM hepatotoxicity has yet to be elucidated. (Supported by ES-00002, OH-00315, AM-16183, and HL-06370.)

28. *Acute Inhalation Toxicity of Vinyl Chloride Monomer: Morphologic and Biochemical Effects of Pretreatment with Inducers of Hepatic Mixed Function Oxidase System.* M. T. MOSLEN, R. J. JAEGER, S. SZABO and E. S. REYNOLDS, Departments of Pathology, Peter Bent Brigham Hospital and Harvard Medical School, and Departments of Physiology, Kresge Center for Environmental Health, Harvard School of Public Health, Boston, Massachusetts.

Prior studies have shown that the acute hepatotoxicity of vinyl chloride monomer (VCM) is markedly enhanced by phenobarbital (PB) pretreatment and that injury may primarily involve the endoplasmic reticulum of liver parenchymal cells. Since one of the major effects of PB pretreatment is induction of certain components of the mixed function oxidase system (MFOS), we attempted to correlate induction of specific MFOS components with enhancement of hepatic injury by VCM. Male Sprague-Dawley (200 g) rats were pretreated with PB, Aroclor 1254 (1254), hexachlorobenzene (HCB), 3-methylcholanthrene (3-MC), spironolactone (SNL), or pregnenolone-16  $\alpha$ -carbonitrile (PCN), in isomolar doses (400  $\mu\text{mol/kg}$ ), po,

once daily for 7 days. Since pretreatment with 1254 at this dose caused loss of body weight and striking enlargement and pallor of the liver, lower doses were included. Controls were given H<sub>2</sub>O by mouth. On the eighth day, after a 16-hr fast, animals were either sacrificed for determination of microsomal cytochromes *b*<sub>5</sub>, P-448, P-450, NADPH-P-450 reductase and NADH-cytochrome-*c* reductase, oxidative-*N*-demethylase, and glucose-6-phosphatase, or exposed to 5% VCM for 6 hr and sacrificed 24 hr later. Liver injury as indicated by centrilobular vacuolization, focal midzonal necrosis, and increased serum transaminase activity was not apparent in H<sub>2</sub>O-, SNL-, 3-MC-, or PCN-pretreated rats, while slight, moderate, and severe injury were consistently found in HCB-, PB-, and 1254-pretreated animals, respectively. In animals pretreated with 150 μmol 1254/kg, a dose which nearly tripled P-450, VCM produced the most severe injury of the combinations tested. Although liver injury was only found in animals pretreated with an agent which induced P-450, induction of P-450 alone did not correlate with injury. PCN which induced P-450 did not potentiate VCM injury. Other cellular components in addition to P-450 may be important in the potentiation of VCM toxicity. (Supported by NIH Grants ES-00002, OH-00315, AM-16183, and HL-06370.)

29. *Results of a Vinyl Chloride-Teratology Study in Mice, Rats, and Rabbits.* B. A. SCHWETZ, B. K. J. LEONG, F. A. SMITH, M. BALMER and P. J. GEHRING, Toxicology Research Laboratory, Health and Environmental Research, The Dow Chemical Company, Midland, Michigan.

The potential of inhaled vinyl chloride monomer (VCM) to have a deleterious effect on the developing embryo and fetus of laboratory animals has been evaluated. In an initial experiment, CF-1 mice, Sprague-Dawley rats, and New Zealand white rabbits were exposed to 500 ppm VCM 7 hr daily during organogenesis. Some of the VCM-exposed mice were also treated with 15% ethanol (EtOH) in the drinking water as their only source of liquid to determine if concomitant ingestion of ethanol would alter the toxicity and/or teratogenicity of VCM. No signs of toxicity were observed in the adult rats or rabbits during exposure or at the time of necropsy and caesarean section. Among the rats, there was a significant decrease in fetal body weight but the incidence of external, soft tissue and skeletal malformations was not different than among control litters. There was no effect on fetal body measurements or the incidence of external, soft tissue or skeletal malformations in rabbits. Mice were more susceptible to VCM than either of the other two species. There was a slight decrease in the weight gain among mice and maternal deaths were observed (6 deaths/30 exposed mice). The percentage of pregnancy among mice was slightly lower than among controls. There was a slight increase in the incidence of resorptions and fetal body weights were slightly lower than control values but the incidence of external, soft tissue and skeletal malformations was not different than among controls. Concomitant treatment of mice with EtOH in the drinking water accentuated some of the toxic effects associated with exposure to VCM alone. The maternal weight gain and the pregnancy rate was further decreased, but the incidence of maternal deaths was not increased by concomitant EtOH ingestion. The incidence of cleft palate and certain skeletal anomalies was significantly increased among mice receiving EtOH plus VCM. Thus, based on the results of this initial study, exposure of bred mice, rats, and rabbits to 500 ppm VCM during organogenesis did not result in a teratogenic response. Mice were slightly more susceptible to the toxic effect of VCM than either rats or rabbits. Simultaneous exposure of mice to VCM by inhalation and EtOH in the drinking water resulted in toxic effects greater than that observed among mice exposed to either one alone. These results are being used to design additional studies to assess the toxicity of VCM.

30. *A Laboratory Aerosol System for Conducting Inhalation Toxicity Studies.* B. S. BRAR, C. E. TRAITOR, C. R. BOSHART and J. F. NOBLE, Toxicology Research Section, Lederle Laboratories Division of American Cyanamid Company, Pearl River, New York.

An aerosol system has been developed for the safety evaluation of drugs to be used in the respiratory tract. Design features of the equipment include: (1) a spinning disc generator

capable of producing a monodisperse aerosol with constant concentration of known particles from 0.3  $\mu\text{m}$  to 10  $\mu\text{m}$  in diameter; (2) a cylinder 120 liters in volume to receive the product aerosol and provide breathing connections for two dogs, four monkeys, and/or ten rats; (3) a sampler for aerosol characterization; (4) an inflatable face mask with mouthpiece through which the dogs or monkeys breathe; (5) a respiratory resistance unit to facilitate measurement of respiratory parameters; and (6) a sodium iodide scintillation detector, pulse height-analyzer for bronchial clearance studies. The equipment produces high quality monodisperse aerosols for particle deposition and clearance studies in the respiratory tract. Further, for safety evaluation of drugs, it provides a dynamic flow of fresh aerosol at ambient temperature and pressure. Its usefulness is enhanced by the fact that the animals breathe through the mouth or, as in rats, through the nasal passages. This method has advantages over the common approach of whole body or head exposure which makes it impossible to separate the toxicological effects of inhalation from those due to ingestion and cutaneous absorption.

31. *Bronchial Clearance of Radioactive Iron Oxide Particles in Normal Cynomolgus Monkeys.* B. S. BRAR, J. MORRISON, C. E. TRAITOR and C. R. BOSHAUT, Toxicology Research Section, Lederle Laboratories Division of American Cyanamid Company, Pearl River, New York.

The potential usefulness of the kinetics of bronchial clearance as a means of assessing the deleterious effects of inhalants on mucociliary action in the monkey has been demonstrated. The clearance of radioactive monodisperse ferric oxide aerosol particles was studied in normal adult cynomolgus monkeys. Test aerosol consisted of 2.0 or 3.2  $\mu\text{m}$  iron oxide particles tagged with technetium (Tc 99m) produced by a spinning disc generator. Each monkey, while restrained in a chair, inhaled aerosol for 5 min through a mouthpiece. Immediately following completion of particle inhalation, the first chest radioactivity measurements were made using a 3  $\times$  3 in. sodium iodide crystal scintillation detector. Radioactivity was monitored for 6–24 hr after inhalation of the radioactive aerosol. About one-half of the total bronchial clearance occurred during the first hour followed by a more gradual clearance phase. The same general clearance characteristics were found in all monkeys, although some monkeys cleared more rapidly than others. The methodology described has the potential for evaluating the effects of aerosols on mucociliary activity.

32. *In Vitro Studies of Rat Pulmonary Alveolar Macrophages Subsequent to Intrapulmonary Instillation of Lead (PbO).* P. AGOSTA, C. A. FISCHER, E. J. KAMINSKI and J. C. CALANDRA, Industrial Bio-Test Laboratories, Inc., Northbrook, Illinois.

The effects of lead particulate on rat pulmonary alveolar macrophages (PAM) were studied. Two groups of four Long-Evans Strain Rats received one intrapulmonary dose of PbO—1.0 mg/rat and 0.25 mg/rat. Two groups of eight rats were used as controls (untreated and vehicle control). Rats were sacrificed at 7, 10, 15, and 40 days. Counts of PAM values and viability were done by pulmonary lavage. Total and tissue lead concentrations were determined. Cells from the 40-day sacrifice were maintained in supplemented MEM or McCoys modified 5a with HEPES buffer. Both media were supplemented with rat serum, antibiotics, and a fixed number of formalin-fixed lymphocytes and combinations thereof. Formalin-fixed lymphocytes were used to stimulate PAM immune responsiveness. Counts were made to determine the PAM viability over time. Although the overall viability of the PbO exposed PAM values appeared to be slightly less than controls, the PbO exposed PAM values were harvested in greater numbers per rat than controls.

33. *Studies on the Mechanism of Tolerance Development to the Pulmonary Toxicity of 4-*Ipomeanol*.* M. R. BOYD, L. T. BURKA, B. A. OSBORNE and B. J. WILSON, Center in Environmental Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee.

4-*Ipomeanol* (1-[ $\beta$ -furyl]-4-hydroxypentanone) produces a selective acute pulmonary toxicity in several animal species, including the rat. In previous studies, we have shown that the

mechanism of production of the lung lesions probably involves mixed-function oxidase (MFO) catalyzed covalent binding of the toxin to pulmonary macromolecules. Pretreatment of rats with sublethal doses of 4-ipomeanol (10 mg/kg) produces a state of remarkable tolerance to subsequent challenge with normally lethal doses (>30 mg/kg) of the compound. We have found that significantly less radioactivity accumulates and becomes covalently bound in lungs of tolerant rats compared to nonpretreated controls, when the animals are challenged with [<sup>14</sup>C]4-ipomeanol. The amount of radioactivity accumulating and binding in other tissues is relatively unchanged. In vitro we have observed a significant decrease in the NADPH-dependent covalent binding of [<sup>14</sup>C]4-ipomeanol to pulmonary microsomes from tolerant rats. We have concluded that tolerance results from a decrease in pulmonary MFO activity produced by the pretreatment doses of 4-ipomeanol. Presumably, therefore, on subsequent challenge with larger doses of the toxin, insufficient concentrations of the alkylating metabolite are formed to produce the usual pathology.

34. *Embryo- and Fetotoxicity of Inhaled Trichloroethylene, Perchloroethylene, Methylchloroform, and Methylene Chloride in Mice and Rats.* B. K. J. LEONG, B. A. SCHWETZ and P. J. GEHRING, Toxicology Research Laboratory, Dow Chemical USA, Midland, Michigan.

The effect of inhaled trichloroethylene, perchloroethylene, methylchloroform, and methylene chloride on embryonal and fetal development has been evaluated at a concentration two times the maximum allowable excursion limit for human industrial exposure. Pregnant mice and rats inhaling 1250 ppm of methylene chloride during the gestation period had carboxyhemoglobin contents as high as 12.6% (controls average about 0.5%). However, no significant maternal, embryonal or fetal toxicity, or teratogenic effects were observed. Inhalation of other chlorinated solvents (methylchloroform at 875 ppm, trichloroethylene at 300 ppm, or perchloroethylene at 300 ppm) caused neither elevation of carboxyhemoglobin concentrations nor any significant adverse effects on embryonal and fetal development.

35. *The Accumulation of Paraquat by the Lung and the Relevance of this to the Treatment of Paraquat Poisoning.* M. S. ROSE, L. L. SMITH and I. WYATT, Biochemical Mechanisms Unit, Imperial Chemical Industries Limited, Central Toxicology Laboratory, Alderley Park, Nr Macclesfield, Cheshire, United Kingdom. (A. A. B. Swan)

Paraquat and diquat are bipyridilium herbicides which have similar chemical structures and chemical properties. They have the same intravenous LD<sub>50</sub> values but, whereas paraquat-poisoned rats die with extensive lung damage, diquat-poisoned rats do not develop lung damage. After oral administration, paraquat is accumulated by the lung whereas diquat is not. An energy-dependent accumulation process in slices of rat lung has been demonstrated which is selective for paraquat. It has been shown that other tissues from rats do not accumulate paraquat in vitro to the same extent as the lung. Lung tissues from other species, including man, have also been shown to accumulate paraquat in vitro. The accumulation of paraquat by slices of rat lung is inhibited by a variety of compounds including histamine, 5-hydroxytryptamine and noradrenaline. It is concluded that paraquat might be accumulated into lung tissue by a system which normally removes these compounds from the circulation. Less toxic derivatives of these amines have been investigated as possible antidotes to paraquat poisoning.

36. *Phospholipase C from Aspergillus flavus.* D. S. SAWHNEY, Food and Drug Administration, Washington, D.C. (J. M. Taylor)

During the investigation of growth and metabolism of *Aspergillus flavus* NRRL-2999 on egg yolk, it was observed that after 3–4 days of incubation at 25°C the inoculated samples of egg yolk showed a severalfold increase in viscosity. The cause of this increase was investigated and found to be related to the production of phospholipase C. The mycometabolite, which might be of public health significance, was isolated and purified 972-fold by ammonium sulfate fractionation, and successive Sephadex G-200 gel filtration which gave 90–95% purification. The



structural and ultrastructural alterations produced in the egg yolk by the enzyme were investigated by scanning and transmission microscopy. The enzyme was extracellular, inducible, stable, and hemolytic in nature. Molecular weight, isoelectric point, and substrate specificity were also studied. The investigation showed that *A. flavus* produced phospholipase C in addition to aflatoxins. The documented carcinogenicity of aflatoxins could be affected by the enzyme which produced membrane changes. The findings emphasize the significance of the enzymatic mycometabolites in addition to aflatoxins in animal and human health.

37. *A Proposed Mechanism of the Action of Carbon Disulfide on Dopamine- $\beta$ -Hydroxylase.* M. J. MCKENNA and V. DiSTEFANO, Department of Pharmacology and Toxicology, University of Rochester School of Medicine and Dentistry, Rochester, New York. (D. R. Taves)

Alterations in adrenal, brain, and cardiac catecholamine concentrations in rats after exposure to carbon disulfide (CS<sub>2</sub>) vapor have been attributed to the inhibition of dopamine- $\beta$ -hydroxylase (DBH). The ability of CS<sub>2</sub> to react with endogenous amines has led to the hypothesis that inhibition of this metalloenzyme may occur via the formation of dithiocarbamate intermediates which bind to enzymic copper. This hypothesis was tested in vitro with purified bovine adrenal DBH and with the enzyme as found in isolated bovine catecholamine storage granules. CS<sub>2</sub> had no effect on purified DBH. However, pretreatment of the enzyme with CS<sub>2</sub> in the presence of an added amine or amino acid resulted in a significant decrease in DBH activity. Synthesized dithiocarbamate derivatives of several amino acids were effective inhibitors of DBH activity in both systems. In the granule preparation, however, CS<sub>2</sub> treatment inhibited DBH more effectively than equivalent concentrations of the dithiocarbamates. This was attributed to the possibility that CS<sub>2</sub> diffuses rapidly across the granule membrane and reacts with intragranular catecholamines to form dithiocarbamates. Analysis of the intragranular content of CS<sub>2</sub>-treated preparations by gas chromatography provided evidence in support of this possibility. Therefore, inhibition of DBH activity by CS<sub>2</sub> may be mediated by the action of dithiocarbamates formed from endogenous amino acids or from intragranular catecholamines. (Supported by NIH Grants GM-01781 and GM-15190.)

38. *Antihemolytic and Anticonvulsant Activity of Some Substituted Thiosemicarbazides and Their Inhibition of NAD-Dependent Oxidations and Monoamine Oxidase.* C. DWIVEDI, S. S. PARMAR and R. D. HARBISON, Department of Pharmacology and Center in Toxicology, Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee.

Some 1-(2,4-dichloro and 2,4,5-trichlorophenoxy acetyl)-4-alkyl/aryl thiosemicarbazides were synthesized and evaluated for their ability to protect against hypoosmotic hemolysis and to inhibit the oxidations of pyruvate,  $\alpha$ -ketoglutarate, NADH, and succinate and monoamine oxidase (MAO). Assay of hypoosmotic hemolysis was carried out using dog blood without oxygenation. Respiratory activity of rat brain homogenate was determined by measuring oxygen consumption in the Warburg manometric apparatus. MAO activity was determined spectrophotofluorometrically using kynuramine as substrate. All these thiosemicarbazides protected against in vitro hypoosmotic hemolysis and inhibited NAD-dependent oxidations of pyruvate and  $\alpha$ -ketoglutarate while NAD-independent oxidation of succinate was not affected. These compounds also inhibited MAO activity and the degree of inhibition ranged from 26.5 to 89.2% at a final concentration of 0.33 mM. Almost all of these thiosemicarbazides possessed anticonvulsant activity when tested against pentylenetetrazol (PTZ)-induced seizures. Protection against PTZ-induced seizures ranged from 10 to 70% at a dosage of 100 mg/kg. A relationship appears to exist between membrane-stabilizing property of these thiosemicarbazides and their ability to exhibit selective inhibition of NAD-dependent oxidations and inhibition of MAO. On the other hand, anticonvulsant activity possessed by these thiosemicarbazides was found to be unrelated to their in vitro antihemolytic and enzyme inhibitory properties. (Supported by USPHS Grants ES 00267, ES 00782, and DA 00141.)

39. *Stimulation and Suppression of Hepatic and Extrahepatic Microsomal Mixed-Function Oxidases (MFOs) by 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD)*. G. E. R. HOOK, B. A. FOWLER, T. C. ORTON and G. W. LUCIER, Pharmacology Branch and Environmental Toxicology Branch, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North Carolina. (J. Fouts)

TCDD is one of the most toxic compounds known to man. The ability of TCDD to alter hepatic microsomal MFOs, although perhaps unrelated directly to the toxic action of the compound, is without precedent as far as dose-response relationships are concerned and indicates that the ingestion of minute amounts of TCDD could radically alter the metabolism of other simultaneously ingested foreign compounds such as drugs or chemicals of environmental origin. The response of microsomal MFOs following oral ingestion of TCDD was investigated in both hepatic and extrahepatic tissues of the rat, rabbit, and guinea pig. Cytochrome P-450 concentrations and benzpyrene hydroxylase activities were increased in rat liver microsomes by oral doses of less than 1  $\mu\text{g}$  TCDD/kg. However, *N*-demethylase and testosterone 2 $\beta$ - and 16 $\alpha$ -hydroxylase activities of hepatic microsomes from male rats were suppressed. The stimulating effect of TCDD on rat hepatic microsomes was considerably more persistent than the suppressive effect. Following a single oral dose of only 25  $\mu\text{g}$  TCDD/kg, benzpyrene hydroxylase of male rat liver microsomes remained significantly elevated after 73 days but the suppression of benzphetamine *N*-demethylase had gone by 35 days. In microsomes from extrahepatic tissues of the rat, MFO stimulation by TCDD occurred only in the kidney. However, UDP-glucuronyl transferase was increased in microsomes from the lung, kidney, intestine, and brain but not testes. The response of the rabbit and guinea pig to TCDD differed considerably from that of the rat. Benzpyrene hydroxylase was unaffected in hepatic microsomes from guinea pig and suppressed in microsomes from rabbit liver. Benzphetamine *N*-demethylase was also suppressed in rabbit liver microsomes. The only pulmonary microsomal MFO responsive to TCDD was biphenyl 4-hydroxylase of the rabbit and guinea pig. Suppression of MFO activity was not observed in any of the extrahepatic tissues studied and may be confined to only certain hepatic systems. The results indicate that the most dramatic TCDD effects on microsomal MFOs are limited to the liver and kidney.

40. *Species Difference in the Induction and Inhibition of Drug Metabolizing Enzymes by Rifampin*. D. PESSAYRE and P. MAZEL, Department of Pharmacology, George Washington University Medical Center, Washington, D.C.

Rifampin is used in conjunction with other drugs in the long-term therapy of tuberculosis. The drug has been shown to affect liver function and in combination with other anti-tubercular agents the incidence of hepatitis is much greater than with rifampin alone. The effects of rifampin on hepatic microsomal enzymes has therefore been investigated. Thirty minutes after the administration of rifampin (100 mg/kg) ip to male Swiss-Webster mice hexobarbital sleeping time was increased 110% and zoxazolamine paralysis by 130% above controls. In vitro hydroxylation of zoxazolamine ( $2.5 \times 10^{-5}$  M) and the demethylation of ethylmorphine ( $5 \times 10^{-4}$  M) were inhibited by 50% by concentrations of rifampin of  $6 \times 10^{-4}$  M and  $5 \times 10^{-5}$  M, respectively. Administration of rifampin (50 mg/kg) daily to mice for 6 days resulted in an increase in liver weight (20%), ethyl morphine demethylation (84%), zoxazolamine hydroxylation (78%), benzpyrene hydroxylation (157%), cytochrome P-450 content (50%), and NADPH cytochrome *c* reductase activity (43%). Aniline hydroxylase, *p*-nitrophenol glucuronyl transferase, and microsomal protein were unaffected. Only partial induction was present after 3 days of rifampin treatment. In rats (Sprague-Dawley 150-200 g) pretreatment with rifampin (100 mg/kg) ip daily for 6 days failed to increase hepatic microsomal enzyme activity. Ethyl morphine demethylase ( $K_m$ - $6.0 \times 10^{-4}$  M) was competitively inhibited by rifampin ( $K_i$ - $3.0 \times 10^{-5}$  M). These results suggest: (1) that in mice rifampin has a biphasic effect, inhibiting microsomal enzymes on acute administration and inducing on chronic treatment; (2) that the induction is species specific; (3) that in vitro inhibition is similar and thus rifampin probably binds to microsomes in an identical manner, and (4) that one should be alerted to the possible effects of rifampin on the metabolism and toxicity of other drugs in patients undergoing initial

and chronic rifampin therapy. (Supported in part by Merck Sharp and Dohme-France and Pfizer International-France.)

41. *The Effects of Fenthion and Some of its Metabolites on Enzymic and Functional Activities in the Cat.* G. E. GROBLEWSKI, S. L. VITOROVIĆ, J. NGATIA, J. H. WILLS and F. COULSTON, Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York.

The intrinsic potencies of the organophosphorus insecticide, fenthion, and three of its principal metabolites were compared using relatively modest doses of the substances in the cat. These compounds had been studied by intraperitoneal injection into the rat of large fractions of the LD<sub>50</sub> doses by DuBois and Kinoshita (*Toxicol. Appl. Pharmacol.* 6, 86-95, 1964). In vivo, fenthion sulfone was about twice as inhibitory of ChE of the cat's liver as oxofenthion and nearly four times as active as fenthion and fenthion sulfoxide. Fenthion and its sulfone and sulfoxide were more actively inhibitory of ChE of the liver than of its triacetinesterase (TACE). Oxofenthion seemed to be the most potent inhibitor of the ChE's of the blood, skeletal muscle, auricular cardiac muscle, and brain stem, whereas fenthion seemed to be the most potent inhibitor of ChE in ventricular cardiac muscle. None of the four compounds in iv doses up to 10 mg/kg, or up to 12 mg/kg for fenthion, had a significant effect on neuromuscular transmission, ventilatory activity, the length of the PR interval of the ECG, or the duration of the QRS complex. Fenthion may have reduced slightly the heart rate and the blood pressure; the other three compounds produced moderate elevations of the blood pressure. Oxofenthion and fenthion sulfone increased the contractile force of the duodenum. All four compounds elevated the threshold voltage for electrical excitation of the inspiratory area of the medulla. Fenthion, the only compound to lower rather than to elevate blood pressure, was the most potent inhibitor of ChE in ventricular muscle. Otherwise, the correlation of functional alterations with ability to inhibit hydrolytic enzymes was not evident in this series of experiments.

42. *In Vitro Inactivation of Dichlorvos (DDVP) and Inhibition of Mouse Tissue Esterases.* M. EHRLICH and S. D. COHEN, School of Pharmacy, University of Connecticut, Storrs, Connecticut.

Organophosphate (OP) inhibition of esterases other than acetylcholinesterase (ACHE) may serve as a detoxification mechanism in that any OP bound to such esterases would not be available to inhibit the more critical enzyme, ACHE. Malaoxon (MX), paraoxon (PX), and DDVP bind to and inhibit esterases. Prior inhibition of esterase binding by triorthocresyl phosphate (TCP) potentiates the anticholinesterase action of both MX and PX (*Toxicol. Appl. Pharmacol.* 27, 537, 1974). To determine the importance of DDVP's esterase binding, we compared it and DDVP's anti-ACHE action on control and TCP-treated mice. Eighteen hour pre-treatment with 125 mg/kg TCP reduced mouse liver binding detoxification of DDVP to 5% of control activity, but did not potentiate the in vivo anti-ACHE action of subsequently administered DDVP. This suggests that even though DDVP binds to and inhibits esterases, the binding and inhibition do not represent important detoxification mechanisms for this compound. To compare the interaction of DDVP with esterases with those of MX, we studied the inhibition of esterases by each OP, in vitro. The concentrations ( $\times 10^{-7}$  M) of DDVP required for 50% inhibition were  $0.084 \pm 0.02$ ,  $0.43 \pm 0.02$ ,  $6.9 \pm 0.9$ , and  $8.4 \pm 0.4$  for liver diethylsuccinate (DES), triacetin (TA), and procaine (Proc) esterases and cholinesterase (CHE), respectively. In similar studies with MX, the 50% inhibition concentrations ( $\times 10^{-7}$  M) were  $26 \pm 6$ ,  $0.28 \pm 0.05$ ,  $40 \pm 3$ , and  $1900 \pm 300$  for liver DES, TA, Proc and CHE, respectively. Fifty percent inhibition concentrations of DDVP and MX for brain ACHE activity were  $3.3 \pm 0.3$  and  $1.6 \pm 0.5$  ( $\times 10^{-7}$  M), respectively. Thus, for brain ACHE and liver TA esterase the sensitivity to inhibition by DDVP was similar to that for MX. There was, however, a marked difference in the sensitivities to these OP's for both liver CHE and DES esterase. Both of these enzymes were much more sensitive to inhibition by DDVP, the OP which was not potentiated by TCP treatment. Thus, OP affinity for specific esterases may determine whether or not OP toxicity will be potentiated by prior esterase inhibition. (Supported by University of Connecticut, Research Foundation Grant.)

43. *Aldrin Epoxidase and Dihydroisodrin Hydroxylase in Hepatic Needle Biopsy Specimens: Assay and Properties.* R. I. KRIEGER, S. J. GEE, J. L. MILLER and T. THONGSINTHUSAK, Department of Environmental Toxicology, University of California, Davis, California.

Aldrin and dihydroisodrin are chlorinated cyclodienes that are metabolized by hepatic microsomal mixed-function oxidases. The substrates and their respective metabolites, dieldrin and monohydroxy dihydroisodrin, are relatively stable, can be easily extracted from aqueous media using hexane, and are analyzed in picogram quantities using electron capture gas chromatography. Using 0.2 ml incubation media containing a glucose-6-phosphate NADPH-generating system, parameters such as substrate concentration, time, cofactors, and enzyme concentration have been evaluated. *Macaca mulata*, *M. radiata*, and white rat hepatic needle biopsy specimens from mature males have been used. When 4-min incubation periods are used with protein levels (*M. radiata*) between 1 and 8 mg the apparent  $K_m$  and  $V$  for the epoxidase is  $1.4 \times 10^{-5}$  M and 214 pmol/mg/min and for the hydroxylase  $2.3 \times 10^{-5}$  M and 151 pmol/mg/min, respectively. The levels of each are elevated by microsomal enzyme inducers such as phenobarbital and DDT. Aldrin epoxidation and dihydroisodrin hydroxylation can be studied in needle biopsy specimens, and they can provide a readily obtained measure of hepatic oxidative activity. (Supported in part by NIH ES 00054.)

44. *Inhibition of Neural Membrane Adenosinetriphosphatases by Organophosphates.* H. R. BROWN and R. P. SHARMA, Toxicology Program, Utah State University, Logan, Utah.

The delayed neurotoxicity of organophosphorus compounds has not been satisfactorily explained on the basis of inhibition of cholinesterases. The critical role of  $\text{Na}^+$ ,  $\text{K}^+$ -stimulated  $\text{Mg}^{2+}$ -dependent ATPase in maintaining energy metabolism and its location within neuronal membranes coupled with reports indicating inhibition of certain ATPases by DFP suggest the possible involvement of ATPases in the observed neurotoxic paralysis. Tri-*o*-tolyl phosphate (TOTP) and mevinphos were examined for time dependent inhibition of ATPases. These compounds inhibited not only the  $\text{Na}^+$ ,  $\text{K}^+$ -stimulated enzyme but also the ouabain-insensitive  $\text{Mg}^{2+}$ -dependent activity of ATPases in a synaptosomal fraction from chicken spinal cord. Inhibition curves obtained from these experiments indicate the inhibition process to occur in an exponential fashion following an initial drop. The bimolecular inhibition constants, affinity constants, and binding constants were determined from the log-linear portion of the curves. A comparison of these constants with those found for inhibition of acetylcholinesterase revealed the inhibitory potential of mevinphos to be several orders of magnitude greater for cholinesterase. On the other hand TOTP, which is not a direct inhibitor of cholinesterases and is a well-known neurotoxic organophosphate, inhibited ATPases at a much lower concentration as compared to mevinphos. The inhibition of  $\text{Mg}^{2+}$ -dependent ATPase by TOTP was potentiated by addition of  $\text{Ca}^{2+}$ . Interference in the ability of affected neurons to respond to their energy requirements as a result of inhibition of various ATPases appears to provide a possible explanation for TOTP neurotoxicity.

45. *Hearing Loss Associated with Chronic Lead Poisoning in Squirrel Monkeys.* C. WILPIZESKI, Jefferson Medical College, Philadelphia, Pennsylvania.

This study was done to confirm the possible relationship between plumbism and hearing impairment suggested in the clinical literature. Adult squirrel monkeys were fitted with permanent recording electrodes having tips located in the cochlear nucleus region and on the dural surface of the parietal and frontal areas of the brain. Two of the subjects were trained to track their detection thresholds behaviorally by means of an automated conditioned avoidance procedure. Each monkey was given approximately 500 mg/kg of lead oxide (in aqueous suspension) po per day, 5 days per week until near death (2-22 wk). Hearing was monitored electrophysiologically at monthly intervals using computer-averaging techniques or at least weekly by the behavioral response method. There was great intersubject variability in survival time, weight loss, hematocrit, blood lead concentration, and auditory response. Data recorded during the terminal measurement session revealed only slight hearing losses (5-14 dB) as measured at the peripheral neural level and moderate impairment (10-24 dB) at the brain level. When viewed in

the context of the behavioral audiometric data of this and other studies, the changes in cortical evoked response threshold would appear to reflect CNS dysfunction not related to hearing processes. Lead, therefore, is a relatively benign ototoxic agent for the adult monkey.

46. *Retinal Degeneration in Rats with Elevated Body Temperature Exposed to Fluorescent Illumination.* W. G. BUSH and R. L. SWARM, Department of Biology, Lincoln University, Pennsylvania, and Department of Experimental Pathology and Toxicology, Hoffmann-La Roche Inc., Nutley, New Jersey.

Irreversible loss of photoreceptor cells occurred in pyrexia rats which had been exposed to an average cool white fluorescent illumination of moderate intensity (84 fc). Histologically observable retinal degeneration was apparent in animals which were sacrificed 21 days after a 6-hr exposure at a body temperature of 101°F, a 2-hr exposure at 102.5°F, or a 30-min exposure at 104°F. Animals sacrificed 21 days after exposure showed a greater degree of retinal degeneration than animals sacrificed 72 hr after exposure. This indicates that deterioration of photoreceptor cells continues for longer than 72 hr following exposure.

47. *Effects of Ethyl and Methyl Cyanoacrylate Adhesives on Albino Rabbit Eyes.* V. M. SEABAUGH, G. W. BIERBOWER, C. A. HOHEISEL and J. McLAUGHLIN, JR., Division of Biological Science, Bureau of Biomedical Science Consumer Product Safety Commission, Washington, D.C.

Cyanoacrylate adhesives have been marketed since 1959 in the United States for industrial and medical use. Since 1973, the cyanoacrylates were sold for household use, and are subject to the Federal Hazardous Substances Act Regulations (FHSA). The purpose of this investigation was to see if the eye precautionary labeling was adequate for cyanoacrylate adhesives. Nine trade name cyanoacrylate adhesives were tested. For each of the test products, the eyes of six New Zealand albino rabbits, weighing 2.0–2.5 kg, were randomly selected and tested according to the FHSA. One-tenth milliliter of the sample was instilled into the right eye of each rabbit. The other eye served as a control. An additional six rabbits per test product were similarly tested with the following exception: After 30 sec exposure, the right eye of each rabbit was rinsed with 300 ml of tap water delivered in 2 min. The polymerized glue film was removed from all test eyes immediately after the 30-sec or 24-hr exposure. Each test and control eye was examined grossly prior to testing, and at days 1, 2, 3, and 7 after instillation. At sacrifice, the control and test eyes were enucleated from each rabbit, fixed in Formol solution, processed for histological examination, and stained with hematoxylin and eosin. Histopathology was performed on all eyes. Upon gross observation, all of the nine products were severe eye irritants: i.e., iritis, conjunctivitis, and opacity (1–7 days). Histologically, an adverse ocular tissue reaction was elicited by all products tested. A notice published in the *Federal Register* May 9, 1974 (39 CFR 16511) states that the Commission concludes at this time that the adhesive properties of these glues do not subject the consumer to an unreasonable risk; however, these adhesives are eye irritants and as such require proper labeling.

48. *Ocular Toxicity in Beagle Dogs with an Experimental Sympathomimetic Agent.* G. R. STEFFEN, J. D. HENDERSON, JR., R. A. NELSON, M. T. CASE and D. R. SAUNDERS, Riker Laboratories, Inc., 3M Company, 3M Center, St. Paul, Minnesota.

Ocular lesions were detected in Beagle dogs receiving high doses of R-804, an experimental fluoromethane sulfonanilide sympathomimetic agent, during a preclinical safety evaluation study. The dose of R-804 was increased during the 14-day study from 20 mg/kg bid po for days 1–3 to 30 mg/kg bid for days 4–6 and finally to 40 mg/kg bid po for days 7–14. Two males and two females received R-804 while one male and one female were not dosed. Complete physical examinations including indirect ophthalmoscopy and intraocular pressure determinations were conducted predose and on days 6 and 13. Intraocular pressure in the untreated controls fluctuated during the study between 17 and 22 mmHg. Intraocular pressure decreased in 3/4 of R-804 dogs from an initial mean value of 16 to a final mean value of 7 mmHg. Severe ocular lesions consisting of conjunctival hyperemia, corneal opacities, epiphora, mydriasis followed by myosis, corneal herniation, hyphema, ptosis, and anterior synchia also developed in three of the

R-804 animals. Other clinical signs observed in the treated animals included tachycardia, anorexia, skin erythema, piloerection, hypoactivity, ataxia, salivation, polydipsia, and anhelation. One R-804 animal was euthanatized on day 8 following several days of total anorexia, severe depression, icterus, and oral hemorrhages.

