

# ABSTRACTS OF PAPERS

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Abstracts of Papers for the Twelfth Annual Meeting of the  
Society of Toxicology, New York, New York  
March 18-22, 1973

1. *Fertility and Reproduction of Rats Treated with Hexachlorophene.* J. B. PLANK, P. L. WRIGHT, SANDRA L. HALEY, M. L. KEPLINGER, G. L. KENNEDY, JR., and J. C. CALANDRA, Industrial BIO-TEST Laboratories, Inc., Northbrook, Illinois.

A series of studies to assess the effect of hexachlorophene (HCP) on fetal development and pup survival have been completed using Charles River strain albino rats. Three levels (12.5, 25.0 and 50.0 ppm) were fed in a three-generation reproduction study. Two levels (5 and 10 mg/kg) were dosed po in a study to determine the effects on mating performance and fertility (Phase I) and two higher levels (15 and 30 mg/kg) were utilized in a perinatal and lactation performance study (Phase III). Mating and fertility indices were not affected by HCP in the Phase I study nor was there any indication of impaired fertility within any generation of the reproduction study. There was no evidence of increased fetal mortality resulting from prenatal exposure to HCP in any of the studies. Pup survival, during the lactation period, was reduced in the groups from dams given 15 or 30 mg/kg but was not affected in the groups from dams given lower levels of HCP. No unusual behavioral reactions were observed among pups from any group.

2. *Behavioral Toxicity of Hexachlorophene in Rats.* L. R. WEISS, J. T. WILLIAMS, and S. KROP, Food and Drug Administration, Washington, D.C.

Neurotoxic effects have been reported for hexachlorophene (HCP) in man and animals. Pathologic lesions associated with these effects in animals have been described as reversible. The reported return of neurologic function following recovery from severe intoxication prompted us to study the effects of HCP on behavior using three methods. (1) After establishing stable bar-pressing avoidance responding, adult female rats were treated daily with HCP (1, 5, 10 and 25 mg/kg, po) and tested 5 days a week. Below 25 mg/kg no adverse effects on behavior were seen for periods over 30 days. Rapid deterioration of avoidance responding occurred 1-2 days prior to onset of hindlimb paralysis. After withdrawal, bar-pressing escape behavior returned rapidly, but avoidance was delayed. A block in the recall process is suggested which requires a relearning of the conditioned behavior, although animals were capable of reaching original responding values. (2) Similar effects were produced by HCP (25 mg/kg) in rats conditioned to avoid a shock by movement to another arm of a T-maze. Increases in avoidance response times, decreases in response to conditioned tone with increases in performance errors and in response times to the shock were seen at this dose but not at 5 mg/kg. Behavioral recovery was complete in 5-7 days after drug withdrawal. (3) HCP (25 mg/kg, daily) causes hindlimb paralysis in about 12 days. Seven days after drug withdrawal and the disappearance of paralysis, a third group of (naive) rats were examined for their ability to acquire bar-pressing avoidance behavior. The data indicate a delay in the rate of learning to escape and to avoid, and a lag of long duration in "transfer" of escape to avoidance responding, although the rats eventually attained the avoidance level of controls. These results show that severe intoxication with HCP induces effects on behavioral processes which outlast the recovery from neurological deficits.

3. *Attenuation of CNS Toxicity of Hexachlorophene in the Rat and the Cat.* JOSEPH P. HANIG, J. MICHAEL MORRISON, JR., and STEPHEN KROP, Food and Drug Administration, Washington, D.C.

Reports of hexachlorophene (HCP) neurotoxicity include hindlimb paralysis with tonic extensor rigidity, paralysis of gut and bladder, brain edema, temperature elevation, hyper-ventilation, anorexia, nausea and vomiting. Vacuolated spongy degeneration of white matter

in brain and cord has been found, and is reversible several months after abstention from HCP. These findings suggested a pressure lesion resembling that seen in animals deficient in vitamin A and prompted us to determine the effect of vitamin A on HCP toxicity. Male weanling rats (90–110 g) were administered massive oral doses of vitamin A (2.5 or 30 mg/kg every third day; or 0.83 or 10 mg/kg daily) by gavage for 2 weeks with control animals receiving an equal volume of corn oil. HCP (50 or 75 mg/kg) was then given daily and vitamin treatment continued. Examination for changes in gross behavioral and neurological function was performed twice daily using a 6-point scale. Our results indicate that development of toxicity and death were significantly delayed by 1–2 weeks in rats receiving vitamin A. The severity of the lesions in brain paralleled the severity of the observed toxicity in both groups. In a similar study on 12 male cats, age 1½–2 yr, of 6 treated with vitamin A orally all survived with only 1 showing serious signs after 4 daily oral doses of HCP (20 mg/kg), while of 6 cats receiving HCP only, 3 died and 3 survivors showed moderate to serious signs. Some cats vomited shortly after HCP, but blood HCP concentrations correlated well with the severity of toxicity. Cerebrospinal (cisternal) fluid pressures (CSFP) under barbiturate anesthesia in cats showing moderate to severe neurological signs appeared elevated (180–225 mm H<sub>2</sub>O compared with normals of 50–70 mm H<sub>2</sub>O). Vitamin A deficiency has long been known to be a potent elevator of CSFP accompanied by a pressure lesion in white matter. Our findings indicate that vitamin A reduces toxicity of HCP; possibly by slowing the elevation of CSFP.

- ✓ 4. *Biological Responses of the Rat to Polychlorinated Biphenyls II.* J. V. BRUCKNER, K. L. KHANNA, and H. H. CORNISH, School of Public Health, The University of Michigan, Ann Arbor, Michigan.

Polychlorinated biphenyls (PCB's) have been detected throughout the world in tissues of a large number of biological species, including man. However, at present little toxicological basis exists for assessing the potential harm posed by PCB's to humans and to other mammals. The present series of investigations was undertaken to delineate significant biological manifestations of acute and prolonged exposure to PCB's. Based on previous studies in this and other laboratories, emphasis was placed upon renal and hepatic function, levels of certain heme compounds, and microsomal drug metabolism. A range of doses of a commercial PCB mixture (Aroclor 1242) was administered for varying periods of time by oral intubation and by ip injection to Sprague-Dawley rats. Principal findings included lipid vacuolation of renal and hepatic parenchyma, elevation of serum glutamic oxalacetic transaminase (SGOT) activity, depression of hematocrit, enhancement of liver weight, and elevation of hepatic microsomal enzyme activity. Within 24 hr after a single ip injection of 100 mg/kg of aroclor 1242, significant increases in the following hepatic parameters were observed: total weight, microsomal protein, hydroxylation of acetanilide and N-demethylation of aminopyrine, NADPH-cytochrome c reductase activity, and levels of cytochromes *P*<sub>450</sub> and *b*<sub>5</sub>. Hydroxylation activity remained elevated over control values for as long as 40 days. These results suggest that PCB's may alter the rate of metabolism of both endogenous and exogenous substances, thus affecting the biological responses of the mammal to drugs and to environmental stresses.

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The highly heterogeneous commercial polychlorinated biphenyls (PCB's) have been reported to alter mammalian hepatic function. Are the effects due to the biphenyl nucleus, the number of chlorine groups per molecule or the position of the chlorine groups on the ring structures? To answer these questions, pure biphenyl and isomerically-pure mono-, di-, tetra-, hexa-, octa- and decachloro biphenyls with known chemical position were injected ip (50 mg/kg/day) into young male Wistar rats (50–60 g) for 3 days, the animals being killed 96 hr after the last injection. The potency of the pure PCB's was compared to that of *o,p'*- and *p,p'*-DDT and commercial Aroclors (1254, 1260) administered at the same concentrations. Hepatic function was assessed by pentobarbital sleeping times and *in vitro* assays of hepatic microsomal *O*-demethylase, *N*-demethylase, aniline hydroxylase, nitro-reductase and carboxylesterase

activities and the cytoplasmic bromosulphophthalein (BSP)-glutathione conjugating enzyme system. The biphenyl nucleus played little role since biphenyl caused no induction of hepatic drug-metabolizing enzymes. Monochlorobiphenyl (4-Cl) was without influence; of the di- and tetra- chlorobiphenyls, substitution of chloro groups at the 3 and 4 positions resulted in enhanced induction of microsomal monooxygenases. The more highly chlorinated hexa- and octa- chlorobiphenyls caused an enhanced induction of monooxygenases, though differing little from one another. Nitro-reductase and carboxylesterase activities were not significantly affected by any of the agents whereas all agents, including biphenyl, caused a marked induction of the BSP-conjugating system.

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Five human male volunteers ingested a single dose of 5 mg/kg 2,4,5-T without incurring detectable clinical effects. Concentrations of 2,4,5-T in plasma and its excretion were measured at intervals after ingestion. The clearances from the plasma, as well as the body, occurred via apparent first order rate processes with half-lives of 23.1 and 29.7 hr respectively. Essentially all of the 2,4,5-T was absorbed into the body and excreted unchanged via the urine. In the body, 65% of the 2,4,5-T resided in the plasma where 98.7% was bound reversibly to protein. The volume of distribution was 0.097 liter/kg. Utilizing the kinetic constants from the single dose experiment, the expected concentrations of 2,4,5-T in the plasma of individuals receiving repeated daily doses of 2,4,5-T were calculated. From these calculations, it was determined that the plasma concentrations would essentially reach a plateau value after 3 days. If the daily dose ingested in mg/kg is  $A_0$ , the concentrations in the plasma after attaining plateau would range from 12.7  $A_0$  to 22.5  $A_0$   $\mu\text{g/ml}$ . This range would converge to approximately 17  $A_0$   $\mu\text{g/ml}$  as the daily dose  $A_0$  is distributed throughout the day.

- ✓ 7. *Effects of 2,4,5-T and other organochlorides on the metabolism of [1,2- $^3\text{H}$ ]testosterone by male organs of reproduction.* J. W. LLOYD, J. A. THOMAS, M. G. MAWHINNEY, and C. S. DIERINGER, Department of Pharmacology and Urology, West Virginia University Medical Center, Morgantown, West Virginia.

Male mice treated with varying doses (6.25, 12.5 or 25 mg/kg daily  $\times$  10, po) of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) led to a significant reduction in the ability of the prostate gland to assimilate radioactive testosterone ( $\text{T-H}^3$ ). Regardless of the dosage, the average reduction in the uptake of  $\text{T-H}^3$  by the prostate gland was about one-third. This treatment period did not cause any significant changes in the relative amounts of  $\text{T-H}^3$  metabolites in the prostate, but the absolute amounts of a least three radiosteroids were lowered by this herbicide. The highest dose regimen of 2,4,5-T (viz. 25 mg/kg daily  $\times$  10) caused a 33% reduction in the levels of dihydrotestosterone- $\text{H}^3$ , a 37% reduction in androstenediol- $\text{H}^3$  and a 14% reduction in prostatic androstenedione- $\text{H}^3$ . Acute dose regimens failed to alter the assimilation of labeled testosterone by the mouse prostate gland. The 10-day dose regimen of 2,4,5-T had no significant effect upon the bioconversion of  $\text{T-H}^3$  to its radiosteroid metabolites in homogenates of liver. Treatment with 2,4,5-T did not alter either the gravimetric responses of the male sex accessory organs nor did it affect prostate gland fructose levels. Although sex accessory organ cAMP- $\text{H}^3$  and ATP levels were unaffected by the 10-day administration of 2,4,5-T, preliminary data indicate that either dieldrin or DDT can alter these biochemical parameters. (Supported in part by a grant from the Environmental Protection Agency—EPA 801650-02.)

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The herbicides, 2,4-D and 2,4,5-T, are both weak organic acids and might be expected to be transported actively by renal tissue. This proposition has been examined using the tissue slice

technic, wherein slice uptake is equated to renal tubular secretion. Both rat and rabbit renal cortical slices were prepared by the free-hand procedure. All incubations were performed at 25°C with 100% oxygen unless otherwise stated. The time-course of accumulation by rabbit tissue was characterized by a rapid initial phase and a slower secondary phase of uptake, although no steady state was reached after 4-5 hr of incubation. Usually 2,4,5-T was accumulated to a greater extent than 2,4-D. For example, at 3 hr, the tissue slice:medium distribution ratio (S/M) for 2,4,5-T was 46 and for 2,4-D, 29. The uptake of both compounds was stimulated by acetate, pyruvate, and lactate, and inhibited by succinate. The metabolic inhibitors, 2,4-dinitrophenol and iodoacetamide, decreased uptake by 90%, as did nitrogen. The competitive inhibitor of renal organic acid transport, probenecid, reduced the S/M by 80%. Similar transport characteristics were noted for rat renal cortical slices. In addition a significant potassium requirement was noted for the uptake of both acids. These data are interpreted to mean that both herbicides are secreted actively by the mammalian kidney. Such a process may account for the relatively rapid plasma clearance reported in some *in vivo* studies. (Supported by PHS grant AM 13020).

9. *Non-teratogenicity of 2,4,5-Trichlorophenoxyacetic Acid in Monkeys (Macaca mulatta)*. W. H. DOUGHERTY, F. COULSTON, and L. GOLBERG, Albany Medical College, Albany, New York.

Reports that 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) affects embryonic development in various species, mainly rodents, have left unresolved the question of potential hazard to man resulting from exposure to 2,4,5-T containing a minimal amount of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). An answer to this problem was sought by studying the action of 2,4,5-T in the pregnant nonhuman primate *M. mulatta*. Mature female rhesus monkeys were mated with males of proven fertility. Forty pregnant females were assigned to either the control group, or one of the dose-level groups: 0.05, 1.0, and 10.0 mg/kg. Technical Grade 2,4,5-T containing less than 0.05 ppm TCDD was administered in a No. 5 gelatin capsule by stomach tube daily from day 22 through day 38 of pregnancy. Of the 10 control monkeys, 9 delivered live offspring. (The spontaneous abortion rate among untreated females in our colony is approximately 12%). Of the 10 monkeys in the 0.05 mg/kg group, all gave birth to normal babies. Seven of the monkeys receiving 1.0 mg/kg doses delivered live offspring; there was 1 stillborn fetus and 1 abortion, and 1 female has yet to deliver. Six of the monkeys receiving 10.0 mg/kg doses had live offspring; there were 2 abortions, and 2 females have not delivered. Hematology, clinical chemistry, and urinalysis data obtained on all females prior to and immediately following the administration of the 2,4,5-T and at 2-month intervals until parturition indicated no toxicity to the female. Detailed examination of the live infants and the stillborn fetus, including skeletal X-ray studies, revealed no gross developmental abnormality in any of the groups. Under the conditions of this experiment there is no evidence that 2,4,5-T is teratogenic in the rhesus monkey, nor that it interferes in any way with normal development of the young. (Supported by Research Grant 2PO1-ES00226-06 from the National Institute of Environmental Health Sciences, NIH, and by the National Institutes of Health Training Grant 2T01-ES00103-06.)

10. *The Significance of an Adaptation Phenomenon in Toxicity Studies*. T. BALAZS, Food and Administration, Washington, D.C.