49. *Acute Toxicity and Effect of Dithiobiuret and Acrylamide on Rotorod Performance in Rats and Mice.* R. E. PETERSON and N. K. SHETH, The Medical College of Wisconsin and Wood Veterans Administration Center, Wood, Wisconsin. (J. M. Fujimoto)

Acrylamide (Ac) has been reported to produce peripheral nerve damage and failure of rats to remain on a rotating rod (Kaplan and Murphy, *Toxicol. Appl. Pharmacol.* **22**, 259, 1972). The purpose of this study was to compare Ac neurotoxicity with that induced by dithiobiuret (Dtb). More specifically, rotorod performance, peripheral nerve damage, lethality, and secretion around the eyes of red porphyrin pigment from the Harderian glands were assessed in rats and mice treated with Dtb and Ac alone and in combination. In rotorod experiments with rats Dtb (1 mg/kg/day) or Ac (50 mg/kg/day) was given ip until there was failure of every animal in the group to stay on the rod. The onset of failure was similar for Dtb (5-7 days) and Ac (6-8 days) but recovery was more prolonged with Ac. For both compounds, axons of posterior tibial nerves appeared histologically normal at the onset of motor failure but tended to be fragmented and swollen later during the recovery period. When given in combination, Dtb prolonged the duration of Ac induced motor failure in rats and potentiated the lethality of Ac in mice. Continuous treatment with phenobarbital (75 mg/kg/day) in rats delayed the onset of both Dtb and Ac induced motor failure; however, phenobarbital pretreatment in mice (75 mg/kg/day for 4 days) did not alter the 48-hr ip LD50 of Dtb (218 mg/kg) or Ac (170 mg/kg) in mice. On gross observation Dtb increased the secretion of red porphyrin pigment from the Harderian glands in both rats and mice whereas Ac had no effect. In conclusion, Dtb and Ac produce motor failure and peripheral nerve damage in rats but only Dtb increased Harderian gland secretion. (Supported by a PMA Foundation Research Starter Grant.)

50. *The Effects of Low-Level Dieldrin Exposure on the EEG and Learning Ability of the Squirrel Monkey.* G. A. VAN GELDER and W. L. CUNNINGHAM, Behavioral Toxicology Laboratory, College of Veterinary Medicine, Iowa State University, Ames, Iowa.

Dieldrin, a persistent chlorinated hydrocarbon insecticide, acts as a neurotoxicant. Two studies examined the effects of subclinical exposure on acquisition of a nonspatial successive discrimination reversal task and on onset of dieldrin-related EEG alterations in adult male squirrel monkeys. The first study used a reversal criterion of 15 consecutive correct responses. A 0.1 mg/kg group ( $N = 3$ ) achieved significantly fewer reversals than either 0.01 mg/kg ( $N = 4$ ) or control ( $N = 3$ ) groups over a 55-day exposure period. Subsequent exposure termination and increase to 0.1 mg/kg, for 54 days, did not affect performance in the high and low groups, respectively. The second study recorded four nonexposed EEG's from each of another nine monkeys before exposure began for 60 days at three levels: 0.1 mg/kg ( $N = 3$ ), 0.01 mg/kg ( $N = 3$ ), and no exposure ( $N = 3$ ). EEG recordings were then taken 4 and 24 hr postdosing on days 10, 30, 50, and 20, 40, 60, respectively. High amplitude (200-300  $\mu$ V), slow waves (4-6 Hz) occurred more often in the 4-hr recording condition. Most changes occurred on day 50 in both exposure groups. No dieldrin-related EEG changes occurred in the control group. Together, continuous daily 0.1 mg/kg exposure impaired acquisition and altered the EEG in about the same time frame. Also, continuous exposure at 0.01 mg/kg, below the threshold for enzyme induction (0.05-0.1 mg/kg), altered the EEG. Preliminary data from a third study indicate that 1 yr accumulated 0.01 mg/kg exposure slows acquisition, relative to the rate of acquisition at that level in the first study.

51. *Acute Behavioral Changes Induced in the Rat by the Intracerebroventricular Administration of Thyrotropin Releasing Factor (TRF) and Somatostatin.* M. L. COHN, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania. (M. Eisler)

TRF has been utilized in man for the diagnosis and treatment of hyper- and hypothyroidism. Somatostatin most recently has been given in the treatment of diabetes mellitus, acromegaly,

and giantism. While little attention has been given to the direct action of the hypothalamic factors on the brain directly as modulators of behavior, it has been shown that TRF, a tripeptide, induces the secretion of thyrotropin stimulating factor from the pituitary and somatostatin inhibits the secretion of growth hormone. The present study is concerned with the profound psychotropic effects of both TRF and somatostatin. Male Sprague-Dawley rats, 80-120 g, intact and thyroidectomized, were injected centrally with TRF and somatostatin by a method previously described (Cohn *et al.*, *Neuropharmacology*, 1973). Pharmacologic doses of TRF (7.50-50  $\mu$ g) administered centrally to the unanesthetized rat resulted in intermittent sedation and hyperactivity with tearing, squirrel upright position, arched back, and staggering gait. In intact and thyroidectomized rats, higher concentrations of TRF induced hyperthermia. Combinations of TRF and phentolamine resulted in head-to-tail rotation on a flat surface. The rats were unusually aggressive. In anesthetized rats, TRF shortened the sleeping time but not dose-relatedly; the addition of TRF to dibutyryl cyclic AMP further shortened narcosis but with persistent wide ranges of sleeping times. Upon arousal, a severe and acute cerebral locomotor disorder lasting several hours was observed. Lower doses of somatostatin (5-45  $\mu$ g) in the nonanesthetized rat resulted in profound sedation and hypothermia. Higher doses induced "barrel rotation." Anesthetized rats slept three- to fourfold longer than the control group. Because TRF and somatostatin administered peripherally cross the blood-brain barrier, the behavioral effects seen in the rat should be thoroughly investigated before patients are subjected to long-range treatment with these compounds. (Supported by NIMH Grant DA-00605.)

52. *Studies of the Mechanism of the Negative Inotropic Action of Halogenated Methanes.*  
E. W. VAN STEE, P. A. TOY, A. M. HARRIS, M. L. HORTON and K. C. BACK, 6570th Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio.

Exposure of laboratory animals to halogenated methanes impaired myocardial performance. The mechanism of the reduction in the vigor of myocardial contraction was investigated in intact guinea pigs and isolated, Langendorff, rabbit heart preparations perfused with Krebs-Henseleit solution. Open-chested, anesthetized guinea pigs were exposed to bromotrifluoromethane, bromochlorodifluoromethane, and chlorobromomethane in oxygen for 2, 5, or 10 min. Hearts were freeze-clamped in situ and myocardial ATP content was found not to correlate with changes in ventricular performance. Left ventricular mechanical activity was monitored in approximately isometrically contracting isolated hearts by means of a fluid-filled balloon connected to a strain-gauge pressure transducer. At the same time action potentials were recorded from left ventricular myocardial fibers using glass microelectrodes filled with 2.5 M KCl and with a tip impedance of 20-25 Mohms. A loss of the phase 2 plateau and prolongation of the duration of the action potentials corresponded to the decrease in the vigor of myocardial contraction. The results of these experiments were consistent with the hypothesis that the decrement observed in myocardial performance during halomethane exposure was the consequence of the impairment of subcellular translocation of  $Ca^{2+}$  to the contractile apparatus rather than a lack of availability of ATP for hydrolysis during contraction.

53. *Mammalian Toxicity and Degradation of Fenitrooxon and S-Methyl Fenitrooxon.* D. J. ECOBICHON, G. L. MYATT and R. GREENHALGH, Department of Pharmacology, Dalhousie University, Halifax, Canada and Chemistry and Biology Research Institute, Canada Department of Agriculture, Ottawa, Canada.

Commercial fenitrothion [*O,O*-dimethyl-*O*-(3-methyl-4-nitrophenyl)phosphorothioate] is known to be contaminated by an inherent impurity *S*-methyl fenitrooxon [*O,S*-dimethyl-*O*-(3-methyl-4-nitrophenyl)phosphorothiolate], the quantity of this isomer being dependent upon the purity of starting materials and product technology. The similarity of this impurity to fenitrooxon [*O,O*-dimethyl-*O*-(3-methyl-4-nitrophenyl)phosphate] prompted a comparative study of the two agents, examining (1) the I50 for cholinesterase inhibition in vitro (2) the LD50 in the mouse, and (3) the rate of enzymatic degradation by mammalian plasma and hepatic arylesterase. In vitro inhibition studies of mammalian plasma pseudocholinesterase

and erythrocyte and eel tissue acetylcholinesterase revealed comparable I50 values for the two agents. The LD50 (ip) values in male CD strain mice were 112 and 44.5 mg/kg for *S*-methyl isomer and fenitrooxon, respectively. The rate of spontaneous hydrolysis of these esters in an aqueous buffer, pH 7.4, was 20.4 and 5.8 nmol/min at concentrations of  $2 \times 10^{-3}$  M *S*-methyl isomer and fenitrooxon, respectively. The instability of *S*-methyl isomer was also reflected in its extremely rapid enzymatic hydrolysis by plasma and hepatic arylesterases of the mouse, rat, guinea pig, and rabbit, compared to the rate of hydrolysis of fenitrooxon. It would appear that the *S*-methyl isomer impurity will not pose an environmental problem, being very unstable in aqueous solution and in mammalian tissues.

54. *The Morphological Effects of Maternally Administered Phenobarbital and DDT on Hepatic Cell Structure in Young Rats.* M. M. HANSELL and D. J. ECOBICHON, Departments of Anatomy and Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada.

The purpose of this study was to determine when, during the period of perinatal development, the liver would respond to a xenobiotic inducing agent. Hepatocytes of rat pups at various stages of pre- and postnatal development, receiving either phenobarbital (PB) or DDT transplacentally or via the milk, were examined for morphological alterations and changes in activities of drug-metabolizing enzymes. PB (75 mg/kg/day for 3 days) or DDT (50 mg/kg/day for 3 days) were administered orally via a feeding needle and syringe to pregnant or lactating dams, the pups being killed 24 hr after the last dose, at 3 days prepartum, at term, and at 4, 7, 14, 21, and 28 days of age. Samples of liver were fixed and embedded for light and electron microscopy and the remaining tissue was used for measurement of enzyme levels. Enzymatic studies showed no significant induction prior to birth, although chemical analysis identified the presence of residues in fetal tissues. No morphological differences were observed in hepatocytes from experimental and control animals before birth. Hepatocytes from experimental pups 4 days and older showed increased smooth endoplasmic reticulum, lipid inclusions and microbodies, morphological alterations which are associated with increased drug metabolism. Myelin whorls were observed in 4-day-old pups. These changes could be correlated with induction of hepatic mixed function oxidases (*O*-demethylase). The alterations resulting from DDT treatment were generally greater than those resulting from PB treatment.

55. *Studies on the Toxicity of Phosalone.* M. A. GALLO, Rhodia Inc., Somerset, New Jersey. (A. A. Stein)

Phosalone (*S*-[6-chloro-3-(mercaptomethyl)-2-benzoxazolinone] *O,O*-diethyl phosphorodithioate) is the active ingredient in the insecticide Zolone. Experiments were conducted to determine the acute and chronic toxicity of the compound to rats and dogs and to investigate percutaneous absorption in young pigs. The acute oral LD50 of phosalone in rodents is approximately 100 mg/kg and the dermal LD50 is 1500 mg/kg or greater depending on the formulation employed. The oral and dermal LD50 of the oxygen analog in rats is 35 mg/kg and 4000 mg/kg, respectively. Percutaneous absorption studies in young pigs indicate only a slight translocation of the  $^{14}$ C-labeled material which may account for the marked difference between the oral and dermal toxicity. Feeding phosalone to rats at dietary concentrations of 25, 50, and 250 ppm and to dogs at 100, 200 and 1000 ppm resulted in dose dependent inhibition of RBC and plasma cholinesterase with an apparent level of no observable effect at 50 ppm. Brain cholinesterase was inhibited 40% at 1000 ppm in the dogs but little or no inhibition was observed in rat brain at any dietary concentration. No other signs of cholinergy were observed. The results of these experiments indicate that phosalone is less toxic than many other widely used organophosphates.

56. *Report of Toxicological Tests on 152 Detergents.* G. W. BIERBOWER, V. M. SEABAUGH, R. E. OSTERBERG and C. A. HOHEISEL, Division of Biological Science, Bureau of Biomedical Science, Consumer Product Safety Commission, Washington, D.C. (R. M. Hehir)

The Consumer Product Safety Commission has an ongoing Detergent Survey with both chemical and biological analyses. The biological tests are for eye irritation, dermal irritation, a provisional test for screening potentially corrosive compounds and oral LD50. The rabbit is



used for all but the LD50 (oral) where the rat is used. To date, 152 detergents have been tested. The results of the eye irritation test showed responses of 36 (slight) and 72 (severe) eye irritations. Of the 152 detergents tested for skin irritation, 89 were under a Primary Irritation Index (PII) of 5.0 and 55 are 5.0 or above. For the dermal irritation test, the PII of 5.0 or more requires a warning label on the product. For LD50 (oral) of the detergents tested, we found 106 under 5 g/kg and 46 over 5 g/kg. An LD50 under 5 g/kg requires a cautionary label. With the provisional test for screening potentially corrosive compounds, 27 detergents were considered corrosive, 90 irritant and 28 nonirritant. An evaluation of the results of each of the four biological tests indicate no clear-cut relationship between the tests. The eye irritation test could be considered the most sensitive test of the four.

57. *Mortality from Pesticides in 1969*. W. J. HAYES, JR., Center in Environmental Toxicology, Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee.

Through cooperation of the National Center for Health Statistics and the state departments of health, copies of certificates for deaths that occurred in the United States in 1969 were obtained for all categories under which accidental poisoning by pesticides could be classified. In order to investigate further each death in which association with a pesticide was established or could not be excluded, a letter was sent to the person who signed the certificate or to the hospital involved, or both. Most accidental deaths caused by pesticides now are reported under category E865, adopted in 1968 for that purpose. There was, in 1969, a substantial reduction in the number of pesticide-associated deaths compared to the annual numbers during the decade and a half prior to 1962 and even some reduction compared to 1968. This is true even though a few accidental deaths caused by pesticides still are reported in categories other than E865. As in the past, more than half of the victims are children; most are males; and a disproportional fraction are nonwhite.

58. *Alkyl Ethoxy Sulfates. Absorption, Distribution, Excretion, and Metabolite Identification Studies in Rats and Man*. J. A. McDERMOTT, D. H. HUGHES and P. M. QUINLIN, The Procter and Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio. (E. V. Buehler)

Alkyl ethoxy sulfates (AES),  $R-(CH_2CH_2O)_n-OSO_3Na$ , where  $R = C_{12}H_{25}$  to  $C_{18}H_{37}$  and  $n = 1-12$ , are utilized as anionic surfactants in household detergent products. Two representatives of this class of surfactants,  $C_{16}E_3S$  and  $C_{16}E_9S$ , have been studied in rats using  $^{14}C$  samples labeled in the alkyl or ethoxylate groups, or  $^{35}S$  in the sulfate portion of the molecules. Only the  $^{14}C$  alkyl labeled compounds were employed in human studies. In man and rat an oral dose of [ $^{14}C$ -1-hexadecyl]- $C_{16}E_3S$  is extensively absorbed and biotransformed, and is excreted primarily in the urine with lesser amounts appearing in the feces and expired air (65, 15, and 11%, respectively, of the dosed activity). Conversely a dose of [ $^{14}C$ -1-hexadecyl]- $C_{16}E_9S$  is poorly absorbed by the rat or man and appears mainly in the feces with smaller amounts in the urine and expired air (85, 5, and 5%, respectively, of the dosed activity). Oral doses of either [ $^{35}S$ ]- $C_{16}E_3S$  or [ $^{14}C$ -1-ethoxylate]- $C_{16}E_3S$  are handled alike by the rat (62, 26, and 0% of the dosed activity appears, respectively, in the urine, feces, and expired air) indicating little metabolism occurs in the sulfate and ethoxylate portions of the molecule. The major urinary metabolite, accounting for 75% of the activity in urine, in rat and man following an oral dose either of  $^{14}C_{16}E_3S$  or  $C_{16}^{14}E_3S$  was found to be  $-OOCCH_2(OCH_2CH_2)_3OSO_3^-$ .

59. *Carboxyhemoglobin Levels of Humans Exposed to Methylene Chloride*. C. L. HAKE, R. D. STEWART, A. WU and S. A. GRAFF, Department of Environmental Medicine, The Medical College of Wisconsin, Milwaukee, Wisconsin.

Since the discovery in this laboratory of an increase in blood carboxyhemoglobin (COHb) concentrations during exposure of humans to methylene chloride (dichloromethane, DCM), several studies have been carried out wherein this phenomenon was investigated. In a 6-wk study, ten healthy volunteer male subjects breathed air containing 0, 50, 100, 250, or 500 ppm DCM for 7.5, 3, or 1 hr daily under sedentary conditions. Nine healthy volunteer female subjects were similarly studied during a week of exposure to 250 ppm DCM in the environmentally

controlled chamber. Comprehensive studies regarding the acute effects of these exposures upon the health and behavioral performance of the subjects were carried out. Blood COHb saturations determined prior to, during, and after exposure to DCM exhibited the expected increases, but no behavior or performance decrements were found. Four female and three male nonsmokers had a mean increase of over 8% COHb saturation upon their first daily 7.5 hr exposure to 250 ppm DCM. Increases on the third and fifth days of exposure at this level were slightly lower. The COHb values peaked less than 3 hr postexposure. In a third study, a DCM-based paint and varnish remover was used to demonstrate the effects of this commonly used household product on COHb concentrations under actual use conditions. In a large room with a low ventilation rate ( $t_{1/2} = 33$  min), COHb values reached 4.5% saturation after 3 hr of active paint removal, and further increased to 9% after breathing noncontaminated air for 4 hr. The increased time necessary to reach peak blood COHb saturation after exposure to the paint and varnish remover was attributed to the simultaneous exposure to DCM and methanol, also present in the remover mixture with a probable competition between the two chemicals for an enzymatic site responsible for their degradation. The results of these studies further confirm the hazards of uncontrolled consumer use of DCM. (Portions of this work supported by Contract No. HSM-99-72-84, National Institute of Occupational Safety and Health.)

60. *Uptake and Pharmacodynamics of Vinyl Chloride Administered to Rats by Different Routes.*

R. J. WITHEY, Branch of Chemical Safety, Health and Welfare, Canada, Ottawa, Ontario, Canada. (I. C. Munro)

The discovery in May 1973, of vinyl chloride monomer in alcoholic beverages which had been packaged in rigid polyvinylchloride containers led to the examination of a variety of foodstuffs packaged in this material. The finding of at least 2-3 ppm and on occasions as high as 10-20 ppm of vinyl chloride monomer in a wide range of foodstuffs has given rise to concern with respect to the human health hazard which this could present. The recognition of vinyl chloride as a carcinogen in April 1974, following the discovery of angiosarcoma as the cause of death in at least 25 workers who had been engaged in the manufacture of polyvinylchloride, has enhanced the concern with respect to the presence of vinyl chloride monomer in foods. In order to assess the hazard presented by the oral ingestion of vinyl chloride monomer, rats which have been surgically prepared with an indwelling jugular canula have been dosed by intragastric intubation with solutions containing up to 2 mg/ml of aqueous vinyl chloride and sequential samples of blood have allowed time concentration curves to be obtained. The uptake of vinyl chloride by this route is extremely rapid, peak concentrations being achieved less than 10 min after administration of the dose. Elimination from the blood compartment appears to be biexponential, the slower terminal phase having a half-life of about 6 min. Studies with the same animal model, in a single restraint cage, exposed to concentrations of vinyl chloride of up to 2000 ppm in the gas phase have shown a similar rapid uptake followed by a plateau blood concentration during several hours of exposure. On removal from the vinyl chloride atmosphere, blood concentrations fall rapidly to barely detectable concentrations after 30 min. Since most studies of the oncogenic response to vinyl chloride have been carried out with gas phase exposures and the appearance of a variety of forms of carcinoma appears to be related to the vasculature, these comparative uptake and pharmacokinetic studies should assist in the assessment of the health hazard posed by vinyl chloride.

61. *Placental Transfer of Halogenated Benzenes (Pentachloro-, Pentachloronitro-, and Hexabromo-) in Rats.* D. C. VILLENEUVE and K. S. KHERA, Health Protection Branch, National Health and Welfare, Ottawa, Canada.

The present study was carried out to provide information on the placental transfer of three organohalogens of environmental concern. Pentachloro-, pentachloronitro-, and hexabromobenzene were administered (po) to rats daily on days 6-15 of gestation at doses of 50, 100, and 200 mg/kg. On day 22, the dams were killed and fetuses removed by caesarean section. Maternal brain, heart, kidney, liver, spleen, and adipose tissue as well as the whole fetus were analyzed for organohalogen residue by glc. Pentachlorobenzene accumulated in the fetus to a greater

degree than hexabromobenzene. In maternal tissues pentachlorobenzene accumulated in adipose tissue to the greatest extent followed by liver, spleen, brain, heart, and kidney. With hexabromobenzene, greatest accumulation was observed in adipose tissue followed by spleen, liver, heart, kidney, and brain. Pentachloronitrobenzene was not detected (0.01 ppm) in any maternal tissue or whole fetus.

62. *Preclinical Safety Evaluation of 7-Chloro-2-Methyl-3,3a-Dihydro-2H,9H-Isoxazolo(3,2-b)-(1,3)Benzoxazin-9-one (W-2395), A New Anti-Inflammatory Agent.* B. N. BANERJEE, N. J. IVINS and R. D. SOFIA, Wallace Laboratories, Cranbury, New Jersey.

W-2395 is a new Wallace Laboratories compound which possesses analgesic, antipyretic, and anti-inflammatory properties. The preclinical toxicity of this compound was evaluated in rodents, dogs, and monkeys. The oral LD50 values in dogs, mice, and rats were >2500, 2250, and 1160 mg/kg, respectively. One-month comprehensive toxicologic studies were carried out in Long-Evans rats and in rhesus monkeys. The evaluation in rats was accomplished by mixing the compound in the diet to provide approximate daily doses of 200, 100, and 50 mg/kg; the monkeys received single daily doses by gavage, the levels being 300, 150, and 50 mg/kg. No behavioral changes or definitive drug-related mortalities were noted in either experiment. One of the 30 rats receiving the highest dose died during the third week on study; however, the autopsy did not reveal anything that could be attributed to W-2395. One monkey, also in the highest dose group, died on day 6 of the study with severe rectal bleeding. However, this was not drug related but due to strongyloidiasis. Hematology and clinical chemistry values were generally within normal ranges in all groups of both species; the few abnormal determinations included elevated SGPT in one female monkey in each of the high and middle dose groups, but hepatic histopathology was normal in both of these animals. Gross and microscopic examinations of the tissues and organs revealed no lesions attributable to W-2395.

63. *Fate of [<sup>14</sup>C]Hexachlorobenzene in the Rhesus Monkey and the Rat.* R. S. H. YANG and K. A. PITTMAN, Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York. (J. H. Wills)

A study of the comparative metabolism of HCB has been initiated in several mammalian species, including the rhesus monkey. To assess the possible loss of [<sup>14</sup>C]HCB and <sup>14</sup>CO<sub>2</sub> in the expired air, each of two male rats was injected iv with approximately 1.3 μCi of [<sup>14</sup>C]HCB (13.4 μCi/mg) and was held in a closed-metabolic system for 48 hr. The level of radioactivity was determined on urine, feces, various tissues, and the contents in different traps for moisture, volatile chemicals, and CO<sub>2</sub>. The results indicated that little or no radioactivity was exhaled by the animal. Approximately 0.7 and 0.1 % of the radioactivity was recovered from feces and urine, respectively. Fat contained the highest amount of radioactivity, followed by adrenal gland. The radioactivity found in the other tissues was about 1/30 of that of fat. To study the disposition of [<sup>14</sup>C]HCB in rhesus monkeys, a female monkey (4.9 kg) was injected iv with 24.7 μCi of [<sup>14</sup>C]HCB and was held in a metabolic cage for 100 days prior to sacrifice. Radioactivity in plasma, feces, and urine was measured at various intervals throughout the experiment. There was a rapid decline of plasma radioactivity in the first 6 hr after the injection followed by a second phase of slow decline. Approximately 17.1 and 1.8 % of the administered radioactivity was excreted in feces and urine, respectively. Of some 30 tissues analyzed for radioactivity, fat contained the highest activity followed by adrenal gland and other tissues. Two additional monkeys have been under study along similar lines for up to 8 mo. (Supported by National Institute of Environmental Health Sciences Grant 2P01-ES00226-08, by NIH Training Grant 2T01-ES00103-08 and by a Grant from Gesellschaft für Strahlen- und Umweltforschung, Neuherberg, West Germany.)

64. *Species-Specific Binding of <sup>14</sup>C-Hexachlorobenzene to Animal Blood Cells.* R. S. H. YANG, Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York. (E. W. McChesney)

In the course of comparative metabolic studies of [<sup>14</sup>C]hexachlorobenzene (<sup>14</sup>C-HCB) in mammalian species, it was observed that, in the rat, the level of radioactivity in whole blood

was about six times greater than in plasma; this was not the case in the monkey. In vitro experiments showed that human blood behaved similarly to that of the monkey. These findings suggest that there is a species-specific binding of [ $^{14}\text{C}$ ]HCB to erythrocytes and possibly other cells in animal blood. A comparative study was conducted to examine the in vitro binding of [ $^{14}\text{C}$ ]HCB with blood cells of some 16 species of animals. [ $^{14}\text{C}$ ]HCB was bound to blood cells of rats, mice, and rabbits; there was little or no binding in the case of other species: human, monkey, pig, cow, horse, donkey, sheep, goat, dog, guinea pig, hamster, turkey, and chicken. In the case of the rat, there is rapid binding of [ $^{14}\text{C}$ ]HCB, clearly associated with erythrocytes. The bound radioactivity can be removed by toluene extraction but not by dialysis or repeated washing with saline. (Supported by National Institute of Environmental Health Sciences Grant 2P01-ES00226-08, by NIH Training Grant 2T01-ES00103-08 and by a Grant from Gesellschaft für Strahlen- und Umweltforschung, Neuherberg, West Germany.)

65. *Acute Toxicity of Penitrem A in Dogs.* A. W. HAYES, D. B. PRESLEY, J. A. NEVILLE and J. R. GRIDER, The University of Alabama, University, Alabama.

The effect of ip administered penitrem A, a tremogenic fungal metabolite, on dogs of both sexes and mixed breeding was determined by serum test, by survival times and by evaluation of gross lesions. All dogs were treated for routine canine diseases and observed for 2–3 wk prior to the experiment. Alkaline phosphatase, glutamic-oxaloacetic transaminase, lactic dehydrogenase, and creatinine phosphokinase activities and survival time varied in relation to duration of exposure and total dose of mycotoxin administered. Consistent clinical features of the mycotoxicosis were anorexia, weight loss, emesis, tenesmus, rectal temperature to 108°F, and dehydration. Tremors appearing as early as 5 min after dosing and ending in clonic or tetanic convulsions were observed in dogs receiving 0.5 mg/kg or higher penitrem A. Concentrations of blood uric acid and cholesterol exceeded the normal range. Gross pathologic changes frequently observed were subserosal and submucosal hemorrhages in some of the organs of the thoracic and peritoneal cavities. The severity of the lesions was dependent on the amount of toxin administered. Pentobarbital treatment was successful if administered within 1–2 hr after exposure to penitrem A.

66. *Cromolyn Sodium Toxicity Studies in Primates.* J. E. BEACH, A. M. J. N. BLAIR, A. J. CLARKE and C. T. BONFIELD, Fisons Ltd. Pharmaceutical Division, Loughborough, Leicestershire, England, and Fisons Corporation, Bedford, Massachusetts. (R. J. Weir)

An extensive animal program—including approximately 20 separate toxicity studies in 5 species of primates—was undertaken to evaluate the safety of cromolyn sodium (disodium cromoglycate). The studies were done to support a New Drug Application for use of the drug by inhalation in the long-term prophylactic treatment of asthma and by application to the eyes and nose for the treatment of allergic conjunctivitis and/or rhinitis. This paper will review the results of the primate toxicology program including studies using inhalation, subcutaneous, intravenous, intranasal, and ocular routes of administration. While conducting the basic safety evaluation program, a previously unreported proliferative arterial lesion was encountered in several primate studies. The sequence of events and the research program which was required to show that this lesion was not related to drug administration but was genus specific and part of the background pathology of the macaque monkey will be described. The drug showed no significant toxicity when given by any of the proposed clinical routes.

67. *Dietary Factors Influencing Carrageenan-Induced Cecal Ulceration in the Guinea Pig.* R. ABRAHAM and R. MANKES, Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York.

Guinea pigs given 2% degraded carrageenan (C16), derived from *Eucheuma spinosum*, in drinking water, develop cecal ulceration within 2 wk. A significant feature in the ulcerative process is the ability of the macrophages in the lamina propria of the guinea pig to engulf and

store C16, an event that brings about stimulation and release of lysosomal enzymes with damage to surrounding tissue. Cecal ulceration was not seen in guinea pigs given a 2% solution of C16 in milk, or in the diet for 1, 2, 4, and 18 wk, nor was there uptake or storage of C16 in macrophages of the lamina propria. Instead, pigment (whose properties are being elucidated), was present at these sites. These experiments suggest that the site of storage of the macromolecular material is significant in relation to pathogenesis of cecal ulceration, and that the absence of C16 in macrophages of the lamina propria leaves the cecum un ulcerated. However, carra-geenan-laden macrophages were found in the submucosa in all of the experimental animals, the effects of which must await further investigation. (Supported by Research Grant 2-P01-ES00226-08 from the National Institute of Environmental Health Sciences, NIH, and by National Institutes of Health Training Grant 2-T01-ES00103-08. Dr. Abraham is the recipient of a Research Career Award No. 1 K04 ES70607-02.)

68. *Comparative Study of Methadone Toxicity and Distribution in Adult, Pregnant, and Newborn Animals.* M. W. STEVENS, B. MANTILLA-PLATA and R. D. HARBSION, Department of Pharmacology and Center in Toxicology, Vanderbilt Medical Center, Nashville, Tennessee.

Drug concentrations not considered harmful to adults may have toxicological effects on the fetus and newborn due to their increased susceptibility. Distribution of methadone in pregnant mice was determined by administration of [<sup>14</sup>C]methadone (5 mg/kg). Day 13 pregnant animals showed plasma and tissue distributions similar to that of nonpregnant adult female mice. However, after iv administration, fetal concentration in day 17 pregnant animals was significantly greater than the fetal concentrations of day 13 animals when compared at 1, 2, and 4 hr after drug administration. Maternal plasma concentrations were not significantly different. Oral drug administration showed similar results with day 17 fetal concentration being twice that of day 13, and increasing the fetal exposure to drug and possible risk to the developing fetus. Methadone was also shown to be significantly more toxic to newborn rats when compared to adult animals; 3- or 4-day-old newborn rats were also more susceptible to methadone induced toxicity than 1- to 2-wk-old animals. Phenobarbital (PB) (60 mg/kg twice daily for 4 days) or SKF-525A (SKF) (40 mg/kg 1 hr before drug) altered methadone induced toxicity in male mice. At a dosage of 60 mg/kg, methadone produced 100% mortality. This was reduced to 20% by PB pretreatment. At 30 mg/kg methadone was lethal to 40% of the mice. SKF pretreatment increased the incidence of death to 90%. Pretreatment also altered distribution in the pregnant female mice. SKF pretreatment increased fetal concentration and PB pretreatment decreased fetal concentration at all gestational periods studied. (Supported by USPHS Grant DA 00141.)

69. *The Acute Toxicity of Dimethylformamide and Its Combined Effects with Ethanol in the Mouse.* J. BURGUN, R. MARTZ, R. B. FORNEY and G. F. KIPLINGER, Department of Toxicology, Indiana University School of Medicine, Indianapolis, Indiana.

Dimethylformamide (DMF) has widespread use as an industrial solvent. It has been reported (Wink, *Ann. Occup. Hyg.* **15**, 211-215, 1972) that exposure to DMF causes intolerance to alcohol. The purpose of this study was to determine the acute toxicity of DMF and whether DMF had any effect on ethanol sleep time. The ip LD<sub>50</sub> was 1.54 ml/kg. The ip LD<sub>50</sub> of DMF diluted with distilled water was 5.61 ml/kg, indicating that dilution greatly decreased the toxicity of DMF. Doses of 2 ml/kg and greater produced signs of depression. Mice were injected ip with either distilled water or 1, 2, or 4 ml/kg of DMF diluted in water, followed by a hypnotic dose (4 g/kg) of ethanol. The two higher doses significantly increased mean sleep time. Another group of mice was treated with the same dosage regimen except that ethanol was administered 48 hr after DMF. Mice were also treated with either distilled water or 1 ml/kg DMF for 5 days, followed by ethanol, or treated for 5 days, left untreated on day 6 and again dosed on day 7, followed by ethanol administration. In none of these studies were mean sleep times significantly different from control. DMF increased ethanol-induced sleep time at doses

approximately one-third and greater of the LD50 of DMF, suggesting an additive response to the depressant effects of the two compounds. (Supported by PHS T01 GM 1089-11.)

70. *Cadmium Chloride Administration in Rats and the Onset of Liver Pathology.* E. J. FAEDER, L. C. KING, S. Q. CHANEY, R. BRUCE, T. HINNERS and B. FOWLER, Bioenvironmental Laboratory Branch, Environmental Protection Agency, Research Triangle Park, North Carolina; National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. (F. Andrew)

It is well documented that cadmium exposure in animals promotes liver and kidney damage. Studies correlating detectable changes in blood enzymes with ultrastructural alterations of affected organs in cadmium-treated animals have not been previously reported. Cadmium chloride was administered subcutaneously 3 days per week to groups of male Wistar strain rats at 0, 0.25, 0.5, or 0.75 mg Cd/kg. Five animals per dose group were killed after 2, 4, 6, and 8 wk. Blood, liver, kidney, and spleen were removed. Cadmium and zinc concentrations were determined in these tissues by atomic absorption spectroscopy. At the times sampled, the fraction of total cadmium administered remained constant in the liver and kidney; 40-50% in the liver, and 6% in the kidney. Measurements were made on plasma aspartate amino-transferase (AAT),  $\gamma$ -glutamyl transpeptidase ( $\gamma$ -GT), and ornithine transcarbamylase (OTC) levels. Until week 6, no groups showed values significantly different from controls. At week 6, GT and AAT activities became elevated. Red blood cell carbonic anhydrase activity was measured and no significant change was observed in samples up through week 8. Electron microscopic examination of liver tissue indicated proliferation of the rough endoplasmic reticulum at week 6. Connective tissue fiber bundles also became prominent at week 6. Observable liver changes correlated with the elevations in plasma enzymes usually considered to reflect chronic liver damage.