Long term toxicity studies in animals may overlook what happens in the very early stages of the treatment. Following the repeated administration of various chemicals, certain degenerative tissue changes may occur rather early; but under continued dosage healing occurs and an apparent resistance of the tissue to the toxic effect develops. W. de B. McNider (1928) and others described this phenomenon for renal and hepatic tissues; others described it for the gastric and alveolar epithelium and for the myocardium. This type of tissue resistance has several common features: (1) It develops only after an induced lesion. (2) It is not specific to the inducing agent, but extends to agents causing similar lesions. (3) It lasts during the period of dosing as well as for varying times thereafter. (4) Its duration after the interruption of the dosing appears to be a function of the turnover rate of the affected morphological or biochemical

system. (5) Pharmacokinetic change does not seem to play a role in its development. This phenomenon is of significance in the safety evaluation of chemicals. The present practice of performing gross and histopathology examinations solely in long term studies may overlook toxic effects of the compound due to the development of tissue resistance or to other adaptation mechanisms. Such examinations appear to be necessary also in acute toxicity studies as well as periodically in the early stage of the long term study to detect those toxic effects to which resistance develops.

11. *Central Nervous System Effects of Some Dopamine Derivatives in the Mouse.* R. J. BORG-MAN, I. J. GOODMAN, J. J. MCPHILLIPS and R. E. STITZEL, Departments of Medical Chemistry, Psychology and Pharmacology, West Virginia University, Morgantown, West Virginia.

A series of dopamine analogs were synthesized and evaluated for their potential utility in the treatment of Parkinsonian-like states. The drugs were tested in mice using model systems as simulators of Parkinson's disease. These included the ability of the compounds to protect against oxotremorine-induced tremors or to reverse reserpine-induced depression. The compounds were also tested for their ability to decrease body temperature, a central dopaminergic response. The dopamine derivatives tested were: 3,4-diacetoxyphenyl-2-*N,N*-dimethylamino-propane (DD-1); 3,4-dihydroxy- $\beta$ -phenethylmethylamine (DD-2); *N,N*-dimethyl-3,4-dihydroxy- $\beta$ -phenethylamine (DD-3); *N,N*-dimethyl-3,4-diacetoxy- $\beta$ -phenethylamine (DD-4), and 3,4-diacetoxy- $\beta$ -phenethylamine (DD-5). Two compounds, DD-1 and DD-4, showed slight activity against tremors induced by oxotremorine. The same two compounds had a marked ability both to reverse reserpine-induced depression and to produce a fall in body temperature. The other compounds were relatively ineffective in the model systems. Apomorphine was moderately effective in reversing reserpine depression while both apomorphine and dopamine produced decreases in body temperature. It is tentatively concluded that both DD-1 and DD-4 gain access to the central nervous system, are active as potential antiparkinsonian drugs in animal models, and possess intrinsic dopaminergic activity.

12. *The Effect of Industrial Solvents on Adrenergic Transmitter Mechanisms.* TORBJÖRN MALMFORS, Astra Pharmaceuticals AB, Toxicology Laboratories, Södertälje, Sweden. (J. J. McPhillips.)

The biogenic amine, noradrenaline (NA), is a transmitter substance involved in many important functions of both the peripheral and central nervous system. As many transmitter mechanisms of the adrenergic nerves and the NA-neurons in the CNS are well known it was thought to be worthwhile to look into the effect of industrial solvents on these mechanisms in order to throw some light on the effect of these compounds on at least one type of neurons. The effect on the peripheral adrenergic nerves was studied *in vitro*. Mouse vas deferens was incubated in Krebs-Ringer solution containing varying concentrations of various solvents. The effect on the electrically elicited, nerve-mediated contractions was studied as well as other parameters. It was found that some solvents, but not all, interfered with the adrenergic transmission. Trichloroethylene potentiated the transmission by, among other things, increasing the sensitivity of the effector cells to NA. The effect of industrial solvents on the CNS was estimated by measuring the turnover rate of NA in animals exposed to vapours of organic solvents. This parameter is considered to be a relevant sign of an effect on the CNS. It was found that some solvents, particularly trichloroethylene, increased the NA turnover rate, while solvents with similar technical properties did not show the same stimulating effect on the CNS. These results indicate that some industrial solvents form a potential hazard to man by sensitizing effector cells to NA and by affecting the CNS. The latter effect might partly explain the addiction-like effect of some industrial solvents.

13. *Preclinical Toxicological Studies with AP-237, A New Analgesic.* L. E. GONGWER, A. G. WHEELER, G. E. FILLMORE, and S. J. RZUCIDLO, ICI America Inc., Wilmington, Delaware.

AP-237, or 1-*n*-butyryl-4-cinnamyl piperazine HCl, a novel compound originally synthesized by the Kyorin Pharmaceutical Co., Ltd. of Japan, is awaiting government approval in Japan following extensive clinical trials as a potent analgesic, and is currently under investigation at ICI America. Various species exhibited acute oral toxicity sensitivities to AP-

237 that followed a pattern: guinea pig (1000 mg/kg) < mouse < young rat < day-old and old breeder rat < dog (260 mg/kg). Species sensitivity to subcutaneous toxicity increased from young rat and guinea pig (400 mg/kg) < dog < old rat and mouse < day-old rat (100 mg/kg). AP-237 appeared to be slightly more toxic to female rodents than to male when administered po and less toxic when administered sc. A 90-day study in male and female Wistar rats fed *ad libitum* dietary levels of AP-237 to provide 190, 120, 75, 47.5, 30, or 0 mg/kg/day showed a significant dose-related reduction in the efficiency of feed utilization as indicated by decreased body weight gains and increased feed consumption. Other parameters (femur length, organ weights, clinical laboratory tests, and histopathology) were not affected by the drug. In a 35-day study, male and female beagle dogs (3/sex/level) were administered oral dosages of 48, 24, 12, or 0 mg AP-237/kg/day with no significant changes in growth, appearance, survival, and clinical laboratory tests. Liver and adrenal weights were significantly larger in the group receiving 48 mg/kg/day, but other organ weights were unaffected. Histologic observations were considered incidental or artifactual. These data encourage continued investigations with AP-237.

14. *Preclinical Toxicology of Fenoprofen*. J. L. EMMERSON, W. R. GIBSON, E. C. PIERCE, and G. F. KIPLINGER, Toxicology Division, Eli Lilly and Company, Greenfield, Indiana.

The sodium and calcium salts of fenoprofen [*d*l-2-(3-phenoxy-phenyl)propionic acid], a new anti-inflammatory drug, were evaluated for toxicological effects in animals. LD50 values for fenoprofen sodium (FS) in mice and rats were 500 mg/kg iv, 500–700 mg/kg sc and 800–1300 mg/kg po. The acute oral toxicity of the less soluble fenoprofen calcium (FC) was equivalent to that of FS. Large parenteral doses of FS induced clonic convulsions; the cause of death was attributed to respiratory depression. There were no effects noted in dogs given single doses of FS of 100 mg/kg iv or 200 mg/kg po. Rats tolerated dietary levels of 0.1, 0.2 or 0.4% FS for 90 days without significant mortality. While rats given 0.1% drug-diets were normal, the highest dosage (about 325 mg/kg/day) produced a necrotizing papillitis of the kidney in about 20% of the animals. FS, in daily oral doses of 5, 10 or 20 mg/kg/day for 90 days, produced dose-related erosions and ulceration of the gastric mucosa in dogs. Doses exceeding 30 mg/kg/day resulted in death of dogs from gastrointestinal ulceration within 14–21 days. No tetratological effects were observed in rats or rabbits given daily oral doses of 50 or 100 mg/kg of FC. In pre/postnatal studies the drug inhibited parturition in about 50% of rats given daily doses of 50 or 100 mg/kg/day. No drug effects on fetal development were observed nor were they evident during the postpartum or lactation periods.

15. *The Toxicological Properties of Seclazone (W-2354), 7-chloro-3,3a-dihydro-2H,9H-isoxazolo (3,2-b)(1,3) benzoxazin-9-one, a new anti-inflammatory agent*. N. B. BANERJEE, J. MOCK, and N. IVINS, Wallace Laboratories, Cranbury, New Jersey.

W-2354 is a new heterocyclic compound which possesses diuretic, uricosuric, and anti-inflammatory properties. The preclinical toxicity of this compound was evaluated in 520 (260 male–260 female) mature Long Evans Rats for a period of 48 weeks at dose levels of 200, 100, and 50 mg/kg/day. The compound was incorporated in the diet by mechanical mixer with levels adjusted weekly to maintain a relatively constant intake of test material. Water was available *ad libitum*. A reduction in food intake and body weight was observed for the animals in the 200 and 100 mg/kg/groups. There were some variation in the hematological values at 3 and 6 months, such as an elevated hematocrit in males and a higher neutrophil and lower monocyte counts in females in the 200 mg/kg group at 3 months, and a high RBC count in males and females receiving 200 mg/kg for 6 months. However, at termination, all hematological values were within the normal range. The evaluation of clinical biochemistry values indicated a lowered total protein value for the 200 mg/kg animals of both sexes and a decrease in sodium and chloride values in males as compared with controls. Nine males and 11 females of the 200 mg/kg group and 1 male of the 100 mg/kg group died during the study. However, at autopsy, no untoward effects were observed. At necropsy most of the organ weights when expressed as a percent of body weight were significantly increased in both sexes at the 200 mg/kg/day level. On the basis of these data, it may be concluded that W-2354 did not show any significant toxicity in the Long Evans rat up to a dose level of 200 mg/kg/day, which is approximately 10 times the human dose.

16. *Absorption, Distribution, and Excretion of Metiapine in Rats and Dogs.* R. H. HOOK, J. M. WILLIAMS, and G. J. WRIGHT, Merrell-National Laboratories, Division of Richardson-Merrell, Inc., Cincinnati, Ohio.

Metiapine (2-Methyl-11-(4-methyl-1-piperazinyl)-dibenzo[b,f] [1,4] thiazepine) has been characterized as a major tranquilizer. Absorption, excretion, and tissue distribution studies have been performed in Long-Evans rats and Beagle dogs dosed with [ $^{14}\text{C}$ ] metiapine (2 mg/kg, po). Rats excreted approximately 72% of the dose in the feces and 12% of the dose in the urine within 24 hr. Biliary secretion studies indicated that metiapine was well absorbed by rats and that a large part of the absorbed dose was returned to the gastrointestinal tract through biliary secretion as metabolites. Absorption and excretion studies in dogs gave results similar to those observed in rats, with feces being the major route of excretion. Peak plasma concentrations of  $^{14}\text{C}$  were reached 2 hr after dosing. Tissue  $^{14}\text{C}$  concentrations in rats 24 hr after dosing were highest in the liver and eyes. In dogs,  $^{14}\text{C}$  concentrations 72 hr after dosing were highest in liver, gall bladder, bile and heavily pigmented eye tissues (choroid and iris). Chronic toxicity studies failed to reveal any drug-induced corneal, lens, or retinal changes in albino rats or Beagle dogs (Gibson, J. P., Rohovsky, M. W., Newberne, J. W. and Larson, E. J., Toxicity studies with metiapine. *Toxicol. Appl. Pharmacol.*, in press.)

17. *Primate Absorption and Elimination Balance Studies Including Pulmonary, Urinary, Biliary and Fecal Excretion of *t*-Butanol, Trimethylsilanol, Dimethylsilanediol and Hexamethyldisiloxane.* DONALD R. BENNETT and WAYNE H. STATT, Biomedical Research, Dow Corning Corp., Midland, Michigan.

The simplest monomeric structural units, and potential hydrolysis products, of polydimethylsiloxanes ("silicones") have not been evaluated toxicologically. Trimethylsilanol ( $\text{Me}_3\text{SiOH}$ ) represents the end-blocking unit for linear siloxanes, and dimethylsilanediol [ $\text{Me}_2\text{Si}(\text{OH})_2$ ] is the internal monomeric unit of cyclic and linear siloxanes.  $\text{Me}_3\text{SiOH}$  can easily condense to form hexamethyldisiloxane ( $\text{Me}_3\text{SiOSiMe}_3$ ) and thus should also be evaluated in any study of  $\text{Me}_3\text{SiOH}$ .  $\text{Me}_3\text{SiOH}$  could be considered a bioisostere of *t*-butanol ( $\text{Me}_3\text{COH}$ ), but no stable carbon analog of  $\text{Me}_2\text{Si}(\text{OH})_2$  is available.  $\text{Me}_3\text{COH}$ ,  $\text{Me}_3\text{SiOH}$ ,  $\text{Me}_3\text{SiOSiMe}_3$  and  $\text{Me}_2\text{Si}(\text{OH})_2$  are all completely absorbed orally.  $\text{Me}_3\text{SiOH}$ , and to a lesser extent  $\text{Me}_3\text{SiOSiMe}_3$  but not  $\text{Me}_2\text{Si}(\text{OH})_2$ , is in fact a CNS depressant similar to  $\text{Me}_3\text{COH}$  in the mouse, rat, rabbit, and monkey except that  $\text{Me}_3\text{SiOH}$  is about three times as potent as  $\text{Me}_3\text{COH}$  on an equimolar basis. Specially constructed primate metabolic chambers were used for 3-4 days for continuous monitoring of expired air, and separate collection of urine and feces without volatile  $^{14}\text{C}$  losses. In addition each monkey underwent cholecystectomy 2-3 weeks prior to being used for the initial metabolic study, and a chronic indwelling common bile duct cannula was implanted which drained into the duodenum and which could be exteriorized or replaced through a non-draining intestinal fistula as desired. The common bile duct cannula and intestinal fistula allowed continuous monitoring of bile flow and sampling of  $^{14}\text{C}$  activity. After monitoring and sampling, the bile was immediately returned to the duodenum. Efficiency of recovery of administered dose was  $99.3 \pm 4.1\%$  ( $n = 17$ ). At dose levels of 20 to 80 mg/kg given po or iv to rats or monkeys,  $\text{Me}_3\text{SiOH}$  and  $\text{Me}_3\text{COH}$  were both eliminated similarly, i.e., 10-30% pulmonary, 70-85% urinary, 1-5% in bile but ultimately less than 1% in feces. Rates of excretion were similar and 90% of the dose was eliminated in 24 hr. The values for  $\text{Me}_3\text{SiOSiMe}_3$  po were similar; however, the values for pulmonary and urinary excretion were reversed after iv administration.  $\text{Me}_2\text{Si}(\text{OH})_2$  po or iv was different only in that no pulmonary excretion was evident and the urinary value was correspondingly increased.

18. *Primate Absorption and Elimination Balance Studies Including Pulmonary, Urinary, Biliary and Fecal Excretion of Octamethyl-cyclotetrasiloxane and 2,6-cis-Diphenylhexamethylcyclotetrasiloxane.* JOSEPH E. LEBEAU and STANLEY J. GORZINSKI, Biomedical Research, Dow Corning Corp., Midland, Michigan. (D. R. Bennett.)

In a continuing study involving the metabolism of organosilicon compounds, two specific low molecular weight cyclic oligomers have been examined in the unanesthetized monkey (M.



mullata). 2,6-*cis*-Diphenylhexamethylcyclotetrasiloxane, 2,6-*cis*-[(PhMeSiO)<sub>2</sub>-(Me<sub>2</sub>SiO)<sub>2</sub>], is an antigonadotrophin (Toxicol. Appl. Pharmacol. **21**, 12-88, 1972). Octamethylcyclotetrasiloxane, (Me<sub>2</sub>SiO)<sub>4</sub>, is the corresponding all-methyl analog. Specially constructed primate metabolic chambers were used for 3-4 days for continuous monitoring of expired air, and separate collection of urine and feces without volatile <sup>14</sup>C losses. In addition each monkey underwent cholecystectomy 2-3 weeks prior to being used for the initial metabolic study, and a chronic indwelling common bile duct cannula was implanted which drained into the duodenum and which could be exteriorized or replaced through a non-draining intestinal fistula as desired. The common bile duct cannula and intestinal fistula allowed continuous monitoring of bile flow and sampling of <sup>14</sup>C activity. After monitoring and sampling the bile was immediately returned to the duodenum. At dose levels of 1 mg/kg, efficiency of recovery of administered dose was 89 ± 5% (n = 6). Both compounds were well absorbed orally, and 80% of the dose was eliminated in the first 48 hr. After po and iv administration, recovery of radioactivity from 2,6-*cis*-[(PhMeSiO)<sub>2</sub>-(Me<sub>2</sub>SiO)<sub>2</sub>] was found to be respectively 3.3 and 2.8% in expired air, 60 and 65% in urine and 28 and 20% of total dose, in feces, while 45% of the total dose had passed through the bile after the iv dose. Similarly, recovery of radioactivity from (Me<sub>2</sub>SiO)<sub>4</sub> was found to be 23.5 and 18.9% in expired air, 55.5 and 71.9% in urine and 8.4 and 1.8% in feces, while 11.5% had passed through the bile. Thus enterohepatic circulation was evident for both compounds.

- ✓ 19. *Chronic Toxicity and Carcinogenicity of Industrial Chemicals and Pesticides*. B. ULLAND, E. K. WEISBURGER and J. H. WEISBURGER, Litton-Bionetics Inc., Kensington, Maryland, and National Cancer Institute, Bethesda, Maryland.

Two-year studies were conducted in Charles River CD rats to determine the chronic toxicity or carcinogenicity of environmental chemicals. The maximal tolerated dose and half that level, experimentally determined for each chemical, were either incorporated into ground diet or given by gastric intubation twice weekly to male and female rats. In most instances, experimental materials were administered for 18 months followed by a 6-month observation period. *N*-2-Fluorenylacetamide was used as a positive carcinogenic control. At the termination of these studies, there was a high incidence of benign tumors in surviving negative control and experimental animals. These benign tumors principally affected the pituitary and mammae. Under the conditions of this experiment, the following chemicals were determined to be noncarcinogenic: Sodium azide, bis(2-chlorethyl)-ether, Mirex, saccharin (o-benzoic sulfimide), semicarbazide, and ethylene carbonate. Semicarbazide, however, proved to be a lathyrogen and affected the high-dose animals starting at the 10th week of the study. These animals suffered severe skeletal deformities. Ethylene carbonate caused severe nephrotoxicity, especially to the high-dose males, which was not observed in the preliminary toxicological experiments. There was an increased incidence of tumor-bearing animals when compared to negative control animals with the potassium salt of bis(2-hydroxyethyl)dithiocarbamic acid and Avadex. However, a wide variety of organs was affected and there was no definitive increase in tumors at one site.

20. *Further Studies on Carcinogenicity of Environmental Chemicals Including Simple Aromatic Amines*. A. B. RUSSFIELD, F. HOMBURGER, E. K. WEISBURGER, and J. H. WEISBURGER, Bio-Research Consultants, Inc., Cambridge, Massachusetts, and National Cancer Institute, Bethesda, Maryland.

Ortho- para- and meta-toluidine are used as chemical reagents and in the dye industry. Six-year studies with large doses of each of the three compounds in dogs (Deichman, 1967) were negative. Moradami and Nisimura (1940) had obtained bladder tumors in rabbits with ortho-toluidine given by sc injections. In the present study male Charles River (Sprague-Dawley derived) CD rats and male and female Charles River (Ha/ICR) CG-1 mice were given ortho-, meta- or para-toluidine at two dose levels in the diet. Rats given the ortho compound showed significant incidences of urinary bladder cancers as well as of subcutaneous tumors. In mice vascular tumors occurred in both sexes receiving the ortho compound and hepatomas were seen in those animals receiving the para compound. Neither bladder, vascular

or hepatic tumors were seen in rats and mice receiving the meta compound. The previously described carcinogenicity of ortho-toluidine for bladder epithelium is thus confirmed. Paratoluidine also appears to have carcinogenic potency but does not affect the rodent bladder.

21. *Studies on the Toxicity and Carcinogenicity of Some Commonly Used Chemical Agents in Mouse and Rabbit Skin.* F. STENBACK AND P. SHUBIK, Eppley Institute for Cancer Research, University of Nebraska Medical Center, Omaha, Nebraska.

Because little is known about the toxicity or carcinogenicity of many commonly used cosmetics, detergents, solvents and fixatives, longterm studies on their effect in mouse and rabbit skin were performed. Each chemical was applied topically with a graduated pipette (0.02 ml, 2 times weekly for life) on the backs of Swiss mice and on the inner side of the ears of rabbits. Animals were inspected and weighed weekly. Autopsies were performed on all animals and gross lesions, as well as all tumors, were studied histologically. In experiments using hexachlorophene a significant percent of the animals died at the beginning of the experiment; however, the survivors did not exhibit an excessive mortality during the later part of the experiment. Gross, visible neurologic abnormalities were common though most were reversible. Histologically hemorrhage in the brain was seen as well as perivascular lymphocyte infiltration and necrosis of the vascular wall in the dermis, lungs, liver, and kidney. Large ulcerations in the epidermis were common. Of the other substances studied, only benzophenone-treated animals showed an increased mortality compared to the acetone-treated and untreated control animals. A very small number of skin tumors occurred; 2 squamous cell papillomas, 1 squamous cell carcinoma and a few tumors of dermal origin (dermatofibromas, neurofibromas and fibrosarcomas) occurred. The incidence of spontaneous tumours did not increase.

22. *Acute Toxicity of 2-Fluorenylacetamide (2-FAA) in Mice.* THOMAS J. HALEY, K. L. DOOLEY, and J. R. HARMON, National Center for Toxicological Research, Jefferson, Arkansas.

Wilson *et al.* (Cancer Res. 1941, 1, 595-608) reported little or no acute toxicity of 2-FAA in C57 mice but did not conduct an LD50 determination. Moreover, no signs of acute toxicity were described. Determination of the oral LD50 of 2-FAA in CD-1 strain mice gave the following results: males 2.02 (1.7-2.38) g/kg and females 1.87 (1.57-2.25) g/kg. The slopes of the curves were 1.19 (1.07-1.32) and 1.20 (1.06-1.36), respectively. Sedation, lachrymation, rigid paralysis of all four legs, secretion of a red-orange urine, and circling both clockwise and counterclockwise during recovery were observed. Histopathological examination of survivors revealed concretions in the urinary bladder 30 days after dosing. Observations on dead animals indicated the appearance of concentration 48 hr after dosing. The oral LD50 in C57BL/6SP was: males 1.22 (1.16-1.28) g/kg and females 1.02 (0.94-1.10) g/kg. The slopes of the curves were 1.11 (0.98-1.25) and 1.13 (0.96-1.33), respectively. With both strains of mice, the females were more susceptible. Potency comparisons between strains showed the C57BL/6SP strain to be more susceptible, but this was related to the use of sonication for suspending the 2-FAA in the latter determination.

✓ 23. *Tumorigenesis of Dimethylnitrosamine in the Pekin Duck.* M. D. MCCracken, G. D. BOTTOMS, and W. W. CARLTON, School of Veterinary Science and Medicine, Purdue University, West Lafayette, Indiana.

The carcinogenicity of dimethylnitrosamine (DMN) has been well established for a number of animal species. The acute toxic effects of DMN have been previously described in Pekin ducks. In this study DMN was fed continuously at a level of 0.005% to male Pekin ducks for 8 to 10 months. Approximately 40% of the animals died during the first 8 months, and these deaths were usually attributable to the hepatotoxic effect of DMN. Hepatic lesions consisted of the formation of hematocysts and marked fibroplasia, often with rupture of the hematocysts. Hepatic neoplasms were first seen at 6 months. By 9 months hepatic neoplasms were present in 71% of the ducks. Grossly the neoplasms were from a few mm to several cm in diameter and were either solid white masses or cystic nodules filled with blood. Histologically the neoplasms were highly anaplastic hemangiosarcomas. DMN was not observed to be carcinogenic

in other organs. The addition of prednisolone to the diet reduced the incidence of hepatic neoplasms to 51%.

- ✓ 24. *Effect of Diet on Carcinogenesis by Nitrosamines or Dimethylhydrazine.* A. E. ROGERS and P. M. NEWBERNE, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Certain carcinomas in man, those of lung, esophagus, colon, bladder and liver, apparently result from exposure to environmental carcinogens. Diet is a major environmental factor which may either contain carcinogens or influence the body's production or metabolism of them. We have found that lipotrope (choline) deficiency enhances hepatocarcinogenesis in rats by aflatoxin B<sub>1</sub> (AFB<sub>1</sub>). Male, Sprague-Dawley rats were fed semisynthetic control or lipotrope-deficient diet containing diethylnitrosamine (DEN), 40 ppm for 18 weeks or one of the diets without added DEN and given dibutyl nitrosamine (DBN), 200 mg/kg/wk sc for 19 weeks. At 7 months, 50% of lipotrope-deficient, DEN-treated rats were dead of hepatocarcinoma, whereas 16% of controls had died. Eighteen percent of deficient rats had esophageal squamous carcinoma or polyp, which were not found in controls. In DBN-treated rats, 20% of deficient and 4% of normal rats developed hepatocarcinoma. The incidence of lung and bladder tumors was the same in both groups, 33 and 35% respectively. In a separate study, we examined the effect of vitamin A on dimethylhydrazine (DMH) carcinogenesis in the colon. Vitamin A deficiency enhances induction of colon tumors by AFB<sub>1</sub>. Rats were fed semisynthetic diet containing 500, 30 or 0-1 IU vitamin A/g and given DMH 15-30 mg/kg intragastrically once a week. Colon carcinoma developed in 60-100% of rats at 6 months. Rats fed excess vitamin A had fewer tumors per animal than controls but the same incidence. Vitamin A-deficient rats had a slightly greater incidence of tumors than controls. (Supported in part by PHS-NCI contract 72-2076.)

- ✓ 25. *Induction of Tumors by Simultaneous Feeding of Nitrite and Morpholine.* R. C. SHANK and P. M. NEWBERNE, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Recent studies have indicated that under appropriate conditions the food additive sodium nitrite may be involved in the *in vivo* nitrosation of certain amines to form carcinogenic *N*-nitrosamines. A study was begun in September 1971 in which rats and hamsters were fed a semisynthetic diet containing 5 to 1000 ppm sodium nitrite and/or morpholine; animals fed 5 or 50 ppm *N*-nitrosomorpholine served as positive controls. For over a year rabbits have been fed similar diets at fewer levels of nitrite and morpholine. While nitrite and morpholine produced no detectable nitrosamine in the diet, feeding the combination resulted in high incidences of liver cell carcinoma with a high rate of lung metastases. Angiosarcoma primary to both lung and liver were also frequently observed. Diet containing 1000 ppm sodium nitrite and morpholine induced tumors in more rats in a shorter time than did diet containing 50 ppm *N*-nitrosomorpholine. At the high level of sodium nitrite, diets containing as little as 5 ppm morpholine were capable of inducing liver cell carcinoma. The studies support the conclusion that nitrite can react *in vivo* with morpholine to form the carcinogenic product, *N*-nitrosomorpholine. (Supported by the US Public Health Service Food and Drug Administration Contract 71-81.)

26. *Effects of 2-aminoanthraquinone on the kidneys and hemochemistry of rats.* J. R. BAKER, E. R. SMITH, T. N. FREDRICKSON, H. ROSENKRANTZ, J. H. WEISBURGER, and E. K. WEISBURGER, Mason Research Institute, Worcester, Massachusetts. National Cancer Institute Bethesda, Maryland.

In a chronic toxicity test 100 male and 100 female Fischer 344 rats were fed 1 or 2% 2-aminoanthraquinone mixed in ground Wayne Laboratory Meal. Following 6-8 weeks of treatment, clinical signs of drug-related changes were noted mainly in females. During the next 12-14 weeks, toxicity was manifested sequentially by weight loss, alopecia, urethral discharge, dehydration, ataxia, and finally, convulsions prior to death or sacrifice. Toxic signs and lethality occurred 2 weeks sooner in females at high dose than at low dose. Blood

chemistry studies of females revealed a depression of hematocrit, a decline in hemoglobin and RBC, while both sexes showed an elevated WBC. Blood glucose increased by 40% in females and 159% in males and both sexes were hyperkalemic. In female rats BUN values were increased by 525%. Nephrotoxicity was confirmed by histopathological observation of deposits of yellow-brown crystals of the parent compound and/or its metabolites in medullary and cortical tubules. Metaplasia of tubular epithelial cells, multinucleated giant cells, marked interstitial fibrosis and round cell infiltration was observed. Bone marrow was hypoplastic with depression of leukoblasts and erythroblasts. In males there were no histopathological abnormalities. Thus, 2-aminoanthraquinone initiated renal uremia and bone marrow hypoplasia which were sex-related.

- ✓ 27. *Biochemical, Mutagenic and Pathological Effects of Nitrosamines in Rats.* FRANCES SAURO, LEONARD FRIEDMAN and SIDNEY GREEN, Food and Drug Administration, Washington, D.C.

Biochemical cytogenetic (bone marrow cells) and mutagenic determinations (dominant lethal test) were performed in rats receiving chronic dietary administration of sodium nitrite and morpholine given separately or in combination and acute administration of nitrosomorpholine (NM) or dimethylnitrosamine (DMNA). No dominant lethal or cytogenetic effects were seen in rats receiving 1% dietary sodium nitrite for 31 weeks, or 1% dietary sodium nitrite for 15 weeks followed by the combination of 1% sodium nitrite and 0.5% morpholine for 16 more weeks. The rats in the latter group grew at a slower rate than the rats of the other groups and the livers of all the surviving rats in this group had tumors and a lower protein and RNA content and higher DNA content than those of the non-treated rats. Single oral doses of 117-470 mg NM/kg or 50 mg DMNA/kg 29 hr prior to sacrificing produced inconsistent cytogenetic effects (only gaps and non-significant chromatid breaks were seen). The administration of NM and DMNA resulted in liver and kidney enlargement, an increase in plasma transaminase and a decrease in incorporation of [<sup>3</sup>H]leucine into plasma protein. These effects were more pronounced with DMNA than NM. Preliminary results with DMNA (7.4 mg/kg/day for 5 days) indicated no dominant lethal effects.