71. *Protective Role of Liver Metallothionein Against Inhibition of Microsomal Proteins in the Rat.* S. Q. CHANEY, E. J. FAEDER, L. C. KING and T. HINNERS, Bioenvironmental Laboratory Branch, Environmental Protection Agency, Research Triangle Park, North Carolina. (W. F. Durham)

A recent literature report indicates that a single, intraperitoneal injection of cadmium acetate into a rat can result in the inhibition of hepatic drug metabolism. We have undertaken this study to evaluate the effects of chronic dosing, where the total liver metal burden is gradually increased. Male rats of the Wistar strain were set up in groups of five. Each group received 0, 0.25, or 0.75 mg Cd/kg, as the chloride, in isotonic sterile saline. Subcutaneous injections were administered three times per week. One group at each dose level was killed each week for 3 consecutive weeks. Liver and blood were removed from each animal. Half of each liver was used to prepare microsomes; the other half was used for cadmium and zinc analysis. Aminopyrine demethylase, cytochrome P-450, and cytochrome P-450 reductase were assayed by standard methods. No difference among controls and dosed animals was observed. Liver cadmium concentrations gradually rose to 60  $\mu$ g/g wet weight in the higher dose animals. Approximately 80-90% of all cadmium measured in the liver was bound to soluble proteins. Elution chromatography on Sephadex G-75 indicated that the binding protein had a molecular weight of about 10,000. Thus, in the case when cadmium is administered slowly to rats, a low molecular weight protein in liver appears to be its primary binding site. Microsomal liver enzymes do not seem to be initially affected.

72. *Effect of Animal Pretreatment on the In Vivo Covalent Binding of Halothane Metabolites in Rat Hepatic Lipids and Protein.* A. J. GANDOLFI and R. A. VAN DYKE, Department of Anesthesiology, Mayo Clinic, Rochester, Minnesota.

Halothane (1,1,1-trifluoro-2-bromo-2-chloroethane), a common volatile anesthetic, has previously been shown by this laboratory to be metabolically activated by isolated perfused rat livers and rat hepatic microsomal incubations to intermediates which bind to proteins and phospholipids (*Anesthesiology* 38, 328, 1974; *Drug Metab. Disp.*, Sept.-Oct. 1974). This study evaluated the effects of diet, animal pretreatment, and time on the covalent binding in vivo of

labeled halothane to hepatic subcellular proteins and phospholipids of the phenobarbital-induced rat. Following pretreatment and induction, the rats were injected ip with 100  $\mu$ l of [ $^{14}$ C]- or [ $^{36}$ Cl]halothane. Selected time periods following the dosing the animals were sacrificed and their livers removed, and homogenized; mitochondria and microsomes isolated, and the specific radioactivity associated with microsomal and mitochondrial phospholipids and residual protein was determined. Partial characterization of the sites and type of binding to the phospholipids and proteins was performed using various hydrolytic and chromatographic procedures. In addition, any hepatic histopathological changes were noted. It was found that maximum binding to mitochondrial and microsomal phospholipids was 1-2 hr after dosing while the binding to their proteins was maximal at 12-24 hr. Studies with [ $^{36}$ Cl]halothane indicated that the metabolite bound to phospholipids retained the Cl atom while that involved in protein binding does not. Increased binding was seen following pretreatment by a diet high in polyunsaturated fatty acids, diethylmaleate-induced glutathione depletion, or a 7% oxygen atmosphere. Decreased binding was found after exposure to halothane or treatment with either cobalt chloride or the antioxidant DPPD. The metabolite bound to the phospholipids is attached through the fatty acid moiety. These results indicate that a partial metabolism of halothane to reactive intermediates is responsible for this binding. (Supported in part by USPHS Grants GM-15178 and ES-00743.)

73. *Effect of Alteration of Hepatic Mixed Function Oxidase Activity on the Toxicity of 2,3,7,8-Tetrachlorodibenzodioxin (TCDD) to Rats.* P. W. BEATTY and R. A. NEAL, Center in Environmental Toxicology, Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee.

TCDD, a contaminant of various products involving the use of the polychlorinated phenols, is one of the most toxic synthetic chemicals yet discovered. In spite of an extensive general knowledge of the toxicity of TCDD to a number of animal species, the mechanism by which it exerts its lethal effects is not yet known. It is also not clear whether the parent compound or some metabolite is responsible for the toxic effects seen. A possible metabolite or metabolites which may be responsible for the toxicity of this compound is an epoxide or a phenol resulting from the mixed function oxidase catalyzed metabolism of TCDD. If a mixed function oxidase catalyzed product or products is responsible for the toxicity of TCDD, it would appear possible that an increase in the activity of the mixed function oxidase enzyme system might increase the toxicity of TCDD. In these studies, we have altered the activity of the hepatic mixed function oxidase enzymes in weanling male, adult male, and adult female rats by administration of inducing agents or by castration of adult male rats and examined the effect of these treatments on the toxicity of TCDD. The results of these studies have indicated that pretreatment of weanling rats with phenobarbital, a treatment which increases the activity of the hepatic mixed function enzyme system, decreases the toxicity of TCDD as compared to untreated controls. In addition, castration of adult male rats, a treatment which decreases the activity of the mixed function oxidase enzyme system, increases the toxicity of TCDD in comparison to noncastrated controls. Similar effects have been observed in female rats in which the activity of the mixed function oxidase enzymes have been increased by pretreatment with testosterone or phenobarbital. The results of these studies imply that an increase in mixed function oxidase activity is associated with a decreased toxicity of TCDD. Further, these data imply that the parent form of TCDD is the more toxic compound and that metabolism by the mixed function oxidase enzyme system results in a less toxic metabolite.

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

A series of experiments have been carried out examining the toxicological implications of the mixed function oxidase catalyzed metabolism of carbon disulfide. The results of these studies have indicated that the decrease in the activity of the hepatic mixed function oxidase

enzyme system and the concentration of cytochrome P-450 seen on incubation of CS<sub>2</sub> with rat liver microsomes in the presence of NADPH is the result of the binding of the sulfur atom released in the mixed function oxidase catalyzed metabolism of CS<sub>2</sub> to COS. Moreover, it appears that COS is further metabolized by the mixed function oxidase enzyme system to CO<sub>2</sub> and that, analogous to the metabolism of CS<sub>2</sub> to COS, the sulfur atom released in this reaction also binds to the microsomes and inhibits benzphetamine metabolism and decreases the concentration of cytochrome P-450 detectable as its carbon monoxide complex. The results of these studies also suggest that the decrease in the concentration of cytochrome P-450 and the liver damage seen on in vivo administration of CS<sub>2</sub> to phenobarbital pretreated rats, is due to the mixed function oxidase catalyzed release and binding of the sulfur atoms of CS<sub>2</sub>. The decrease in the concentration of cytochrome P-450 seen on incubation of CS<sub>2</sub> with rat liver microsomes in the presence of NADPH does not appear to be the result of destruction of the heme group or its dissociation from the apoenzyme since the total amount of protoheme is unchanged in microsomes which have been incubated with CS<sub>2</sub> and NADPH as compared to those not incubated with these compounds.

75. *Effects of a Variety of Narcotics and Narcotic Antagonists on Liver Microsomal Oxygenases.* D. R. BOWMAN, M. C. BRAUDE and R. P. MAICKEL, Department of Pharmacology, Medical Sciences Program, Indiana University, Bloomington, Indiana, and National Institute on Drug Abuse, Rockville, Maryland.

As part of an overall project comparing several pharmacological aspects of synthetic narcotics and narcotic antagonists, the effects of a selection of these drugs on rat liver microsomal oxygenases have been compared to phenobarbital. Rats were given daily sc injections of cyclazocine, methadone, naloxone, pentazocine or phenobarbital for 7 or 14 days. The livers were removed and liver microsomal oxygenase activity was determined in fortified microsomal fractions by measuring aniline hydroxylase (production of *p*-aminophenol) and *p*-nitroanisole demethylase (production of *p*-nitrophenol). Under these conditions, phenobarbital produced significant induction of liver microsomal oxygenase activity, both on a per gram liver basis and in terms of activity per milligram of microsomal protein. Cyclazocine, when given for 14 days, caused a significant increase in activity of both enzyme systems on a per gram of liver basis without a corresponding elevation per milligram of microsomal protein. Naloxone induced both total activity and specific activity of aniline hydroxylase after 7 days of treatment, although the drug had no effect on *p*-nitroanisole demethylase. After 14 days, only the activity of aniline hydroxylase per gram liver was elevated. Methadone and pentazocine had no consistent significant effects on either enzyme system, nor did any of the drugs interfere with the enzyme-inducing activity of phenobarbital. (Supported in part by USPHS Contract HSM-42-73-226.)

76. *Comparative Metabolism of Environmentally Significant Chlorinated Aromatic Compounds in Hepatic Microsomal Systems.* S. M. GHASUDDIN, J. O. NELSON, R. E. MEHZER, P. C. KEARNEY and J. R. PLIMMER, Department of Entomology, University of Maryland, College Park, Maryland, and Pesticide Degradation Laboratory, A.E.Q.I., A.R.S., U.S.D.A., Beltsville, Maryland.

The polychlorinated biphenyls (PCB) and the chlorinated dibenzo-*p*-dioxins are important environmental contaminants. The degree and position of chlorination of these compounds were studied in rat liver microsomes to assess the structure-degradation relationship. The 2,5,2'-, 2,5,2',5'-, and 2,4,5,2',5'-chlorinated biphenyls and dibenzo-*p*-dioxin, its 2,7-dichloro, and 2,3,7,8-tetrachloro analogs were incubated in microsomal systems prepared from phenobarbital-pretreated rats with an NADPH-generating system. Metabolites were isolated, purified by thin-layer chromatography, and identified by spectral methods. With the PCBs the number and quantitative significance of metabolites decreases with increasing degree of chlorination. A similar relationship was observed with the dioxins. The approximate percentages of the applied dose recovered as metabolites after 4 hr incubation were for trichlorobiphenyl, 50%; tetrachlorobiphenyl, 30%; pentachlorobiphenyl, 5%; percentages of the recovered dose for dibenzo-*p*-dioxin, 70%; dichloro- and tetrachlorodibenzo-*p*-dioxin were essentially unmetabolized. Chromatographically the metabolites behave as hydroxylated products. These



studies indicate that the highly chlorinated biphenyls and dioxins will be resistant to metabolic attack in mammalian systems.

77. *Assessment of Hepatic Mixed-Function Oxidase Induction in Rhesus Monkeys using In Vivo, In Vitro and Histological Parameters.* J. L. MILLER, R. I. KRIEGER, M. RORVIK, B. RUEBNER, S. J. GEE and T. THONGSINTHUSAK, Departments of Environmental Toxicology and Medical Pathology, University of California, Davis, California. (W. W. Kilgore)

The exposure of our population to a diversity of environmental chemicals may result in the altered ability of the hepatic microsomal mixed-function oxidase (MFO) system to metabolize lipophilic compounds. The development of rapid and sensitive *in vivo* parameters to quantitate MFO activity will facilitate assessment of the hepatic effects of foreign chemicals. [<sup>14</sup>C]Antipyrone plasma half-lives (APH) were determined in rhesus monkeys after exposure to ethanol, phenobarbital and *p,p'*-DDT. Needle biopsies provided liver tissue for characterization of ultrastructure changes using light and electron microscopy. Rhesus monkeys, tube-fed 100 calories/kg of a liquid diet based on casein in which 41% of the calories were derived from grain alcohol, showed an 18% decrease in APH. Control animals fed the same diet containing isocaloric amounts of glucose showed no significant change. Transient changes in liver ultrastructure were observed. In another study, treatment of these animals with 15 mg/kg phenobarbital twice daily for 4 days resulted in a 36% decrease in APH which was associated with a marked proliferation of liver endoplasmic reticulum. Urinary cortisol:6 $\beta$ -hydroxycortisol ratios were not significantly elevated following phenobarbital treatment. Monkeys fed diets containing 0, 10, 100, and 500 ppm *p,p'*-DDT for consecutive 30-day periods showed no dose related changes in APH. Significant increases in APH were noted following trauma during feeding studies. *In vitro* MFO activity was measured in liver homogenates prepared from needle biopsies. *O*-demethylase activity showed dose related increases. Aldrin epoxidase activity was not significantly altered. [<sup>14</sup>C]Antipyrone plasma half is a useful *in vivo* indicator of MFO activity in rhesus monkey. Induction studies employing needle biopsy material hold considerable promise for evaluation of hepatic effects of environmental chemicals. (Supported in part by NIH ES00054.)

78. *Tissue Toxicity of Polymers: The Importance of Sample Standardization.* B. F. MATLAGA, L. P. YASENCHAK and T. N. SALTHOUSE, Ethicon Research Foundation, Somerville, New Jersey.

Studies were designed to demonstrate the necessity for a standard geometry and surface in evaluating polymer implants for histotoxicity. Increased use of polymers as surgical materials and as drug release agents requires standardization of samples and procedures for valid *in vivo* toxicity evaluations. Samples of medical grade polyethylene, polypropylene, polyurethane, PTFE, silicone, and PVC with and without 0.4% dibutyltin bis(isooctyl acetomercaptide) stabilizer were prepared as 1 mm diam. rods, triangular and star-shaped cross sections in a C. W. Brabender modular extrusion instrument to minimize effects of material variables in sample preparation. Hand cut and abraded samples were also prepared. Samples were implanted in rat gluteal muscles for 7, 14, 21, and 35 days; samples with adjacent tissue were then examined histologically, and the lysosomal hydrolytic enzyme activity (acid phosphatase and aminopeptidase) quantitated in cryostat sections by microspectrophotometry (Salthouse *et al.*, *Toxicol. Appl. Pharmacol.* **25**, 201, 1973). The level of hydrolase activity at the implant sites has been applied as an index of polymer-tissue compatibility. The 1-mm extruded samples demonstrated a uniformly low tissue reaction and hydrolase activity. However, the triangular and star-shaped cross sections, hand cut and abraded samples were associated with significantly higher hydrolase activity and cell populations at the implant sites. PVC with 0.4% organotin stabilizer induced high hydrolase activity in adjacent cell populations. Results reinforce our opinion that quantitation of lysosomal enzyme activity provides a valuable numerical index of tissue irritation and histotoxicity caused by implanted polymers. For valid comparative studies, the necessity for standardization of sample geometry and surface was confirmed.

79. *An Evaluation of the Cardiac Sensitizing Potential of Scotchgard Brand Fabric Protector.* J. L. EGGLE, JR., J. F. BORZELLECA and J. E. LONG. Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia, and the 3M Company, St. Paul, Minnesota.

In view of the recent history of the intentional and unintentional misuse (not used according to directions stated on the label) of consumer aerosol products containing halogenated hydrocarbons, concern has been expressed about the potential cardiac sensitizing effects associated with the unintentional misuse of Scotchgard. Experiments were therefore designed to assess the cardiac sensitizing potential of Scotchgard Brand Fabric Protector FC-4101 containing 1,1,1-trichloroethane (TCE) under simulated misuse conditions. Beagle dogs were prepared for telemetric monitoring of their electrocardiogram (EKG) and placed in a 290-liter exposure chamber. This system allowed observation of possible effects on the EKG of exposure to Scotchgard alone and in combination with intravenous epinephrine (8 and 16  $\mu\text{g}/\text{kg}$ ) and stress induced by a 2-sec blast from an air horn. Changes seen in heart rate were of relatively small magnitude and consisted primarily of a presumed reflex bradycardia to epinephrine and stress and a slight tachycardia following exposure to Scotchgard producing a TCE concentration of 5000 ppm. Inhalation of Scotchgard did not act synergistically with exogenous epinephrine 8  $\mu\text{g}/\text{kg}$  or endogenous epinephrine released in response to stress to induce cardiac arrhythmias. Also, the arrhythmias induced by 16  $\mu\text{g}/\text{kg}$  epinephrine or 16  $\mu\text{g}/\text{kg}$  epinephrine and stress were not intensified by the concurrent inhalation of 5000 ppm TCE generated by spraying Scotchgard into the exposure chamber. It is concluded that under the simulated misuse conditions utilized in this study, Scotchgard did not cause cardiac sensitization.

80. *Use of z Scores in Characterizing Concentrations of Lead in Human Tissues.* S. B. GROSS and S. J. RASCHE, Department of Environmental Health, University of Cincinnati, Cincinnati, Ohio.

Twenty-nine tissues from each of 46 white males from the Cincinnati area have been analyzed for lead to characterize normal tissue values. Needed was a method to compare the lead concentrations between the different tissues from the same individual and to compare the body burdens of lead from individual to individual. The z score statistic satisfies these requirements. The z score is one of several standard scores frequently used in educational and psychological statistics to express the relative position of measurements obtained on different individuals or qualities with reference to a standard basis. It expresses the deviation from the mean ( $X - \bar{x}$ ) in standard deviation (SD) units:  $z = (X - \bar{x})/\text{SD}$ . The concentrations of lead from the various tissues varied widely, many of them increasing or decreasing with age. The z score was used to compare the differing tissue concentrations and to automatically adjust for concentration changes with age. Using averaged z scores for soft and hard tissues, an individual's body burden could be classified into four exposure categories: long-term low level, recent high level, past high level, and long-term high level exposures. By adjusting for age the z score allows the expression of statistically significant correlations between the lead concentrations of different tissues, not apparent using lead concentrations alone.

81. *Spermic Cholinergic System and Occurrence of Acetylcholine (ACh) and Other Quaternary Ammonium Compounds in Mammalian Spermatozoa.* M. R. BISHOP, B. V. R. SASTRY, D. E. SCHMIDT and R. D. HARBISON, Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee.

The existence of a cholinergic system consisting of the acetylcholine (ACh) cycle (synthesis-release and stimulation at a receptor and hydrolysis by cholinesterase) at certain sites of the nervous system has been well established. Recent investigations have indicated that ACh-cycle plays a significant role in non-nervous tissues, one of which is the motility of spermatozoa. However, the substances in spermatozoa which exhibited ACh-like activity were not identified. In our studies the spermatozoa from the fresh ejaculate of the bull were washed with calcium-free Krebs-Ringer phosphate solution, and extracted with acetonitrile. The quaternary ammonium compounds from the acetonitrile extract were subjected to pyrolysis gas chromatography.

In the gas chromatogram, two peaks which represented 2-dimethylaminoethyl acetate and 2-dimethylaminoethyl propionate were identified. This suggests that ACh and propionylcholine (PCh) occur in bull spermatozoa. According to our estimation bull spermatozoa contained  $4.27 \pm 1.41$  (mean  $\pm$  SD) pmol ACh/ $10^6$  cells, and  $1.47 \pm 0.48$  pmol PCh/ $10^6$  cells. (Supported by USPHS Grants NS-04699, RR 05424, ES-00782, and ES-00267.)

82. *Effects of a Single Infusion of Canine Whole Blood on the Canine Gall Bladder.* D. ABRUTYN, G. BRIZIARELLI, J. TORNABEN and E. SCHWARTZ, Warner-Lambert Co., Morris Plains, New Jersey.

Canine whole blood was infused into dogs and selected parameters in hematology, clinical chemistry, and tissue morphology were measured. The purpose of this paper is to describe an unusual effect in the gall bladder of some dogs given whole blood, a finding not previously reported in the literature. A series of studies were undertaken in which mongrel or Beagle blood collected in Fenwal® packs containing ACD anticoagulant was typed for seven of the known canine erythrocyte antigens and/or cross-matched with the blood of mongrel or Beagle recipients. In each experiment normovolemic or hypovolemic recipients were divided into antigenically similar (SWB-compatible) or disparate (DWB-incompatible) groups and were given a single intravenous infusion of blood at 25 ml/kg in a constant rate of 10 ml/min. Four of the normovolemic dogs were given citrated blood into which 1 mg of heparin was added to each 100 ml of blood infusate immediately prior to infusion. Dogs were made hypovolemic by removing 25 ml/kg of blood within 4 min; within 1 hr of blood withdrawal, they were infused with a similar amount of blood from a donor. After complete autopsy, gall bladder lesions were noted grossly and microscopically. Some of the gall bladders were examined for the presence of IgG by direct immunofluorescence. Hemorrhagic gall bladders were seen in most of the normovolemic mongrels (4/4) and Beagles (3/4) given SWB mongrel blood and in a 1/4 mongrel and 1/4 Beagle dogs each given DWB. Three of the four dogs given the heparinized blood also showed the effect. In those bladders in which immunofluorescence studies were performed, the presence of IgG correlated well with those showing hemorrhages. The data suggest the hemorrhage is due to an immunologic factor; however, the reason for gall bladder predilection is unknown.

83. *Toxicity of Citrinin and Ochratoxin A in Guinea Pigs.* H. L. THACKER, W. W. CARLTON and G. N. SANSING, School of Veterinary Medicine, Purdue University, West Lafayette, Indiana and Commercial Solvents Corp., Terre Haute, Indiana.

Citrinin (25 and 50 mg/kg) and ochratoxin A (OA) (10 and 20 mg/kg) were tested for toxic effects in guinea pigs; citrinin was dissolved in 1:1 mixture of 50% ethanol and DMSO and administered via intragastric intubation. Ochratoxin A dissolved in 0.1 N NaHCO<sub>3</sub> was administered po via dosing needle. Clinical signs were few but oliguria and anuria were noted in some principals given OA. Activities of enzymes isocitric dehydrogenase, glutamate oxaloacetate transaminase, and glutamate pyruvate transaminase were elevated in the urine. Serum activities of lactic dehydrogenase, GOT, and ICDH were elevated. Gross lesions found in both citrinin and OA individuals were pale mottled kidneys, friable pale livers, enlarged mesenteric lymph nodes, and localized hyperemic to hemorrhagic segments of the small and large intestines. In guinea pigs given citrinin, discrete pale foci of apparent necrosis of variable size were found in the liver. Histopathologic alterations in OA principals were confined primarily to the kidneys; tubular nephrosis was characterized by cloudy swelling and necrosis with desquamation of the epithelial cells, primarily of but not limited to, the proximal convoluted tubular portion of nephrons. In citrinin principals, renal changes were similar but were more extensive with involvement of the distal segment and loop of Henle. Desquamation was very limited, but calcification of damaged tubular cells was common. Hepatic changes were focal areas of parenchymal cell necrosis and early degenerative change involving primarily midzonal regions. (Supported by NIH Grant ES00463.)

84. *Ochratoxin A and Citrinin-Induced Nephropathy in Beagle Dogs*. D. N. KITCHEN, W. W. CARLTON and G. N. SANSING, School of Veterinary Medicine, Purdue University, West Lafayette, Indiana, and Commercial Solvents Corp., Terre Haute, Indiana.

Previous studies in our laboratory have established the renal toxicity of ochratoxin A and citrinin in young Beagle dogs. Synergistic toxicity were observed when young Beagle dogs were given combined daily doses of ochratoxin A and citrinin. Ochratoxin A was administered orally via capsule and citrinin dissolved in ethanol was given by intraperitoneal injection. Clinical signs of toxicosis included anorexia, retching, tenesmus, polydipsia, polyuria, weight loss, prostration, and death. Activities of glutamate-oxaloacetate transaminase, isocitrate dehydrogenase, and lactic dehydrogenase (LDH) were increased in the urine as was serum LDH activity. Cellular and granular casts were present in the urine. Serum concentrations of sodium, potassium and chloride were decreased. At necropsy, ileocolic intussusception was common, but other tissues were grossly normal. Histopathologic alterations were those of renal tubular necrosis, especially in the straight segments of the proximal and distal convoluted tubules and in the collecting ducts. Necrosis was found in the mesenteric lymph nodes and in Peyer's patches of the gut, especially in the ileum and colon. (Supported by NIH Grant ES00463.)

85. *Effect of Maternally Administered Minocycline on Embryonic and Fetal Development in the Rhesus Monkey (Macaca mulatta)*. B. A. JACKSON, D. E. RODWELL, L. A. KANEGIS and J. F. NOBLE, Toxicology Research Section, Lederle Laboratories Division of American Cyanamid Company, Pearl River, New York.

Pregnant rhesus monkeys (*Macaca mulatta*) received minocycline orally during embryogenesis and/or during the period of skeletal ossification to study the effect of this antibiotic on embryonic and fetal development. Some pregnancies were interrupted and the fetuses collected before term by caesarean section while others were allowed to proceed to term. No apparent maternal toxic effects occurred in treated animals. Measurements, 4 or 5 hr after dosing showed that the doses administered (8.7–17.4 mg/kg/day) produced average blood concentrations of 6  $\mu$ l/ml; on occasion they reached 9  $\mu$ l/ml and higher. All fetuses and neonates were alive at the time of sacrifice and manifested no gross external malformations. There were no internal malformations (skeletal or soft tissue) nor were there apparent effects on body weights or organ weights of any fetus or neonate from treated mothers. Thus minocycline, administered to pregnant rhesus monkeys during embryogenesis and fetal skeletal ossification at doses yielding blood concentrations which were in excess of the expected human therapeutic level, produced no effects on development of offspring.

86. *Correlation of Histologic and Serum Enzyme Changes of Isoproterenol-Induced Myocardial Lesions in Dogs*. S. E. SADEK and E. A. PFTZER, Department of Experimental Pathology and Toxicology, Hoffmann-La Roche Inc., Nutley, New Jersey.

The present investigation was undertaken to determine the nature of altered enzyme activities in serum of dogs with chemically induced myocardial lesions. Isoproterenol hydrochloride in sterile water (25 mg/ml) was injected subcutaneously into 15 Beagle dogs at a dosage of 2.5 mg/kg or lower either once or twice at a 24-hr interval. No mortality occurred. With myocardial infarction in humans, important diagnostic tests include peak elevations of enzyme assays for CPK and GOT after 1 day and for LDH and HBD after several days. In contrast, chemically induced myocardial lesions in the dog were best correlated with peak elevations in CPK activity that reached 20–40 $\times$  normal after 8 hr and GOT activity that increased to 10 $\times$  normal after 8–12 hr; both enzyme activities returned to normal after 3 days. LDH and HBD activities increased to 3–4 $\times$  normal after 24 hr and then fluctuated irregularly when measured for up to 14 days. Autopsy at either 3 or 14 days after the initial injection showed grayish-white lesions (3–30 mm in greatest dimension) that were focally disseminated in heart muscle especially prominent in ventricular walls and papillary muscle. Microscopic examination of the 3-day lesions showed focal myocardial degeneration characterized by swelling of myofibers, eosinophilic granular cytoplasm, and nuclear fragmentation. The hearts from dogs killed 14 days after

initial injection showed scar formation. The results showed that serum CPK was the more reliable indicator of chemically induced myocardial damage in the dog. The increase in enzyme activity after 6-12 hr correlated well with the degree of heart injury.

87. *Subacute Toxicity of Hexachlorobenzene in the Rat.* T. KUIPER-GOODMAN, D. L. GRANT, C. A. MOODIE, G. KORSRUD and I. C. MUNRO, National Health and Welfare, Health Protection Branch, Ottawa, Ontario, Canada.

Hexachlorobenzene (HCB) is a persistent fungicide and industrial contaminant which has been used for over 25 yr. There have been several reports of HCB residues in man, cattle, poultry, and wildlife and not enough is known about the toxicity of this compound. Therefore, 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, 8, or 32 mg/kg body wt. Groups of four rats of both sexes were killed at 21, 42, 62, 89, and 104 days of feeding and at various times after feeding with HCB was discontinued. Residues of HCB reached a plateau before 104 days and were dose related, with concentrations in adipose tissue > liver > brain > serum. At the two highest dose levels relative liver weight was increased in both sexes. Pathological examination showed that mainly the liver was affected, with acentrilobular increase in hepatocyte size due to proliferated smooth endoplasmic reticulum. This was correlated to increased activities of drug metabolizing enzymes, which persisted long after the animals were on a HCB-free diet. Females developed porphyria, with high porphyrin concentrations persisting after the rats were placed on a HCB-free diet. Pathological examination of the other organs indicated no changes with the possible exception of the kidney.

88. *Parathion-Dieldrin Interactions on Androgen Metabolism in the Mouse.* L. G. SCHEIN and J. A. THOMAS, Department of Pharmacology, West Virginia University Medical Center, Morgantown, West Virginia.

Previous studies have revealed that dieldrin can interfere with androgen metabolism (*Toxicol. Appl. Pharmacol.* **26**, 523, 1973), but that parathion exerts little effect upon male sex hormone levels (*Toxicol. Appl. Pharmacol.* **29**, 53, 1974). The present studies reveal that dieldrin (2.5 mg/kg daily  $\times$  5 or 10, po) and parathion (2.6 mg/kg daily  $\times$  5 or 10, po) can interact so as to cause even greater disturbances in testosterone dynamics in mouse prostate glands. The most pronounced inhibitory effect upon the *in vivo* assimilation of [<sup>3</sup>H]testosterone by the prostate gland occurred when parathion was administered for 5 days followed by a similar treatment period with dieldrin. The total radiosteroid concentrations detected in the prostate glands of mice receiving the aforementioned dose sequence of pesticides were decreased 54%. Hepatic microsomes, like the prostate glands, revealed significant reduction in [<sup>3</sup>H]testosterone concentrations following the simultaneous administration of dieldrin and parathion. These findings reveal that organochlorine and organophosphate pesticides can interact and produce exaggerated biological changes in the male reproductive system. (Supported in part by the Environmental Protection Agency.)

89. *Effect of Low Dietary Levels of DDT on Acute CCl<sub>4</sub> Hepatotoxicity in Rats.* M. T. KOEFERL and R. E. LARSON, Dept. of Pharmacology and Toxicology, Oregon State University, Corvallis, Oregon.

Acute pretreatment of rats with DDT has been shown to potentiate acute CCl<sub>4</sub>-induced hepatotoxicity. The present investigation was undertaken to quantify the DDT-CCl<sub>4</sub> interaction from the standpoint of a chronic dietary exposure to DDT, and to compare acute and chronic pretreatments. Rats fed 6-65 ppm for 3 wk and 65 ppm for 24 wk attained body burdens of DDT and its metabolites ranging from 6.1 to 30.6 ppm, compared to 0 ppm for controls. In the acute study rats were treated with 35-150 mg DDT/kg po 24 hr prior to CCl<sub>4</sub> exposure. If all of the 35 mg/kg dose were absorbed, it would represent a total body burden of 35 ppm and would be comparable to a chronic exposure of 65 ppm for 24 wk. CCl<sub>4</sub> was administered by gavage at dosages of 0.125-1.0 ml/kg. A potentiation of serum transaminase (SGPT and SGOT)

responses to  $\text{CCl}_4$  was noted in all groups pretreated with DDT. This potentiation effect was dose-related, both to  $\text{CCl}_4$  and to the total body burden or dose of DDT.  $\text{CCl}_4$ -induced BSP retention was also greatly enhanced following prior dietary exposure to DDT. The onset and development of potentiated hepatic dysfunction paralleled that of parenchymal cell destruction. All results were confirmed by histopathology. About 75% of the induced cytochrome P-450 concentration in DDT-fed rats was destroyed within 6 hr after  $\text{CCl}_4$ , at which time the onset of potentiation was noted. Hepatic regeneration was impaired in DDT-fed rats, since the P-450 concentration continued to decline between 24–48 hr after  $\text{CCl}_4$ . The amount of P-450 destruction appeared to correlate with the degree of hepatic damage observed. More P-450 was destroyed in DDT-fed rats as a result of the induced microsomal concentration of the cytochrome present initially before  $\text{CCl}_4$  was given. The  $\text{CCl}_4$ -P-450 interaction could play an integral role in the potentiation phenomenon, as McLean and McLean (1969) have suggested. Since rats and man respond similarly to  $\text{CCl}_4$ , these results indicate that man's chronic exposure to DDT could render him highly susceptible to a secondary exposure to  $\text{CCl}_4$ .

90. *Dose- and Age-Dependent Metabolic Disposition of the Herbicide 1-(5-tert-butyl-1,3,4-thiadiazol-2-yl)-1,3-dimethylurea in the Rat.* D. G. HOFFMAN, J. L. EMMERSON and D. M. MORTON. Toxicology Division, Lilly Research Laboratories, Greenfield, Indiana.

As an extension of previously reported studies the metabolic disposition of 1-(5-tert-butyl-1,3,4-thiadiazol-2-yl)-1,3-dimethylurea (EL-103) was investigated at doses employed in chronic toxicity studies. [ $^{14}\text{C}$ ]EL-103 was administered by gavage to young (2 mo) and old (15–18 mo) male rats. After solvent extraction, plasma and urine metabolites were separated by tlc and quantified by scintillation counting. In young rats the apparent  $t_4$  for plasma  $^{14}\text{C}$  was 4 and 20 hr after doses of 10 and 160 mg/kg, respectively. The major circulating metabolites were the 3-hydroxymethyl-, 3-desmethyl-, and 1,3-di-desmethyl compounds. The relative proportions of the three circulating metabolites were not affected by dose. Approximately 85% of a 10-mg/kg dose was excreted in the urine of both young and old rats within 24 hr. Young rats excreted 40, 80, and 82% of a 160-mg/kg dose in 24, 48, and 72 hr, respectively, while old rats excreted 20, 62, and 86% of the dose during the same time periods. The pattern of urinary metabolites was similar in young and old rats given 10 mg/kg but that pattern differed slightly from the one obtained from rats of either age given 160 mg/kg. The major urinary metabolites were hydroxylated as well as demethylated. Dose appears to have had more effect than the age of the animal on the metabolic disposition of EL-103.

91. *Toxicity Studies of a Short Acting Semisynthetic Cardiac Glycoside.* R. D. HEMM, J. J. POLLOCK and L. L. AUTHIER, Ayerst Research Laboratory, Animal Health Laboratories, Animal Health Division, Chazy, New York.

Since the characterization and clinical development of the natural cardiac glycosides, semi-synthetic compounds with greater margins of safety have been eagerly sought. The present study was conducted to define the toxicologic potential of a new cardiac glycoside (Actodigin) in Beagle dogs. This glycoside differs in the position of attachment of the lactone ring to the steroid nucleus and allows a rapid onset and dissipation of action. Actodigin was administered intravenously to young adult male and female Beagle dogs for 14 consecutive days at doses of 150 and 300  $\mu\text{g}/\text{kg}/\text{day}$ . Ouabain was administered as a positive control at a loading dose of 48  $\mu\text{g}/\text{kg}/\text{day}$  and at a maintenance dose of 9  $\mu\text{g}/\text{kg}/\text{day}$ . Physical examinations, electrocardiograms, ophthalmoscopic exams, and clinical laboratory analyses were performed periodically. Gross and microscopic examinations of tissues and organ weight measurements were performed terminally. Survival, ophthalmologic, clinical chemistry, urinalysis, organ weights, and gross and microscopic tissue observations were comparable in all groups. Electrocardiogram changes (bradycardia, A-V blockade, ventricular escape beats), decreased erythroid values, and emesis occurred in the high dose Actodigin and ouabain groups. The electrocardiogram changes in the Actodigin-treated groups were of shorter duration than in the ouabain group and dissipated rapidly after cessation of treatment. Decreased weight gain occurred in all drug-treated groups. It was concluded that Actodigin had a rapid onset and dissipation of action and a relatively lower toxicologic potential than ouabain.