28. *The Effect of Natural and Synthetic Delta-9-tetrahydrocannabinol on Reproductive and Lactation Performance in Albino Rats.* M. L. KEPLINGER, P. L. WRIGHT, SANDRA L. HALEY, J. B. PLANK, MONIQUE C. BRAUDE, and J. C. CALANDRA, Industrial BIO-TEST Laboratories, Inc., Northbrook, Illinois.

Experiments to determine the effects of synthetic delta-9-tetrahydrocannabinol ( $\Delta$ -9-THC) and of a crude marijuana extract (CME) containing 16%  $\Delta$ -9-THC upon mating performance, fertility, fetal viability, lactation performance and pup survival were conducted. Charles River strain albino rats were dosed po with 0.5, 1.5 or 5.0 mg  $\Delta$ -9-THC/kg from either source daily. Dosing was initiated 60 days prior to mating for males and 14 days prior to mating for females in the Phase I studies and on day 15 of gestation in the Phase III studies. Drug administration to the parental animals was continued until sacrifice. A slight depression in body weight gain was observed among rats given 5 mg/kg. Behavioral reactions observed among animals from all treatment groups included inactivity, irritability, pallor and pupillary constriction. The incidence and severity of these reactions were dose-related. An apparent tolerance was manifested by a decrease in the severity and duration of these reactions. There were no treatment-related mortalities. Mating and fertility indices were similar for the control and all treatment groups. At the interim sacrifice, on day 14 of pregnancy, the numbers of corpora lutea, implantation sites, and viable fetuses were similar for all groups. The numbers of live fetuses/100 implantation sites for the control and each of the three dose levels of synthetic  $\Delta$ -9-THC and CME respectively were 93, 93, 98, 97, 94, 93, and 97. The average numbers of pups delivered and viable at birth did not differ among treatment and control groups. There were no statistically significant differences in number of live pups on lactation days, 1, 4, 12 or 21. The average numbers of pups weaned for the control and each of the three dose levels of synthetic  $\Delta$ -9-THC and CME respectively in the Phase III studies were 7.3, 7.2, 6.3, 4.7, 7.6, 6.5 and 6.4. (Supported by NIMH contract HSM-42-71-106.)

29. *The Effect of Natural and Synthetic Delta-9-tetrahydrocannabinol on Fetal Development.*

SANDRA L. HALEY, P. L. WRIGHT, J. B. PLANK, M. L. KEPLINGER, MONIQUE C. BRAUDE, and J. C. CALANDRA, Industrial BIO-TEST Laboratories, Inc., Northbrook, Illinois.

New Zealand albino rabbits and Charles River strain albino rats were used in experiments to determine the teratogenic potential of synthetic delta-9-tetrahydrocannabinol ( $\Delta$ -9-THC) and of crude marijuana extract (CME) containing 16%  $\Delta$ -9-THC. Pregnant rabbits were dosed po from days 6 through 18 of gestation with 0.5, 1.5, 5.0 or 15.0 mg  $\Delta$ -9-THC/kg from either source. All females were sacrificed one day prior to term. Sedation lasting 1 to 2 hr was noted among all rabbits given 15 mg/kg; sedation was less apparent at the lower dose levels. There were no deaths among treated or control rabbits. A significant depression in body weight gains of females given 15 mg/kg from CME was observed. The numbers of live rabbit fetuses/100 implantation sites were 90, 91, and 75 for 3 control groups, 84, 90, 72, and 78 for the four levels of synthetic  $\Delta$ -9-THC and 82, 90, 75, and 56 for the four levels of CME. External abnormalities were observed in 2 of 348 fetuses from control rabbits, in 3 of 445 fetuses from rabbits given synthetic  $\Delta$ -9-THC and 3 of 349 fetuses from rabbits given CME. Similar findings were observed in pups from treated and control dams. Examinations of internal and skeletal development disclosed no effects which could be related to prenatal exposure of rabbit fetuses to synthetic  $\Delta$ -9-THC or CME. Pregnant rats were dosed po from Days 6 through 15 of gestation with 5, 15 or 50 mg  $\Delta$ -9-THC/kg from either synthetic  $\Delta$ -9-THC or CME. Abnormal behavioral reactions observed among animals from all groups included inactivity, irritability, pallor and pupillary constriction. The severity and duration, but not the incidence, of these reactions were dose-related. An apparent tolerance was manifested by a decrease in the severity and duration, but not complete absence, of these reactions. There were no treatment-related mortalities. Maternal weight gains were depressed in all treatment groups. The numbers of live rat fetuses/100 implantation sites were 96 and 96 for two control groups, 94, 96 and 96 for the three levels of  $\Delta$ -9-THC and 95, 94, and 97 for the three levels of CME. Examinations of external, internal and skeletal development disclosed no effects which could be related to prenatal exposure of rat fetuses to synthetic  $\Delta$ -9-THC or CME. (Supported by NIMH contract HSM-42-71-106.)

30.  *$\Delta$ <sup>9</sup>-Tetrahydrocannabinol and defensive behavior in rats.* KLAUS A. MICZEK and HERBERT BARRY, III, Department of Psychology, Carnegie-Mellon University and Department of Pharmacology, University of Pittsburgh, School of Pharmacy, Pittsburgh, Pennsylvania.

Contradictory reports of effects of  $\Delta$ <sup>9</sup>-tetrahydrocannabinol (THC) on aggressive behavior in laboratory animals prompted us to investigate this drug in a situation which reliably generates the full complement of fighting behavior in rats without preceding painful stimulation. Rats maintained at 90% of their free-feeding weight were trained to eat food pellets in a chamber (30 × 30 × 28 cm). Defensive behavior was quantitatively measured in 15-min tests in the same chamber with an experienced, dominant, stimulus animal, in the absence of food. A total of 36 test animals and 8 stimulus animals were used. The test animals were injected ip, 30 min before the start of the test, with THC (1, 2 or 4 mg/kg) or its vehicle (20% propylene-glycol—1% Tween 80-isotonic saline) in a volume of 1 ml/kg. After injections with the vehicle, the test animals showed the ritualistic defensive-submissive behavior pattern usually observed in response to a dominant animal, including defensive-upright, submissive-supine, and immobile-crouching postures, urination, defecation, and absence of grooming behavior, while being threatened and attacked, but usually without injuries. The highest THC dose increased greatly the duration of immobile-crouching postures, while tending to decrease the duration of defensive-upright postures. Also, the drug increased the frequency of attacks and, especially, the severity of injuries. The lower doses had smaller effects in the same direction. With all three doses of THC there was no significant effect on other components of the submissive-defensive behavior pattern (submissive-supine posture, urination, defecation, grooming behavior) or on running speed to a food pellet in a control test. Contrary to some other reports, THC did not increase aggressive behavior, but apparently impaired the defensive-submissive behavior pattern which effectively prevents or minimizes injury in normal intraspecies fighting.

31. *Some Cardiovascular Actions of  $\Delta^9$ -Tetrahydrocannabinol in the Rat.* BARBARA R. MANNO and JOSEPH E. MANNO, Veterans Administration Hospital and Department of Pharmacology and Therapeutics, Louisiana State University Medical Center in Shreveport, Shreveport, Louisiana. (Charles L. Winek.)

The mechanism of the highly reproducible,  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC) dose-related tachycardia in humans who have smoked marijuana remains obscure. This work was initiated as one phase of a study to differentiate the centrally mediated versus direct myocardial and vascular effects of  $\Delta^9$ -THC. Hearts from decapitated rats were perfused by a modified Langendorf technique with a modified Krebs-Henseleit bicarbonate medium, pH 7.4. A 10-g diastolic tension was maintained on the spontaneously beating heart for the duration of the experiment. Force of contraction and coronary perfusion pressure changes were also monitored. Twelve doses of  $\Delta^9$ -THC ( $1.04 \times 10^{-9}$  to  $1.04 \times 10^{-5}$ M) were infused into the hearts for 3-min intervals. No chronotropic change was observed at any dose of  $\Delta^9$ -THC used. Alterations in both inotropic response and coronary vasculature resistance were observed. As the concentration of  $\Delta^9$ -THC increased, a negative inotropic effect of as much as -26% of control was observed. The vasculature resistance denoted by changes in perfusion pressure produced a biphasic response. Concentrations of  $\Delta^9$ -THC from  $1.04 \times 10^{-9}$  to  $2.91 \times 10^{-6}$ M were vasoconstrictive with increases in pressure as much as +33% of control.  $\Delta^9$ -THC concentrations of  $2.65 \times 10^{-6}$  to  $1.04 \times 10^{-5}$  M produced a decrease in pressure of as much as -34% of control indicating vasodilation. These studies using varied concentrations of  $\Delta^9$ -THC in isolated perfused hearts indicate that (1) the  $\Delta^9$ -THC dose-related tachycardia is not a direct effect on the myocardium; (2) a direct myocardial inotropic effect was induced by low concentrations of  $\Delta^9$ -THC and (3) a direct coronary vasculature effect was produced by  $\Delta^9$ -THC and (3) a direct coronary vasculature effect was produced by  $\Delta^9$ -THC. (Supported in part by NIMH Grant MH21383-01 and Veterans Administration Research Grant #2-72.)

32. *Induction of Aryl Hydrocarbon Hydroxylase in Rat Lung by Inhalation of Marijuana. Smoke.* JACQUES MARCOTTE and HANSPETER WITSCHI, Department of Pharmacology, Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada.

Peroral administration of a crude cannabis preparation to rats has been found to enhance the activity of aryl hydrocarbon hydroxylase (benzpyrene hydroxylase) in liver and lung. It seemed therefore desirable to investigate whether the inhalation of smoke from cannabis sativa plant material would have a similar effect. Male Sprague-Dawley rats (110-140 g) were exposed in a small inhalation chamber to a mixture of air and smoke produced by burning cigarettes rolled from dried cannabis sativa (0.8 g of material/cigarette). The exposure to the smoke given off by 4 cigarettes, burnt within 1 hr, produced significantly increased activity of pulmonary aryl hydrocarbon hydroxylase; peak activity (up to 3 times control values) occurred between 6 to 12 hr after exposure and then fell off. Induction was dose-dependent if examined 6 hr after exposure to 2 to 8 cigarettes and persisted up to 24 hr after 6 and 8 cigarettes, but not after 2 or 4 cigarettes. In these experiments, only negligible enzyme induction was observed in the livers of the same animals. Pretreatment of the rats for 1 hr with actinomycin D (2 mg/kg) prevented to 50%, and with cycloheximide (2 mg/kg) prevented to 100%, the appearance of enhanced pulmonary aryl hydrocarbon hydroxylase activity, measured 6 hr after exposure to 4 cigarettes. In a parallel series of experiments, rats were exposed to the smoke of a marijuana placebo; this was plant material from which the cannabinoids had been removed by solvent extraction. Smoke produced by the placebo proved to be as effective an inducer of pulmonary aryl hydrocarbon hydroxylase as was the material containing the cannabinoids. Since pulmonary aryl hydrocarbon hydroxylase is also readily induced by carcinogenic polycyclic hydrocarbons, it might be appropriate to consider in future pharmacological and toxicological studies on marijuana not only the cannabinoids and their derivatives, but also all the other products occurring in the crude plant material. (Supported by the Canadian Medical Research Council, MRC Group in Drug Toxicology.)

33. *Methadone-Induced Aggressive Behavior*. MELBRA DIANE SINGH and JASBIR M. SINGH, Department of Pharmacology, Xavier University College of Pharmacy, New Orleans, Louisiana.

Methadone substitution program for the treatment of narcotic addiction are being tried widely in the U.S.A. and other parts of the world. This approach has caused considerable concern because of increased behavioral toxicity, patient death, swelling of the ankle, feet, and face and hepatic changes. As a matter of fact no study dealing with the behavioral toxicological effect has been reported. We have developed a model to study the behavioral toxicological effects of methadone. Rats, weighing 150–175 g, are fed 0.1 % methadone in the drinking water for 180 days. Methadone is dissolved in distilled water. The drug is withdrawn on the 181st day and the signs of physical dependence (tremors, change in weight and temperature) appear within 24 to 72 hr after drug withdrawal. Nalorphine, 2.5 mg/kg ip produced tremors only and no intense aggression in methadone-withdrawn (24–72 hr) animals. Reserpine, 2.5 mg/kg, is injected 24 hr after the drug withdrawal. Another injection of reserpine is given 48 hr after the drug withdrawal. In order to induce aggressive behavior, the methadone-withdrawn reserpine-treated animals are injected with 2.5 mg/kg of *d*-amphetamine sulfate 72 hr after the drug withdrawal. The injection of amphetamine sulfate will induce signs of abstinence such as, loss of body weight, change in body temperature, tremors, vocalization when handled and stereotype fighting postures with actual fighting (mild) and biting attacks. In addition to methadone-induced behavioral toxicity loss of hair and scratching behavior were induced in 20 % of the animals. (In part supported by Scholl Foundation.)

34. *EEG Correlates of Behavioral Toxicity of Neuroleptic Drugs*. S. FIELDING, M. CORNFELDT, T. MCGREEVY, B. OUTWATER, and L. PACIFICO, Research Department, CIBA-GEIGY Pharmaceutical Co., Summit, New Jersey. (H. Lal.)

At the preclinical level the continuous shock avoidance procedure is routinely used for detecting antipsychotic compounds. The blockade of continuous avoidance behavior is indicative of neuroleptic activity and may be viewed as behavioral toxicity. The purpose of the present study was to compare in the squirrel monkey the antiavoidance effects of haloperidol and thioridazine with electrophysiological changes observed in the EEG. Animals were implanted bilaterally under halothane anesthesia with concentric bipolar stainless steel electrodes aimed at the substantia nigra, globus pallidus, and putamen. Stainless steel screws served as electrodes for areas 4 and 6 of the motor cortex. EEG was recorded on an 8-channel Grass 78 polygraph while the animal was working on a continuous shock avoidance procedure with response shock and shock intervals set at 24 and 8 sec respectively. The results indicate that the electrophysiological correlates of behavioral toxicity produced by a 5 and 10 mg/kg oral administration of haloperidol and thioridazine respectively, have two major distinctions. First, during control every bar press and shock is associated with a period of low voltage fast activity (LVFA). This activity resembles a general activation pattern. Following the administration of either drug LVFA is present but the duration gradually begins to shorten until it lasts only as long as the shock presentation (0.5 sec). Second, approximately 20 min following avoidance blockade a persistent high voltage slow activity (HVSA) pattern was observed in all areas with the exception of the globus pallidus. A major difference between the two compounds occurs in the putamen during HVSA. A particular rhythmic pattern occurring frequently was noted under haloperidol. With thioridazine such a pattern was observed only occasionally. In conclusion, the HVSA pattern may be a manifestation of neuroleptic depression.

35. *Behavioral Aspects of Morphine-Amphetamine Interaction*. HARBANS LAL, JOSEPH ZABIK, and SURENDRA K. PURI, Department of Pharmacology and Toxicology, College of Pharmacy, University of Rhode Island, Kingston, Rhode Island.

Pretreatment with *d*-amphetamine sulfate (2.5–10 mg/kg, ip) or SKF 525-A (12.5–50 mg/kg, ip) caused a dose-dependent increase in the lethality of morphine sulfate (400 mg/kg, ip) both in grouped and individually housed mice. Systemic amphetamine did not produce appreciable effect on *N*-demethylation of morphine. The rats made dependent on morphine by chronic

administration readily acquired ability to emit operant responses for self-ingestion of water or ethanol (40–80% v/v). The operant responding for amphetamine was decreased when amphetamine solutions were substituted for water. Similar amphetamine solutions were readily ingested by non-addicted rats. Therefore, amphetamine increases the acute toxicity of morphine and produces aversive effects in the morphine-dependent animals.

36. *Some Inhibitory Actions of Morphine on Sex Accessory Gland Metabolism.* J. A. THOMAS, M. G. MAWHINNEY, and J. W. LLOYD, Department of Pharmacology and Urology, West Virginia University Medical Center, Morgantown, West Virginia.

The administration of different doses of morphine (10, 20 or 40 mg/kg daily  $\times$  10, sc) led to significant decreases in prostate gland fructose concentrations. Fructose concentrations, an index of androgenic function, were markedly lower in mice treated with 40 mg/kg  $\times$  10. This highest dose increment resulted in prostatic fructose values comparable to those seen in the castrate animal. Similarly, testicular weights were significantly lowered by this dose of morphine suggesting an inhibition of pituitary gonadotrophins. The 10-day treatment period (20 mg/kg) resulted in a significant reduction in the levels of [ $^{14}$ C]-sorbitol and [ $^{14}$ C]-fructose metabolized from [1- $^{14}$ C]glucose by the prostate gland *in vitro*. Such findings indicate that morphine also exerts a direct inhibitory action upon carbohydrate metabolism in mouse sex accessory organs. Prostate gland ATP concentrations were also observed to be reduced following the administration of morphine. Preliminary experiments indicate that the ability of the prostate gland to assimilate radioactive testosterone is impaired by the previous administration of morphine. It is also possible that morphine treatment alters the metabolism of [ $^3$ H] testosterone by the prostate gland. (Supported in part by grants from the West Virginia University School of Medicine.)

37. *The Teratogenicity of Diazepam Metabolites in Swiss-Webster Mice.* R. P. MILLER and B. A. BECKER, Department of Pharmacology, University of Iowa, Iowa City, Iowa.

GLC and mass spectrometer analyses of fetal extracts prepared 4 hr after maternal administration of diazepam (DAP), 400 mg/kg, po, revealed the presence of *N*-demethyl-diazepam (NDD) and diazepam (DAP). These substances and oxazepam (OX), a delayed metabolite, were administered to pregnant mice at 400 mg/kg, po, a DAP dosage known to produce in utero deaths (resorptions) and malformations (cleft palates) in Swiss-Webster mice, for the purpose of determining whether these substances could cause resorptions and/or malformations. In addition, the effects of restraint and fasting were also tested. Mice were treated on day 14 of pregnancy (plug = day 1) as follows: Group A, DAP, 400 mg/kg, po; Group B, NDD, 400 mg/kg, po; Group C, OX, 400 mg/kg, po; Group D, restraint and 26-hr fasting; or Group E, carboxymethylcellulose sodium (CMC), 0.5%. In utero deaths manifested on day 19 as 2 or more resorptions/litter (no. resorbed/no. implants; values in parentheses are no. of litters with resorbed/no. litters) were: Group A, 27/116 (6/9); Group B, 7/108 (2/9); Group C, 49/218 (8/15); Group D, 27/200 (5/14); and Group E, 7/93 (2/8). Cleft palate, the principal malformation, was distributed as follows (no. per no. of live fetuses; values in parentheses are no. of litters with 1 or more cleft/no. litters): Group A, 22/89 (5/8); Group B, 19/101 (5/9); Group C, 7/169 (5/13); Group D, 5/173 (4/14); and Group E, 1/81 (1/9). Restraint plus 26-hr fasting did not cause significant ( $p < 0.05$ ) increase over controls in resorptions or malformations. OX and DAP caused significant numbers of resorptions above controls. NDD and DAP caused significant numbers of malformations above controls. (Supported by NIGMS 12,675.)

38. *The Teratogenic Interaction of 5-Diazouracil and 5-Iododeoxyuridine in the Mouse Embryo.* RICHARD G. SKALKO, DONNA ANNE CANIANO, and DAVID S. PACKARD, JR., Birth Defects Institute, New York State Health Department and Institute of Experimental Pathology and Toxicology, Albany Medical College, Albany, New York. (L. Golberg.)

The pyrimidine base analog, 5-diazouracil (DU), is carcinostatic to many animal tumors and possesses both antiviral and antibacterial activity. It is also an irreversible inhibitor of



the soluble liver enzyme dihydrouracil dehydrogenase and, as such, inhibits the catabolism of [5-<sup>125</sup>I]iododeoxyuridine *in vivo* and *in vitro* (Cancer Res. **30**, 2937, 1970). Iododeoxyuridine (IUdR), a thymidine analog, possesses similar biological properties and is incorporated into the DNA of susceptible tissues. The interaction of these compounds on the 10-day (stage 18) ICR mouse embryo was investigated. DU is embryolethal but non-teratogenic at a dose range of 5–20 mg/kg with a calculated ED50 of 12.4 mg/kg. IUdR (in 0.5% CMC) is highly embryolethal and teratogenic, producing 94% resorptions (500 mg/kg) and 46% resorptions with 95% CP in survivors (300 mg/kg). When 10 mg/kg DU was administered between 3 hr before and 3 hr after either dosage of IUdR, a definite and significant reduction in embryolethality was observed. Two malformations (CP, ectrodactyly) were reduced but increased in incidence dependent upon time, the greatest reduction occurring when DU was given 3 hr before IUdR. Correlative biochemical studies indicate that DU prevents catabolism of 300 mg/kg [<sup>3</sup>H]-IUdR when administered 3 hr before but has no effect on the level or rate of incorporation of IUdR into embryo DNA. A preliminary interpretation suggests that DU is interfering with the ability of incorporated IUdR to produce congenital malformations.

39. *Reproductive, Teratogenic and Egg Production Studies in Animals Fed SO<sub>2</sub>-Treated Activated Sewage Sludge*. A. L. PALANKER, J. W. KEATING, M. S. WEINBERG, A. L. SHEFFNER, and R. DEAN, Foster D. Snell, Inc., Florham Park, New Jersey, and Robert A. Taft Water Research Center, Water Quality Office, Environmental Protection Agency, Cincinnati, Ohio.

The effects on reproductive performance of an organic feed concentrate (OFC) derived from SO<sub>2</sub>-treated activated sewage sludge were investigated in rats, rabbits, and chickens. The organic feed concentrate was incorporated into nutritionally balanced diets at levels of 5 and 10% at the expense of sucrose and fed to rats in 3 separate experiments: before, during and after mating; during gestation; during gestation and lactation. Rabbits were fed during gestation only. Fertility, gestation, viability and lactation performances were evaluated. Laying hens were fed 5, 10, 15 and 20% OFC in synthetic and commercial diets for 6 weeks. Effects on egg production performance were determined. No teratogenic effects were seen in rats or rabbits. No alteration in reproductive parameters were noted in rats fed 5% OFC. Increased food intake and body weight gains were incidentally recorded (5% OFC male and female rats) during the pre-mating feeding period. Reduced fertility, viability and lactation indexes were seen at 10% OFC levels. Egg laying performance was suppressed in the 5 and 10% OFC levels. Egg laying ceased entirely in birds fed 15 and 20% OFC. The OFC preparation used in this study contained very little calcium and high levels of phosphate, which could account for the observed depression in egg laying.

- ✓ 40. *Teratogenic Studies with Pentachloronitrobenzene in Rats*. ROBERT L. JORDAN and JOSEPH F. BORZELLECA, Departments of Anatomy and Pharmacology, Medical College of Virginia, Health Sciences Division, Virginia Commonwealth University, Richmond, Virginia.

The soil fungicide pentachloronitrobenzene (PCNB) was given to pregnant rats at dosages from 100 ppm to 1563 ppm to assess its effects on embryogenesis. PCNB, dissolved in corn oil, was administered to timed pregnant Charles River Strain Albino rats by oral intubation on days 6 through 15 of gestation. On day 20, dams were sacrificed and fetuses removed and examined for gross malformations. In addition, the number of corpora lutea, position and number of dead and/or resorbed fetuses, fetal weights and sex-ratios were recorded. None of these indices differed significantly from control values at any level of treatment with PCNB. Following gross examination, one-half of the fetuses were cleared and stained with alizarin red-S for visualization of potential skeletal defects and the remainder either fixed in Bouin's solution prior to free-hand razor blade sectioning for visceral examination or stored in formalin. Examination of fetal skeletons revealed little or no difference between controls and treated groups in either the number or type of skeletal malformations or in the incidence of minor skeletal variations such as rudimentary accessory ribs. The number of soft tissue anomalies detected during sectioning of PCNB-treated fetuses did not differ significantly from that of

controls. The most common defects, dilated renal pelvis, hydronephrosis and hydroureter, were found in control and treated groups and appeared unrelated to treatment. These data indicate that PCNB, given in oral dosages up to 1563 ppm daily, during the period of embryogenesis, is nonteratogenic in the rat.

- ✓ 41. *The Effect of Pentachloronitrobenzene on Fetal Kidneys*. DIANE COURTNEY, Environmental Protection Agency, Perrine Primate Laboratory, Perrine, Florida. (J. F. Borzelleca).

Pentachloronitrobenzene, a fungicide, inhibits kidney formation in the C57B1/6 mouse fetus. Oral administration of 500 mg/kg, daily, from day 7 to 11 of gestation produced effects in 80% of the litters. Renal agenesis occurred unilaterally about two times more often than it occurred bilaterally. Samples of blood, urine, liver, kidney, fat, placenta and fetuses were analyzed for pentachloronitrobenzene and its possible metabolites: pentachlorobenzene, pentachloroaniline, pentachlorophenylsulfide and hexachlorobenzene. Fat contained the highest concentration of all compounds at all times studied. Fetuses contained higher concentrations of the metabolites than pentachloronitrobenzene. By 24 hr the tissue content of pentachloronitrobenzene in the maternal tissues was very low or non-detectable while the metabolites were readily detectable.

- ✓ 42. *Fetotoxic Effects of Pentachlorophenol in the Golden Syrian Hamster*. DONALD K. HINKLE, Environmental Protection Agency, Perrine Primate Laboratory, Perrine, Florida. (F. K. Kinoshita.)

Pentachlorophenol or its derivatives are widely used as contact herbicides and/or for control of termites and other wood boring insects. Oral administration of levels ranging from 1.25 to 20.0 mg/kg daily from day 5 to 10 of gestation was done to assess possible fetotoxic effects of this compound. Fetal deaths and/or resorptions were observed in 3 of 6 test groups. Samples of maternal blood and fat, as well as entire fetuses, were analyzed for pentachlorophenol. There was close correlation between the concentrations of PCP recovered from the maternal blood and the fetuses. The highest measured concentrations in the blood, fat and fetuses occurred within 3 hr following the last administered oral dose. Concentrations in fat persisted in measurable amounts up to 120 hr following the last oral dosing and exceeded the blood and fetal concentrations at that time.

- ✓ 43. *The Effect of Tetrachlorophenol and Pentachlorophenol on rat Embryonal and Fetal Development*. B. A. SCHWETZ and P. J. GEHRING, Chemical Biology Research, The Dow Chemical Company, Midland, Michigan.

This study evaluated the effects of 2,3,4,6-tetrachlorophenol and pentachlorophenol on rat embryonal and fetal development. Dose levels up to and including a maximally tolerated dose (30 mg tetrachlorophenol/kg/day, 50 mg pentachlorophenol/kg/day) were administered orally to pregnant Sprague-Dawley rats on days 6 through 15 of gestation. Additional groups of rats received 30 mg pentachlorophenol/kg on days 8 through 11 or 12 through 15 of gestation. The only fetal anomaly associated with the administration of tetrachlorophenol was delayed ossification of the skull bones, evidence of fetal toxicity. Following the administration of pentachlorophenol, signs of toxicity to the embryo and fetus such as subcutaneous edema, dilated ureters and minor anomalies of the skull, ribs, vertebrae and sternbrae were observed at an incidence which increased with increasing dose levels. The developing rat embryo is most susceptible to the toxic effects of pentachlorophenol during early organogenesis. Neither tetrachlorophenol nor pentachlorophenol were truly teratogenic in the rat. The dose level of these materials which caused no toxicity to the embryo or fetus was 10 mg tetrachlorophenol/kg/day and 5 mg pentachlorophenol/kg/day.

- ✓ 44. *Teratogenic Effects of Ethylenethiourea in Rats and Rabbits*. K. S. KHERA, Food Research Laboratories, Health Protection Branch, National Health & Welfare, Ottawa, Canada.

Ethylenethiourea (ETU), a degradation product of the ethylenebisdithiocarbamate group of fungicides and identified recently as a crop residue, was studied for teratogenic activity.

Single daily po doses of 0, 10, 20, 40 or 80 mg/kg of ETU in aqueous solution were administered to nulliparous rats and does. Three experiments in rats with varied treatment-duration and a single experiment in does were conducted. Dosing was programmed in rats from pre-pregnancy (21 days or more) to pregnancy day 15, from 6-15 days or from 7-20 days of pregnancy and in does from 7-20 days of pregnancy. All experiments in rats revealed ETU induced meningoencephalocele, meningorrhagia, meningorrhea, hydrocephalus, agenetic cerebellum, disoriented ependymal layer surrounding an obliterated neural canal, abnormal pelvic limb posture with equinovarus and short or kinky tail but no adverse effect on fetal survival. Incidence of most of the deformities was dose-dependent, minimal effect only, being observed at 10 mg/kg. Results from the rabbit study suggested an increased incidence of resorption sites and decreased brain weight in the group treated with 80 mg ETU/kg.

- ✓ 45. *The Effect of 2,4-dichlorophenyl-p-nitrophenyl ether on the Rat Fetus.* R. D. KIMBROUGH, T. B. GAINES, and R. E. LINDER, Chamblee Toxicology Laboratory, Environmental Protection Agency, Chamblee, Georgia.