92. *Preclinical Toxicity of 3-Deazauridine in Dogs and Monkeys.* T. R. CASTLES, J. L. SANYER and C. C. LEE, Pharmacology and Toxicology, Midwest Research Institute, Kansas City, Missouri.

The intravenous toxicity of 3-deazauridine, a pyrimidine nucleoside which has been reported to be effective against arabinosylcytosine-resistant leukemia cells, was studied in 30 beagle dogs and 14 rhesus monkeys. In dogs, the highest single dose (1 g/kg) that could be given produced no toxicity. Five daily doses of 3 mg/kg were tolerated, while 5 daily doses of 250 mg/kg caused death in 9 days. In monkeys, 5 daily doses of 416 mg/kg produced death in 7 days. The toxicity was dose-related in both species. Toxic signs included severe emesis (dogs only), anorexia, weight loss, and bloody diarrhea. Severe reticulocytopenia, mild leukopenia, and slight increases in BUN occurred. Tissue lesions included degenerative changes in the gastrointestinal tract, depression of bone marrow, and dermatitis (monkeys only). The target organs were tissues with a high rate of cell division. (Supported by a subcontract from the Battelle Columbus Laboratories, under Contract No. N01-CM-43746 from the Division of Cancer Treatment, National Cancer Institute, NIH.)

93. *Another Hypolipidemic Failure.* T. MALMFORS, Astra Pharmaceuticals AB, Toxicology Laboratories, Södertälje, Sweden.

In animal studies, SAB 432, an aryloxyacetic acid, was shown to be an agent considerably more hypotriglyceridemic and slightly more hypocholesterolemic than clofibrate. The preclinical toxicological studies were performed in rats and dogs. From the results of these studies it was concluded, however, that SAB 432 was too toxic to be used as a hypolipidemic agent in man. In the rats hemolytic anemia, hepatotoxicity, and testicular degeneration were observed. The same effects, as well as involution of the thymus and atrophy of the prostate, were noticed in the dogs. The results will be discussed with regard to possible mechanisms of the toxic effects. Particular attention will be paid to the relation between the hypolipidemic effect, the biotransformation, and the changes observed.

94. *The Acute Oral Toxicity of Several Munition Compounds and Their Synthetic Intermediates in Rodents.* J. V. DILLEY, B. S. ANDERSON, D. N. ROBERTS and C. C. LEE, Pharmacology and Toxicology, Midwest Research Institute, Kansas City, Missouri.

Mammalian toxicity of several munition compounds and their synthetic intermediates that may find their way into wastewater from manufacturers has been under investigation. The acute oral toxicity of trinitroglycerin, trinitrotoluene, five dinitrotoluene isomers, white phosphorus, and nitrocellulose were determined in rats and mice. White phosphorus was highly toxic (LD50 = 2.5–3.5 mg/kg) while nitrocellulose was relatively nontoxic (LD50 greater than 5 g/kg). Nitroglycerin produced lethality within a few hours or not at all. Trinitrotoluene and 3,4-dinitrotoluene produced convulsions within 10–20 min after treatment. The nitrotoluenes readily produced methemoglobin and heinz bodies. White phosphorus produced fatty livers as its most prominent gross pathological feature. (Supported by the USAMRDC, Department of the Army, under Contract No. DAMD-17-74-C-4073.)

95. *11-Hydroxy- $\Delta^9$ -Tetrahydrocannabinol Induced Changes in the Perfused Rat Heart.* B. R. MANNO and J. E. MANNO, General Medical Research, Veterans Administration Hospital, Shreveport, Louisiana, and Department of Pharmacology and Therapeutics, Louisiana State University School of Medicine in Shreveport, Shreveport, Louisiana.

The effects of 11-hydroxy- $\Delta^9$ -tetrahydrocannabinol (11-OH- $\Delta^9$ -THC) on the myocardium and the coronary vasculature have been evaluated using the isolated perfused rat heart. The 11-OH- $\Delta^9$ -THC was suspended in a vehicle of 1.5% Tween 80, 5% ethanol and water and doses ranging from 0.1 to 28  $\mu$ g were infused over a 3-min period. Hearts were monitored for changes in rate, force of contraction, and perfusion pressure continuously during the infusion period and for 20 min after the infusion of 11-OH- $\Delta^9$ -THC ceased. Heart rate fluctuations ranging

from 3 to 5% of control occurred but were not considered to be significant. With low concentrations of 11-OH- $\Delta^9$ -THC, an initial slight increase in the inotropic response (less than 5% of control) occurred followed by a negative inotropic response (less than -6% of control). Only a negative inotropic response occurred at high concentrations of 11-OH- $\Delta^9$ -THC (up to -20% of control). Perfusion pressure, an index of coronary vasculature resistance, increased slightly with most doses of the drug (up to 10% of control). With doses of 25.5 and 28  $\mu$ g of 11-OH- $\Delta^9$ -THC, a biphasic response occurred. Initially, a decrease (vasodilation) was observed (-15 and -9% of control) followed by an increase in perfusion pressure (vasoconstriction) not exceeding 10% of control. The data indicate that 11-OH- $\Delta^9$ -THC has little direct effect on heart rate, however, it does produce a negative inotropic response on the myocardium with a subsequent decreased coronary vasculature resistance followed by increased vasculature resistance. (Supported in part by NIH Grant DA 00136.)

96. *Cardiovascular Actions of 11-Hydroxy- $\Delta^9$ -Tetrahydrocannabinol in the Rat.* J. E. MANNO and B. R. MANNO, Department of Pharmacology and Therapeutics, Louisiana State University School of Medicine in Shreveport and General Medical Research, Veterans Administration Hospital, Shreveport, Louisiana.

Eleven-hydroxy- $\Delta^9$ -tetrahydrocannabinol (11-OH- $\Delta^9$ -THC), dissolved in a vehicle of 5% ethanol, 1.5% Tween-80, and water (Olsen *et al.*, 1973, *J. Pharm. Pharmac.* **25**, 344) was administered intravenously or subcutaneously to male, albino rats in doses of 0.025, 0.05, and 0.15 mg/kg. Direct arterial blood pressure and heart rate were continuously monitored via indwelling femoral arterial catheters from the unanesthetized, unrestrained animals for 90 min after drug administration. A similar series of experiments was conducted to investigate the effect of pretreatment with the metabolic inhibitor, SKF-525A ( $\beta$ -diethylaminoethyl diphenylpropylacetate) administered 30 min before the 11-OH- $\Delta^9$ -THC. The administration of 11-OH- $\Delta^9$ -THC by either the intravenous or subcutaneous route did not produce any significant alteration in cardiovascular response in animals not pretreated with SKF-525A. In animals that were pretreated with SKF-525A there was an immediate positive chronotropic effect up to +10% of control at the 0.05-mg/kg 11-OH- $\Delta^9$ -THC dose. There was a negative chronotropic action at the 0.15-mg/kg dose that returned back to control values after the intravenous dose. The positive and negative chronotropic actions occurred when the drug was administered either intravenously or subcutaneously. The data indicate a biphasic action of 11-OH- $\Delta^9$ -THC on heart rate and support implication of the compound in the marijuana-induced tachycardia observed in man. (Supported in part by NIH Grant DA 00136.)

97. *Subacute Oral Toxicity of ABBOTT-41988, A Synthetic Cannabinoid Congener.* G. R. THOMPSON and S. LEVIN, Abbott Laboratories, North Chicago, Illinois.

The preclinical toxicity of ABBOTT-41988 (5,5-dimethyl-8-[5-(4-fluorophenyl)-2-pentyl]-10-hydroxy-2-(2-propenyl)-1,2,3,4-tetrahydro-5h-[1]-benzopyrano[3,4-d]pyridine) was evaluated in 50 male and 50 female Long-Evans rats that were treated po for 29 days. The compound was suspended in 0.5% Methocel for administration at 2, 10, and 50 mg/kg/day. All animals survived treatment. Generalized dose-related depression commenced 15-30 min after the initial treatment and included decreased activity, hypothermia, bradypnea, anorexia, adipsia, slight hypouresis, and subsequently decreased growth rates. Simultaneously, the rats exhibited irritability, aggressiveness, and hypersensitivity to tactile stimuli. Female rats were consistently more susceptible to the drug as indicated by a faster onset and greater intensity of behavioral effects. Hypersensitivity and tolerance to depression in both sexes were evident at 24-72 hr, but the time of onset was inversely related to the dosage. A "stimulatory" effect was evident in female rats on day 30 as indicated by decreased hexobarbital sleeptimes. Moderately decreased 24-hr excretion of creatinine correlated with decreased growth rates. Hematological, hemochemical, urinary, and histopathological parameters were generally unaffected by treatment. Toxicity produced by ABBOTT-41988 was similar to that produced by other cannabinoids despite pharmacological differences.



98. *Sequential Histopathologic Changes Induced in Mice by a Technical and a Purified Preparation of 2,4,5-Trichlorophenoxyacetic Acid.* B. HIGHMAN, T. B. GAINES and H. J. SCHUMACHER, University of Arkansas School of Medicine, Little Rock, Arkansas, and the National Center for Toxicological Research, Jefferson, Arkansas.

It was reported previously (Highman and Schumacher, *Toxicol. Appl. Pharmacol.* **29**, 134, 1974) that maternal mice given 4-8 doses of 120 mg/kg of a technical preparation containing 97.9% 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) often developed myocardial lesions, hypocellularity of the bone marrow, and depletion of lymphocytes in the thymus, spleen, or lymph nodes. To determine if impairment in maternal health associated with such lesions was a significant factor contributing to an increase in fetal abnormalities (unpublished findings), 474 dihybrid mice received po, as in the earlier study, 0, 60, or 120 mg/kg of 2,4,5-T on 9 successive days, beginning 6 days after mating; 325 received the technical and 64 a purified preparation of 2,4,5-T, and 85 were untreated controls. Mice were sacrificed when they became moribund and at 1, 4, 6, 8, and 11 days after beginning treatment. All mice given 60 mg/kg and some given 120 mg/kg of 2,4,5-T appeared normal at sacrifice and showed little or no pathological changes. Mice susceptible to 120 mg/kg became ill or moribund after 1-8 doses and few survived 11 days; 34 of 66 moribund mice given the technical and 23 of 31 given the purified preparation of 2,4,5-T showed myocardial lesions, and more showed lesions in other organs. Mice appearing ill had fewer lesions than moribund mice. These findings support the view that the lesions are primarily due to the 2,4,5-T rather than to dioxins and other impurities in the technical preparation and that impaired maternal health is not the primary cause of the increased incidence of fetal abnormalities in maternal mice given 2,4,5-T.

99. *Cytogenetic Evaluation of Several Dioxins in the Rat.* S. GREEN and F. S. MORELAND, Food and Drug Administration, Washington, D.C.

Cytogenetic investigations were performed with three dioxins to determine what potential these substances have for producing chromosomal aberrations in the bone marrow of male rats. The substances investigated were 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 2,7-dichlorodibenzo-*p*-dioxin (DCDD), and dibenzo-*p*-dioxin (DD). In the first study, the three compounds were dissolved in DMSO and were administered by intubation at 10 µg/kg each day for a 5-day period. All rats were sacrificed 6 hr after the last administration. Colchicine was injected ip at 2 mg/kg 2 hr before sacrifice. No significant increases in chromosomal aberrations were observed. The second study was restricted to TCDD, the most potent of the compounds. TCDD in an anisole corn oil solvent (1.5% v/v) was administered to rats ip at 5, 10, or 15 µg/kg. One group received TCDD orally at 20 µg/kg. The positive control substance, triethylenemelamine (TEM), was administered ip at 0.375 mg/kg. The following groups were sacrificed 24 hr after injection: positive control, 20 µg/kg and 15 µg/kg. The remaining groups were sacrificed 29 days after injection. Microscopic examination of the slides revealed no evidence of chromosomal aberrations in any of the dioxin-treated groups. The group administered TEM showed a statistically significant increase in abnormalities over the control. Accordingly, with the treatment regimen employed in this investigation, TCDD, DCDD, and DD appear to possess no potential for producing chromosomal aberrations in the bone marrow of male rats.

100. *Fate of [<sup>14</sup>C]Mirex in the Female Rhesus Monkey.* M. W. KENNEDY, K. A. PITTMAN and V. M. STEIN, Center of Experimental Pathology and Toxicology, Albany Medical College, Albany, New York. (E. W. McChesney)

[<sup>14</sup>C]Mirex (5.23 mCi/mmol) was given iv to female rhesus Monkeys 1 and 2 and orally to Monkey 3 in a dose of about 1 mg/kg. Blood, plasma, urine, and fecal samples were analysed for <sup>14</sup>C content by oxidation and liquid scintillation counting. Monkey 1 was autopsied 106 days and Monkey 3 autopsied 23 days after receiving [<sup>14</sup>C]Mirex. Tissue samples were analysed for <sup>14</sup>C content. Monkey 2 is still under study. In the monkeys treated iv, plasma <sup>14</sup>C showed a rapid decrease over the first few hours from initial high values. In the monkey treated orally, <sup>14</sup>C first appeared in the plasma at 2 hr and reached a maximum at 5 hr. Thereafter the decline in plasma radioactivity paralleled that found in the iv animals. After 2 wk, the rate of decline

decreased considerably and  $^{14}\text{C}$  could be measured reliably for at least 8 mo. Less than 0.4% of the dose was excreted in the urine in the first week, after which urine collections were discontinued.  $^{14}\text{C}$  was present in the feces for at least 8 mo, with a cumulative excretion of <5%. At autopsy of Monkeys 1 and 3, all tissues analysed contained  $^{14}\text{C}$ . The highest concentration of  $^{14}\text{C}$  was found in the fat, which was calculated to contain about 90% of the dose. Analysis of fat and feces for the chemical nature of the  $^{14}\text{C}$  showed almost all to be present as unchanged Mirex. There was a small amount of a compound more polar than Mirex in the feces, which comprised less than 3% of the fecal radioactivity. Urinary  $^{14}\text{C}$  could not be extracted by solvents which extract Mirex. Mirex, given intravenously or orally, rapidly leaves the plasma, primarily going into the fat where it remains for a long period of time. Metabolism plays a minor role in disposition of this compound. (Supported by Research Grant 2P01-ES00226-08 from the National Institute of Environmental Sciences NIH and by National Institutes of Health Training Grant 2T01-ES00103-08.)

101. *Adriamycin-Induced Myopathies in the Rhesus Monkey with Emphasis on Cardiomyopathy.* E. P. DENINE and L. H. SCHMIDT, Southern Research Institute, Birmingham, Alabama.

The toxicity of adriamycin, delivered alone or in combination with cyclophosphamide has been studied in the rhesus monkey. Responses to both single and multiple courses of these agents have been examined. Doses of 3.0, 4.5, 6.0, 7.5, and 9.0 mg adriamycin/kg were utilized in single course regimens; doses of 3.0, 4.5, and 6.0 mg in multiple course regimens, delivered at 28-day intervals. In combination studies, the cyclophosphamide dose was 60.0 mg/kg. Responses to the above regimens were both immediate and delayed. The former, encompassing acute injury to bone marrow, lymphoid tissue, mucosa of the gastrointestinal tract, and skin (alopecia), were rapidly reversible. Delayed responses, progressive during treatment courses and generally irreversible thereafter, included severe weight loss, muscle wasting, and evolution of a clinical syndrome indistinguishable from that of congestive heart failure (anasarca, ascites, pleural and pericardial effusions). These reactions were associated with distinctive lesions of the myocardium and diaphragmatic muscle (myocytolysis). The incidences and severity of these lesions were clearly related to the total dose of adriamycin. The cardiotoxicity of adriamycin was enhanced slightly but significantly by concomitant delivery of cyclophosphamide. (Supported by Contract No. N01-CM-02083, National Cancer Institute.)

102. *Differential Regional Effects of the Convulsant Methionine Sulfoximine on Serotonin Turnover in the Rat Brain.* D. D. DIETZ and O. Z. SELLINGER, Department of Environmental and Industrial Health, Toxicology Division and Laboratory in Neurochemistry, Mental Health Research Institute, The University of Michigan, Ann Arbor, Michigan. (J. E. Fitzgerald)

Regional changes in 5-HT concentrations and turnover rate have been implicated in the genesis of many seizure states. Previous work from this laboratory has shown that the convulsant agent L-methionine-DL-sulfoximine (MSO), significantly reduces 5-HT concentrations in the hypothalamus, cerebral cortex, and brainstem after a convulsant dose of MSO and in the hypothalamus and striatum after repeated administration of subconvulsant doses of MSO. MSO has also been shown to potentiate pCPA induced depletions of 5-HT in the midbrain and hypothalamus, while antagonizing this depleting effect in the hippocampus. This along with experiments demonstrating a protection from MSO seizures by the 5-HT precursor 5HTP prompted further study of MSO as it affects 5-HT turnover. The type A monoamine oxidase inhibitor clorgyline (*N*-methyl-*N*-propargyl-3 (2,4-dichlorophenoxy) propylamine) was given to adult male rats in a dose of 2 mg/kg iv, 3 hr after a subconvulsant dose of MSO (150 mg/kg) or saline ip. The rats were sacrificed at 0 time, 10 min, 30 min, and 2 hr after clorgyline. 5-HT and its deaminated metabolite, 5HIAA were fluorimetrically determined in seven neuroanatomical regions of the rat brain after 2 hr and in the striatum, cerebral cortex, brainstem, and midbrain at the early time points. Our results show that MSO antagonized the clorgyline induced buildup of 5-HT in the cerebral cortex and the brainstem, while it was without effect in

the midbrain, in the face of no corresponding changes in 5-HIAA concentrations in these regions. On the contrary in the striatum, MSO antagonized the clorgyline induced 5-HIAA depletion while having no effect on 5-HT concentrations. The above results suggest that MSO is altering the turnover rate of 5-HT differentially in the various discrete areas of the rat brain and they therefore implicate the involvement of the serotonergic system in the mediation of the MSO seizure. (Supported by USPHS, Grant 06294 and the Epilepsy Foundation of America and Environmental Health Science Research Training Grant 5 T01 ES 00138-4.)

103. *Growth and Endocrine Alterations After Neonatal Monosodium L-Glutamate in the Rat: Evaluation of Relationship to Arcuate Dopamine Neuron Damage.* C. B. NEMEROFF, G. N. ERVIN, G. BISSETTE, L. E. HARRELL and L. D. GRANT, The Neurobiology Program and the Biological Sciences Research Center, University of North Carolina, School of Medicine, Chapel Hill, North Carolina. (R. L. Dixon)

Systemic injections of monosodium L-glutamate (MSG) into neonatal rodents causes lesions in the arcuate nucleus (ARC) region of the hypothalamus. Controversy currently exists over whether a subsequent syndrome occurs in adulthood, consisting of stunted skeletal growth, hypophagia, obesity, altered locomotor activity, and atrophic changes in endocrine glands—all likely due to disruption of basal hypothalamic control of anterior pituitary secretion of trophic hormones. The present study reexamined behavioral and endocrine organ changes after neonatal MSG administration in rat and also examined possible damage to ARC dopamine neurons thought to be important in regulation of anterior pituitary function. One group of 2-day-old neonatal rats received a single ip injection of MSG (4 g/kg) in saline (MSG-I group), while another group received four additional MSG-injections during the first 10 days of life (MSG-V group). Control animals received equivolume injection of vehicle (0.9% NaCl, pH 7.5). Periodic assessment over 24 wk of body weight gain, 24-hr food and water consumption, locomotor activity and gonadal development revealed various signs of the syndrome previously reported. While MSG-treated animals continually weighed less than sex-matched controls, they developed an obvious obesity that was most apparent in MSG-V females. Later autopsy examination revealed massive accumulation of adipose tissue in the MSG-treated rats. Both MSG groups showed an apparent hypophagia which, however, was statistically insignificant when 24-hr food intake was expressed on a per gram body weight basis. About half of the MSG-treated animals engaged in self-mutilation consisting of tail biting that often led to eventual amputation of the tail. At sacrifice, MSG animals were found to have significantly smaller pituitaries, adrenals, ovaries, and testes than control animals, the latter two changes suggesting disruption of anterior pituitary gonadotrophin secretion similar to that seen after ARC dopamine depletion. Fluorescence microscopy of MSG-treated animals revealed a disappearance of perikarya of ARC dopamine neurons with no evident effect on catecholamine neurons in surrounding or distal brain areas. Thus neonatal MSG may have potential use as a tool in destroying a single monoamine system in the brain, the tuberulo-infundibular dopamine system, without damage to other monoamine pathways. (Supported by NIMH Grant MH1110 and an Alfred P. Sloan Foundation Grant to the Neurobiology Program.)

104. *The Usefulness of Drug Blood Level Studies During Toxicological Safety Evaluation.* C. B. COUTINHO, J. A. CHERIPKO, T. CREWS, B. H. MIN and A. C. LEVY, Division of Experimental Therapeutics, Hoffmann-La Roche Inc., Nutley, New Jersey.

The usefulness of blood level data in providing indications of adequate absorption, reproducibility, and proportionality to dose, accumulation, induction, or steady state conditions, differences between species and within the same species and the relationship of drug concentrations to adverse symptomatology are illustrated by blood level patterns obtained during evaluation of two anti-inflammatory, structurally related, carbazoles: C-5720 and C-4632. Mean peak blood concentrations in dogs dosed with C-5720 at 1, 5, and 25 mg/kg/day po were 2.5, 10, and 48  $\mu\text{g}/\text{ml}$ , respectively, with a half-life of elimination of 5–8 hr. No toxicity was evident in these dogs. Mean peak blood concentrations in rats intubated with 5, 10, and 25 mg/kg/

day were 12, 24, and 50  $\mu\text{g}/\text{ml}$ , respectively, with a  $t_{\frac{1}{2}}$  similar to the dog. No toxicity occurred at the 5 and 10 mg/kg doses. However, at 25 mg/kg (peak blood concentration of 50  $\mu\text{g}/\text{ml}$ ) intestinal perforations, peritonitis, and death occurred, suggesting a greater susceptibility of the rat to this carbazole. The nontoxic dose in the rat was 10 mg/kg which produced a peak blood concentration of 24  $\mu\text{g}/\text{ml}$ . Blood levels of C-4632 were monitored in two separate studies in dogs at doses of 25–100 mg/kg. In the first study, mean peak blood concentrations at the 100 mg/kg dose were 112  $\mu\text{g}/\text{ml}$  and declined rapidly ( $t_{\frac{1}{2}} = 3\text{--}7$  hr). Mild inflammatory changes of the liver occurred in these animals. In the second study, peak blood concentrations at 100 mg/kg averaged 157  $\mu\text{g}/\text{ml}$  and declined more slowly ( $t_{\frac{1}{2}} = 9\text{--}25$  hr). Unlike the first study, serious toxicity occurred and was related to the higher blood concentrations of drug. No toxicity was observed in either study at the 25-mg/kg dose where peak blood concentrations were between 36–52  $\mu\text{g}/\text{ml}$ . Blood concentrations associated with anti-inflammatory activity at 10 and 30 mg/kg oral doses of C-4632 in adjuvant-induced arthritic rats were 20 and 48  $\mu\text{g}/\text{ml}$ . The inhibition of the inflammatory response was 33 and 59%, respectively. A 400-mg oral dose to human subjects resulted in an average blood concentration maximum of 18  $\mu\text{g}/\text{ml}$ , the level associated with a pharmacologic response in the rat.

105. *Estimating the Maximum Test Dose in Carcinogenesis Bioassays using Computerized data Handling and Analysis.* E. GORDON, C. RIGGS, P. BOSTIAN, B. M. ULLAND and L. CALLAHAN, NCI Frederick Cancer Research Center, Frederick, Maryland.

It is necessary to establish the maximum test dose (MTD) for long-term carcinogenesis bioassay. Excessive levels will jeopardize the continuation of a study, while low levels may give false negatives. A preliminary toxicity data system (PTDS) has been developed to evaluate the MTD. Animal groups are placed on varying dose levels and body weights are recorded bi-weekly. When possible, the initial range of doses is chosen from previous studies. Where no data are available, doses are started at 50,000 ppm and serially halved to 775 ppm. The PTDS generates body weight graphs and mortality counts for each group on a weekly basis. At 12 wk the animals are necropsied. Least square regressions of weight versus days are computed for each group and evaluated at day 84. The respective percent control weight values are plotted on log-probit paper, least squares line calculated, and a MTD predicted to produce a 10% weight loss. The final MTD is based on the PTDS weight analysis, clinical signs of the animals during the study, findings at necropsy, and a thorough histologic examination. This method has been used on Fischer 344 rats and  $\text{B}_6\text{C}_3\text{F}_1$  mice. Data generated from chronic studies compared with PTDS-predicted results show that rats follow the predicted patterns while mice show greater variability. Subchronic studies in mice are now being extended to 26 wk to improve the MTD estimating procedure. This system is particularly helpful when a number of subchronic studies must be routinely evaluated. It offers a fast, objective method of analyzing data and may be applicable to other laboratories faced with the problem of MTD determination. (Sponsored by the National Cancer Institute under Contract No. N01-CO-25423 with Litton Bionetics, Inc.).

106. *A Statistical Approach to the USP Biological Evaluation of Plastics.* K. A. MILLER, J. E. DOYLE and J. G. SCHMIDT, Baxter Laboratories, Inc., Morton Grove, Illinois. (P. J. Garvin)

Interpretation of the USP tests for biological evaluation of plastics is based on the difference between the local or systemic responses produced by a blank or negative control and the test plastic or an extract of the plastic. Accordingly, the requirements of the test are met if the average response to the test sample is not significantly greater than the average response to the control (USP XVIII, 926–929). Computerized statistical evaluation offers a precise method of analyzing these data and controlling both Type I errors, i.e., declaring a nontoxic plastic to be toxic; and Type II errors, i.e., declaring a toxic plastic to be nontoxic. In order to facilitate a statistical analysis the following procedural modifications have been implemented: number of test and control sites or animals per test are balanced, number of animals tested is increased, test sites are randomized, and the Draize scoring method is used. Scores from the grading of coded test and control samples in the Intracutaneous Reactivity Test are compared using an

analysis of variance which adjusts for time dependent effects and differences due to biological variability of response. The computer program analyzes the scores for each test variable individually and collectively to determine whether there is significant difference in reactions produced by the test material as compared with the control. The requirements of the test are met if there is no significant difference between the response to the test and control samples at an  $\alpha$  level of 0.05, the probability of a Type I error. The  $\alpha$  level indicates the degree of toxicity, with  $\alpha = 0.05-0.025$  designating a mildly toxic material, and  $\alpha < 0.025$  a severely toxic one. Similar procedures are used for analyzing the results for the Systemic Toxicity and Intramuscular Implantation Tests. The major advantages in the computerized statistical analysis and the accompanying procedural modifications lie in reducing observer bias in grading, controlling Type I and II errors, and providing a uniform method for comparison of plastics.

107. *Advances in Automation for Experimental Pathology*. C. H. FRITH, B. HIGHMAN and A. J. KONVICKA. University of Arkansas School of Medicine, Little Rock, Arkansas, and the National Center for Toxicological Research, Jefferson, Arkansas.

The establishment of the National Center for Toxicological Research (NCTR), Jefferson, Arkansas, to study long-term effects of low doses of potential toxic substances, including carcinogens, resulted in the setting up of chronic experiments requiring histopathologic studies of large numbers of animals. The processing of the tissues and the recording and analysis of the massive amount of pathologic data generated from these studies necessitated the development and implementation of a number of advances in automation for experimental pathology. This has resulted in a unique computerized pathology data system at NCTR. The system encompasses the development of training programs for necropsy technicians, histology technicians, and pathology tissue screeners; the use of automated equipment for processing, embedding, sectioning and staining tissue; the use of prelabeled cassettes, slides, and pathology forms; the use of mark sensitive forms for the collection of gross and microscopic findings; the use of data collection terminals for animal identification, animal weights, and organ weights; the collection of gross and microscopic data on a Mod Comp III minicomputer; and the storage, analysis, and generation of pathology reports and tables over a data link from an IBM 370-158 computer. Some of the features of this system may be applicable to other experimental pathology laboratories as well as medical pathology departments.

108. *The Role of Electron Microscopy in Toxicologic Assessment Past, Present, and Future*. T. KUIPER-GOODMAN and H. C. GRICE, Health Protection Branch, National Health and Welfare Canada, Ottawa, Canada.

Since electron microscopy became popular about 15 yr ago, it has been widely used to increase our morphological perspective in pathology and cellular toxicology. In this presentation we will review some of the more recent techniques which have helped us in our toxicologic assessment. The use of a stereologic approach has made it possible to quantify sizes, surface areas, and numbers of cells and organelles in an organ, so that measurements can be statistically evaluated and more readily correlated with biochemical changes. In this way a dose-response curve for a morphological change such as smooth endoplasmic reticulum increase can be established. Image analyzing computers may be able to automatically perform some of these measurements. The addition of energy dispersive detectors to electron microscopes (scanning or transmission) has made it possible to do a semiquantitative analysis in ultrathin sections of elements from sodium upward. Scanning electron microscopy has added a third dimension and new perspective. Methods are now available whereby both scanning and transmission electron microscopy can be performed on the same tissue sample.

109. *A Comparative Study of Dermal Reactions Using the Intact Rabbit Skin*. J. B. SULLIVAN, J. C. STRAUSBURG and R. W. KAPP, JR., Hazleton Laboratories, Inc., Vienna, Virginia. (J. L. Gargus)

Comparative studies have been conducted in the past using methods that are part of federal regulatory acts. These studies have clearly demonstrated variation in interpretation of results

between laboratories. One of the reasons for the variation could well be the manner in which the material is applied to the skin. For this reason six commercial powder detergent products were studied and applied to the intact skin of rabbits following the Department of Transportation corrosivity protocol as well as the Primary Skin Irritation protocol which is part of the Federal Hazardous Substances Act. These products were administered to unmoistened intact skin in the dry form as defined by the Department of Transportation design and in several different ways that laboratories use for the Primary Skin Irritation procedure. These included application of the powdered compounds to premoistened skins, to skins covered with a moist gauze patch, in the dry form with distilled water injected through the patch, and also as a solution or slurry to the skin. In all cases a gauze patch was used which was held securely in place with adhesive tape. Observations were made at 4 hr, 24 hr, and 72 hr for each application. The treated sites were scored for erythema and edema and observed for necrosis. The resultant observations demonstrated that the two test procedures gave a range of no response to necrosis of the skin and clearly showed the effect of premoistening the skin. Variation was also demonstrated in the Primary Skin Irritation procedure which was clearly indicative of the manner in which the material was applied to the skin.

110. *Detection of Chlorinated Dioxins: Induction of Aryl Hydrocarbon Hydroxylase Activity in Rat Hepatoma Cell Culture.* J. A. BRADLAW, L. H. GARTHOFF, D. M. GRAFF and N. E. HURLEY, Food and Drug Administration, Washington, D.C. (S. Green)

Induction of aryl hydrocarbon hydroxylase (AHH) activity was used to detect low levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and other chlorinated dioxins. Monolayer cultures of Reuber rat hepatoma cells in late log phase of growth were challenged for 48 hr with seven halogenated dibenzo-*p*-dioxins. Homogenized cell extracts from pooled cultures were assayed for AHH activity by the enzymatic conversion of benzo(a)pyrene to 3-hydroxybenzo(a)pyrene as described by Nebert and Gelboin (*J. Biol. Chem.* **243**, 6242, 1968). Enzyme activity, expressed as picomoles product formed/milligram of protein/minute at 37°C, was between 10 and 24 in control cell extracts. The assay was linear for enzyme concentration and time under the conditions employed. TCDD was the most potent inducer tested. A threefold induction was produced by 0.5 pmol/culture (161 pg) and maximal induction by 50 pmol/culture of TCDD as compared with control. In comparison, a threefold induction was produced by 1,2,3,4,7,8-hexachloro- and 1,2,3,4,7-pentachlorodibenzo-*p*-dioxin with 5 and 50 pmol/culture, respectively. Little or no induction of AHH activity was observed with 500 pmol/culture of dibenzo-*p*-dioxin; 2,7-dichloro-; 1,3,6,8-tetrachloro-, and 1,2,4,6,7,9-hexachlorodibenzo-*p*-dioxin. With 96 hr exposure to TCDD, a threefold induction of AHH activity was produced by 0.078 pmol/culture (25 pg). The three chlorinated dioxins that induced AHH activity had halogen atoms in at least three of the four lateral ring positions which is consistent with observations reported in chick embryo liver (Poland and Glover, *Mol. Pharmacol.* **9**, 736, 1973). However, the cell culture bioassay has potentially greater sensitivity and may serve as a practical method for the detection of minute amounts of chlorinated dioxins in animal tissues and food samples.

111. *A New Technique: Serial Bone Marrow Biopsies for Monitoring Chromosomal Changes.* J. H. NEMENZO, A. PASI and C. H. HINE, Department of Pharmacology, University of California, San Francisco, California.

With currently available techniques, somatic cytogenetic studies in bone marrow cells can only be performed in cells sampled from sacrificed experimental animals. When studying chemically induced mutations in chromosomes of somatic cells it is advantageous to ascertain the evolution of the chromosomal changes induced. Continuous monitoring of chromosomal changes in each individual experimental animal is possible only when samples are obtained through serial biopsy. The study reported shows that serial bone marrow biopsy is feasible in the rat. Convenient sampling times were found to be 24 hr before, 1, 7, and 30 days after treatment. The first (24 hr) sample permits establishment of the base line level of spontaneous aberrations, so that each individual experimental animal can serve as its own control. Additional

control is provided by animals treated with the solvent alone. The study shows also that arrest of mitosis in bone marrow cells can be achieved also when colcemide is given, instead of to the animal, directly to the bone marrow suspension. Extracorporeal treatment of bone marrow cells with colcemide permits avoidance of the toxic effects of the antimetabolic agent to the experimental animal. After fixation and staining each metaphase is photomicrographed and each photomicrograph scored for chromosomal aberrations. Each photomicrograph can be traced to the original metaphase by appropriate microllocation. The validity of the use of serial bone marrow biopsies and extracorporeal colcemide treatment for testing the mutagenicity of chemicals will be demonstrated with the results obtained with triethylene melamine.