In a reproduction study conducted in our laboratory and a similar study reported by Ambrose *et al.* (Toxicol. Appl. Pharmacol. **19**, 263-275, 1971) dietary levels of 100 ppm or greater of technical 2,4-dichlorophenyl-p-nitrophenyl ether (TOK<sup>®</sup>) fed to rats increased the number of dead pups. To determine whether the pups were stillborn, or died immediately after birth, 8 male and 8 female rats were fed 500 ppm technical TOK<sup>®</sup> in the diet. They were pair-mated after 61 days and, counting day of insemination as day 0, the fetuses were removed by Cesarean section on day 20 of pregnancy. All of the fetuses, a mean of 11.8/dam, were alive when taken but died during the next 12 hr. To determine whether a contaminant, or the TOK<sup>®</sup> itself, produced the effect, 3 groups of 11 pregnant rats each were dosed with TOK<sup>®</sup> in peanut oil, or peanut oil alone, by stomach tube on days 7 through 15 of pregnancy. Day of insemination was counted as day 0. Group 1 rats, given 50 mg/kg/day of pure TOK<sup>®</sup>, produced 10 litters with an average of 2.2 live and 7.3 dead pups. Group 2, given 50 mg/kg/day, produced 11 litters with an average of 2.7 live and 9.5 dead pups. Group 3, given peanut oil alone produced 11 litters with an average of 12.7 live pups and none dead.

Fetuses from dams exposed to TOK<sup>®</sup> were cyanotic but formed normally. Hemoglobin and erythrocyte counts of fetuses from both TOK<sup>®</sup>-treated and control dams were normal and methemoglobin was not elevated. Electron microscopic examination of the lungs of the pups suggested that poor expansion of the lungs in combination with less well developed lamellar bodies in alveolar epithelium and a possible effect on surfactant may cause the rapid death after birth of the offspring of the TOK<sup>®</sup>-treated dams.

- ✓ 46. *Ochratoxicosis in Domestic Swine.* G. M. SZCZECZ, W. W. CARLTON, and J. TUIITE, Departments of Veterinary Pathology and Botany and Plant Pathology, Purdue University, West Lafayette, Indiana.

The toxicity for SPF female domestic swine of an ochratoxin A-containing rice culture of *Aspergillus ochraceus* and of purified ochratoxin A was studied. When daily doses of 2.0 mg/kg of ochratoxin A were given, swine were moribund or dead by day 3, and at daily doses of 1.0 mg/kg survival was for 6 days. Clinical features of the toxicosis included anorexia, depression, diarrhea, elevated rectal temperatures, polydipsia, polyuria, dehydration, prostration and death. No clinically significant elevations occurred in the concentrations of the enzymes glutamic pyruvic transaminase, glutamic oxalacetic transaminase, alkaline phosphatase, leucine amino peptidase and lactic dehydrogenase in serum or in dialyzed urine which was tested every second day. There was a marked terminal elevation of concentrations of isocitric dehydrogenase in the urine but not in the serum of test swine. Blood urea nitrogen was increased to a high of 120 mg/100 ml. Alterations in the urine included increased amounts of protein and glucose, decreased specific gravity and the presence of numerous granular casts and necrotic tubular epithelial cells. At necropsy the mesenteric lymph nodes were edematous and hyperemic and the kidneys were pale. Histopathologic alterations of necrosis of renal tubular epithelium, the presence of granular casts and proteinaceous debris and tubular

dilatation were especially prominent in the proximal and distal convoluted tubules. The germinal centers in most lymph nodes contained one to several necrotic cells and there was marked lymphoid depletion in the spleen. Slight cytoplasmic vacuolation and necrosis of occasional hepatocytes occurred in the center of hepatic lobules. Focal erosions due to necrosis of epithelial cells occurred at the tips of many villi in the jejunum and ileum. (Supported in part by National Institutes of Health Grant ESCA-00463, United States Department of Agriculture Cooperative Agreement 12-14-100-9091 (51) and by National Institutes of Health Post Doctoral Special Fellowship No. RR S1419.)

- ✓ 47. *Toxicosis Produced in Dogs by Culture of Penicillium citrinum*. W. W. CARLTON, G. M. SZCZECZ, and J. TUITTE, Departments of Veterinary Pathology and Botany and Plant Pathology, Purdue University, West Lafayette, Indiana.

Some isolates of *P. citrinum* are toxic because of the production of a mycotoxin, citrinin, and previous studies have demonstrated renal toxicity for this mycotoxin, but data for the dog are lacking. In this study, an isolate of *P. citrinum* known to produce citrinin was grown on rice and fed to young Beagle dogs at dietary concentrations (w/w) of 25 and 50%. Certain hematologic and renal function parameters were examined and tissues were studied histopathologically. The dogs consumed small amounts of the fungal diets, became dehydrated, polyuric and polydipsic. Blood urea nitrogen concentrations were increased. Alterations in the urine included low specific gravity, presence of large amounts of glucose and presence of many necrotic renal epithelial cells in the urinary sediment. Gross and microscopic lesions were confined to the kidneys which were pale and slightly swollen at necropsy. Microscopic changes were those of tubular dilatation and degeneration and necrosis of renal tubular epithelial cells. Citrinin (culture *P. citrinum*) is nephrotoxic for the dog but the pathoanatomic changes in the kidney differ from those produced by ochratoxin A. (Supported in part by National Institutes of Health Grant FSCA 00463 and United States Department of Agriculture Cooperative Agreement No. 12-14-100-9091 (51).)

- ✓ 48. *Effects of Diet and Light on the Response of Rats to Aflatoxin and Monocrotaline*. P. M. NEWBERNE and A. E. ROGERS, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Spontaneous photosensitization and toxic responses in man and animals are relatively common. These pathological disturbances are often associated with exposure to known or suspected drugs or toxins, liver injury and sunlight. In animals, the condition is most often due to circulating porphyrin or its derivative phylloerythrin and is dependent on toxic injury to the liver. We have conditioned rats on a control diet or a diet marginal in lipotropes for 2 weeks, administered an LD50 dose of either aflatoxin B<sub>1</sub> or monocrotaline and then exposed half of each group to simulated sunlight provided by a long-arc Xenon light source. The 2-week mortality indicated (1) that the marginal lipotrope diet protected against the toxic effects of both toxins and (2) that exposure to the light source enhanced the toxicity of aflatoxin but decreased the toxicity of monocrotaline. (Supported in part by grants from NIEHS and the Duro-Test Corporation.)

- ✓ 49. *Mycotoxin Induced Developmental Abnormalities in Mice*. A. WALLACE HAYES and RONALD D. HOOD, Departments of Microbiology and Biology, The University of Alabama University, Alabama.

The rubratoxins and ochratoxins are metabolites produced by storage molds that are toxic either by contact or by inadvertent ingestion of the toxins when present in foods or feeds. Since information on teratogenic effects of mycotoxins is limited, this work was undertaken to determine if these metabolites were teratogens in mice and if their effects were similar to those reported for aflatoxin. Albino mice were injected, ip, with either rubratoxin or ochratoxin on various days of pregnancy. Animals were examined on gestation day 18 for evidence of embryonic mortality and fetal malformation. Treatment with rubratoxin on each of gestation days 6-12 at dose levels of 0.4, 0.6, 0.9, or 1.2 mg/kg resulted in increased mortality on all days

and at all dose levels. Fetal weights were decreased at all day-dose combinations except day  $9 \times 0.6$  or  $0.9$  mg/kg. Developmental abnormalities, such as exencephaly, malformed jaws and pinnae, umbilical hernia and open eye, were seen in fetuses treated on days 6, 7, 8, 9 and 11. Fetuses from females treated on day 8 did not survive to day 18 except at the dose level of  $0.4$  mg/kg. No skeletal defects were seen. Ochratoxin dosage was  $5$  mg/kg on each of gestation days 7–12 which resulted in increased embryonic mortality and decreased fetal weights in all cases. Developmental anomalies including severe cranio-facial malformation, exencephaly, anophthalmia, syndactyly, polydactyly, short tail and micrognathia were noted on all days but were particularly severe on days 8 and 9. Some skeletal defects, such as rib abnormalities, in addition to those defects associated with the external gross malformations were observed in fetuses from mice exposed to ochratoxin. Our results demonstrate that single doses of either mycotoxin that were not lethal to pregnant females produced growth and developmental retardation, congenital malformations and death in fetuses. Differential effects of these mycotoxins were dependent on dosage and on the stage of embryonic development. (Supported by Grant No. ES-00464.)

- ✓ 50. *Aflatoxin B<sub>1</sub>—induced biochemical lesion in Rat Liver Mitochondria.* E. A. BABABUNMI and O. BASSIR, University of Ibadan, Ibadan, Nigeria. (F. Oehme.)

Since high concentrations of aflatoxin occurred in the liver after a single-dose injection in the rat it was desirable to investigate the effect of aflatoxin B<sub>1</sub> (AFL) on mitochondrial ATPase activity. In accordance with the low distribution of AFL in the heart and testis after an ip injection essentially no swelling was produced by AFL on the mitochondria obtained from these tissues; however swelling produced by AFL,  $3 \times 10^{-3}$  M, with liver mitochondria was quite marked as indicated by a spectrophotometric measurement at  $520$  nm. Also, the addition of ATP,  $0.15$  M, completely reversed the swelling of the liver mitochondria to which AFL had been added. AFL produced an activation of DNP-induced ATPase in the liver mitochondria at a concentration of  $20 \times 10^{-5}$  M but produced no further stimulation with increasing concentration of the toxin. No effect was noticeable in the mitochondria obtained from kidney, heart and testis. Toxicity studies have shown that the ip LD<sub>50</sub> is about  $6.0$  mg/kg for the male rat. Since the distribution throughout body fluids is not uniform it is not possible to estimate the concentrations of AFL which may affect the processes of energy conservation in tissues. However, these observations suggest that uncoupling of mitochondrial oxidative phosphorylation may be a primary lesion in aflatoxin poisoning.

- ✓ 51. *Carrageenan-Induced Squamous Metaplasia of the Rectal Mucosa in the Rat.* R. FABIAN, R. ABRAHAM, L. GOLBERG, and F. COULSTON, Albany Medical College, Albany, New York.

Degraded iota-carrageenan (C16), derived from *Eucheuma spinosum*, was administered to young adult Sprague-Dawley rats as a solution in the drinking water (5%, daily intake  $6$ – $10$  g/kg) or by stomach tube ( $0.5$  or  $5$  g/kg/day). Except for loss of hair around the anus, no effect upon appearance, behavior, or growth was observed. At daily doses of  $5$  g/kg and higher the stools were soft to semi-fluid and occult blood was detected in 3–7 days. After 2 weeks, grossly visible blood was sporadically observed on the surface of the stool and on the anal margins. Examination of rats given the 5% solution or  $5$  g/kg daily for 6 months revealed a gray-white coating of the distal rectum. Histologically this coating was found to consist of keratinizing stratified squamous epithelium. At 6 months the metaplastic epithelium extended approximately  $0.5$  cm from the anorectal junction and by 15 months involved as much as  $3.0$  cm of the distal rectum. No such metaplasia was observed with a daily dose of  $0.5$  g/kg. Studies of the pathogenesis of the squamous metaplasia revealed the earliest change to be aggregation of macrophages in the lamina propria and submucosa of the anorectal region. Loss of mucosal glands and replacement of the luminal epithelium by stratified squamous epithelium occurred in areas of macrophage aggregation. The presence of ulceration, and the accompanying acute inflammatory response, resulted in a more intense proliferation of stratified squamous epithelium involving the glands as well as the luminal epithelium. Most of these macrophages contained material which stained metachromatically with toluidine blue, and was believed to be carrageenan. A striking increase in histochemically-demonstrable acid phosphatase activity

within the macrophages paralleled the accumulation of this material. Death and disintegration of the macrophages appeared to precede the changes which led to metaplasia. Two adenomatous polyps occurred in the rectum of a rat given 5 g/kg of C16 daily by tube for 15 months. One polyp contained islands of squamous epithelial cells; in the second adenomatous glands predominated. Although some metachromatic material accumulated within macrophages in the cecum and proximal portions of the colon, lesions were not found. At a daily dose of 500 mg/kg small amounts of metachromatic material accumulated in macrophages at the anorectal junction, but metaplasia did not occur. (Supported by FDA Contract 69-7, by Research Grant 2P01-ES00226-06 from the National Institutes of Environmental Health Sciences, NIH, and by National Institutes of Health Training Grant 2T01-ES00103-06.)

- ✓ 52. *The "Lung Edema Factor" from Mold-Damaged Sweet Potatoes: Further Studies and Characterization.* MICHAEL R. BOYD and BENJAMIN J. WILSON, Department of Pharmacology and Center in Environmental Toxicology, Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee.

We recently ascribed the pulmonary toxicity of mold-damaged sweet potatoes (*Ipomoea batatas*) to the presence of a "lung edema factor" (LE) which is produced in the tubers in response to microbial infection (*Nature* 227, 521 (1970)). The first lung-edemagenic component found in toxic extracts was 4-ipomeanol (1-[3-furyl]-4-hydroxy-1-pentanone) (*Nature* 231, 52 (1971)). We now present data which suggests that the LE factor comprises a group of at least four closely related compounds which exhibit similar lung-toxic properties: 4-ipomeanol, the isomeric 1-ipomeanol (1-(3-furyl)-1-hydroxy-4-pentanone), the corresponding diketone (1-(3-furyl)-1,4-pentanedione), and the diol (1-(3-furyl)-1,4-pentandiol). The acute pulmonary toxicity of all these 1,4-dioxygenated-1-(3-furyl) pentanes is grossly similar to that already described for 4-ipomeanol. Toxicity of some of the compounds, especially 1-ipomeanol and the diol, to other organs, such as the kidney, has also been noted. This preliminary observation suggests that pathological responses other than lung damage should be considered as possible features of moldy sweet potato toxicity.

53. *Toxic Interaction of Ethanol and Morphine in Mice.* D. J. MCCOY, D. J. BROWN, and R. B. FORNEY, Indiana University School of Medicine, Indianapolis, Indiana.

This study was designed to investigate the toxic interaction of ethanol with morphine. Estimations of the ip LD50 for ethanol and morphine were established in mice as 6.4 g/kg  $\pm$  0.6 and 480 mg/kg  $\pm$  30, respectively. Seven mixtures of the two drugs, based on weight/weight ratios which ranged from 50:1 to 1:5 (ethanol:morphine), were administered and an LD50 for each mixture was determined. These observed LD50 values were statistically compared to theoretical LD50 values calculated assuming either additive or independent interaction. The comparison indicates additive interaction in all ratios greater than 2:1. At ratios of 2:1, 1:1 and 1:5 an antagonistic interaction was suggested (i.e. a greater amount of morphine was required with the mixture than with morphine alone to achieve an LD50). This antagonism reflects the possibility that independent stimulant and depressant properties combine to produce the lethal effect of morphine.

54. *Quantitative Relationship Between Blood Alcohol Concentration and Psychomotor Performance.* M. A. EVANS, R. MARTZ, B. E. RODDA, G. F. KIPLINGER, and R. B. FORNEY, Department of Toxicology, Indiana University Medical Center and Lilly Laboratory for Clinical Research, Marion County General Hospital, Indianapolis, Indiana.

Fifteen male volunteers ingested fruit juice solutions of ethanol mixed to produce a blood alcohol concentrations of approximately 0, 25, 50, 75 and 100 mg/100 ml. Each subject received in random order 1 treatment per week for a total of 5 weeks. Actual blood alcohol concentrations were estimated by means of the Borkenstein breathalyzer at selected intervals during the experimental session. Psychomotor performance, as evaluated by delayed auditory feedback and pursuit meter, demonstrated impairment with increasing blood alcohol concentrations. The Wobble Board, a device for estimating stability of stance, showed a linear increase in sway with increased blood alcohol concentrations. Similar results were seen in subjective responses as evaluated by the Cornell Medical Index Questionnaire.

55. *Effects of Ethanol on Glutethimide Distribution in Relationship to a Mechanism for Toxicity Enhancement.* L. B. HETLAND, and D. COURI, The Ohio State University College of Medicine, Columbus, Ohio.

The combined ingestion of two or more CNS-depressant drugs has been reported to produce a potentiated toxicity which is responsible for a large number of human fatalities annually. In studies aimed at elucidating the underlying mechanism of action of this type of toxicity, we have investigated the effects of ethanol on the distribution of glutethimide (Doriden) in serum, whole brain, and brain regions in young adult male Wistar rats. Initial experiments indicated that glutethimide toxicity was enhanced in a greater than additive manner following the administration of subtoxic concentrations of ethanol. In similar experiments, measurements of ataxia and sleep times were also enhanced. Orally administered glutethimide (500 mg/kg) in combination with ethanol treatment (2 g/kg, ip) resulted in a shift in the appearance of maximum mean whole brain glutethimide concentrations from 2 hr in controls to 4 hr in experimental animals throughout an 8-hr time course. In addition, serum and whole brain glutethimide concentrations of animals also given ethanol ranged from 2- to 5-fold over their controls at 4 and 8 hr, respectively. Serum ethanol concentrations at these latter times were markedly diminished vis, 85 and 5 mg/100 ml as compared to levels of 200-250 mg/100 ml during the first 2 hr. Furthermore, analysis of brain regions after glutethimide and ethanol treatment resulted in glutethimide concentrations in cerebellum and pons-medulla ranging from 200-2000% of controls (i.e. glutethimide without ethanol) at 4 and 8 hr, while in the remaining brain tissue, the values were as low as 5% of controls. These findings suggest that the demonstrated alterations in the distribution of glutethimide after the combined administration of ethanol may account, at least in part, for the observed enhancement of toxicity. (Supported in part by grant GM 01417 from the National Institutes of Health.)

56. *Effect of Elevated Carboxyhemoglobin Levels on Driving Related Tasks.* MUKUL M. MEHTA, GARY D. HERRIN, THOMAS H. ROCKWELL, and FRANCIS W. WEIR, The Ohio State University, Columbus, Ohio.

Four series of experiments, involving 25 different human subjects, were conducted over a 2-year period to study the effects of up to 20% carboxyhemoglobin (COHb) on driving related tasks. The subjects were given the following tests: (1) Psychophysical tests involving gross and fine coordination, spatial relations, and a battery of tests designed to measure the performance of visual function; (2) Simulated driving tests in a laboratory environment to measure (a) visual pursuit tracking, and (b) visual choice reaction time; (3) Highway driving tasks designed to investigate the effects of COHb on driving performance. A quadratic relation was established between performance decrement and COHb level for brightness discrimination tests. The regression equation is significant at the 95% level. No change in gross/fine coordination, or spatial relational abilities, was noticed. Significant negative correlations were observed between mean tracking lag-errors obtained from laboratory tasks and blink rates obtained during open highway driving ( $P > 0.95$ ), and constant car-following protocols ( $P > 0.8$ ). Blink rates decrease under 20% COHb, while tracking lag-error increased. The relationship suggests that carbon monoxide acts as a mild psychological stressor. The effects of 20% COHb might interact synergistically with the fatigue of driving. (Supported in part by the Coordinating Research Council and the Environmental Protection Agency, under Contract Number 68-02-0329 and CRC APRAC Project Number CAPM-9-69.)

57. *Neuropharmacological and Behavioral Toxicity Studies with Prostaglandin A<sub>2</sub>.* E. I. DOBRIN, J. L. BLOSS, and W. J. POTTS, Searle Laboratories Division, G. D. Searle & Co., Chicago, Illinois (J. Singh.)

Prostaglandin A<sub>2</sub> (PGA<sub>2</sub>) has been reported in the literature to have CNS depressant effects similar to those of the major tranquilizers such as chlorpromazine (Potts & East, The Pharmacologist, 13, Fall, 1971.) In these studies, we attempted to further define the neuropharmacological and behavioral toxicity of PGA<sub>2</sub> by utilizing a battery of tests designed to elucidate the mechanisms of action of compounds affecting the CNS. In the initial studies, groups of male albino mice were injected ip with 25, 50, 100 and 200 mg/kg of PGA<sub>2</sub> dissolved in a

phosphate buffer. One control group of mice was treated with buffer alone. Clinical signs were observed and toxic manifestations were recorded at 30, 60, 120 min and 24 hr post treatment. The LD50 was calculated by computer to be 93 mg/kg. Doses approximating 20% and 1% of this LD50 (19 and 1 mg/kg respectively) were then given to mice in order to test for the following properties: anticonvulsant, analgesic, central anticholinergic, dopaminergic, serotonergic, antidepressant, MAOI, muscle relaxant, major and minor tranquilizer. Two avoidance procedures were employed in rats to determine specific effects on behavior of  $PGA_2$ : one-way shuttle active avoidance (Tenen, *Psychonomic Science*, 6, 407-408, 1968, Potts & McKown, *Psychological Reports*, 24, 959-964, 1969) and a two-way shuttle conditioned avoidance procedure in trained rats (Potts, *Life Sciences*, 10 (Pt. I): 655-660, 1971). In the latter test, the rats were treated both ig and ip. The ip doses were 1.12, 2.25, and 4.5 and 9 mg/kg and the ig doses were 4.5, 9.0 and 18.0 mg/kg. The results of these studies indicate that  $PGA_2$  has potent muscle relaxant activity and some antidepressant effects as well as major tranquilizing properties. One possible mode of action of this drug may involve effects on serotonin since the diarrhea induced by  $PGA_2$  was blocked by methysergide, a serotonin antagonist. Some of the signs observed might indicate that the depressant action of  $PGA_2$  could be slightly different from that of the known major tranquilizers.

58. *Behavioral Toxicity of Some Industrial Chemicals*. S. N. PRADHAN and BIJALI GHOSH, Department of Pharmacology, Howard University College of Medicine, Washington, D.C. (S. L. Schwartz.)

Behavioral toxicity of some industrial chemicals was investigated on several schedules of spontaneous and learned behavioral schedules in rats following their chronic administration. Rats were subjected to the behavioral schedules (e.g. spontaneous motor activity, maze running, fixed-ratio food reinforcement, fixed-ratio water reinforcement, fixed-interval food reinforcement) in daily sessions of 0.5-1 hr for 5 to 6 days a week up to 3-4 months in some cases, until the base-line level of their performance became relatively stable, when treatment with a chemical was started. One of the three chemicals, e.g. xylene (0.2, 0.5 or 1 ml/kg), benzene (0.1, 0.2, 0.5 or 1 mg/kg) or carbon tetrachloride (0.05, 0.1, 0.2, 0.4 or 0.8 ml/kg) was injected sc after the daily session 6 days a week for a number of weeks. Saline was injected in control subjects concurrently. Changes in behavioral activity of each animal due to chemical treatment was expressed as percent of the control, the latter being the average of the data from 3 daily sessions preceding the treatment. Decrease in behavioral activity was considered as an indication of behavioral toxicity produced by these chemicals. Changes in the saline-treated rats, if any, were also taken into consideration for proper evaluation of toxicity. Under this experimental condition, behavioral toxicity was evident in 1 of the 2 schedules involving xylene, in all of the 5 schedules with benzene, and 3 of the 4 schedules with carbon tetrachloride. Variations were observed in the sensitivity of different schedules. In some schedules the chemicals also showed greater effect with higher doses showing a rough dose-response relationship. From this investigation it appears that the behavioral methods can be used for determining the toxic effects of chemicals even when these chemicals may not affect the central nervous system directly. (Supported by a USPHS grant No. EC 00229.)

59. *Biochemical Toxicology of 1,4-Butanediol*. JOSEPH E. ZABIK, DAVID P. VAN DAM, and ROGER MAICKEL, Department of Pharmacology, Medical Sciences Program, Indiana University, Bloomington, Indiana.

The compound, 1,4-butanediol (1,4-BD), is widely used in applications ranging from industrial syntheses to food and pharmaceutical preparations. Despite this widespread use, relatively little is known of the toxicology of 1,4-BD, a molecule that may be considered as a dimeric form of ethyl alcohol. On administration of single ip doses of 1,4-BD to adult, male rats, the LD50 was found to be 1370 mg/kg with 95% confidence limits of 1298-1445 mg/kg. The compound caused a significant depression; at 800 mg/kg, loss of righting reflex occurred in 32 min, at 1000 mg/kg in 27 min. An LD100 was seen at 1600 mg/kg, with loss of righting reflex occurring in 20 min and death in 2-3 hr. Chronic dosage for 10 days at 500 mg/kg showed no deaths and no significant depression of weight gain; similar treatment at 1000 mg/kg



slightly depressed weight gain. Plasma and liver FFA and triglycerides showed small abnormalities. Interaction studies of 1,4-BD and ethyl alcohol will also be presented. (Supported by USPHS grants K02-MH-41083 and MH-18852 and NASA grant NGL-15-003-117.)

60. *Teratogenic Studies with Oxisuran (A Differential Immunosuppressive) and Azathioprine in the Rat.* E. P. HORNYAK, S. M. JONES, W. WILLIAMS, A. CERNISKI, and E. SCHWARTZ. Warner-Lambert Research Institute, Morris Plains, New Jersey.

Studies were undertaken to compare the effects of a differential immunosuppressant, oxisuran with azathioprine on a number of reproductive parameters and the developing fetus. Both compounds were administered by gastric gavage to pregnant rats from days 6 through 15 of gestation. Oxisuran was administered at daily doses of 100, 200 and 400 mg/kg and azathioprine at 1, 10 and 25 mg/kg. Necropsy was performed on day 20 of gestation. Following the examination of each fetus for gross soft tissue abnormalities,  $\frac{1}{3}$  of the total number were autopsied and examined for internal soft tissue defects and the remainder were cleared and stained with alizarin red for the detection of osseous defects. No differences between oxisuran-treated animals and control animals were noted with regard to pregnancy maintenance; numbers of corpora lutea or implantation sites; number, size or viability of fetuses *in utero*; or the number of fetal resorptions. The mean food consumption and the body weights of oxisuran-treated animals at all dose levels were significantly lower during the treatment period than those of the untreated and vehicle control groups. Rats treated with 10 and 25 mg/kg of azathioprine when compared to control animals showed significantly lower body weights and food consumption values, as well as higher incidence of abortions and/or resorptions. No viable fetuses were obtained from these two groups. The 1 mg/kg group was not significantly different from control animals with regard to the parameters investigated. The results indicate oxisuran produced no alterations in any reproductive parameters, while azathioprine did. Neither compound produced adverse behavioral signs nor was there evidence of drug induced teratogenicity.

- ✓ 61. *A Comparison of Teratogenic Response in Four Laboratory Species.* KENNETH MORGAREIDGE, DAVID BAILEY, and JOSEPH McLAUGHLIN, Food and Drug Research Laboratories, Inc., Maspeth, New York, and Division of Toxicology, Food and Drug Administration, Washington, D.C.

The responses of four animal species to sham-dosage and to known teratogens were compared in teratologic screening studies on a series of 42 so-called GRAS compounds, which included a series of gums, curing agents, BHT, BHA and talc among others. Each substance was administered ig to groups of pregnant mice, rats, hamsters, and rabbits with concurrent sham-treated groups and equivalent groups receiving either aspirin or 6-aminonicotinamide (6-AN) as positive controls. From over 420 litters per species, some 5000 fetuses have been examined for both the sham and positive control treatments in mice, rats, and hamsters. Half as many rabbits have been used. All fetuses were obtained by laparotomy 24 hr prior to term and examined for both soft and skeletal tissue anomalies. The performance of the dams was also recorded with respect to all important parameters.

Confirming earlier reports, it has been found that aspirin is a consistent teratogen only for the rat at a dose level of 250 mg/kg per day for 11 days. Maternal toxicity prevents this drug from showing positive effects in the mouse and it has virtually no effect in hamsters. Rabbits respond to 6-AN as a single dose on Day 9 of gestation. However, its extreme toxicity leads to erratic performance. It is expected that these studies will lead to the establishment of "normal" ranges for nidation, fetal maturation, viability, and occurrence of abnormalities in untreated animals of these four common laboratory species. (The work was conducted under contract for the Food and Drug Administration, FDA Contract 71-260.)

62. *Effect of Three Antiinflammatory Agents on Parturition in the Rat and Rabbit.* J. W. NOVEROSKE, R. J. HESSINGER, and P. A. MATTIS, Merck Institute for Therapeutic Research, West Point, Pennsylvania.

Indomethacin at doses of 0.05, 0.10, 0.50, and 1.00 mg/kg, bid, phenylbutazone at doses of 5, 15, and 45 mg/kg, bid, and acetylsalicylic acid at doses of 25 and 75 mg/kg, bid, were given to rats (CRCD) by gavage from day 18 of gestation till parturition and to rabbits (NZW)

by stomach tube from day 28 of gestation till parturition as suspensions in 0.5% methylcellulose. Doses selected were based on both antiinflammatory activity and toxicity in the rat. Each drug was given at 9:00 am and 1:00 pm. All three antiinflammatory agents displayed evidence of maternal toxicity in both species; gastrointestinal lesions were occasionally seen in animals that had died before or during parturition. The time to onset of parturition was delayed in rats given 0.50 and 1.00 mg/kg of indomethacin, 5, 15, and 45 mg/kg of phenylbutazone, and 25 and 75 mg/kg of acetylsalicylic acid. In contrast, indomethacin and phenylbutazone, but not aspirin, treatment shortened the time to onset of parturition in the rabbit. The duration of parturition (from onset to completion) was slightly lengthened in rats given indomethacin at doses of 0.50, and 1.00 mg/kg, phenylbutazone at a dose of 5 mg/kg, and acetylsalicylic acid at a dose of 25 mg/kg. In rabbits, the duration of parturition was not lengthened by the three antiinflammatory agents. There was an increase in the number of dead pups delivered by rats given 1.00 mg/kg of indomethacin, 45 mg/kg of phenylbutazone, and 75 mg/kg of acetylsalicylic acid, and by rabbits given 75 mg/kg of acetylsalicylic acid.

63. *A Computerized System for Evaluating Data from Reproduction Studies*. P. L. WRIGHT, JO ANN KNUTSON, SANDRA L. HALEY, M. L. KEPLINGER, and J. C. CALANDRA, Industrial BIO-TEST Laboratories, Inc., Northbrook, Illinois.