112. *Development of a Smoke Toxicity Test.* D. P. DRESSLER, W. A. SKORNIK and S. B. BLOOM, Department of Surgery, Harvard Medical School, Cambridge Hospital, and Youville Hospital, Cambridge, Massachusetts. (R. M. Hehir)

Pulmonary complications, often related to smoke inhalation, have become the major cause of death from fire, yet there is no generally accepted laboratory method for determining smoke toxicity. It was the objective of this study to develop a method for determining the relative toxicity of commonly available building and decorating materials. Using a specially designed smoke exposure apparatus, which permits on-going environmental and physiological monitoring, 800 rats were exposed, 16 at a time, to various fuel loads, either smouldered or ignited, at predetermined temperatures. The resultant smoke was inhaled at ambient or controlled temperatures. Smoke toxicity was evaluated by determining the mortality, carboxyhemoglobin, and pulmonary pathology following exposure to smoke produced by various amounts of fuel in relation to the volume of the environment (milligrams/liter). Toxicity was also dependent on the temperature of the fuel, temperature of the animal exposure chamber, and the duration of exposure. If fuel is allowed to ignite, and the animals are permitted to breathe the pyrolysates for 15 min at 25°C, then the toxicity, as reflected by mortality, is 7.1% for white pine, 95.8% for vinyl wallpaper, 43.5% for regular wallpaper, 7.14% for acrylic carpet, 40.6% for nylon carpet, and 100% for vinyl flooring, when studied at 128 mg/liter fuel load concentrations. Similar results are found with other materials when animals are exposed to varying concentrations. The addition of heat to the animal chamber proportionately increased the mortality. Greater survival generally resulted from only the smouldering of equal concentrations of fuel loads. These studies have shown that (1) relative toxicity data is possible, thus permitting identification of particularly toxic materials, and (2) the smoke inhalation model can be used to develop preventive, protective, and therapeutic measures.

113. *Effect of Hexachlorobenzene on Rat Reproduction.* D. L. GRANT, G. V. HATINA and W. E. J. PHILLIPS, Bureau of Chemical Safety, Food Directorate, Health Protection Branch, Ottawa, Canada. (W. A. Mannell)

This study was designed to study the effect of hexachlorobenzene (HCB) on rat reproduction. Dietary concentrations of HCB (0, 10, 20, 40, 80, 160, 320, and 640 ppm) were fed to Sprague-Dawley rats and four generations of rats were raised. Pregnancy (number of dams whelping/number of dams mated), viability (pups surviving 5 days/pups born alive) and lactation (pups weaned/live pups on day 5 less culls) indices were measured. Body weight gains of parents and pups were monitored. HCB residues were measured in the 21-day-old pups of the F<sub>1</sub>a generation. The two highest dietary concentrations were toxic to the F<sub>0</sub> generation and 50 and 20%, respectively, of the females died. Suckling pups were particularly sensitive and many died prior to being weaned. The viability index was zero in the F<sub>1</sub>a and F<sub>1</sub>b generations for rats fed 320 and 640 ppm HCB and only 55% for the 160 ppm group. The lactation index decreased from 30% for the F<sub>1</sub>a and F<sub>1</sub>b generation pups to 0% for the F<sub>2</sub>a and F<sub>2</sub>b generation pups in the 160 ppm group. The lactation index decreased from 93% in the F<sub>1</sub>a generation to 40% in the F<sub>3</sub>b generation in the 80 ppm group. Body weight gain was affected by treatment. No gross abnormalities were present in pups. Residues were present in the weanling rats in a dose-dependent manner.

114. *The Placental Transfer of Methotrexate in Rats*. R. M. McCLAIN and J. J. SIEKIERKA, Department of Experimental Pathology and Toxicology, Research Division, Hoffmann-La Roche Inc., Nutley, New Jersey. (S. E. Sadek)

The placental transfer of [<sup>3</sup>H]methotrexate (MTX) was studied in pregnant rats on day 20 of gestation. Rats were injected iv with MTX (0.1–10 mg/kg) and its presence determined in fetuses, placentas, yolk sacs, and maternal plasma and livers. The highest concentration of MTX was found in maternal liver where the percentage of the administered dose bound by liver decreased markedly as the dosage increased. The binding of MTX by placenta also decreased as the dosage increased; however, the concentration of MTX in the placenta was only 3–8% of the concentration found in liver at a given dosage. The transfer of MTX to the fetus was found to be very limited but proportional to the administered dose over the range of 0.1–10 mg/kg. Net influx was observed at doses of 0.1 and 1 mg/kg; however, net efflux from the fetus was observed at a dosage of 10 mg/kg beyond 0.5 hr after administration. Unidirectional flux was assumed to occur until MTX saturated fetal dihydrofolate reductase binding sites. The observed efflux suggests that at high MTX dosages, after the binding sites are saturated, unbound MTX is available for back diffusion or countertransport. The fetal yolk sac was found to rapidly accumulate MTX against an apparent concentration gradient via a process that did not appear to be saturable. These studies demonstrate that the fetal yolk sac accumulates substantial amounts of MTX and that the placental transfer of MTX is limited. This latter observation may, in part, account for the decreased toxicity in the developing rat and decreased inhibition of dihydrofolate reductase (in vivo) with MTX after placentation is established.

115. *Interactive Effects of 5-Fluorouracil and Endotoxin on Mouse Spleen and Bone Marrow DNA Synthesis and Content*. K. M. WATT, M. A. FRIEDMAN and A. E. MUNSON, Department of Pharmacology, Medical College of Virginia, Health Sciences Division, Virginia Commonwealth University, Richmond, Virginia.

Toxicity of 5-fluorouracil (FU) in mice is markedly synergized by pretreatment with bacterial endotoxin (ET). The purpose of this study was to pinpoint the biochemical mechanism of this synergy. Male Balb/c mice were treated with 2 mg/kg ET, iv and 24 hr later received 100 mg/kg FU, iv. [<sup>3</sup>H]thymidine was injected ip 30 min before killing. Marrow DNA was depressed 57 and 27% at 2, and 3 days after ET. Similarly marrow DNA was depressed 56 and 93% at 1 and 2 days after FU. In the combination group recovered DNA was lowered by 71 and 83% at 1 and 2 days after FU. In contrast, [<sup>3</sup>H]thymidine uptake into marrow DNA was depressed only slightly by 5-FU and slightly stimulated by ET. It is noteworthy that the magnitude of DNA synthesis inhibition was greater in the combination group and the recovery was much less marked. In the case of spleen, DNA content, 5-FU blocked the mitogenic effects of ET. The spleens from the combination groups contained significantly less DNA than either the controls, FU or ET treated mice. Similarly, the stimulation of [<sup>3</sup>H]thymidine uptake into spleen DNA by ET was suppressed by treatment when mortality was starting to occur and uptake in the three experimental groups was two to seven times control. (Supported by NIEMS Grants ES000713 and ES000701 and American Cancer Society Institutional Grant.)

116. *Physiologically Solubilized Di-2-Ethylhexyl Phthalate and its Effect on the Intact Rhesus Monkey: A Preliminary Report*. M. S. JACOBSON, L. N. BUTTON, W. H. WATSON, B. I. BARWICK, R. J. JAEGER and S. V. KEVY, Blood Preservation Laboratory and Transfusion Service, Children's Hospital Medical Center; Department of Pediatrics, Harvard Medical School; Department of Physiology, Harvard School of Public Health, Boston, Massachusetts.

It is impossible to establish the significance in man of animal studies based upon the parenteral administration of a relatively insoluble substance such as di-2-ethylhexyl phthalate (DEHP). Studies from our laboratory have demonstrated that the quantity of DEHP extracted by storage of platelets and lipemic plasma in a polyvinyl chloride (PVC) blood bag is: (1) in a true solution, (2) dependent upon the temperature, duration of storage, and the lipid content of the medium. We therefore chose platelet transfusion of the rhesus monkey for 1 yr as an ideal



clinically significant toxicological model. Our controls were: (a) nontransfused animals, (b) monkeys transfused with platelets stored in siliconized glass or polyethylene containers, and (c) transfusion of platelet-poor plasma stored in PVC. The cumulative amount of DEHP infused in the PVC platelet-transfused monkeys was 76.80–96.00 mg or 24.04–30.05 mg/kg. Comparable amounts of DEHP are normally received by transfused leukemic or aplastic patients in the same time period. As compared to the nontransfused controls the (PVC) platelet monkeys demonstrated: (1) detectable DEHP (3–5% of the total amount infused) by quantitative analysis of biopsy material, (2) a decrease in hepato-splenic ratio using isotopic techniques, (3) normal splenic function as measured by the ability to form antibodies to platelets and sheep erythrocytes, (4) an alteration in compartmental analysis of BSP kinetics, and (5) subtle morphologic changes in liver histology. Similar pathophysiological parameters are being evaluated in the transfused controls and will be repeated 3 and 6 mo after cessation of transfusion therapy in all monkeys. Judgment of the toxicity of phthalates must take into consideration the risk-benefit ratio that component therapy provides. (Supported by a contract with the National Heart and Lung Institute, RFP-NHLI-72-6.)

117. *Effects of Vehicle on the Response to Intravenous Di-(2-Ethylhexyl) Phthalate (DEHP) in Rats.* J. G. SCHMIDT, P. J. GARVIN and J. E. LEESTMA, Baxter Laboratories, Inc., Morton Grove, and Northwestern University, Chicago, Illinois.

Investigations of the biological effects following intravenous injections of DEHP in animals are complicated by the insolubility of DEHP in aqueous vehicles. DEHP can be dispersed as micelles in nonionic surfactants such as polysorbate-80 (P-80) with extensive sonication or with heat and can be solubilized in organic solvents such as ethanol. However, consideration must be given to the inherent toxicity of the vehicle and to the stability of DEHP in the vehicle upon contact with blood. Recent reports have described hemorrhagic pulmonary lesions attributed to DEHP dispersed in P-80; however, we have found that P-80 alone in saline, which is also a micellar dispersion, was toxic. The LD<sub>50</sub> of a 13.3% (v/v) P-80 dispersion in 0.9% saline injected iv into male Sprague-Dawley rats was 13.3 ml/kg at 24 hr and 11.1 ml/kg at 7 days post-injection. Necropsy revealed hemorrhagic lesions of the lungs and kidneys with severity proportional to dose. The iv LD<sub>50</sub> of a 5.0% (v/v) DEHP-13.3% (v/v) P-80 dispersion in saline was 4.93 ml/kg (246.5 mg of DEHP/kg) at 24 hr and 4.77 ml/kg (238.5 of DEHP mg/kg) at 7 days. The combination resulted in greater toxicity and more severe lesions of lung tissue at equivalent volume doses than did the P-80 vehicle alone; however, the increased toxicity was attributed to an additive physical effect resulting from the combined effect of the DEHP and P-80 micelles in contact with blood. DEHP dissolved as a 50% true solution in absolute ethanol was well tolerated by rats at acute iv doses as high as 500 mg of DEHP/kg; mortality at higher volume doses was due directly to the toxicity of the ethanol vehicle. Such a system is not physiologically stable, though. In vitro studies have shown that DEHP dissolved in ethanol readily precipitated out when added to plasma. In vivo, such instability was associated with pathologic effects but at much higher doses of DEHP than when dispersed in the P-80 vehicle. These results indicate that the toxicity and pathologic changes resulting from the iv injection of DEHP dispersed in P-80 were due to the physical effects of globular or micellar load. Similarly, when a sufficient globular load of DEHP alone was administered as a result of in vivo precipitation from ethanol solutions in the blood, similar pathologic lesions resulted. (Supported by NHLI Contract NOI-HB-2-2990-B.)

118. *Pediatric Study of Chronic Morphine Treatment in Rats.* M. S. DANILOVITZ, G. E. LIGHTKEP and A. H. MOSHER, Department of Pathology/Toxicology, McNeil Laboratories, Inc., Fort Washington, Pennsylvania. (R. L. Steelman)

Two generation rat studies using chronic drug treatment have been suggested as one means of determining the safety of agents proposed for human pediatric use. This protocol was designed to determine the effect of chronic treatment on growth and development of rats using mortality, body weight, reproductive capacity, open-field behavior, gross necropsy observations, and brain/body weight ratios as parameters, and to evaluate the practicality and appro-

priateness of the methods selected. Morphine was given orally at 20 mg/kg/day via gavage (10 ml/kg). Primary dams (12) were treated from day 0 of gestation to day 6 of lactation; litters were reduced to 8 pups each at birth; F<sub>1</sub> generation pups (96) were selected on the basis of maximum body weight, apparent viability and optimum litter sex ratio. Treatment of pups via gavage was begun at 7 days of age and continued until completion of the mating interval (male rats) or weaning of F<sub>2</sub> generation pups (female rats). Morphine-treated dams had litter sizes which were slightly smaller than control dams; pups had similar body weights in control and morphine-treated litters. After 1 wk of treatment via gavage, obvious growth retardation was present in morphine treated pups, and by weaning age (21 days), these pups were only one-half the body weight of control pups. Tremors were observed before daily treatment at 2 wk of age and disappeared within the following week. Although morphine-treated pups had retarded body weight gain, there was no alteration in the age at which eyes and ears opened, male rat pup testes descended, and female rat pup vaginas opened. Mating and fertility of F<sub>1</sub> generation rats was similar to control levels, but F<sub>2</sub> generation morphine-treated litters had pups with smaller body weights and higher mortality than control litters. Again, eye and ear opening occurred at a similar age in control and morphine-treated pups. Open-field activity was not altered by morphine treatment of F<sub>1</sub> generation rats. No gross lesions attributable to drug treatment were observed in F<sub>1</sub> or F<sub>2</sub> generation rats. The ratio of brain/body weight was similar in control and morphine-treated F<sub>1</sub> and F<sub>2</sub> generation rats.

119. *Effect of Narcotics on the Male Reproductive System.* J. A. THOMAS, J. T. DOMBROSKY and M. G. MAWHINNEY, Department of Pharmacology, West Virginia University Medical Center, Morgantown, West Virginia.

The daily injection of methadone (5, 10, or 20 mg/kg daily  $\times$  5 or 10, sc) to sexually mature mice led to losses in testicular weights, particularly at the higher doses and longer durations of drug administration. A reduction of male sex hormone secretion, as evidenced by significant decreases in the weights of the prostate and seminal vesicles, was also observed following the methadone regimens. Morphine (20 mg/kg daily  $\times$  10), unlike methadone, was unable to produce any significant changes in these gravimetric responses. Whether the CNS acting properties of morphine correlate with cyclic adenosine monophosphate (cAMP) remains unresolved, but this analgesic can significantly elevate endogenous concentrations of this nucleotide as well as accelerate the conversion of [<sup>3</sup>H]adenosine to [<sup>3</sup>H]cAMP in reproductive organs. Likewise, methadone is capable of increasing cAMP concentrations in sex accessory organs. In vitro, either methadone or morphine ( $2 \times 10^{-3}$  M) can enhance prostatic concentrations of cAMP.

120. *The Toxicity of Decabromodiphenyl Oxide and Octabromobiphenyl as Determined by Subacute and Chronic Dietary Feeding Studies in Rats.* J. M. NORRIS, R. J. KOCIBA, C. G. HUMISTON and P. J. GEHRING, Dow Chemical U.S.A., Midland, Michigan.

The need for fire-resistant materials has stimulated the development of new compounds for use as fire retardant additives. Two such chemicals, decabromodiphenyl oxide (DBDPO) and octabromobiphenyl (OBBP) were evaluated in comparable 30-day rat dietary feeding studies to elucidate handling hazards and in chronic dietary feeding studies to determine suitability for large scale use. No untoward effects were seen in rats receiving 8 mg DBDPO/kg/day in the 30-day study. Dose-related increased liver weights occurred at 80 and 800 mg DBDPO/kg/day and liver lesions at 800 mg/kg/day. Rats receiving OBBP showed increased liver weight at all dose levels, and increased kidney weights at 80 and 800 mg/kg/day. Kidney and liver lesions were seen at all dose levels and hematological changes at 800 mg OBBP/kg/day. Rats on a 2-yr study on DBDPO were not adversely effected at dose levels of 1.0, 0.1, or 0.01 mg/kg/day. Tissue analyses performed at various times during the study showed no accumulation had occurred. Rats maintained for 8 mo on diets providing comparable dose levels of OBBP showed no overt indications of adverse effects. Liver weights were increased at the 1.0-mg OBBP/kg/day level and tissue analyses showed high level bromine accumulation in adipose and liver tissue. These data indicate suitability of DBDPO in its intended use but not of OBBP because of the likelihood it would accumulate in the environment.

121. *Pharmacokinetic and Toxicologic Studies with Triclocarban*. P. L. WRIGHT, L. G. SCHARPF, G. J. LEVINSKAS, D. E. GORDON and M. L. KEPLINGER, Monsanto Company, St. Louis, Missouri, and Industrial Bio-Test Laboratories, Inc., Northbrook, Illinois.

Triclocarban (3,4,4'-trichlorocarbanilide, TCC<sup>®</sup>) is a bacteriostatic agent present in anti-microbial toilet bar soaps. The single oral LD50 is >34.6 g/kg in rats. Emesis is induced in dogs at doses >4.6 g/kg. Chronic feeding studies with rats at 3000 and 10,000 ppm resulted in degeneration of the germinal epithelium lining the seminiferous tubules, atrophy of the tubules, and oligospermia after 6 mo. No testicular lesions were present in rats fed 1000 ppm (100 mg/kg/day). No testicular lesions were present in monkeys given 300 mg/kg/day orally for 90 days, in rabbits treated dermally with 40 mg/kg/day for 90 days, or in mice treated dermally with 600 mg/kg on alternate days for 18 mo. No other gross, biochemical, hematological, central nervous system or histopathological effects related to TCC were observed. TCC is not mutagenic (300 mg/kg ip, mice), teratogenic, or fetidical (100 mg/kg per os, rabbits and 1000 mg/kg per os, rats) nor carcinogenic (10,000 ppm dietary, 24 mo, rats and 600 mg/kg dermally, 18 mo, mice). Rats fed 1000 ppm [<sup>14</sup>C]TCC reached a steady state blood concentration of 20 ppm [<sup>14</sup>C]TCC equivalent after 15 days. Liver had the highest concentration (70 ppm equiv) followed by kidneys, blood, spleen, and testes. Blood and tissue <sup>14</sup>C half-life were approximately 2.5 days. Blood concentrations in human subjects (Scharpf *et al.*, *Arch. Environ. Health* **29**, in press, 1974) after showering with soap containing 2% [<sup>14</sup>C]TCC were below 10 ppb at all sampling times.

122. *Gamma Glutamyl Transpeptidase Activity as an Indicator of Carcinogenesis in the Mouse*. B. J. GOUGH and K. P. BAETCKE, National Center for Toxicological Research, Jefferson, Arkansas.

S. Fiala and E. Fiala (*J. Nat. Cancer Inst.* **1**, 151-158, 1973) reported that feeding of 2-AAF to rats resulted in an elevation of gamma glutamyl transpeptidase (GGTP) activity in liver tissue. Studies were undertaken to determine the relationship of GGTP elevation to the onset of hyperplasia and to tumor formation in BALB/c mice. Male mice were fed the carcinogen 2-AAF at levels of 0, 25, 50, 75, and 100 ppm in the diet for 42 days. Analysis of GGTP activity in liver and kidney tissue at 42 days revealed little or no correlation between elevation of enzyme activity and feeding of 2-AAF or presence of urinary bladder hyperplasia. Liver and kidney tissues were collected from male and female BALB/c mice fed diets containing 2-AAF at levels of 0, 100, 250, or 500 ppm for 18 mo. Increases in GGTP activity were weakly correlated with the presence of carcinoma of the liver or bladder tumors. It appears that GGTP activity is a poor indicator of the carcinogenic process in BALB/c mice following administration of 2-AAF.

123. *Carcinogenicity of Ethylene Dibromide (EDB) and 1,2-Dibromo-3-Chloropropane (DBCP) after Oral Administration in Rats and Mice*. M. B. POWERS, R. W. VOELKER, N. P. PAGE, E. K. WEISBURGER and H. F. KRAYBILL, Hazleton Laboratories, Inc., Vienna, Virginia, and National Cancer Institute, Bethesda, Maryland.

As part of a program to identify potential environmental carcinogens, lifetime studies were conducted in Osborne-Mendel rats and hybrid B6C3F<sub>1</sub> mice on various agricultural and industrial chemicals. The chemicals were administered in the diet or by gavage at the maximum tolerated dose and half that dose as determined in earlier studies. A preliminary report (Olson *et al.*, *J. Nat. Cancer Inst.* **51**, 1993-1995, 1973) identified two of these chemicals, ethylene dibromide (EDB) and 1,2-dibromo-3-chloropropane (DBCP) to be highly carcinogenic inducing gastric squamous cell carcinoma relatively early in the experiments. We now report our findings at termination of these studies following 62 wk of treatment with EDB and 78 wk with DBCP. Tumor induction was noted as early as 12 wk with EDB and 10 wk with DBCP. Both chemicals induced a high incidence of squamous cell carcinoma of the stomach in rats and mice (in excess of 90 and 70% in EDB-treated rats and mice, respectively; in excess of 60 and 90% in DBCP-treated rats and mice, respectively). The squamous cell carcinomas originated in the cardiac portion of the stomach subsequently metastasizing by direct extension to the adjacent viscera and mesentery and, in some animals, by the hematogenous route to the lungs

and other tissues. DBCP also induced a high incidence (54%) of mammary gland adenocarcinoma in female rats but not in the mice. Both of these chemicals have wide agricultural use as grain and soil fumigants and, in addition, ethylene dibromide is used as a gasoline additive and chemical intermediate. The results of these rodent studies suggest a potential environmental hazard with use of these chemicals.

124. *Drug-Related Thyroid Tumors in the Fischer Rat (F-344)*. B. Y. COCKRELL and F. M. GARNER, Litton Bionetics, Kensington, Maryland. (E. R. Hart)

Nineteen thyroid adenomas and 27 thyroid carcinomas were observed in 200 Fischer rats given chemical (NCI no. C02186) by gavage for 18 mo. Tumors were most common (31 of 46) in female rats given the maximum tolerated dose determined experimentally. Both follicular and parafollicular cell type were recognized in three categories: hyperplasia, adenoma, and carcinoma. Follicular hyperplasia consisted of focal proliferation of follicular epithelial cells. Parafollicular hyperplasias appeared as small clusters of light cells or as a diffuse excess of cells between follicles. Identification of parafollicular cells was verified by a modified silver stain or by electron microscopy. Adenomas were recognized as delineated nodules of either follicular or parafollicular cells. Many of the follicular adenomas had cystic and papillary features. Carcinomas of both cell types were recognized by capsular invasion, hyperchromatism, and occasional mitotic activity. Mitotic activity and amyloid were more common in follicular carcinomas. Regional lymph nodes did not contain metastases nor did routine sections of lungs. Control data on 160 male and female Fischer rats of the same age include two thyroid parafollicular adenomas and three parafollicular carcinomas with no metastases found in the lungs. Statistical comparison of control with experimental data indicate that the carcinogenicity of this chemical is both dose- and sex-related. (Supported by Tracor Jitco, Inc., Subcontract No. 74-25-106002.)

125. *Chemically Induced Hepatic Neoplasms in the Fischer Rat (F-344)*. F. M. GARNER and B. Y. COCKRELL, Litton Bionetics, Kensington, Maryland. (E. R. Hart)

A spectrum of lesions including hyperplasia, hepatic cell adenoma, and hepatocellular carcinoma was induced in rats by administration in the diet of NCI Compound no. C02006. Malignant liver tumors were observed in female rats receiving 1000 ppm in the diet as early as 12 mo. By the end of the study of this feeding group of 50 animals, 28% had hepatic cell adenomas and 60% hepatocellular carcinomas. Few survived to the end of this 2-yr study. A similar feeding group of 50 males and 50 females fed 500 ppm had an equivalent tumor incidence. Approximately 70% of both sexes had benign liver tumors and 20% of each sex had hepatocellular carcinomas. Nearly all animals survived to the end of the experiment. Fifty males fed 250 ppm had a much lower incidence of benign and malignant liver tumors, 28% had benign tumors while only 2% developed hepatocellular carcinoma. Nodular hyperplasia was found in one control female rat. No other hepatic lesions were observed in any of the controls. Clear-cut distinction between hyperplasia and neoplasia could not always be determined with certainty, however, nearly all of the tumors classified as malignant metastasized to other sites, usually the lungs. No other compound-related gross or microscopic lesions were observed in the study. This compound is obviously carcinogenic and the induced neoplasia is dose related. (Supported by Tracor Jitco, Inc., Subcontract No. 74-25-106002.)

126. *Mutagenicity of Ethylene Oxide*. J. W. EMBREE and C. H. HINE, Toxicology Activity, Department of Pharmacology, School of Medicine, University of California, San Francisco, California.

The mutagenicity of ethylene oxide (EO) has been well described in plants and lower animals. Because of the wide usage of this compound it appeared desirable to study possible mutagenic effects of EO in mammalian systems. The mutagenic effect of inhaled EO on germinal cell lines was studied using the rat dominant lethal assay of 10 wk duration. Male Long-Evans rats were exposed 4 hr to 1000 ppm EO. Sampling of the females at weeks 1, 2, 3, and 5, corresponding to postmeiotic and meiotic stages of spermatogenesis, showed a significant

increase in dead implants/total implants over control level. Preimplantation losses were not increased. Somatic cell mutation effects were studied by means of a bone marrow metaphase cytogenetics technique in male Long-Evans rats exposed to 250 ppm EO for 7 hr/day for 3 days. Bone marrow sampled 24 hr after the last exposure showed a significant increase in isochromatid and chromatid gaps and breaks, rearrangements and exchanges, and ring formations. Overall total aberrations went from 7/120 metaphases in the control group to 101/120 metaphases examined in the experimental group. A more extensive somatic cell investigation was performed with the micronucleus test. Tests using the TA 1535 series of histidine mutant *Salmonella typhimurium* strains indicated that EO mutagenicity is not due to the by-product ethylene chlorohydrin and is a base substitution event. The results indicate the importance of further testing of ethylene oxide to assign a risk factor to an exposed population.

127. *Study of the Mutagenic Potential of the Monocalcium Salt of Nitrotriacetic Acid (NaCaNTA) by the Dominant Lethal Test in Mice.* T. A. JORGENSEN, G. W. NEWELL, P. GRIBLING, M. O'BRIEN and D. CHU, Stanford Research Institute, Menlo Park, California.

Previous mammalian mutagenesis investigations of nitrotriacetic acid (NTA) have provided results considered as inadequate in scope or, at best, inconclusive. Because this compound has functional properties which could allow it to replace significant amounts of phosphates in certain detergent formulations, it was considered appropriate that a comprehensive mammalian mutagenesis study of NTA be conducted. Information presented here represents one part of such a program, an extensive dominant lethal test of NaCaNTA. Male mice were distributed into 5 groups of 20 males each. Treatment was by gavage with 0, 0.1, 0.05, and 0.025% solutions, with a 0.05 mg/kg triethylenemelamine group as the positive reference, daily for 5 consecutive days. Following treatment, each male was mated to two adult virgin female mice. When a female was found with a mating plug, she was replaced with a new virgin female. This regimen continued for 7 wk. All data were subjected to rigorous statistical analysis by means of a specifically designed computer program. No consistent responses occurred to suggest that the monocalcium salt of NTA (NaCaNTA) is mutagenic to the mouse by the dominant lethal procedure used in this study. It is concluded that NTA (NaCaNTA), at maximum solubility in water at room temperature (0.1%), and administered over a 5-day period (equiv. to 50 mg/kg/day) does not induce dominant lethal effects in mice.

128. *Reproductive and Teratogenic Studies with Naltrexone in Rats and Rabbits.* J. A. NUTTE, G. L. KENNEDY, S. SMITH, M. L. KEPLINGER and J. C. CALANDRA. Industrial Bio-Test Laboratories, Inc. Northbrook, Illinois, and National Institute on Drug Abuse, Rockville, Maryland.

Following a preliminary dose ranging study, naltrexone was evaluated for effects on fertility and reproductive performance in rats and for effects on embryonic and fetal development in both rats and rabbits. To examine effects on fertility and reproductive performance, oral dose levels of 10, 30, or 100 mg/kg were administered for 63 or 14 days prior to mating male or female animals, respectively, with untreated controls. Dosing continued until mating was accomplished for the males, until day 14 of gestation for one-half of the females and until weaning 21-day-old pups for the rest of the females. Food consumption, behavior, as well as mating and fertility indices were similar for control and treated groups. Females treated with 30 or 100 mg/kg dose levels and males treated with 100 mg/kg gained less weight than controls during the pre-mating period. Test and control females sacrificed on gestation day 14 had similar numbers of implantation sites, resorption sites, corpora lutea, and viable fetuses. Parturition indices, pup survival and pup body weight data also revealed no significant differences which could be correlated with naltrexone exposure. The teratogenic potential was evaluated in Charles River rats and New Zealand rabbits. Rats and rabbits were administered 20, 60, or 200 mg/kg orally days 6-15 or 6-18 of gestation, respectively. Rats were sacrificed on day 20 and rabbits on day 29 to examine numbers of implantation sites, resorption sites, corpora lutea, and viable fetuses as well as any external, internal, and skeletal abnormalities in offspring. No effects correlated with

naltrexone administration were found with respect to body weight gain of dams, number of viable fetuses per 100 implantation sites, fetal body weights, external fetal abnormalities, internal organ malformations, or skeletal anomalies. The data from these studies suggest that naltrexone had no effect on fertility and reproductive performance in rats and was not teratogenic to rats or rabbits at the dose levels examined. (Supported by NIDA contract HSM-42-72-171.)

129. *Teratogenic Potential of Methadone and 1-Alpha-Acetylmethadol (LAAM) in Rats and Rabbits.* G. L. KENNEDY, J. A. NUIE, S. SMITH, L. KEPLINGER, M. C. BRAUDE and J. C. CALANDRA. Industrial Bio-Test Laboratories, Inc. Northbrook, Illinois, and National Institute on Drug Abuse, Rockville, Maryland.

The teratogenic potential of two narcotic agonists, methadone and LAAM, was evaluated in rat and rabbit. Following preliminary dose ranging studies, drug treatment was initiated on day 6 of gestation in both species and continued daily through day 15 (rat) and day 18 (rabbit). Methadone doses of 1, 3, and 10 mg/kg and LAAM doses of 0.2, 0.6, and 2.0 mg/kg were administered orally in aqueous solution to rats or as powder via gelatin capsule to rabbits. Controls were treated with vehicle. Female animals were sacrificed 1 day prior to natural delivery, and fetuses examined for external development, structure of internal tissues and organs, and for skeletal development. With respect to methadone, gravid rats treated with 10 mg/kg during organogenesis gained less weight than did controls and lower dose groups. No increase in resorption sites was observed in the drug treatment group, nor was any increase in the number of fetal abnormalities, either grossly, following internal examination, or following skeletal evaluation, observed in the rat. In the rabbit, two duplicate studies (Expts I and II) were executed with methadone at the previously mentioned dose levels. Rabbits showed reduced body weight gains (Expt. I) or body weight losses (Expt. II) during drug treatment. Although numbers of resorption sites were increased in certain groups, the lack of a dose-response relationship makes the significance of this finding questionable. No terata were observed in the control group or among the 47 fetuses recovered from rabbits exposed to 1 mg/kg. However, 3 of the 130 (2.3%) fetuses exposed in utero to 3 mg/kg and 4 of the 188 (2.1%) fetuses exposed to 10 mg/kg displayed multiple abnormalities. Internal development and skeletal development examination disclosed no effects related to methadone administration. Pregnant rats and rabbits treated with LAAM displayed slightly reduced weight gains during pregnancy. Embryotoxicity, as indicated by an increase in fetal resorptions, was not increased. Fetuses obtained from LAAM-treated females were structurally normal following complete external and internal examination. The findings of these studies indicate that LAAM did not demonstrate teratogenic activity in the rat or rabbit at the dose levels employed; nor did methadone in the rat. The findings with methadone in the rabbit, however, although low in incidence, suggest that the findings be further examined. (Supported by NIDA contract HSM-42-72-171.)

130. *Analysis of Strain Differences in Sensitivity and Reproducibility of Results in Assessing 2,4,5-T Teratogenicity in Mice.* T. B. GAINES, J. F. HOLSON, JR., C. J. NELSON and H. J. SCHUMACHER, National Center for Toxicological Research, Food and Drug Administration, Jefferson, Arkansas.

The teratogenic effect of technical 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) was studied using exceptionally large numbers of pregnant mice of the C57BL/6, C3H/He, BALB/c, and A/JAX inbred strains and CD-1 stock. Dose-response curves were determined for the incidence of cleft palate, embryoletality, and fetal growth retardation. These determinations were replicated 6 to 10 times for each inbred strain and 35 times for the CD-1. The number of litters studied ranged from 236 (BALB/c) to 1485 (CD-1). Treatment was by gavage on days 6-14 of pregnancy and dosage levels of 2,4,5-T ranged from 15 to 120 mg/kg/day. The lowest dosage tested in the A/JAX was 15 mg/kg and this dose was teratogenic. The other strains and CD-1 demonstrated teratogenicity at 30 mg/kg, the lowest dose tested. There were significant differences ( $p < 0.05$ ) in sensitivities between the strains. As an example, the calculated ED50 (the dose that affects 50% of the litters) for the incidence of cleft palate in the BALB/c

mice was 105 mg/kg whereas the ED50 in the A/JAX mice was 25 mg/kg. There was also great variation between replications in the same strain with regard to induction of cleft palate, embryonic death, and fetal weight reduction. These findings indicate that studies using small numbers of animals may fail to demonstrate teratogenicity at the low doses which were shown to be teratogenic in this study. Numerous statistical analyses were performed which have proven beneficial in determining appropriateness of statistical methodology as well as providing information on the interrelationships and/or interactions of monitored parameters.

131. *Effects from Repeated Inhalation of Parts per Billion of bis(Chloromethyl)Ether in Rats.* B. K. J. LEONG, R. J. KOCIBA, G. C. JERSEY and P. J. GEHRING, Toxicology Research Laboratory, The Dow Chemical Company, Midland, Michigan.

Groups of rats were exposed, respectively, to 100, 10, or 1 ppb of bis(chloromethyl)ether vapor for 6 hr per day, 5 days per week for 6 mo. In rats exposed to 100 ppb, 98 of the 111 examined had gross or microscopic evidence of esthesioneuroepitheliomas with the first confirmed case occurring during the sixth exposure month. The majority of cases appeared between the second and the seventh months postexposure. However, in groups of rats exposed to 10 or 1 ppb, no indication of esthesioneuroepithelioma formation has been observed as of this date (20 mo postexposure). All rats in the 100-ppb group died within 12 mo postexposure while the mortality rates of both 10- and 1-ppb groups were comparable to that of control. Hematological examination at 3, 6, 12, and 24 experimental months, together with exfoliative cytological examination of the lung and cytogenetic evaluation of chromosomes of bone marrow cells immediately after 6 mo of vapor exposure revealed no abnormalities.

132. *Influence of Ozone on Nembutal Anesthesia in Mice.* D. E. GARDNER, J. W. ILLING, F. J. MILLER and D. L. COFFIN. Environmental Protection Agency, Experimental Biology Laboratory, Pathobiology Research Branch, Research Triangle Park, North Carolina.

The effect of repeated exposure of female mice to ozone (1.0 ppm  $\times$  3 hr) on pentobarbital sodium (50 mg/kg) sleeping time has been studied. Sleeping time with pentobarbital sodium for control mice exposed to clean filtered air was  $21 \pm 3$  min. There was a significant increase in sleeping time as a result of prior treatment of ozone. The difference was manifested consistently when pentobarbital sodium anesthesia followed the second and third ozone exposure. For example, when compared to control, those exposed for 2 days increased their sleeping time by 18 min. No difference was discernible following a single exposure, or from 4 or more successive daily exposures to this same concentration. But after 7 days of exposure to 1.0 ppm, a further increase in sleeping time could be induced by raising the concentration of the ozone to 5 ppm  $\times$  3 hr. This one exposure increased the sleeping time to 59.5 min, as compared to control mice.

133. *Inhalation Toxicity Studies with Di(C<sub>7</sub>C<sub>9</sub>C<sub>11</sub>-Alkyl) Phthalate and Di-2-Ethyl Hexyl Phthalate.* G. J. LEVINSKAS, M. W. DIETRICH, K. S. SCHADEBURG, J. W. GOODE, M. L. KEPLINGER and J. C. CALANDRA, Medical Department, Monsanto Company, St. Louis, Missouri and Industrial Bio-Test Laboratories, Inc., Northbrook, Illinois.

Di-2-ethyl hexyl phthalate (DEHP) has a Threshold Limit Value (TLV) of 5 mg/m<sup>3</sup>. Separate 1-mo inhalation studies with rats were conducted at 5 or 20 mg/m<sup>3</sup> with DEHP and di(C<sub>7</sub>C<sub>9</sub>C<sub>11</sub>-alkyl) phthalate (SANTICIZER® 711). This preliminary study was undertaken to provide assurance that the response of rats was similar to each of these products, that these air concentrations could be generated and maintained, and these levels would be satisfactory for a chronic inhalation study with S-711. For the latter, groups of male and female monkeys, rats and guinea pigs were exposed to 5 or 20 mg/m<sup>3</sup> of S-711 for 5 hr a day for a total of 130 days over a 6-mo interval. No deaths occurred among monkeys, and only 1 female rat from the 20 mg/m<sup>3</sup> group died from causes judged unrelated to inhalation of S-711. Several deaths occurred among guinea pigs with the highest incidence occurring in the control group. On the basis of data obtained in this study, it is concluded that 5 mg/m<sup>3</sup> represents a realistic TLV for S-711. It is further concluded that this inhalation study adds indirect support to the current TLV for DEHP.

134. *The Relationship of the Total Dose and Duration of Methoxyflurane Anesthesia to Renal Toxicity in Fisher 344 Rats.* L. E. ARTHAUD and T. A. LOOMIS, Toxicology Laboratory, Department of Pharmacology, School of Medicine, University of Washington, Seattle, Washington.

Methoxyflurane (MOF) anesthesia has been reported to produce a dose-related nephrotoxicity in both man and animals. The nephrotoxicity is aggregated by prolonged administration of MOF. This report investigates the role of anesthetic duration and total exposure dose to the production of renal abnormalities and alteration of serum and urinary inorganic fluoride levels in Fisher 344 rats. Each of three exposure doses (calculated by multiplying the chamber concentration in volumes percent by the duration of exposure in hours) varying from 1.0 concentration-hr to 3.0 concentration-hr were given to two groups of rats over different time intervals. Alterations in body weight, food intake, water intake, urinary output, urinary pH, glucose, blood ketones and protein, urinary fluoride, urinary osmolality, serum fluoride, serum osmolality, and serum biochemistries were used as indices of toxicity. Methoxyflurane produced a dose-related change in food intake, water intake, urinary osmolality, and serum and urinary fluoride concentrations. It is tentatively concluded that low MOF concentrations for relatively long periods of time are more toxic than high concentrations of MOF for shorter periods of time as measured by food intake, water intake, urinary volume, and urinary osmolality.

135. *Phlorizin Binding to Renal Cortical Tissue as an Indicator of Nephrotoxicity.* J. L. ECKER, J. E. GIBSON, G. K. MOORE and J. B. HOOK, Department of Pharmacology, Michigan State University, East Lansing, Michigan.

Phlorizin is an alkaloid that binds tightly and specifically to the renal transport system for glucose. The purpose of this investigation was to examine the binding of phlorizin to renal cortical tissue from animals administered various nephrotoxic agents.  $^3\text{H}$ -labeled phlorizin was administered to Swiss-Webster mice at doses of 0.5, 2, 8, 32, 64, and 96  $\mu\text{mol}/\text{kg}$  via tail vein. Samples of renal cortical tissue were collected 2 hr later and radioactivity determined. Phlorizin concentrations in plasma and kidney cortex were also determined at various times following 32  $\mu\text{mol}/\text{kg}$  phlorizin iv. In other experiments, phlorizin binding to renal cortical tissue was examined in mice poisoned 24 hr previously with a nephrotoxic dose of paraquat (30 mg/kg ip) or potassium dichromate (40 mg/kg ip). The concentration of phlorizin in renal cortical tissue 2 hr following phlorizin was directly related to the dose injected. Phlorizin concentration in renal cortical tissue 10 min following 32  $\mu\text{mol}/\text{kg}$  phlorizin was 3.7  $\mu\text{mol}/100$  g of cortical tissue. Between 10 and 60 min the phlorizin concentration decreased to 0.8  $\mu\text{mol}/100$  g of cortical tissue and remained constant the next 2 hr. Plasma phlorizin concentrations followed a similar pattern, with an initial rapid decline followed by relatively constant levels between 1 and 3 hr. Following an LD50 dose of paraquat, phlorizin binding to renal cortical tissue was markedly enhanced. Two hours following 32  $\mu\text{mol}/\text{kg}$  iv renal cortical concentration of phlorizin was  $0.46 \pm 0.05$   $\mu\text{mol}/100$  g in control tissue compared to  $1.33 \pm 0.29$   $\mu\text{mol}/100$  g in tissue from paraquat-poisoned mice. After poisoning with potassium dichromate, the 2-hr phlorizin concentration in renal tissue was  $4.60 \pm 1.81$   $\mu\text{mol}/100$  g compared to  $0.48 \pm 0.02$   $\mu\text{mol}/100$  g in control tissue. The binding of phlorizin to mouse renal cortical tissue may serve as a sensitive indicator for the nephrotoxic potential of various agents.

136. *Tetrachlorodibenzo-p-Dioxin Induction of Renal Microsomal Enzyme Systems.* B. A. FOWLER, G. E. R. HOOK and G. W. LUCIER, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. (R. L. Dixon)

The toxicities of many xenobiotics are known to be altered by microsomal drug metabolizing enzyme systems. Knowledge concerning levels of microsomal enzyme activities and the distribution of these enzymes among specific cell types within an organ is essential to an understanding of chemical toxicity. Kidney microsomal drug metabolizing activities are normally low but can be markedly stimulated by administration of tetrachlorodibenzo-*p*-dioxin (TCDD). The current study was designed to measure the level and duration of TCDD induction of renal



microsomal systems and to correlate these changes with ultrastructural changes in kidneys of the same animals. Adult male rats were given a single oral dose of 5  $\mu\text{g}$  2,3,7,8-TCDD by gavage in 0.5 ml acetone-corn oil. Controls were given acetone-corn oil only. Experimental animals plus controls were killed at 18 hr and 3, 7, or 16 days after treatment. Kidneys were excised from each animal and tissue blocks processed for electron microscopy. Renal microsomes were prepared from the same kidneys evaluated by electron microscopy. A massive increase in smooth endoplasmic reticulum (SER) within cells of the pars recta ( $S_3$ ) segments of the proximal tubules was observed in the kidneys of TCDD-treated animals at 3, 7, and 16 days. Renal microsomal benzpyrene hydroxylase activities in these animals was increased 55-fold at 18 hr while that of glucuronyl transferase was doubled. At all other subsequent time points, the activity of benzpyrene hydroxylase was increased 80-fold and that of glucuronyl transferase 4-fold. These findings suggest that TCDD induction of microsomal enzyme activities occurred primarily within a specific segment of the renal proximal tubule.

137. *Toxicology Studies with Cytembena—an Anticancer Drug with a Multispecies Renal Toxic Effect.* E. J. GRALLA, G. L. COLEMAN, G. W. OSBALDISTON and M. KASHGARIAN, Yale University School of Medicine, New Haven, Connecticut.

The toxic effects of cytembena in beagle dogs and rhesus monkeys were investigated with the drug given as single or daily iv injections in doses ranging from 12.5 to 200 mg/kg/day to dogs and 6.25 to 50 mg/kg/day to monkeys. Renal tubular damage was a major drug and dose-related finding in both species and was clinically indicated by an accompanying uremia, elevated serum creatinine, and proteinuria. In the kidney, the primary lesion was cellular necrosis and desquamation of the distal tubular epithelium in animals given the lowest toxic doses. More severe, but similar, histological changes which involved both the proximal and distal tubules were found following lethal or sublethal doses in both species. The early subcellular renal changes produced by this drug were further characterized by single dose studies in mice which demonstrated renal mitochondrial swelling and disruption plus generalized cell swelling as progressive, subcellular developments which were well established by 24-hr posttreatment. Cellular regeneration in the renal tubular epithelium was found in the dogs and monkeys retained 6 wk for posttreatment observation, although functional recovery was inconsistent. A toxic effect to lymphoid tissues was an additional finding which is described.

138. *Air Pollution and Childhood Lower Respiratory Disease: Exposure to Oxides of Nitrogen.* D. I. HAMMER, F. J. MILLER and C. G. HAYES, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina. (D. Coffin)

Previous epidemiologic research associating acute lower respiratory disease with elevated ambient nitrogen dioxide exposure was restricted to a limited age of children and considered neither parental education nor indoor air pollution. Acute lower respiratory disease morbidity was surveyed retrospectively among children aged 1-12 yr by questionnaires completed by their parents in three areas in Chattanooga, Tennessee, representing a gradient for nitrogen dioxide. Response rates were over 90% in all three areas and less than 4% of the records were unusable because of missing information. Lower respiratory disease morbidity decreased with age and was reported more frequently among households with a high school or better education. Rates of "any lower respiratory disease," a combined category, and bronchitis were significantly higher in the High exposure community than in the Low exposure community. In general, rates of "any lower respiratory disease" and bronchitis in the Intermediate exposure community were higher than those in the Low community but did not differ from those of the High exposure community. Rates of pneumonia, croup, and "other" chest infections were similar in all communities. Differences in questionnaire reliability, family size and composition, crowding, parental cigarette smoking habits, or the use of gas stoves or gas space heaters were unlikely explanations for the excess morbidity associated with ambient air pollution. In this study, levels of nitrogen dioxide and total suspended particulate matter, rather than suspended nitrates, were the best pollutant indices of excess acute lower respiratory disease morbidity in children.

139. *Dichromate Nephrotoxicity and Renal Dichromate Accumulation.* W. O. BERNDT, Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, Mississippi. (R. P. Smith)

Precise mechanistic explanations for chromium nephrotoxicity are lacking. The present report constitutes a part of a long-range study on this problem. These studies have employed renal slice techniques and have been directed at an understanding of the effects of the chromium ion on various renal transport processes. This ion has been shown to disrupt certain renal transport functions, while having little effect on others. In addition the  $^{51}\text{Cr}$ -labeled compound distributes to renal tissue when injected as dichromate into the rat. In addition, the transport of chromium (chromate or dichromate) by renal cortex slices has been investigated using the  $^{51}\text{Cr}$ -labeled material, and marked accumulation under in vitro conditions has been noted. The  $^{51}\text{Cr}$  label (added as dichromate to an in vitro buffer system) often was accumulated to slice:medium ratios in excess of 80 by 120 min of incubation. The uptake was significantly reduced by iodoacetamide, but not by dinitrophenol or azide. In addition, significant reductions in uptake were noted when either lactate or succinate was present in the bathing solution. Neither acetate nor glucose had any effect. Because the predominant ion species under these in vitro conditions is probably chromate, and relatively little effect on renal transport processes is noted, it is likely that the effect seen in vivo when dichromate is injected is due to the dichromate species.

140. *On the Metabolism of the Carcinogen 1,2-Dimethylhydrazine in Rats.* E. S. FIALA and J. H. WEISBURGER, American Health Foundation, New York, New York.

1,2-Dimethylhydrazine (DMH) is a powerful carcinogen with a high degree of specificity for the large intestine. In order to elucidate its mechanism of action, we undertook to study its metabolism in 7- to 9-wk-old male Fischer rats. Commercially synthesized [ $^{14}\text{C}$ ]DMH was purified and separated from its major (21%) contaminant, [ $^{14}\text{C}$ ]monomethylhydrazine (MMH- $^{14}\text{C}$ ), by preparative thin-layer chromatography on Avicel plates using 2-propanol:HCl:H<sub>2</sub>O (13:3:4, v/v). Radiochemically homogeneous [ $^{14}\text{C}$ ]DMH was administered sc at 21 mg (free base)/kg and excretion of metabolites in the bile, urine, feces, and the expired air was followed over a 24-hr period. At least six different metabolites including [ $^{14}\text{C}$ ]azoxymethane (AOM- $^{14}\text{C}$ ) were detected in the bile by Sephadex LH-20 chromatography. While biliary excretion accounted for only 0.7% of the dose, a significant increase was found after pretreatment of the animals with unlabeled DMH. AOM- $^{14}\text{C}$ , DMH- $^{14}\text{C}$ , and MMH- $^{14}\text{C}$  were found in the urine (12–15% of dose). Fecal excretion accounted for 0.6–0.9%; 45–60% of the dose radioactivity was found in the expired air in the form of  $^{14}\text{CO}_2$  (5–15%) and the basic metabolites MMH- $^{14}\text{C}$ , [ $^{14}\text{C}$ ]methylamine (trapped in dilute H<sub>2</sub>SO<sub>4</sub>) and  $^{14}\text{CH}_2\text{O}$  (identified as its Dime-done derivative). After 24 hr, the greatest residual  $^{14}\text{C}$ -activity was found in the liver (1.6%). The kidneys contained 0.17–0.19% and the colon 0.07–0.09%. These results indicate that the systemic as well as the biliary route may be operative in the mechanisms responsible for the organospecificity of DMH. (Supported by PHS Research Grant CA-15400 from the National Cancer Institute.)

141. *Interactions of Drugs with [ $^{14}\text{C}$ ]Isoniazid Metabolism in the Rat.* B. H. THOMAS and G. SOLOMONRAJ, Drug Research Laboratories, Health Protection Branch, Health and Welfare Canada, Ottawa, Canada. (B. B. Coldwell)

There have been several reports of isoniazid causing hepatotoxicity in humans. This study in rats was intended to investigate possible drug interactions with isoniazid metabolism that would affect its toxicity. [ $^{14}\text{C}$ ]isoniazid was administered orally at a dose of 20 mg/kg. The following interacting drugs were studied: acetylsalicylic acid (ASA, 100 mg/kg, po), phenobarbital (3 days at 40 mg/kg, ip), ethambutol (100 mg/kg, po), rifampin (30 mg/kg, po), and ethanol (3 g/kg, po). Tail blood and urine were collected and estimated for  $^{14}\text{C}$ . Urine was also subjected to paper chromatography in order to determine the various metabolites. Compared to controls ASA, ethambutol and ethanol caused lower blood concentrations of  $^{14}\text{C}$  during the absorptive phase, suggesting delayed isoniazid absorption. Rifampin and ethanol slightly increased the  $^{14}\text{C}$

activity during the postabsorptive phase but none of the treatments had a significant effect on the excretion of  $^{14}\text{C}$  in urine. Isonicotinic acid and occasionally isonicotinoyl glycine excretion was lower in rats given ethambutol, rifampin, and ethanol but *N*-acetylisoniazid tended to be higher. Phenobarbital pretreatment had little effect on any of the parameters studied. Serum transaminases were measured following several of the treatments but in no case were the values outside the normal range. In general the changes observed in this study were small with little evidence of potentially toxic interactions.

142. *The Effect of Acetylsalicylic Acid on the Distribution and Metabolism of [ $^{14}\text{C}$ ]Acetaminophen in Mice.* L. W. WHITEHOUSE, C. J. PAUL and B. H. THOMAS, Drug Research Laboratories, Health Protection Branch, Health and Welfare Canada, Ottawa, Canada. (B. B. Coldwell)

Large doses of acetaminophen have been responsible for a number of human fatalities attributable to hepatic necrosis. A metabolite of acetaminophen, which is believed to be detoxified by conjugation with glutathione, has been alleged to be the causal agent. The glutathione conjugate, following further metabolism, is excreted in the urine as either the cysteine or mercapturate conjugate. The objective of this study was to determine if acetylsalicylic acid (ASA) changed the tissue distribution and urinary metabolite profile of acetaminophen. Male mice were divided into two groups. The treated group (AA') received ASA (200 mg/kg po) 30 min prior to [ $^{14}\text{C}$ ]acetaminophen (150 mg/kg po) while the control group (A') was pretreated with vehicle followed by [ $^{14}\text{C}$ ]acetaminophen. Animals were killed at 0.25, 0.50, 1.0, 2.0, and 4.0 hr and blood, heart, kidney, and liver tissues collected. Urines were collected at 1.5, 4, and 8 hr. Urinary metabolites of [ $^{14}\text{C}$ ]acetaminophen were separated by paper and Sephadex G-10 chromatography and quantitated by liquid scintillation counting. At 0.25 and 0.50 hr tissues from the AA' group tended to possess lower concentrations of  $^{14}\text{C}$  than control tissues, whereas at 2 hr the trend had reversed. Determination of urinary metabolites indicated that ASA reduced sulfate conjugation but increased glucuronidation and cysteine conjugation. From this investigation it was concluded that ASA could possibly have a protective effect against acetaminophen-induced hepatic necrosis.

143. *Metabolism of 2,4-Toluenediamine in the Rat.* P. H. GRANTHAM, T. GLINSUKON, L. C. MOHAN, T. BENJAMIN, P. P. ROLLER, F. E. MITCHELL and E. K. WEISBURGER, Carcinogen Metabolism and Toxicology Branch, National Cancer Institute, Bethesda, Maryland.

2,4-Toluenediamine, a polyurethane intermediate, has induced hepatocellular carcinomas in rats. During a metabolism study with  $^{14}\text{C}$ -labeled 2,4-toluenediamine in male Fischer rats, the excretion was followed up to 120 hr after an ip dose. Within 24 hr 81% of the  $^{14}\text{C}$  was recovered (73% in urine, 8% in feces). By 120 hr, 98.6% was excreted (76.4% in urine, 22.2% in feces). Fractionation of urinary metabolites revealed that 21% of the dose was unconjugated metabolites; 7.5% glucuronic acid conjugates; 10% acid-hydrolyzable conjugates; and 30% water-soluble metabolites. The major unconjugated metabolites were identified by mass spectrometry, tlc and glc, as 4-acetylamino-2-aminotoluene 5.2% of dose; 2,4-diacetylamino-toluene 1.4%, and 4-acetylamino-2-aminobenzoic acid 4.4%. The glucuronide fraction contained four major metabolites, one of which has been identified as 4-acetylamino-2-aminobenzoic acid. The radioactivity in blood and plasma reached a peak 1 hr after an ip dose of 2,4-toluenediamine, decreased rapidly through 7 hr and gradually through 48 hr. Tissue concentrations were greatest in kidney and liver, and much less in spleen, heart, testis, and brain. Radioactivity was bound to nuclear DNA, to RNA, and to microsomal and soluble protein of liver.

144. *Metabolism and Biological Disposition of Butyl Alcohols in the Rat.* D. BECHTEL and H. CORNISH, University of Michigan, School of Public Health, Ann Arbor, Michigan.

It is known that pretreatment of rats with *sec*- and *t*-butanol results in potentiation of the hepatotoxicity of  $\text{CCl}_4$  as measured by increases of SGOT. *n*- and *iso*-butanol do not seem to alter the toxicity of  $\text{CCl}_4$ . We have shown *sec*- and *t*-butanol to be effective inducers of hepatic

microsomal enzyme activity at an oral dose of 500 mg/kg. *n*- and *iso*-butanol have little effect on microsomal enzyme activity. The present study was undertaken to determine the tissue distribution of orally administered butanols and to investigate the relationship between metabolism of the butanols and their effect on microsomal enzyme activity. After an oral dose of 500 mg/kg, all of the butanols reached peak blood concentrations in 45–50 min with peak serum concentrations of: *n*-BuOH, 240 ppm; *iso*-BuOH, 35 ppm; *sec*-BuOH, 260 ppm; and *t*-BuOH, 450 ppm. Except for *t*-BuOH, which was still detectable 24 hr later, the butanols had disappeared from the blood in 2–3 hr after administration. *n*-Butanol gave rise to detectable concentrations of *n*-butylaldehyde; *iso*-butanol produced *iso*-butylaldehyde, *iso*-butyric acid, and traces of acetone; *sec*-butanol gave rise to butanone which was still detectable 24 hr later. No metabolites of *t*-butanol were found in the blood. Both *sec*- and *t*-butanol were partially excreted as glucuronides in the urine. The major portion of the *t*-butanol dose was exhaled unchanged and a large portion of the *sec*-butanol dose, in the form of butanone, was also exhaled. Inhibition of alcohol dehydrogenase with pyrazole caused a tenfold increase in the peak blood concentrations of *iso*-BuOH, and also significant increases in the peak blood concentrations, and persistence of *n*- and *sec*-BuOH. The butanols all seemed to be distributed in the tissues according to water content. This study seems to show a correlation between the mode of metabolism and biologic persistence of the butanols and their differential ability to affect hepatic microsomal enzyme activity. (Supported by Environmental Health Science Research Training Grant 5 TO1 ES 00138-4.)

145. *Excretion and Metabolism of Three [2-6-<sup>14</sup>C]Omadines® Following Intravenous Injection in Female Yorkshire Pigs.* M. ADAMS, J. H. WEDIG, R. JORDAN, L. SMITH, R. HENDERSON and J. BORZELLECA, Medical College of Virginia, Richmond, Virginia; Olin Research Center, New Haven, Connecticut.

A single dose of zinc pyridine-2-thiol-1-oxide (zinc OMADINE®, 5 mg/kg oil-in-water emulsion), sodium pyridine-2-thiol-1-oxide (sodium OMADINE®, 50 mg/kg, aqueous solution), or magnesium sulfate adduct of 2,2'-dithio-bis-pyridine-1-oxide (OMADINE® MDS, 4 mg/kg, aqueous solution), which included 0.5 mCi/100 kg of 2-6-<sup>14</sup>C-labeled material, was injected via a jugular vein cannula into swine in order to study the half-life in plasma, the urinary excretion rate, and the metabolism of these antimicrobial and antifungal agents during a 96-hr study period. Following intravenous administration, all of these agents produced cholinergic manifestations of toxicity including both parasympathetic and somatomotor actions. The initial half-life in plasma approximated 2.80, 2.70, and 2.03 hr for sodium OMADINE®, zinc OMADINE®, and OMADINE® MDS, respectively. The decline of total counts in plasma was represented in each case by a biphasic exponential function. The urinary clearance, in terms of the percentage of the total dose 96 hr post injection, was 94.9 for sodium OMADINE®, 56.3 for OMADINE® MDS, and 54.4 for zinc OMADINE®. The majority of total radioactivity, administered intravenously, was recovered in the urine within the first 24 hr. The principal urinary metabolite of sodium OMADINE® appeared to be OMADINE® disulfide whereas the primary urinary metabolite of both OMADINE® MDS and zinc OMADINE® appeared to be 2-(pyridyl-*n*-oxide) sulfonic acid.

146. *The Urinary and Fecal Metabolites of a Polychlorinated Biphenyl (Aroclor 1016) Excreted by the Rat.* C. C. SMITH, K. LERTRATANANGKOOM, M. G. HORNING and W. W. WEIGEL, Department of Environmental Health, College of Medicine, University of Cincinnati, Cincinnati, Ohio, and The Institute for Lipid Research, Baylor College of Medicine, Texas Medical Center, Houston, Texas.

The urinary and fecal metabolites excreted by the rat after administration of one of the mixtures of polychlorinated biphenyl (Aroclor 1016) have been investigated using tlc, GC, and GC-MS-CIM methods. The urinary metabolites were extracted with ether after acid hydrolysis and separated by tlc into three fractions. Analyses of the fractions (GC-MS) using a quadrupole mass spectrophotometer operated in the chemical ionization mode indicated the presence of a

number of compounds including di-, tri-, and tetrachlorinated biphenyls and monohydroxylated metabolites of dichloro- (M/E 310, TMS), trichloro- (M/E 344, TMS), and tetrachlorobiphenyls (M/E 378, TMS). GC-MS analyses were carried out on two fractions obtained by tlc of fecal extracts. Fraction A contained unchanged di-, tri-, and tetrachlorobiphenyls. Fraction B contained monohydroxylated metabolites of tri-, tetra-, and pentachlorobiphenyls. In addition, metabolites with molecular weights of 389 and 423 were present in significant amounts. These nitrogen-containing metabolites were not present in urinary extracts and were probably formed by the action of microorganisms in the gut. (Supported in part by USPHS Grant 5 010 ES00159, Center for Study of the Human Environment.)

147. *Effects of Pregnancy and Certain Steroids on Demethylation and N-Oxidation by Rabbit Lung Microsomes.* T. R. DEVEREUX and J. R. FOUTS, Pharmacology Branch, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North Carolina.

Rates of lung microsomal dimethylaniline (DMA) demethylation and *N*-oxidation as well as cytochrome P-450 concentrations from 20- and 28-day pregnant rabbits were observed to be as much as two times the values measured in lung microsomes from adult female nonpregnant rabbits. These increases were not seen in lung microsomes from 10-day pregnant rabbits, and only small increases were observed in lung microsomes from 15-day pregnant rabbits. In contrast, no differences were seen in hepatic microsomal DMA demethylase or *N*-oxidase activities at any stage of pregnancy. An attempt was made to correlate high plasma concentrations of steroids during pregnancy with the elevated enzyme activities observed in lung microsomes. Adult nonpregnant rabbits were treated with a variety of steroids that have been shown to increase in plasma during late pregnancy. No effect on lung or liver microsomal DMA metabolism was observed when rabbits were treated with testosterone propionate. However, following animal pretreatment with the glucocorticoids, dexamethasone and hydrocortisone, there was a 40-60% increase in DMA demethylase and *N*-oxidase activities in liver and lung microsomes from adult male and nonpregnant female rabbits. Following pretreatment of adult female nonpregnant rabbits with the mineralocorticoid, deoxycorticosterone, an increase of 50% in rate of microsomal DMA metabolism in lung but not in liver was observed.

148. *Effects of Piperonyl Butoxide and Diethyl Maleate on Toxicity and Metabolism of Parathion and Methyl Parathion.* F. E. MIRER, B. S. LEVINE and S. D. MURPHY, Department of Physiology, Harvard School of Public Health, Boston, Massachusetts.

Pretreatment with piperonyl butoxide (PB), 400 mg/kg, 1 hr prior to challenge, markedly protected mice against methyl parathion (MPS) toxicity while slightly potentiating the effects of parathion (PS). Activation to paraoxon or methyl paraoxon and oxidative degradation may have been inhibited by PB while detoxification of MPS but not PS proceeded by a glutathione-alkyl transferase pathway (Kamienski and Murphy, *Toxicol. Appl. Pharmacol.* **18**, 883, 1971). We confirmed the effect of PB on MPS (LD50 > 325 mg/kg, ip, in PB-pretreated mice vs 8.2 mg/kg in control) and PS toxicity. Pretreatment with diethyl maleate (DEM), 1 mg/kg, 1 hr before challenge, depleted liver glutathione and potentiated PS and MPS toxicity. In vivo, DEM potentiated inhibition of brain cholinesterase by PS and MPS, while PB potentiated inhibition by PS and protected against inhibition by MPS. To compare metabolism of PS and MPS in vivo, plasma and brain pesticide concentrations were measured after ip challenge of pretreated mice. PB increased brain concentrations of PS 7-fold and MPS 11-fold, and plasma concentrations of MPS and PS 5-fold above control. DEM caused a 2-fold increase in brain concentrations of PS and MPS. PS and MPS activation were measured in incubations with fortified, liver homogenates from pretreated animals. PB reduced both activation and degradation of both MPS and PS. DEM caused a large increase in activation of MPS to methyl paraoxon and decreased total degradation. DEM inhibited both activation and degradation of PS. Although the in vitro studies with DEM-pretreated mice support the hypothesis that an active dealkylation pathway for detoxification of MPS exists, in vivo studies do not support this mechanism for the divergent effect of PB pretreatment on MPS and PS toxicity. (Supported by RG ES-00084 and ES-00002 from NIEHS.)

149. *The Influence of Maturation on the Acute Toxicity of Nineteen Drugs in Rats.* D. L. HANE and W. R. POOL, Department of Experimental Pathology and Toxicology, Hoffmann-La Roche, Inc., Nutley, New Jersey.

The acute toxicities of 19 drugs were determined in rats at intervals from day 1 through day 40 after parturition. The drugs were selected to provide a survey of the relationship of different pharmacological activities to their corresponding lethal effects. LD<sub>50</sub> values were determined for each drug by both the oral and intraperitoneal route of administration. Most of the 19 drugs produced their greatest lethality to 1-day-old rats and became increasingly less lethal as the animals matured. At 25 days, the rats were most resistant to the lethal effect of the drugs. However, one drug, strychnine, was least lethal in 1-day-old rats and became more lethal with the maturation of the test animals. In general, the pattern of lethality during maturation was similar for both the oral and intraperitoneal routes of administration. However, the decrease in lethality during maturation was greater by the oral route than by the intraperitoneal route. Little or no correlation could be found between drugs with similar pharmacological activity and their corresponding patterns of toxicity.

150. *A Tumorigenic Evaluation of Aspartame, a New Sweetener, in the Mouse.* F. E. RENO, R. G. MCCONNELL, J. F. FERRELL, J. A. TRUTTER and K. S. RAO, Hazleton Laboratories, Inc., Vienna, Virginia; Searle Laboratories, Chicago, Illinois; and Experimental Pathology Laboratories, Herndon, Virginia.

As part of an extensive safety evaluation program, aspartame (L-aspartyl-L-phenylalanine methyl ester), a new sweetener, was evaluated in this study for its tumorigenic potential in the ICR Swiss mouse following dietary administration of levels of 1, 2, and 4 g/kg/day for 104 wk; a concurrent control group received only the basal diet. Conventional physical, ophthalmoscopic, and clinical pathologic examinations were conducted. Histopathologic examination was performed on all unusual or usual gross lesions from all animals at each treatment level. Additionally, microscopic examination was conducted on 20-27 grossly unremarkable organs from all control and high level animals, and from roughly two-thirds and one-third of the animals at the intermediate and low treatment levels, respectively. Multiple sections of brain and urinary bladder were examined from all animals. Histopathological examination revealed no evidence of any treatment related non-neoplastic changes in any organ or tissue. Tumor data were analyzed by sex employing an actuarial (Life-Table) technique, a method based upon the number of histologically proven tumors and the numbers of animals actually at risk. In no instance did the analysis reveal a tumor incidence in either sex at any treatment level to be significantly higher than that of the controls. Thus, aspartame, administered to the mouse for 104 wk in the diet at levels up to 4 g/kg/day produced no convincing evidence of an effect with respect to the incidence of neoplasms.

151. *2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD): Results of a 13-Week Oral Toxicity Study in Rats.* R. J. KOCIBA, P. A. KEELER, C. N. PARK and P. J. GEHRING, Toxicology Research Laboratory, Health and Environmental Research, Dow Chemical U.S.A., Midland, Michigan.

TCDD is highly toxic and occurs as an unwanted contaminant in the herbicide 2,4,5-trichloro-phenoxyacetic acid. A study was conducted to assess the biologic response of rats given 0, 0.001, 0.01, 0.1, or 1.0 µg TCDD/kg 5 days/wk for 13 wk. Doses of 1.0 µg TCDD/kg/day caused mortality, inactivity, decreased body weights and food consumption, icterus, increased serum bilirubin and alkaline phosphatase, degenerative and inflammatory changes in the liver, lymphoid depletion of the thymus and other lymphoid organs, increased urinary excretion of porphyrins and delta-aminolevulinic acid, minimal alterations of some hematopoietic components, and morphological evidence of a functional suppression of the reproductive organs. Doses of 0.1 µg TCDD/kg/day caused lesser degrees of some of the effects seen in the high dose group of rats. In rats given 0.01 or 0.001 µg TCDD/kg/day, all parameters were essentially unaffected, except for a slight increase in the mean liver-to-body weight ratio in rats given 0.01 µg

TCDD/kg/day. This slight increase in relative liver weight was not considered of any toxicological significance. These data indicate that no discernible ill effects occurred in rats given 0.01 or 0.001  $\mu\text{g}$  TCDD/kg 5 days/wk for 13 wk.

152. *The Fate of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) Following Single or Repeated Oral Doses to Rats.* J. Q. ROSE, J. C. RAMSEY, T. H. WENTZLER, R. A. HUMMEL and P. J. GEHRING, The Dow Chemical Company, Midland, Michigan.

2,3,7,8-Tetrachlorodibenzo-p-dioxin is a highly toxic compound formed as an unwanted contaminant in the synthesis of 2,4,5-trichlorophenol and found in some samples of the herbicide, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Following a single oral dose of 1  $\mu\text{g}$  [ $^{14}\text{C}$ ]TCDD/kg,  $^{14}\text{C}$  activity was detected only in feces and the half-life of  $^{14}\text{C}$  activity in the body was 30.4 days. Twenty-two days after the single oral dose, liver and fat contained concentrations of  $^{14}\text{C}$  activity 10 times greater than any other tissue examined. Following repeated oral doses of 1.0, 0.1, or 0.01  $\mu\text{g}$  [ $^{14}\text{C}$ ]TCDD/kg/day Monday through Friday for 7 wk, the major route of  $^{14}\text{C}$  excretion was via the feces with small amounts in the urine. The half-life of  $^{14}\text{C}$  activity upon repeated administration was 23 days. Assuming a one compartment open model, 73.8% of steady state levels of  $^{14}\text{C}$  activity was achieved in the whole body after 7 wk administration. As concentrations in the tissues approached steady state, concentrations of  $^{14}\text{C}$  activity in liver on day 49 were 3.5 times greater than that of fat and 27 times greater than those concentrations achieved in kidney, thymus and spleen. The  $^{14}\text{C}$  activity in the liver was identified as TCDD by gas chromatography-mass spectrometry and could be quantitatively extracted with organic solvents. The results of this study indicate that TCDD in the body reaches essentially steady state levels after 90 days of repeated daily exposure and the rate at which steady state levels are achieved is independent of the administered dose for the 1.0-0.01  $\mu\text{g}/\text{kg}/\text{day}$  dose range.

153. *The Dose-Dependent Fate of 1,4-Dioxane in Male Rats.* J. D. YOUNG and P. J. GEHRING, The Dow Chemical Company, Midland, Michigan.

Studies on the toxicity of 1,4-dioxane suggest that its fate in rats may depend on the dose administered. In order to determine whether dioxane at low doses poses a hazard which is proportional to the dosage, the pharmacokinetics of single oral doses of 10, 100, or 1000 mg/kg [ $^{14}\text{C}$ ]dioxane has been evaluated in rats maintained in glass metabolism cages. Samples of plasma, urine, feces, expired  $\text{CO}_2$ , and volatile organics in expired air were collected and analyzed as a function of time. The area under the plasma concentration-time plot increased from 40 to 19,200  $\mu\text{g}\cdot\text{hr}/\text{g}$  when the dose was increased from 10 to 1000 mg/kg; i.e., the area increased almost fivefold more than the dose. Even more dramatic was the disproportional increase in the excretion of unchanged dioxane in the expired air as a function of the dose. After a 10-mg/kg dose, 43  $\mu\text{g}/\text{kg}$  were excreted in the expired air, while after a 1000-mg/kg dose the excretion rose to 252,500  $\mu\text{g}/\text{kg}$ , an increase 59-fold greater than the increase in dosage. The percentage of  $^{14}\text{C}$  activity excreted in the urine decreased with increasing dosage. A plot of urinary excretion vs time was nonlinear for the 100- and 1000-mg/kg doses, but was linear with about a 2-hr half-life for the 10-mg/kg dose. Therefore, because of the longer half-lives and altered excretion routes after high dosages, these studies strongly suggest that the hazard from a high dose of dioxane may be disproportionately greater than from a lower dose.

154. *The Effect of Chlorination on the Distribution and Excretion of Polychlorinated Biphenyls.* H. B. MATTHEWS and M. W. ANDERSON, Pharmacology Branch, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North Carolina. (R. T. Drew)

The distribution and excretion of four polychlorinated biphenyl (PCB) isomers, 4-chloro-, 4,4'-dichloro-, 2,4,5,2',5'-pentachloro-, and 2,4,5,2',4',5'-hexachlorobiphenyl, were studied in the rat. The concentration of each of these isomers was determined in major organs and tissues at times varying from 10 min up to 42 days after an intravenous injection of a single 0.6-mg/kg dose. Each of the isomers was rapidly removed from the blood and initially stored largely in the

liver and muscle. The subsequent redistribution of the PCB isomers to the adipose tissue and skin and/or elimination in urine could be related to the degree of chlorination. The peak concentrations of mono- and dichlorobiphenyl in adipose tissue occurred in less than 2 hr and then decayed to less than 2% of the total dose within 7 days. For the pentachlorobiphenyl isomer, the peak concentration in adipose tissue reached approximately 25% of the total administered dose at about 24 hr and decayed biphasically to approximately 4% of the total dose after 42 days. The accumulation of hexachlorobiphenyl in adipose tissue reached a peak value in excess of 60% of the total administered dose at 14 days and this had not decreased appreciably by the end of the 42-day holding period. The accumulation pattern in skin was somewhat similar to that in adipose tissue except that the biphasic decay was much more obvious. The rate of PCB excretion in urine was inversely correlated with the degree of chlorination: 55, 33, 7, and 1% of the total dose for mono-, di-, penta-, and hexachlorobiphenyl, respectively. The remaining portion of the administered mono- and dichlorobiphenyl was excreted in the feces within 7 days. Approximately 90% of the pentachlorobiphenyl dose was excreted within 42 days, whereas only 10% of the hexachlorobiphenyl dose was excreted during the same period. These studies indicate that if an animal can metabolize a PCB isomer, the half-life of that isomer will be a matter of days. However, if a PCB isomer can not be readily metabolized, then long-term storage in the adipose tissue and skin is most probable.

155. *Failure of Thioctic Acid to Cure Mushroom-Poisoned Mice and Dogs.* F. R. ALLEVA, T. BALAZS, A. O. SAGER and A. K. DONE, Food and Drug Administration, Washington, D.C.

We tested thioctic acid (TA) as an antidote for mushroom poisoning in mice and dogs. Dried mushroom (M), *Amanita phalloides*, was suspended in 1% methyl cellulose and given orally to fasted mice at a dose of 4 g/kg. TA was dissolved daily in ethylenediamine, ethanol, and water (pH = 8.2, ethanol = 0.84%) and the solution was kept in a dark glass bottle. The solution was injected ip in doses of 0 (solvent), 0.125, 0.5, 2, or 8 mg/kg hourly 6x daily for 4 days beginning 1 day after M; 16 of 30 controls given M and TA solvent died, compared with 9/10, 8/10, 6/10, and 14/20 mice given M and TA. A dose of 12.5 mg/kg of TA given twice daily for 4 days beginning 1 hr after M also failed to prevent death. Ten fasted beagles were given a lethal dose of 37.5 mg/kg of M orally in gelatin capsules. On the next day, five of the ten received TA, dissolved as described above, at a dose of 1.5 mg/kg (0.3 ml/kg) iv with 5% dextrose in saline (1 ml/kg) hourly 6x daily while the remaining five dogs received only TA solvent and dextrose. By the third day after M, all five controls and four of the five dogs given TA and dextrose died. Emesis, diarrhea, and convulsions preceded death. Blood samples were obtained each morning before injection of TA and dextrose. Serum activities of hepatic enzymes and bilirubin concentrations were very high. Serum concentrations of glucose were very low, in spite of the dextrose injections of the preceding day. Another experiment was conducted to determine whether TA cures M-induced liver damage. Eight fasted beagles received orally a nonlethal but hepatotoxic dose of 21.5 mg/kg of M. On the next day, four of them received 1 mg/kg of TA iv with dextrose hourly 9x daily for 2 days and 2 mg/kg of TA with dextrose 9x daily for the next 5 or 6 days. The remaining four dogs received only TA solvent and dextrose. TA and dextrose had no effect on M-induced elevation of hepatic test values. These experiments revealed no antidotal effect of TA in mushroom-poisoned mice or of TA and dextrose in M-poisoned dogs.

156. *Haloperidol and Propranolol in the Treatment of Acute Amphetamine Intoxication in the Dog.* J. D. CATRAVAS, I. W. WATERS, G. A. BURDOCK and W. M. DAVIS, Department of Pharmacology, School of Pharmacy, University of Mississippi, University, Mississippi

A lethal intravenous dose of *d*-amphetamine sulfate (10 mg/kg) was administered to unanesthetized mongrel dogs. Six stages of toxicity were observed, similar to those described by Zalis *et al.* (*J. Pharmacol. Exp. Ther.* **158**, 115-127, 1967). Amphetamine produced increases in body temperature, systemic arterial systolic and diastolic pressures, left ventricular pressure, mean right atrial pressure, heart rate, cardiac output, stroke volume, total peripheral resistance, oxygen uptake, respiratory rate, tidal volume, minute volume, blood lactate and pyruvate,



plasma urea nitrogen, plasma glutamic oxaloacetic transaminase and glutamic pyruvic transaminase, and a decrease in plasma glucose. The mean survival time was 2 hr. The lethal actions of amphetamine were challenged in a second group of conscious dogs by the intravenous administration of 1 mg/kg haloperidol or 0.5–6.0 mg/kg propranolol at the beginning of stage two. All haloperidol-treated animals survived and their physiological variables remained normal; they were kept under observation for an additional 48 hr. Propranolol showed no appreciable protection and did not change the mean amphetamine survival time. The results indicate a definite protective role of haloperidol against amphetamine lethality and allow an optimistic view for its clinical use in acute intoxication. (Supported by USPHS Grant no. DA 00723-01 from the National Institute on Drug Abuse and in part by the Research Institute of Pharmaceutical Sciences, University of Mississippi.)

157. *The Acute Toxicity of Dimethylnitrosamine*. M. E. ANDERSEN and L. J. JENKINS, JR., U.S. Navy Toxicology Unit, Naval Medical Research and Development Command, Bethesda, Maryland.

Dimethylnitrosamine ( $C_2H_6N_2O$ ) is an intermediate in the synthesis of 1,1-dimethylhydrazine. The carcinogenicity of dimethylnitrosamine, however, has precipitated a search for alternative synthetic procedures. One procedure that has been examined involves the hydrogenation of dimethylnitrosamine ( $C_2H_6N_2O_2$ ). We are now in the process of investigating the acute toxicity of dimethylnitrosamine in the rat. The oral and intraperitoneal LD50 values with their 95% confidence limits were, respectively, 1095 (978–1227) mg/kg and 897 (861–934) mg/kg. These are approximately 20 times higher than the corresponding values for the nitrosamine. Intravenous doses above 700 mg/kg were always fatal to juvenile male rats and doses below 600 mg/kg were never fatal. By all routes of administration the toxic signs were similar. Within minutes after injection rats became ataxic and lethargic but returned to normal within several hours. They were stable for a day or two, then became progressively more lethargic, prostrate for 12–24 hr and died. These signs are similar to those seen with nitrosamine. The majority of nitrosamine-treated rats showed signs of bleeding from the mouth and anus and had areas of hemorrhage within the intestines. Thus the nitrosamine and the nitroamine have different orders of toxicity but produce similar toxic signs following acute administration. In order to determine if this difference in degree of toxicity is due to a different mechanism of toxic action, studies are now in progress to evaluate the histopathologic and biochemical effects of nitroamine and the effect of liver microsomal enzyme effectors on nitroamine toxicity.

158. *Mutagenic Evaluation of Hexachlorophene*. D. W. ARNOLD, G. L. KENNEDY, JR. and M. L. KEPLINGER, Industrial Bio-Test Laboratories, Inc., Northbrook, Illinois.

The possible mutagenic effects of hexachlorophene (HCP) were evaluated with two test systems: the dominant lethal study in mice and host-mediated assay in rats with a histidine auxotroph of *Salmonella typhimurium* (his G46). HCP at dosages of 2.5 or 5.0 mg/kg, administered intraperitoneally as single doses, failed to induce dominant lethal mutagenic changes in the germinal cells of male albino mice. Methyl methanesulfonate at a dose of 100 mg/kg was mutagenic as evidenced by marked increases in early embryonic deaths in utero among female mice bred with treated males during the first 2 wk following treatment. Reversions among histidine-dependent *Salmonella* to their prototrophic form were not induced during a residence time of 3 hr in the peritoneal cavities of male albino rats that had been pretreated for 90 days with HCP at dietary levels of 100 or 200 ppm. Dimethylnitrosamine administered as a single intramuscular injection of 100 mg/kg simultaneously with the bacterial inoculation, was demonstrated to be mutagenic in this system.

159. *Strain-Specific Metabolic Differences in Mice and Their Effect on Mutagen Production from Dimethylnitrosamine in In Vitro Assays*. D. J. BRUSICK, U. WEEKES and D. R. JAGANNATH, Litton Bionetics, Inc., Kensington, Maryland.

The toxic and carcinogenic properties of dimethylnitrosamine metabolites in various rodent species is well documented. Dimethylnitrosamine has also been shown to be activated by

microsomal enzymes to a genetically active intermediate. Using the rate and degree of conversion of dimethylnitrosamine to its genetically active metabolite, *in vitro*, as an indication of dealkylase enzyme activity, we have compared microsomal fractions from various organs of inbred mouse strains possessing variable susceptibilities to the neoplastic properties of dimethylnitrosamine. The results of this study indicate that organs susceptible to the carcinogenic and toxic effects of dimethylnitrosamine are highly active in metabolic activation of this chemical whereas refractile organs possess little or no activating capacities. It was also demonstrated that among mouse strains which are susceptible to dimethylnitrosamine-induced tumors the rate and level of activation for a given organ is related to the *in vivo* tumor induction frequency of the particular strain. Thus it appears that a single active metabolite of dimethylnitrosamine has toxic, carcinogenic and mutagenic properties which tends to support the hypothesis that common steps are involved in all three phenomena.

160. *The Relationship of 2-Acetamidofluorene Mutagenicity in Plate Tests with its In Vivo Liver Cell Component Distribution and its Carcinogenic Potential.* D. MCGREGOR, Section for Mutagen, Teratogen, and Carcinogen Testing, Inveresk Research International, Inveresk Gate, Musselburgh, Midlothian, Scotland. (G. Edward Paget)

Using a plating technique, the mutagenic potentials of 2-acetamidofluorene (AAF) and *N*-hydroxy-AAF were examined after metabolic activation by liver preparations from different animals. Animals used were: male and female rats; male rats treated with methylcholanthrene (MC); male rats treated with AAF; hamsters; guinea pigs; cotton rats; and baboons. Irrespective of the animal susceptibility to AAF carcinogenesis, mutation frequency was always increased in the *Salmonella typhimurium* TA 1538 tester strain. Indeed, the greatest response was found in the presence of liver from cotton rats, a species which is resistant to AAF-induced carcinogenesis. A much better correlation is found between carcinogen binding to cell constituents and carcinogenesis, at least in those species from which information is available. The difficulty which this new information poses for the interpretation of plate tests is discussed.

161. *LD50 Determination and Hypotensive Effect of 3-Methyl-2-Benzothiazolone Hydrazone.* M. A. GREEN and J. L. EGGLE, JR., Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia. (J. F. Borzelleca)

3-Methyl-2-benzothiazolone hydrazone (MBTH) is a compound employed in the colorimetric determination of aliphatic aldehydes. LD50 values based on ip injections of MBTH in rats and mice were found to be 139 mg/kg and 127 mg/kg, respectively. Dose-response studies in the rat indicated that iv injection of MBTH produces a brief fall in mean arterial blood pressure. A maximum decrease in blood pressure of 40-60% was seen following an iv dose of 40 mg/kg. Subsequent 40 mg/kg iv doses 20 min after the initial dose caused an equivalent decline in mean arterial blood pressure. Experiments were carried out with pharmacologic blocking drugs to determine the mechanism of the depressor response. The hypotensive effect was not blocked by atropine, propranolol, or pyribenzamine. This indicates that the hypotensive effect of MBTH is not mediated through stimulation of cholinergic, beta adrenergic, or histaminergic receptors.

162. *Interactions of Tricyclic Antidepressants and Pentobarbital in Pentobarbital-Tolerant and Nontolerant Rats.* I. W. WATERS and S. J. LIU, Department of Pharmacology, School of Pharmacy, University of Mississippi, University, Mississippi and Wood Veterans Administration Hospital, Wood, Wisconsin. (W. M. Davis)

Pretreatment of rats with imipramine (IP), desipramine (DI), amitriptyline (AT), or nortriptyline (NT) at doses of 5 and 25 mg/kg, ip 20 min prior to pentobarbital (25 mg/kg, ip) prolonged pentobarbital sleep time. The effect was dose dependent. Rats treated with DI showed decreases in the rate of disappearance of [<sup>14</sup>C]pentobarbital from both brain and plasma, although the concentration of [<sup>14</sup>C]pentobarbital in these two tissues was not different from controls at return of righting reflex. Pentobarbital-tolerant rats pretreated with either IP, DI,

AT, or NT (25 mg/kg, ip) 20 min prior to pentobarbital (40 mg/kg, ip) slept longer than pentobarbital-tolerant controls, although at awakening, brain and plasma concentrations of [ $^{14}\text{C}$ ]-pentobarbital in the treated animals were not different from control values. Addition of each of the tricyclic drugs to an *in vitro* hepatic microsomal enzyme system showed competitive inhibition of pentobarbital metabolism. Neither of the tricyclic drugs enhanced significantly the development of tolerance to pentobarbital although the combination of pentobarbital and DI stimulated pentobarbital hydroxylase activity. (Supported by the Research Institute of Pharmaceutical Sciences, University of Mississippi.)

163. *Effects of Viral Replication on Drug Metabolism.* W. L. RAGLAND and S. J. BUYNITZKY, Departments of Avian Medicine and Veterinary Pathology, University of Georgia, Athens, Georgia.

Induction and repression of the hepatic drug-metabolizing enzymes can be effected by administration of numerous chemicals by various routes. Consequently, xenobiotic compounds have been assumed to be the major modulators of drug-metabolizing enzymes and the specific effects of certain viruses have been largely ignored. The effect of live Newcastle disease vaccine virus on four enzyme activities (ethylmorphine *N*-demethylase, aryl hydrocarbon hydroxylase, aniline hydroxylase, and nitroreductase) of chicken liver was examined. Vaccination did not alter the enzyme activities in noninduced birds but in birds induced with phenobarbital, the first three enzyme activities were higher and nitroreductase activity lower in the vaccinated birds. Furthermore, adult mallards which had been inoculated with duck hepatitis virus prior to exposure to DDT in the diet accumulated less total pesticide residue than uninoculated birds. Data from these experiments and several published reports will be discussed in order to draw attention to latent or inapparent viral infections as modulators of drug metabolism. (Supported by EPA Grant R 801800.)

164. *The Effects of Lead and Hexachlorophene, Alone and in Combination, in Albino Rats.* D. E. GORDON, G. L. KENNEDY, Jr. and J. C. CALANDRA, Industrial Bio-Test Laboratories, Inc., Northbrook, Illinois.

Hexachlorophene (HCP) has been shown to produce lesions of the central nervous system when fed to albino rats. The possible modification of this response at the tissue level by simultaneous exposure to a second material, lead in the form of lead acetate, was examined. Groups of rats were exposed to up to 200 ppm HCP, to up to 366 ppm lead, and to combinations of the two materials (high exposure 200 ppm HCP and 366 lead). Animals were fed for 30 days with growth and behavior monitored during the exposure. Histologic evaluation of the brain, optic nerve, and spinal cord was conducted on animals sacrificed following 30 days of feeding and on animals fed for 30 days and allowed a subsequent 30-day period on stock diet (recovery period). In the HCP-treated rats a dose-related focal to multifocal vacuolization (status spongiosus) confined to the white matter of the brain, spinal cord, and/or optic nerve sections was observed. The lesion was more severe in the cerebellum and resulted from separation of the myelin strands by edema fluid. Cotreatment with lead did not modify the lesion, either in terms of frequency or severity. The lesions observed among animals sacrificed following the recovery period were less marked but were still discernible. It is concluded that the effect produced by HCP in the central nervous system of the rat is not influenced by simultaneous exposure to lead.

165. *In Vitro Studies of Rat Pulmonary Alveolar Macrophages Subsequent to Intrapulmonary Instillation of Lead (PbO).* P. AGOSTA, C. A. FISCHER, E. J. KAMINSKI and J. C. CALANDRA, Industrial Bio-Test Laboratories, Inc., Northbrook, Illinois.

The effects of lead particulate on rat pulmonary alveolar macrophages (PAM) were studied. Two groups of four Long-Evans Strain rats received one intrapulmonary dose of PbO—1.0 mg/rat and 0.25 mg/rat. Two groups of eight rats were used as controls (untreated and vehicle). Rats were sacrificed at 7, 10, 15, and 40 days. Counts of PAM's and viability were

done by pulmonary lavage. Total and tissue lead contents were determined. Cells from the 40-day sacrifice were maintained in supplemented MEM or McCoys modified 5a with Hepes buffer. Both media were supplemented with rat serum, antibiotics, and a fixed number of formalin-fixed lymphocytes and combinations thereof. Formalin-fixed lymphocytes were used to stimulate PAM immune responsiveness. Counts were made to determine the PAM viability over time. Although the overall viability of the PbO-exposed PAM's appeared to be slightly less than controls, the PbO-exposed PAM's were harvested in greater numbers per rat than controls.

166. *Synergism of the Acute Toxic Effects of 3,4-D Butyl Ester, Dieldrin, Rotenone, and Pentachlorophenol in Rainbow Trout by Carbaryl.* C. N. STATHAM and J. J. LECH, Department of Pharmacology, Medical College of Wisconsin, Milwaukee, Wisconsin.

In static bioassays using fingerling rainbow trout, 1 mg/liter carbaryl (C) decreased the LC50 of 2,4-D butyl ester from 30 to 11 mg/liter. This concentration of C by itself produced no mortality in trout during a 48-hr exposure. Although in vivo studies using plasma and liver extracts from trout exposed to C indicated that esterase activity against 2,4-D butyl ester was decreased by approximately 50%, there was no apparent decrease in the ability of C-treated trout to hydrolyze 2,4-D butyl ester in vivo. When trout were exposed to [<sup>14</sup>C]2,4-D butyl ester in water, the fish which were preexposed to C accumulated approximately twice the amount of <sup>14</sup>C when compared to control fish. Direct analysis of the <sup>14</sup>C residues indicated the presence of only the hydrolysis product, 2,4-D acid in tissues from both control and C-treated trout. Total body washout studies using [<sup>14</sup>C]2,4-D butyl ester showed that the rate of disappearance of <sup>14</sup>C in C-treated fish was not significantly different from control. Enhancement of the toxicity of dieldrin, rotenone, and pentachlorophenol by C in similar experiments indicated that the synergism may be nonspecific. Studies using arecoline (A), a direct acting cholinergic agent, instead of C, showed essentially the same results. The synergism induced by both C and A was blocked by atropine. These studies indicate that both C and A may have increased the toxicities of 2,4-D butyl ester, dieldrin, rotenone, and pentachlorophenol by a cholinergic mechanism, possibly involving uptake of these latter compounds from water. (Supported in part by a NOAA Sea Grant from the University of Wisconsin and by the U.S. Department of the Interior.)

167. *Four-Day Static Fish Toxicity Studies Conducted With Thirteen Cadmium Pigments in Three Species of Freshwater Fish.* G. RAUSINA, J. W. GOODE, M. L. KEPLINGER and J. C. CALANDRA, Industrial Bio-Test Laboratories, Inc., Northbrook, Illinois.

Four-day static fish toxicity studies were conducted with 13 cadmium pigments in three species of freshwater fish. Rainbow trout (*Salmo gairdnerii*), bluegill sunfish (*Lepomis macrochirus*), and channel catfish (*Ictalurus punctatus*) were used as test animals. The pigments contained no greater than 0.1% (w/w) soluble cadmium and were tested at a concentration of 100 mg/liter (100 ppm) in each species. If the 4-day median tolerance limit (4-day TL<sub>50</sub>) of the pigment was greater than 100 ppm, no further testing was done; however, when the 4-day TL<sub>50</sub> was less than or equal to 100 ppm, five additional concentrations were selected in order to permit calculation of the 4-day TL<sub>50</sub>. Soft reconstituted water was used as the test water. Cadmium chloride was used as the positive control. Rainbow trout were most sensitive to all 13 cadmium pigments while bluegill sunfish and channel catfish were not sensitive to 12 of the 13 cadmium pigments at the 100-ppm test concentration. The calculated 4-day TL<sub>50</sub> of cadmium chloride in rainbow trout, bluegill sunfish, and channel catfish was 0.120, 2.53, and 3.53 ppm, respectively.

168. *The Effect of Simazine on the Reproductive Capability of Mallard Ducks.* R. J. FINK, Truslow Farms Inc., Chestertown, Maryland (H. B. Camp)

This study was designed to investigate the effect of Simazine (a triazine herbicide) on the reproductive capability of mallard ducks. The birds were exposed to Simazine at dietary levels of 2.0 and 20.0 ppm from prior to the onset of egg laying through the normal egg production cycle. Reproductive impairment was evaluated by analysis of the following reproductive parameters:

eggs laid, eggshell cracks, viable embryos, live 3-wk embryos, normal hatchlings, 14-day-old survivors, and eggshell thinning. Statistical analysis of the reproductive parameters revealed that Simazine caused no reproductive impairment in mallard ducks.

169. *The Effect of Tricyclohexyltin Hydroxide on the Reproductive Capability of Bobwhite Quail.* R. J. FINK, Truslow Farms Inc., Chestertown, Maryland. (D. D. McCollister)

This study was designed to investigate the effect of tricyclohexyltin hydroxide on the reproductive capability of bobwhite quail. The birds were exposed to tricyclohexyltin hydroxide at dietary levels of 5.0 and 20.0 ppm from prior to the onset of egg laying through the normal egg production cycle. Reproductive impairment was evaluated by analysis of the following reproductive parameters: eggs laid, eggshell cracks, viable embryos, live 3-wk embryos, normal hatchlings, 14-day-old survivors, and eggshell thinning. Statistical analysis of the reproductive parameters revealed that tricyclohexyltin hydroxide caused no reproductive impairment in bobwhite quail.

170. *The Fate of  $^{14}\text{CH}_3^{203}\text{HgCl}$  in the California Sea Lion.* D. R. BUHLER and B. R. MATE, Department of Agricultural Chemistry, Oregon State University, Corvallis, Oregon.

The livers of carnivorous marine mammals contain very high concentrations of mercury (100–200 ppm), almost all of which is in the inorganic state. Since the mercury in their diet is almost entirely methylated, it appears that marine mammals may have developed mechanisms for converting the alkylmercury compound to inorganic mercury. The present studies were, therefore, undertaken to investigate the stability of the carbon to mercury bond and the fate of methylmercury in a representative species of marine mammal, the California sea lion. Two juvenile sea lions received single 0.0805 mg/kg oral doses of  $^{14}\text{CH}_3^{203}\text{HgCl}$  and the animals then were sacrificed after 25 and 102 hr, respectively. No significant excretion of radioactivity occurred during the experiment and a majority of the dose was recovered in the various organs (50–62%) and tissues of the animals. Approximately 35 and 33% of the dose was concentrated in the liver and this organ also showed the highest specific activities. Most of the radioactivity in the brain and blubber was apparently methylmercury since  $^{203}\text{Hg}/^{14}\text{C}$  ratios found in those tissues were similar to those present in the administered dose. In almost all other tissues, particularly the liver, the ratio of  $^{203}\text{Hg}/^{14}\text{C}$  radioactivities observed were appreciably higher. These results confirm that significant quantities of methylmercury are demethylated in the California sea lion, probably by the liver.

171. *Glucuronidation of Diethylstilbestrol as a Steroid or Nonsteroid.* G. W. LUCIER and H. C. SHAH, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. (R. L. Dixon)

The hepatic perinatal development of steroid ( $\beta$ -estradiol and testosterone) and nonsteroid (*p*-nitrophenol and 1-naphthol) glucuronidations was followed to establish possible differences in steroid vs nonsteroid UDP glucuronyltransferase (GT). Glucuronidation of the synthetic estrogen diethylstilbestrol (DES) was also followed to determine if this compound was glucuronidated as a steroid or nonsteroid. In these experiments pregnant rats were administered 3  $\mu\text{g}$  of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)/kg at the fifth, tenth, or sixteenth day of gestation and the perinatal development of UDPGT compared to controls. Hepatic glucuronidation of all substrates tested first appeared 5 days prior to birth with the exception of testosterone UDPGT which first appeared 1 day prior to birth. Nonsteroid UDPGT from controls exhibited a peak in activity around the time of birth so that enzyme activity during this period was higher than that of adults. In contrast, steroid UDPGT did not exhibit high activity around parturition. TCDD treatment during gestation had no effect on fetal glucuronidation rates of any of the substrates tested whereas nonsteroid UDPGT was markedly induced in neonates. Nonsteroid UDPGT was induced approximately eightfold, 21 days after birth and induction was still twofold, 52 days after birth. No significant postnatal induction of steroid UDPGT was observed at any time period. The perinatal development and tissue distribution of DES glucuronidation

was the same as that for  $\beta$ -estradiol in both control and TCDD-treated rats. It is concluded from sheet data and competition experiments that DES is glucuronidated as a steroid.

172. *The Fate of [ $^{14}\text{C}$ ]Diethylstilbestrol in the Pregnant Mouse.* H. C. SHAH, S. GIPSON and J. A. MCLACHLAN, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. (I. P. Lee)

Although prenatal exposure to the synthetic estrogen, diethylstilbestrol (DES), has been reported to reduce fertility (McLachlan and Dixon, *Pharmacologist* 15, 199, 1973) or cause vaginal tumors (Herbst *et al.*, *N. Engl. J. Med.* 284, 878, 1971) in the offspring, very little is known about the physiologic disposition of DES in the pregnant animal. Thus, we have investigated the tissue distribution, metabolism, and excretion of [ $^{14}\text{C}$ ]DES during gestation. [Monoethyl- $^{14}\text{C}$ ]diethylstilbestrol (5.8  $\mu\text{Ci}/\text{kg}$ ; 30  $\mu\text{g}/\text{kg}$ ) was administered intravenously to timed pregnant CD-1 mice on the sixteenth day of gestation. Mice were sacrificed at appropriate times after dosing, and the radioactivity characterized in plasma, liver, muscle, placenta, and fetus; localization of [ $^{14}\text{C}$ ]DES in specific fetal organs was attempted. [ $^{14}\text{C}$ ]DES was rapidly distributed in the body since its concentration in plasma obtained 0.5 min following intravenous administration was half that of the corresponding 0.25-min value. In addition, the parent compound accounted for only 50% of the total plasma radioactivity by 10 min after dosing. The muscle/plasma radioactivity ratio (dpm/g tissue:dpm/ml plasma) was 1.04 at 0.5 min following treatment; muscle  $^{14}\text{C}$  activity paralleled that in plasma throughout the rest of the 4-hr experiment. On the other hand, radioactivity entered the fetus more slowly, the fetus/plasma radioactivity ratio being less than 1 until 30 min after administration. Persistent fetal uptake resulted in a threefold accumulation, relative to plasma, of  $^{14}\text{C}$  activity in the conceptus at 4 hr. The relationship of the distribution of DES to its fetotoxic effects is presently unclear.

173. *Effect of Prenatal Exposure of Mice to Diethylstilbestrol on Reproductive Tract Function in the Offspring.* J. A. MCLACHLAN, H. C. SHAH, R. R. NEWBOLD and B. C. BULLOCK, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. (R. L. Dixon)

Although much is known about the teratogenic activity of drugs and other common environmental chemicals, few studies have been done on the postnatal effects of prenatal drug exposure. Recent reports (e.g., Greenwald *et al.*, *N. Engl. J. Med.* 285, 390, 1971) of the latent appearance of vaginal carcinomas in pubescent girls whose mothers had been given the synthetic estrogen, diethylstilbestrol (DES), during their gestation suggest the importance of such investigations. To assess the effect of prenatal exposure to DES on reproductive tract function in the offspring, timed pregnant CD-1 mice were treated subcutaneously with DES on days 9-16 of gestation, inclusive. The fertility of the offspring was determined postnatally by repetitive breeding techniques at the appropriate times. After in utero treatment with DES, the reproductive capacities of the female offspring were impaired. These effects ranged in a dose-related manner from minimal subfertility (prenatal DES dose: 0.01  $\mu\text{g}/\text{kg}/\text{day}$ ) to complete sterility (10 or 100  $\mu\text{g}/\text{kg}/\text{day}$  dose). A major component of the sterility seen in the high DES dose females was ovarian, since the number of ova recovered after induced ovulation was only 30% that of controls. Sixty percent of the male offspring were found to be sterile following prenatal exposure to 100  $\mu\text{g}/\text{kg}$  DES; no effect was seen at the lower doses. An unusual finding was the presence of unique reproductive tract alterations (including metaplastic and/or neoplastic tissue) in 80% of the males exposed prenatally to the high dose of DES. These results suggest that in utero treatment with DES results in permanent impairment of the offspring's reproductive capacities.

174. *Reproductive Studies of Methadone and 1-Alpha-Acetylmethadol (LAAM) in Rats.* S. SMITH, J. A. NUIITE, G. L. KENNEDY and M. L. KEPLINGER, Industrial Bio-Test Laboratories, Inc., Northbrook, Illinois, and National Institute on Drug Abuse, Rockville, Maryland.

Following a preliminary dose ranging study, the reproductive effects of two narcotic agonists, methadone and LAAM, were evaluated in Charles River strain albino rats. Daily oral doses of

1, 3, and 10 mg/kg of methadone and 0.06, 0.20, and 0.60 mg/kg of LAAM were administered to separate groups of animals; controls received vehicle. Drug administration to males and females was for 63 or 14 days, respectively, prior to mating with untreated controls until sacrifice for the various groups tested. Males were sacrificed after mating. Ten females per group were sacrificed at midgestation (day 14); the remaining females were allowed to litter and carry the young through weaning. Embryotoxicity, growth, fertility, reproductive parameters, and progeny survival and size appeared unaffected at the dosages employed. In addition, a separate study initiating similar daily drug treatment to pregnant females on day 15 resulted in no apparent alterations in pup growth or survival in the methadone treated groups. However, pup survival in the high dose LAAM group was slightly reduced in comparison with other groups. Therefore, it appears that LAAM or methadone demonstrated no significant deleterious effect upon fertility or the various reproductive parameters investigated in the albino rat at the doses employed. (Supported by NIDA contract HSM-42-72-171.)

175. *Aminoaciduria and Proteinuria in Rats after Parenteral Administration of Ni(II)*. P. H. GITLITZ, F. W. SUNDERMAN, JR. and P. GOLDBLATT, University of Connecticut School of Medicine, Farmington, Connecticut.

Pronounced aminoaciduria and proteinuria were found in Fischer female rats after a single ip injection of  $\text{NiCl}_2$  in dosages from 51 to 85  $\mu\text{mol/kg}$ . Amino acids were measured by ion-exchange liquid chromatography and total proteins were measured by the biuret reaction. Illustrative data (mean  $\pm$  SD) for urine amino acids ( $\mu\text{mol/kg/day}$ ) for 39 control rats vs 10 treated rats during the second day after Ni(II) (68  $\mu\text{mol/kg}$ ) were: Ala:  $13 \pm 4$  vs  $83 \pm 51$ ; Arg:  $4 \pm 1$  vs  $30 \pm 18$ ; Gly:  $19 \pm 4$  vs  $64 \pm 37$ ; His:  $5 \pm 1$  vs  $14 \pm 7$ ; Ileu:  $1.4 \pm 0.4$  vs  $16 \pm 10$ ; Leu:  $2 \pm 1$  vs  $28 \pm 18$ ; Lys:  $5 \pm 1$  vs  $68 \pm 44$ ; Met:  $3 \pm 1$  vs  $11 \pm 5$ ; Phe:  $2 \pm 1$  vs  $9 \pm 5$ ; Ser:  $9 \pm 2$  vs  $59 \pm 39$ ; Thr:  $9 \pm 2$  vs  $47 \pm 28$ ; Tyr:  $2 \pm 1$  vs  $14 \pm 8$ , and Val:  $2 \pm 1$  vs  $36 \pm 24$  ( $p$  values all  $< 0.001$ ). Amino acids in plasma were normal or slightly diminished at 24 and 48 hr after injection of Ni(II). Urine protein (mg/kg/day) for 30 control rats vs 7 treated rats during the second day after Ni(II) (68  $\mu\text{mol/kg}$ ) were  $19 \pm 5$  vs  $76 \pm 41$  ( $p < 0.001$ ). Electron microscopy of kidneys at 48 hr after Ni(II) (68  $\mu\text{mol/kg}$ ) revealed fusion of foot processes of glomerular epithelial cells. This study demonstrates that generalized aminoaciduria and proteinuria develop in rats within 24 hr after ip Ni(II). Amino acid and protein excretions consistently returned to normal by the fifth day. (Supported by AEC Grant AT(11-1)-3140 and NIOSH Contract HSM 99-72-24.)

176. *Effects of Lead on the Induction of Hepatic Drug Metabolizing Enzymes by Phenobarbital and 3,4-Benzpyrene*. C. CHOW and H. CORNISH, Interdepartmental Toxicology Program, The University of Michigan, Ann Arbor, Michigan.

There are recent reports that lead inhibits drug metabolism. The objective of this study was to determine the effects of lead on the induction of cytochrome P450, cytochrome  $b_5$ , NADPH-cytochrome  $c$ , and P450 reductase in liver microsomes. Male Sprague-Dawley rats were administered lead acetate (65 mg/kg) and/or phenobarbital (40 mg/kg) ip. Control rats received equimolar sodium acetate, and were sacrificed 12, 24, and 48 hr after dosing. Blood was collected by cardiac puncture, and lead concentration was determined by atomic absorption spectrophotometry with the Delves sampling cup. NADPH-cytochrome P450 reductase was measured in an Aminco Morrow stopped-flow apparatus attached to an Aminco Chance recording spectrophotometer in the dual beam mode. Lead was found to abolish the phenobarbital induction of hepatic microsomal cytochrome P450 at 12 and 24 hr after treatment. However, the inhibitory effect of lead had disappeared and cytochrome P450 concentrations were markedly elevated at 48 hr. Neither lead nor phenobarbital had any significant effect on the concentration of cytochrome  $b_5$ . Lead has no marked effect on either the NADPH-cytochrome  $c$  or P450 reductase activities. Lead was found to partially prevent the stimulation of NADPH-cytochrome  $c$  and P450 reductase activities produced by phenobarbital. In another similar experiment, rats were given an ip injection of lead acetate and/or benzpyrene (20 mg/kg).

Control rats received an equivalent amount of corn oil and/or sodium acetate. Lead was found to inhibit the induction of hepatic microsomal cytochrome P448 produced by benzpyrene. However, a single injection of lead and/or benzpyrene had minimal effects on cytochrome *b*<sub>5</sub>, NADPH-cytochrome *c*, and P450 reductase in liver microsomes during the subsequent 48 hr. During this same time period, there was a negative correlation between the percentage of inhibition of delta-aminolevulinic dehydratase and lead concentration in the blood. The average blood lead concentration increased to 1.89 µg/ml while delta-aminolevulinic acid dehydratase activity dropped to one-third of controls at 48 hr. (Supported by Environmental Protection Agency Grant R-802762-01.)

177. *The Metabolism of [<sup>3</sup>H]Benzene in the Mouse.* L. S. ANDREWS, E. W. LEE, J. KOCIS, C. WITMER and R. SNYDER, Thomas Jefferson University, Philadelphia, Pennsylvania.

Studies on the relationship between benzene metabolism and benzene-induced hemopoietic toxicity have suggested that toxicity may result from the formation of a toxic metabolite (Lee *et al.*, *Toxicol. Appl. Pharmacol.* **29**, 112-113, 1974). Accordingly the metabolism of [<sup>3</sup>H]-benzene in the mouse was investigated in greater detail. The metabolism of 440- and 880-mg/kg doses was complete 24 hr following injection, but this was not true for higher doses (2200-8800 mg/kg). Radioactive compounds were separated into four fractions: [<sup>3</sup>H]phenol, glucuronide, and sulfate conjugates of [<sup>3</sup>H]benzene metabolites and a fraction which consisted primarily of <sup>3</sup>H<sub>2</sub>O. Conjugates comprised the bulk of the radioactivity in all samples analyzed. For most of the dose range (440-8800 mg/kg) <sup>3</sup>H<sub>2</sub>O, glucuronide, and sulfate conjugates represented a relatively constant proportion of the urinary radioactivity. Phenolic glucuronides were the major metabolites of benzene in the urine. The excretion of ethereal sulfate conjugates followed zero-order kinetics. The excretion of free [<sup>3</sup>H]phenol was not related in any simple manner to the administered dose. Following conjugate hydrolysis most of the sample was identified as [<sup>3</sup>H]phenol with a small amount of [<sup>3</sup>H]catechol also present. In summary, the mouse metabolizes [<sup>3</sup>H]benzene to [<sup>3</sup>H]phenol and [<sup>3</sup>H]catechol which are excreted primarily as glucuronide and ethereal sulfate conjugates. The role of phenol and catechol in producing benzene toxicity has yet to be fully characterized. (Supported by USPHS ES-00322.)

178. *Metabolism of Alkyl Polyethoxylates by the In Situ Perfused Rat Liver.* R. B. DROTMAN, The Procter and Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio.

Alkyl polyethoxylates (AEs) of the general formula CH<sub>3</sub>(CH<sub>2</sub>)<sub>x</sub>(OCH<sub>2</sub>CH<sub>2</sub>)<sub>y</sub>OCH<sub>2</sub>CH<sub>2</sub>OH are widely used as nonionic surfactant components of household detergent products. Metabolism of AEs having alkyl groups of *x* = 11, 12, or 13 (C<sub>12</sub>, C<sub>13</sub>, C<sub>14</sub>) and *y* = 5 or 6 (E<sub>6</sub>, E<sub>7</sub>) have been studied with *in situ* liver perfusion. All AEs were radiolabeled with <sup>14</sup>C in the primary carbon atom of the terminal ethoxylate moiety [CH<sub>3</sub>(CH<sub>2</sub>)<sub>x</sub>(OCH<sub>2</sub>CH<sub>2</sub>)<sub>y</sub>-OCH<sub>2</sub><sup>14</sup>CH<sub>2</sub>OH]. When rat livers were perfused with AEs, there was a transient drop of radioactivity during the first 10 min within the recycled perfusion medium. The drop is probably due to an initial tissue distribution of AEs into the liver. By comparing the rate of disappearance of the parent compounds over a 60-min period from the perfusion medium, it is apparent that there is no difference in the rate of metabolism between C<sub>12</sub>\*E<sub>6</sub>, C<sub>13</sub>\*E<sub>6</sub>, or C<sub>14</sub>\*E<sub>7</sub>. Analytical comparison of the metabolites formed from the parent compounds revealed no apparent differences. Evidence from this preliminary study would indicate there are similarities in the metabolism of these AEs.

179. *The Effect of Hyperbilirubinemia on the Plasma Clearance of Indocyanine Green or Sulfobromophthalein.* R. A. YEARY, K. J. WISE and D. R. DAVIS, Department of Veterinary Physiology and Pharmacology, The Ohio State University, Columbus, Ohio.

It is reported that hyperbilirubinemia interferes with hepatic function tests employing the excretion of indocyanine green (ICG) or sulfobromophthalein (BSP). Plasma disappearance of intravenously injected ICG or BSP were compared in hyperbilirubinemic and heterozygous (nonicteric) Gunn rats to determine if the reported alterations in dye excretion were associated



with the interaction of bilirubin and dyes in plasma or the result of underlying hepatic disease. The plasma disappearance of both ICG and BSP were dose-dependant in both icteric and non-icteric rats. However, there were no differences in the first-order rate constant ( $K_e$ ) for the two groups of rats. Similarly there were no differences in the  $K_e$  for ICG and BSP in the two groups of rats after they were pretreated 30 min previously by ligation of the bile duct and iv injection of a bilirubin load of 25 mg/kg. It was concluded that simple hyperbilirubinemia had no effect on the  $K_e$  for the two dyes. However, when ICG (4 mg/kg) and bilirubin (25 mg/kg) were injected simultaneously in either group of rats the  $K_e$  in icteric rats decreased from  $-0.36 \text{ min}^{-1}$  to  $-0.14 \text{ min}^{-1}$  and in nonicteric rats decreased from  $-0.26 \text{ min}^{-1}$  to  $-0.12 \text{ min}^{-1}$ . This effect is believed to be the result of interaction of ICG and unbound bilirubin on hepatic uptake of ICG. Bilirubin measurements revealed that the plasma disappearance of injected bilirubin was significantly greater in bile duct-ligated icteric (UDP glucuronyltransferase deficient) Gunn rats than in bile duct-ligated nonicteric heterozygous Gunn rats.

180. *Dehydrogenation—A Previously Unreported Pathway of Lindane Metabolism in Mammals.* R. W. CHADWICK, L. T. CHUANG and K. WILLIAMS, EPA, NERC, PTSEL, Research Triangle Park, North Carolina.

It is generally accepted that the dehydrochlorination of lindane to  $\gamma$ -pentachlorocyclohexene is the primary initial step in the metabolism of lindane by both plants and animals. Nevertheless, the chemical structure of some recently identified lindane metabolites indicated that there might be important metabolic pathways other than the dehydrochlorination of lindane to pentachlorocyclohexene. This study presents evidence for an alternate initial pathway, specifically the dehydrogenation of lindane to the intermediate metabolite hexachlorocyclohexene. Study of the in vitro metabolism of lindane by liver preparations demonstrates that the dehydrogenase system is associated with the microsomal fraction and has a requirement for reduced pyridine nucleotide coenzyme as well as molecular oxygen. Inhibition by SKF-525-A and CO suggest that the enzyme is cytochrome P-450-dependent. Pretreatment of rats with DDT, which stimulates lindane metabolism, also induces significantly higher dehydrogenase activity. Both the in vivo and in vitro metabolism of the hexachlorocyclohexene intermediate produces previously identified lindane metabolites. Results of this study indicate that dehydrogenation is an important initial pathway in the metabolism of lindane by mammals.

181. *Interaction of Degraded Iota Carrageenans with Model Membrane Systems.* E. P. PITZ, L. GOLBERG and F. COULSTON, Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York.

The capacity of fractions of degraded iota carrageenans (CG) to alter the osmotic fragility of human erythrocytes has been studied in vitro. CG having MW 88,000 or higher had no discernible action, while fractions with lower molecular weights increased osmotic fragility, an effect eliminated by prior incubation of the erythrocytes with bovine serum albumin (BSA). The strong interaction between CG and BSA is indicated by inhibition of metachromasia with toluidine blue. While CG having MW 145,000 neither binds to nor interacts with protein from erythrocytes (in saline, at pH 7.05 for 30 min at 24°C) CG of MW 20,000 either binds to erythrocytes to the extent of 3 mg CG/ml of erythrocytes or withdraws protein from the erythrocyte that repressed metachromasia of toluidine blue. Using human red cell ghosts,  $19.6 \pm 11.0\%$  and  $6.8 \pm 3.0\%$  protein is released by CG's of MW 20,000 and 88,000, respectively. (Supported by Research Grant 2P01-ES00226-08 from the National Institute of Environmental Sciences, NIH and by National Institutes of Health Training Grant 2T01-ES00103-08.)

182. *Cell Proliferation in Mouse Lung Following Intraperitoneal Administration of Butylated Hydroxytoluene.* H. P. WITSCHI and M. G. CÔTÉ, Département de pharmacologie, Université de Montréal, Montréal, Québec, Canada.

Reversible proliferation of alveolar cells in mouse lung after intraperitoneal injection of the antioxidant butylated hydroxytoluene (BHT) was originally described by Marino and Mitchell

(*Proc. Soc. Exp. Biol. Med.* **140**, 122, 1972). We attempted to extend their findings by a histopathologic and biochemical evaluation of BHT-stimulated cell growth in mouse lung. Male Swiss-Webster mice (28–30 g) were injected intraperitoneally with doses of BHT (dissolved in corn oil) ranging from 125 to 1000 mg/kg. A diffuse swelling of alveolar septa was noticed 3 days after BHT. Five days after BHT, more widespread alterations were seen consisting of alveolar wall thickening associated with epithelial and macrophage proliferation. Electron microscopic studies showed that BHT produced essentially a proliferation of type 2 alveolar cells (granular pneumocytes) and their lamellar bodies. Biochemical signs of cell proliferation were a gradual and dose-dependent increase in total lung weight and an increase in total pulmonary RNA, DNA, protein, and lipids (1–5 days after BHT). The smallest effective dose was 250 mg/kg; 5 days after BHT, lung weight, DNA and protein were between 1.5–2.5 times higher than control values, whereas total pulmonary RNA increased 2–4 times. Three days after BHT, thymidine kinase activity was 25 times as high in the lungs of treated animals compared to controls and this was followed on days 4 and 5 by an increased incorporation of thymidine into DNA (5–10 times over normal values). Other enzymes found to increase in activity after BHT included uridine kinase, 5'-nucleotidase, pyruvate kinase, glucose-6-phosphate dehydrogenase, isocitric dehydrogenase, and lactic dehydrogenase. Abnormal proliferation of type 2 alveolar cells is a frequent response of lung tissue to toxic injury. BHT might serve as a model to study the biochemical events preceding and/or accompanying the proliferation of this important cell type in lung.

183. *Modification of the Liver Effects of Two Fluorinated Ethanes.* J. P. F. MURPHY, E. W. VAN STEE and K. C. BACK, 6570 Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio.

The fluoroalkanes dibromotetrafluoroethane and dibromotrifluoroethane are of interest to the USAF as possible fire control agents. Previous studies led to the suggestion that prolonged exposure to either or both of these agents might result in hepatotoxicity. This study was designed to verify whether or not they were hepatotoxic, and if so, to what extent the hepatotoxicity might be related to biotransformation of the compounds. Inhalation of dibromotrifluoroethane at a concentration of 0.25–0.39%, 5 hr daily for 10 days, reduced hexobarbital sleeping time to 77% of control in rats. The increased rate of metabolism of hexobarbital and ethylmorphine by the hepatic 9000g microsomal supernatant fraction prepared from rats exposed to dibromotrifluoroethane (0.25–0.39%, 5 hr daily for 10 days) showed parallel bromide concentrations in rat serum. Treatment of rats with 2,4-dichloro-6-phenyl-phenoxyethyl-diethylamine (SKF 525-A) (25 mg/kg) during the dibromotrifluoroethane exposure period decreased the metabolism of hexobarbital and ethylmorphine by the hepatic 9000g microsomal supernatant fraction, with parallel changes in serum bromide concentrations. Rats treated with sodium phenobarbital (80 mg/kg) during a similar exposure period increased the metabolism of hexobarbital and ethylmorphine with parallel changes in the serum bromide content. No histopathological changes were detected in any of the kidneys or livers from a group that would serve to distinguish any treatment group from any other. Conclusions were that the compounds were debrominated by enzyme systems that were stimulated by phenobarbital and inhibited by SKF 525-A. Neither the parent compounds nor the products of biotransformation were significantly hepatotoxic after 10 days intermittent exposure to high levels of the compounds.

184. *The Toxicity of Inhaled <sup>239</sup>PuO<sub>2</sub> in Immature, Young Adult, and Aged Syrian Hamsters.* C. H. HOBBS, J. A. MEWHINNEY, D. O. SLAUSON, R. O. MCCLELLAN and J. J. MIGLIO, Inhalation Toxicology Research Institute, Lovelace Foundation, Albuquerque, New Mexico.

Although previous studies have shown that inhaled plutonium is carcinogenic to lung and bone, the dose-response relationships and the difference, if any, of the relative susceptibility of animals at various ages have not been defined. In this study, immature (~28 days old), young

adult (~84 days old), and aged (~340 days old) Syrian hamsters were exposed to aerosols of  $^{239}\text{PuO}_2$  to obtain graded initial lung burdens (ILB) which ranged from levels expected to result in relatively early deaths from radiation pneumonitis or pulmonary fibrosis, or both, down to a level equivalent, on a body weight basis, to the accepted maximal permissible lung burden for man. The activity median aerodynamic diameters of the  $^{239}\text{PuO}_2$  aerosols ranged from 1.9 to 2.4  $\mu\text{m}$  with a geometric standard deviation of about 1.5. Desired ILBs for the immature and young adult animals were 240, 60, 15, 3.5, 0.95, 0.24, and 0.029 nCi of  $^{239}\text{Pu}$  irrespective of body weight at exposure. For the aged animals, only the four highest doses were used. A control group was sham exposed for each age group. The  $^{239}\text{PuO}_2$  was retained in the lung with an effective half-life of about 50–60 days in each of the age groups. The small amount of  $^{239}\text{Pu}$  translocated from lung was distributed to bone and liver. At the present time (~60 wk post-inhalation for the animals exposed as young adults), increased mortality over the controls has only been observed in the high (~240 nCi) lung burden group in each of the three age groups. Histopathologic examination of all animals is in progress. Lesions in animals that died at early times were generally severe radiation pneumonitis along with marked pulmonary epithelial hyperplasia, hypertrophy and metaplasia. No pulmonary tumors have been observed to date. (Supported by the Atomic Energy Commission under Contract AT(292)-1013.)

185. *An Evaluation of Petroleum Distillates as Aspiration Hazards in Rats.* R. E. OSTERBERG, R. JOHNSON, G. BIERBOWER and J. McLAUGHLIN, JR., Bureau of Biomedical Science, Division of Biological Science, Consumer Product Safety Commission, Washington, D.C.

Consumer products are continually being created which contain petroleum distillates in new formulations. Thus, the following studies were undertaken to reevaluate available technology which is used to determine potential aspiration hazards among consumer products and to develop information about the physical and toxicological properties of several petroleum distillates and products which contain them. Petroleum distillates and products were administered to rats by intravenous and intratracheal injection, and also by using modifications of the technique utilized by Gerarde (*Arch. Environ. Health* 6, 329–341, 1963). Rat lungs were examined grossly and weighed immediately postmortem or upon sacrifice. Effects seen were edema, congestion, petechiae, and chemical pneumonitis. The severity of lung damage was dose-related to amounts of the petroleum distillates. The incidence of mortality was greatest with viscosities below 90 SUS at 100°F as was severity of damage. Moderate to severe aspiration toxicity was noted with petroleum distillate viscosities up to 360 SUS. Similar effects were observed in subacute studies.

186. *Safety Evaluation of Novel Sources of Protein.* B. L. OSER, 60 East 42 Street, New York, New York.

At the current rate of growth, the populations in certain protein-starved areas of the world (e.g., Latin America, southeast Asia, Africa) will double in 23–30 yr and that of the world at large in 35 yr. Notwithstanding advances in agriculture and food technology, it is predicted that the future need for protein will demand recourse to a variety of novel sources of this critical nutrient for both man and food-producing animals. Improvements in the yield and quality of wheat, corn, and rice, achieved through the "green revolution" and the increasing use of protein concentrates from oil seeds and legumes, offer only partial solutions to the problem inasmuch as the demand will conceivably be greater than can be met by any single approach. In addition to defatted seed meals from which natural toxicants are removed (e.g., cottonseed, rapeseed, linseed), examples of potentially important new sources of protein are genetically selected varieties of corn, wheat, and rice; concentrates from whole fish and from other marine sources (plankton, krill); leaf concentrates; and microcellular biomass (yeasts, molds, bacteria, algae) cultured on chemical substrates and on agricultural or urban waste products. Industrial interest in the production of these products is expanding on a worldwide basis. The Protein Advisory Group of the United Nations Systems has developed guidelines for the nutritional and safety evaluation of novel protein for the use of the industries, professions, and regulatory agencies concerned with the introduction of such products into food channels. Criteria for

safety evaluation of proteins will be outlined for both human food and animal feed, emphasizing the procedures for major foodstuffs as distinguished from trace components.

187. *Transplacental Effects of Drugs on Hearing, Vision, and Behaviour*. P. S. BOURDOIS, J. JUNGHANI, J. PERRAUD and H. REINERT, Centre de Recherche Pfizer, 37400 Amboise, France.

There are little, if any, publications on suitable tests which will measure the effect of drugs, administered during gestation, on the sensory and behavioural function of the offspring. We have begun to test known ototoxic, oculotoxic, or behaviour-modifying drugs in order to validate our procedures. These studies in young animals will form part of drug safety testing and complement the evaluation of drug action on fertility, fetal, peri-, and postnatal development. Methods have obviously to be sensitive and must be suitable for the testing of large numbers of young rats at the same time, otherwise different age groups would be compared. Behavioural tests which require training are unsuitable because of the number of animals involved and the length of time taken until conditioning is achieved. We use the "open field" technique which measures two stereotype responses (ambulation and rearing) and "emotion" (defecation). Amphetamine, iproniazid, chlorpromazine, pemoline, and monosodium glutamate are under investigation in 4-wk-old rats. Preyer's reflex, elicited by audiometer generated sounds at threshold amplitude and different frequencies, has been chosen to test hearing. Gentamicin, kanamycin, dihydrostreptomycin, ethacrynic acid are to be examined in 25-day-old rats and guinea pigs. Cats are being used to test the effects on vestibular function. Vision is tested by recording stroboscope evoked cortical potentials. Thioridazine and chloroquine are under study. Light and electron microscopic examination of the cochlea and retina will be carried out. It is hoped that the presentation of our results and informal discussion at the poster session will assist the development and progression of generally acceptable and reasonably uniform tests for the benefit of interlaboratory comparisons.

188. *Developmental Toxicity of Medroxyprogesterone Acetate After Administration to Pregnant Rabbits*. F. D. ANDREW and R. E. STAPLES, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

The developmental toxicity of medroxyprogesterone acetate (MPA) was investigated in several species following reports that MPA might be teratogenic in humans. MPA is a progestational steroid that is used not only for contraception, but also in the treatment for threatened abortion, and as a test for pregnancy in humans. Sperm detection was termed day 1 in mice and rats; insemination was termed day 0 in rabbits. Mice, rats, and rabbits were sacrificed on day 19, 20, and 28, respectively. Dose levels tested ranged from 0.1 to 3000 mg/kg/day sc once daily for 3, 6, or 9 consecutive days between gestation days 7-15 for mice and rabbits and days 8-16 for rats. Dose-related malformations were rarely seen among the offspring of mice or rats given MPA at the maximum tolerated level. However, cleft palates and other malformations were noted among the offspring of rabbits given MPA. The critical period for administration of MPA to rabbits was days 13-15 of gestation. Administration of 1, 3, or 10 mg/kg/day during this period resulted in 7, 27, or 53% cleft palates, respectively, among living New Zealand offspring. Comparable cleft palate frequencies (5, 37, or 47%) were seen in Dutch Belt offspring after 3, 10, or 30 mg/kg/day MPA, when administered during the critical period. These results are unusual since palate malformations rarely occur spontaneously in rabbits (0.03-0.09%) and have seldom been induced experimentally. Other evidence of developmental toxicity in rabbits included embryoletality (incidence of resorbed and dead fetuses) and retarded development (decreased fetal weight). Studies are in progress to delineate the etiology of developmental toxicity following MPA administration to pregnant rabbits.

189. *Mirex Kinetics in the Rhesus Monkey*. K. A. PITTMAN, M. W. KENNEDY and D. H. TREBLE, Institute of Comparative and Human Toxicology and Biochemistry Department, Albany Medical College, Albany, New York. (F. Coulston)

This study was done to develop a mathematical model to predict the long-term, kinetic behavior of Mirex in animals. [<sup>14</sup>C]Mirex was given (1 mg/kg) iv and po to female rhesus monkeys

(*Macaca mulatta*). Radioactivity was measured in plasma, urine, and feces at intervals after dosing and in tissues when animals were killed. Mirex was analyzed by gas-liquid chromatography. Resolution of semilog plots of plasma concentration-time curves suggested the presence of four or more kinetically distinguishable compartments and provided estimates of rate constants for distribution and elimination. A BASIC-language program, FITKIN, was used to solve the differential equations comprising the model and to adjust the above estimates to obtain a normalized, least-squares fit. Of several models postulated, a mamillary, four-compartment ("central," "fast," "slow," and "very slow"), open system model, providing for the urinary excretion of Mirex from the "central" compartment and for the fecal excretion of Mirex from the "fast" tissue compartment, yielded theoretical data in excellent agreement with observed values for plasma, urinary, and fecal radioactivity. At autopsy the amount of [ $^{14}\text{C}$ ]Mirex found in the liver corresponded to that predicted for the "fast" compartment, and the proportion of the dose (90%) found in the fat corresponded to the predicted accumulation of Mirex in a "very slow" tissue compartment. The model predicted that the accumulation of Mirex into fat would be retarded by the presence of a "slow" tissue compartment, so that distribution equilibrium would take about half a year. From that time to the end of a 10-yr projection, decline in the quantities of Mirex predicted for all compartments was only 2%, implying an extremely long biological half-life. We will continue plasma analyses on an iv dosed monkey for at least 1 yr, in order to check these predictions experimentally. (Supported by Research Grant 2P01-ES00226-08 from NIEHS, NIH, USDHEW, and by NIH Training Grant 2T01-ES00103-08.)

190. *Preclinical Toxicology of Lergotriole Mesylate (D-2-Chloro-6-Methylergoline-8 $\beta$ -Acetonitrile Methanesulfonic Acid Salt)—A New Prolactin Inhibitor.* N. V. OWEN, W. R. GIBSON, W. D. BRODDLE, C. C. KEHR and G. F. KIPLINGER, Lilly Research Laboratories, Toxicology Division, Eli Lilly and Company, Greenfield, Indiana.

Lergotriole mesylate is a semisynthetic tetracyclic compound derived from the ergot alkaloids. It is a dopamine receptor stimulant with potent prolactin inhibiting activity and minimal CNS and blood pressure effects. The toxicity was evaluated in mice, rats, dogs, and monkeys. The oral LD50 values for mice and rats were ca. 275 and 290 mg/kg, respectively, while the ip LD50 in rats was 96 mg/kg. The oral LD0 in dogs and monkeys was greater than 100 mg/kg and the ip LD0 in dogs was greater than 25 mg/kg. Ninety-day oral studies were conducted in rats and dogs. Dose levels were 0, 0.005, 0.015, and 0.050% in the diet of Harlan rats and 0, 5, 10, and 20 mg/kg in gelatin capsules for beagle dogs. Dose-related growth retardation occurred in rats of the 0.015 and 0.050% groups. Female rats of all dose levels except the control had increased ovarian weights, which correlated grossly and histologically with accumulation of the corpora lutea due to inhibition of luteolysis. The main effect in dogs was emesis which occurred shortly following dosing the first few days and only occasionally toward the end of the study. We concluded that lergotriole mesylate has an adequate margin of safety in all species evaluated.

191. *Lipid Peroxidation: A Possible Mechanism for Paraquat Toxicity.* J. S. BUS, S. Z. CAGEN, S. D. AUST and J. E. GIBSON, Department of Pharmacology, Michigan State University, East Lansing, Michigan.

The purpose of this study was to determine the role of membrane lipid peroxidation in the in vivo toxicity of the herbicide paraquat. Adult female Swiss-Webster mice were fed diets deficient in the lipid antioxidants selenium (Se) or vitamin E (Vit E) for 5 wk and then examined for sensitivity to paraquat toxicity by determination of the 7 day ip LD50. The paraquat LD50 in control mice fed laboratory animal chow was 30.0 mg/kg. Se and Vit E deficiency significantly reduced the paraquat LD50 to 10.4 and 9.2 mg/kg, respectively. Liver glutathione peroxidase (a Se-dependent enzyme) activity in the Se-deficient mice was 16% of control activity. Vitamin E deficiency was confirmed by the dialuric acid red blood cell hemolysis test, with 86% hemolysis in deficient mice vs 24% in controls. Administration to control mice of the reduced glutathione (GSH)-depleting agent diethyl maleate at 1.2 ml/kg, 30 min before paraquat treatment

also significantly reduced the paraquat LD50 to 9.4 mg/kg. GSH is a substrate for glutathione peroxidase, which detoxifies lipid peroxides formed during membrane lipid peroxidation. Paraquat alone, 30 mg/kg ip, also reduced liver GSH to 68% of controls 24 hr following treatment. In other experiments, rats exposed for 3 wk to 100 ppm paraquat in the drinking water had significantly elevated lung glucose-6-phosphate dehydrogenase (G6PD) activity compared to controls ( $78.2 \pm 11.7$  vs  $47.0 \pm 2.0$  nmol NADPH oxidized/min/mg protein). G6PD provides reducing equivalents for GSH production and is induced in response to oxidative stress. These data provide in vivo evidence that lipid peroxidation may be involved in paraquat toxicity.

192. *Toxicity and Tissue Distribution of Cyclotrimethylenetrinitramine (RDX)*. N. R. SCHNEIDER and M. E. ANDERSEN, Armed Forces Radiobiology Research Institute, National Naval Medical Center, Bethesda, Maryland; U.S. Navy Toxicology Unit, National Naval Medical Center, Bethesda, Maryland. (L. J. Jenkins, Jr.)

Cyclotrimethylenetrinitramine (RDX) is used primarily as a high explosive. However, RDX has also been used as a drug of abuse and has been implicated in the deaths of some workers in plants where it is manufactured and packaged. In this investigation we have studied the toxicity and tissue distribution of RDX in the rat, with quantitation of RDX in biological materials being accomplished by specific gas chromatography rather than by the traditional, indirect, colorimetric assay of hydrolyzed nitrite. Administration of RDX slurries (500 mg/kg ip) caused severe clonic-tonic seizures in 10/10 rats and killed 8/10. The plasma RDX concentration at the first convulsion was  $5.2 \pm 0.4$   $\mu\text{g/ml}$  of plasma and the peak plasma concentrations reached  $13.8 \pm 2.6$   $\mu\text{g/ml}$ . Brain, heart, liver, and kidney concentrations at death were  $29.5 \pm 2.7$ ,  $25.0 \pm 2.3$ ,  $41.7 \pm 5.8$ , and  $56.8 \pm 5.9$   $\mu\text{g RDX/g wet wt. of tissue}$ , respectively. After doses of 100 mg/kg po rats did not convulse, plasma (RDX) did not exceed 3  $\mu\text{g/ml}$ , and urine (RDX) was generally two to three times the plasma (RDX). The tissue concentrations of RDX in these rats were proportionately lower than those in the 500-mg/kg dose group. However, the tissue distribution patterns were the same for all dosages studied (500 mg/kg ip, 100 mg/kg po, and 50 mg/kg ip), and in no instance was RDX found to accumulate in any specific "target" organ. Studies were also undertaken to assess the toxicity of repeated administration of low doses of RDX. Rats given RDX-saturated water ( $\approx 60$   $\mu\text{g/ml}$ ) ad libitum for 90 days suffered no ill-effects. However, 25% of a group of rats fed 20 mg RDX/kg/day for 90 days died, apparently from an exacerbation of an underlying chronic respiratory disease by the stresses of repeated administration of RDX. Finally, in studies designed to estimate the percentage of RDX excreted in the feces and urine, only 1-2% of the administered RDX was recovered. Yet, no chromatographic evidence for the existence of any metabolite(s) was found. The fate of RDX in vivo is currently being investigated further using  $^{14}\text{C}$ -labeled RDX.

193. *Influence of Chronic Diuretic Therapy on Minoxidil-Induced Necrosis in Dogs*. E. HERMAN, T. BALAZS, F. EARL and S. KROP, FDA, Washington, D.C.

Alterations in myocardial function by diverse groups of drugs represents a critical clinical problem. Digitalis toxicity can be enhanced by simultaneous diuretic therapy. Minoxidil, a long-acting vasodilator, has caused myocardial anoxia in clinical studies. Experimentally, oral administration of minoxidil to beagle dogs at 1-10 mg/kg on 2 consecutive days induced dose-related necrosis. The potential myocardial interaction of minoxidil and diuretic compounds was investigated in dogs receiving oral doses of 250 mg/kg hydrochlorothiazide (8 dogs) or 10 mg/kg furosemide (4 dogs) twice daily. Four control dogs received no diuretic therapy. Serum potassium was depressed from an average of 4.7-3.8 mEq/liter by 14 days. After 14 days, four dogs given hydrochlorothiazide, four dogs given furosemide, and four control dogs were dosed with 3 mg/kg of minoxidil on 2 consecutive days; all dogs were killed 24 hr thereafter. Hemorrhages, both surface and endocardial, were found upon gross observation of the hearts in dogs given minoxidil only and in dogs of the diuretic/minoxidil groups. Focal myocardial necrosis and loss of striations in the left anterior and posterior papillary muscles were seen in both

minoxidil and diuretic/minoxidil groups. No difference in severity of necroses was seen in animals dosed with minoxidil or a combination of hydrochlorothiazide/minoxidil or furosemide/minoxidil. Thus concomitant diuretic therapy did not enhance minoxidil-induced myocardial lesions.

194. *Enhancement of Antibiotic Nephrotoxicity by Furosemide: Studies in Mice.* G. JAMES, W. SMITH, H. BRYANT and T. BALAZS, Food and Drug Administration, Washington, D.C.

The influence of furosemide on the nephrotoxicity of several aminoglycoside and cephalosporin antibiotics was investigated in ICR/FRF2 female albino mice. The mice were injected ip with 20 mg/kg of furosemide 15 min prior to the administration of the antibiotics. The antibiotics were given in comparable fractions of the ip LD50 doses. Forty-eight hours after drug administration, urine samples were obtained and analyzed for protein and glucose, and the mice were then sacrificed and necropsied. Renal tubular injuries were elicited in only the diuretic-pretreated animals given gentamicin and tobramycin at doses as low as 160 and 120 mg/kg, respectively. Furosemide pretreatment also increased the incidence and severity of the tubular injuries induced by doses of 600 and 1200 mg/kg of cephaloridine or 156 and 312 mg/kg of kanamycin. Cefazolin, cephalothin, cefamandole, cephapirin, and cephacetrile, however, failed to produce any signs of nephrotoxicity with or without furosemide. Renal cortical concentrations of antibiotics examined were significantly increased by furosemide pretreatment at 15, 60 and 240 min after dosing. A mechanism for the enhancement of the nephrotoxicity of certain antibiotics by furosemide is postulated on this basis.

195. *The Absorption, Tissue Distribution, and Excretion of Dodecyltrimethylamine Oxide by Selected Animal Species.* D. P. RICE, Procter and Gamble Co., Miami Valley Laboratories, Cincinnati, Ohio. (E. V. Buehler)

Dodecyltrimethylamine oxide (DDAO) is a semipolar surfactant contained in household dishwashing detergents. The investigation of the absorption, tissue distribution and excretion of DDAO by selected animal species used in toxicity testing is a part of a comprehensive toxicity testing program for this surfactant. An oral dose of [<sup>14</sup>C-methyl]- or [<sup>14</sup>C-1-dodecyl]DDAO is rapidly and extensively (88-95%) absorbed by rats. Subsequent excretion of the dosed radioactivity from [<sup>14</sup>C-methyl]DDAO in the urine, feces (bile), and expired air is rapid, accounting for 67, 12(3), and 12%, respectively, over the 72 hr following dosing. Chromatographic investigation of the urinary excretion products reveals only traces of unchanged DDAO. Within 1 hr after oral ingestion of [<sup>14</sup>C-methyl]DDAO the amount of radioactivity in the liver, kidney, and other tissues reaches a maximum and begins to decline. A similar pattern of tissue distribution and excretion of radioactivity is observed when [<sup>14</sup>C-1-dodecyl]DDAO is given. Application of an aqueous solution of [<sup>14</sup>C-methyl]DDAO to the clipped backs of rats, mice, or rabbits results in the absorption of 18, 18, and 47% of the dosed radioactivity, respectively, over a 72-hr continuous contact period.

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