A system for recording, storage, calculation and retrieval of data developed in reproduction studies has been implemented using an IBM 1130 computer. The system employs a single page format for recording dates of male and female pairings, copulations and parturition. Records of numbers of pups delivered and surviving at predesignated days during the lactation period as well as litter and dam weights are recorded on the same document. Computations performed for individual females include estrus periods prior to conception, copulations with fertile males and gestation length. The number of pups born and the numbers surviving at various points during the lactation period as well as average pup weights are also displayed on the individual data output. Computations performed for individual males include copulations with fertile females, litters sired, individual non-pregnant dams exposed, and individual dams impregnated. Appropriate mating, fertility, parturition and pup survival indices are computed by treatment group. Following appropriate statistical treatment, the mean data are printed in structured formats which are acceptable for inclusion in final reports. The system is adaptable for data arising from multi-generation studies, Phase I reproduction studies and Phase III perinatal and lactation performance studies.

64. *The Induction of Egg Laying in Wildfowl Reproduction Studies Through Controlled Lighting Cycles*. ROBERT J. FINK, Hazleton Laboratories, Inc., Vienna, Virginia. (L. W. Hazleton.)

Based upon the frequency of application and the degree of persistence, the potential reproductive impairment of wildfowl may have to be evaluated before an agricultural chemical is registered. The seasonal breeding habits of wildfowl hinder the product development program of an agricultural chemical because the reproduction studies can only be conducted in the spring of the year. Utilizing the mallard duck as a representative waterfowl and the bobwhite quail as a representative upland game bird, it has been demonstrated that through the use of controlled lighting cycles, egg laying can be induced in the fall of the year. Analysis of body weight, food consumption, behavior, egg production, embryonation, embryo survival, normal hatchlings, chick survival, and eggshell thickness indicates that wildfowl reproduction studies can be conducted at different times of the year with comparable results.

65. *Toxicity Studies of a Copper Containing Intrauterine Device in Several Laboratory Animal Species*. G. J. YOUKILIS, R. G. MCCONNELL, R. D. HEMM, and J. M. ANDRESS, Department of Biological Research (Pathology-Toxicology) Searle Laboratories, Chicago, Illinois. (V. A. Drill).

In these toxicity studies, copper containing intrauterine devices (CuIUD) were surgically inserted, fixed and retained within the uteri of rats, rabbits and monkeys for periods of up to 52 consecutive weeks. It was the intent of these studies to evaluate the safety of these devices when retained over such periods of time in an experimental condition related to human usage.

Copper content of the device used in these studies was in excess of the CuIUD human clinical situation when expressed either in mg of Cu/g of exposed uterine tissue or on the basis of body weight. Experimental animals were divided into sham operated, plastic device and CuIUD groups. The plastic device animals received a non-copper containing plastic device providing a chemically inert, foreign object control group. Hematology, clinical chemistry (including serum copper and terminal uterine and vaginal tissue copper determinations), urinalysis, vaginal cytology and general physical examinations were performed on all animals throughout the course of the studies. Antemortem evaluation for all species tested were generally unremarkable. Menstrual cycles were significantly prolonged in the CuIUD monkeys in comparison to controls. No significant variations in monkey serum copper values were noted; tissue (uterus) copper values in the CuIUD were comparable across all groups after 52 weeks of exposure in this species. Serum copper levels were inconsistently elevated in the CuIUD rats over 52 weeks of exposure; uterine tissue copper levels, however, were significantly higher in this species. Microscopic examinations of uterine tissue from all species failed to reveal unusual lesions attributable to the copper material.

66. *Dose Response and No-Effect Levels in 2 Known Mutagens.* D. W. ARNOLD, G. L. KENNEDY, JR., M. L. KEPLINGER, and J. C. CALANDRA, Industrial BIO-TEST Laboratories, Inc., Northbrook, Illinois.

Two alkylating agents and known mammalian mutagens, methyl methanesulfonate (MMS) and ethyl methanesulfonate (EMS), were studied to see if a dose-response and a no-effect level could be obtained using the incidence of early fetal death as the index. Dominant lethal mutagenic studies were conducted using doses ranging from 3.12 to 100 mg MMS/kg and 50 to 300 mg EMS/kg. Doses were administered as a single ip injection to male mice with mutagenic effects monitored by the induction of early embryonic deaths in females that had mated with treated males. A positive response and a no-effect level for each material were obtained.

67. *Effects of Mercury on Mouse Spermatogenesis Studied by Cell Separation and Serial Mating.* I. P. LEE and R. L. DIXON, Pathologic Physiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

In recent years, the potential toxicity of environmental mercury has become a major concern. Normal human tissues contain mercury because there is a daily dietary intake. Nearly all of the mercury in food is in the form of methylmercury due to the biotransformation of inorganic mercury. Because of the levels of methylmercury in the environment and reported cytotoxic and carcinogenic toxicity, we have selected the mouse as a model to compare the effects of organic methylmercury hydroxide (MeHgOH) and inorganic mercuric chloride (HgCl<sub>2</sub>) on male reproductive function. The mercury compounds were administered ip once at a dose of 1 mg/kg, or spermatogenic cells were exposed *in vitro* to mercury concentrations ranging from 10<sup>-3</sup> to 10<sup>-7</sup> M. Spermatogenic cells were separated for biochemical studies using the velocity sedimentation technique (*Toxicol. Appl. Pharmacol.* **23**, 20-41, 1972), and *in vivo* serial mating was used to assess fertility. The effects of MeHgOH or HgCl<sub>2</sub> on the uptake of [<sup>3</sup>H]thymidine by spermatogonia (SG), [<sup>3</sup>H]uridine by early elongated spermatids (EES), and [<sup>3</sup>H]L-leucine by late elongated spermatids (LES) were studied. These experiments indicated that MeHgOH reduced thymidine incorporation by SG by 40.6%, uridine incorporation by EES by 39%, and L-leucine incorporation by LES by 89.8% at 10<sup>-3</sup> M. Similar results were obtained with HgCl<sub>2</sub>, but only at a 4-fold increase in concentration. Fertility profiles obtained from serial mating studies indicated the primary effect of MeHgOH was on spermatogonial cells and premeiotic spermatocytes; no significant effects were seen with HgCl<sub>2</sub> at the same dose. These results suggest an important spermatogenic effect of MeHgOH which could result in reduced male fertility.

68. *Biliary Excretion of Mercury Compounds in the Rat; The Effect of Dose and Time after Administration on the Forms of Mercury in the Bile.* M. G. CHERIAN and J. J. VOSTAL, University of Rochester School of Medicine and Dentistry, Rochester, New York.

Biliary excretion of methyl mercury is higher in the rat than in any other species and favors mercury reabsorption from the intestine. In order to elucidate mechanisms of biliary excretion

of mercury compounds, protein-mercury binding in the bile has been investigated after iv administration of  $^{203}\text{Hg}$ -labeled methyl mercuric chloride in female albino rats and compared with similar experiments after injection of labeled mercuric chloride. Bile samples were collected from anesthetized rats in 30-min intervals for 5 hr after the administration of various doses of methyl mercuric $^{(1+)}$  and mercuric $^{(2+)}$  chlorides and fractionated at once on Sephadex G-75 column. Two distinct forms of mercury complexes were separated from the rat bile: (1) fraction I (FN I) with a molecular weight of approximately 70,000 and (2) fraction II (FN II) with a low molecular weight. Relative amount of mercury bound in biliary FN II increased with the time after injection and with higher doses of methyl mercury; it decreased with time and independently of dose after the injection of mercuric chloride. When lower doses of methyl mercury (0.075 to 0.10 mg Hg/kg) were administered, FN II constituted only a minor fraction (less than 10%) of total mercury in bile. With higher doses of methyl mercury (0.5 mg/kg and higher), the low molecular weight fraction became the prevailing form (about 90%) in biliary excretion of mercury within 2 hr after the injection; more than 95% of mercury in all biliary fractions was still in the form of alkyl mercury. In contrast, FN II did not play any important role in biliary transport of mercuric chloride although cumulative biliary excretions of both studied mercury compounds were comparable. The rate of biliary excretion of inorganic mercury was slightly higher during the first hour and the peak of biliary excretion of mercuric chloride usually preceded that of methyl mercury. Our results indicate that biliary excretion of mercury compounds is determined not only by the ionic and chemical forms of mercury but also by the dose and time elapsed since the administration. (Supported in part by NSF grant GI 300978 and by NIGMS grant GM 105190.)

69. *The Ototoxicity of Methyl Mercury*. S. A. FALK, R. KLEIN, G. M. SANDERS, and D. J. LIM, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina and the Ohio State University Medical College, Columbus, Ohio. (F. Oehme.)

To provide understanding of the etiology of deafness associated with Minamata disease, a study was undertaken to identify and evaluate the pathological effects of methyl mercury hydroxide (MMH) on the nervous tissue and sensory epithelium of the inner ear. Young Hartley guinea pigs (250–500 g) were treated with daily sc doses of 2 mg Hg/kg MMH for 1, 2, 4, 6, and 11 weeks. Hearing was assessed by the Preyer reflex and the cochleas were examined by phase-contrast microscopy and transmission and scanning electronmicroscopy. The presence and distribution of elemental Hg was studied by a nondispersive-X-ray detector attached to the scanning electronmicroscope. After 3 weeks of treatment, treated animals gained weight less rapidly than did controls and by 5 weeks some treated animals began to lose weight. All animals had normal Preyer reflexes prior to sacrifice. By the use of cochleographic reconstructions which record cell for cell pathology of surface preparations of the organ of Corti, the outer hair cells (OHC) were significantly damaged ( $P < 0.05$ ) at the level of  $2\frac{1}{2}$  turns from the cochlear base. Pathology also occurred at  $3\frac{1}{2}$ ,  $1\frac{1}{2}$  and  $\frac{1}{2}$  turns from the cochlear base but was not significant. Cellular pathology included derangement of orderly pattern of stereocilia, cytoplasmic vesiculation, and collapse of cell membranes. Study of the stria vascularis including chromophil, chromophobe and basal cells and capillaries and of the auditory nerve and its spiral ganglion did not reveal any pathology. Thus, MMH exerts an ototoxic effect directly on the OHC. This study is significant in two ways. By mainly affecting the apical turns of the cochlea, MMH is a unique ototoxic agent since aminoglycosidic antibiotics and ethacrynic acid cause initial pathology in the basal turns. Also the sensorineural hearing loss occurring in Minamata disease which was presumed secondary to diffuse CNS pathology, now can be considered secondary to a primary cochlear lesion from organic mercurials.

70. *Evaluation of Chronic Oral Administration of Lead Acetate to Rhesus Monkeys*. J. W. GOODE, S. JOHNSON, and J. C. CALANDRA, Industrial BIO-TEST Laboratories, Inc., Northbrook, Illinois.

Young adult, male and female, rhesus monkeys (*Macaca mulatta*) were administered lead acetate po daily for 30 months. Sixteen animals were divided into 4 groups of 4 animals each. The low dose group (0.05 mg/kg), the medium dose group (0.50 mg/kg), and the high dose

group (5.00 mg/kg) received the lead acetate in coconut milk. The fourth group served as a control and received the vehicle. Total blood lead, urinary  $\Delta$ -aminolevulinic acid (ALA), and  $\Delta$ -aminolevulinic acid dehydrase (ALAD) activity, along with routine hematological, clinical chemical, and urinary parameters were monitored periodically. Two behavioral tests, the delayed response and conditioned response evaluation, were performed weekly to examine cortical integrity. Blood lead proved to be the most significant indicator of exposure with blood values related to total exposure. The average blood lead for the high dose group peaked (82  $\mu$ g/100 ml) during week 8 of dosing, after which period it leveled off and remained between 45 to 60  $\mu$ g/100 ml for the remainder of the study. The average blood lead for the control group ranged between 5 and 21  $\mu$ g/100 ml. Urinary ALA intermittently rose and declined in the high dose group throughout the study while the control group values remained fairly stable. Blood ALAD values for this species proved to be too low to evaluate. All other physiological parameters yielded no evidence of toxic effects due to the lead exposure. The conditioned response test, evaluating the acquisition and retention of learned behavior, indicated that exposure to lead at these dosages was without effect. The delayed response test, examining short-term memory and sensorimotor response, also was unaffected by chronic lead exposure.

71. *Long-term Effects of Lead on Learning and Organ Development in the Growing Rat.* D. R. BROWN, University of Maryland, School of Pharmacy, Baltimore, Maryland. (D. A. Blake.)

Lead poisoning causes encephalitis and permanent brain damage in children but not in adults. The suckling rat may provide an animal model for study of lead induced brain damage since encephalotic changes are produced in the suckling rat, when lead is fed to the lactating mother. (Pentshew and Garro, *Acta Neuropath.* 6, 266, 1966). This does not occur in the adult rat. The objective of this study was to determine the effect of lead on the brain of infant rats through measurements on learning and organ development. Lactating rats were administered lead acetate by gavage for 20 days after parturition; learning was measured in the 8- to 10-week old young using a "T" maze. Organ weights, body weights and blood lead concentrations were measured in suckling, weanling and 8-week-old rats. Learning ability was decreased in the 8- to 10-week-old young, nursed 3 weeks by lactating mothers, administered 17.5, 25, or 35 mg/kg lead daily 1 to 20 days after parturition. Administration of 35 mg/kg lead to mothers 1 to 10 days after parturition similarly decreased learning in the 8-week-old young, but administration of 35 mg/kg lead 11 to 20 days after parturition did not. The blood lead had returned to control values by 8 weeks of age. None of the doses altered growth rate but 35 mg/kg lead increased brain and kidney-to-body weight ratios in the suckling and weanling rats. Liver-to-body weight ratios were unchanged. The increase in liver PCMA demethylase activity, which occurs between 5 and 30 days of age was delayed in the lead-treated rats. No toxic effects were observed in the treated mothers. These results suggest that the brain of the suckling rat is particularly sensitive to lead with effects on learning produced, which are still present in the 8- to 10-week-old adult.

72. *Slowed Learning in Lambs Prenatally Exposed to Lead.* GARY A. VAN GELDER, THOMAS L. CARSON, and WILLIAM B. BUCK, Behavioral Toxicology Laboratory, Veterinary Diagnostic Laboratory, Iowa State University, Ames, Iowa.

The effects of subclinical prenatal and early postnatal lead exposure on the developing nervous system and later intellectual and behavioral development are poorly understood. The effects of in utero lead exposure on postnatal behavior in lambs was investigated in three groups of sheep. Blood lead concentrations in the ewes were determined throughout gestation with resulting mean values for the three groups of 0.06 ppm (control), 0.16 ppm (fed 2.3 mg lead/kg/day), and 0.30 ppm (fed 4.5 mg lead/kg/day). None of the ewes showed any clinical evidence of lead toxicosis throughout gestation. Postnatal behavior in the lambs was evaluated using 4 lambs from the control, 8 from the low-lead, and 6 from the high-lead groups. Beginning at 7 days of age, the lambs were tested in a Hebb-Williams closed field maze. Lead exposure did not affect the rate of learning, number of errors, or trial latencies. At 5 months of age the lambs were trained on a two-choice visual discrimination operant task. A total of 7 problems were used. When correct performance for each subject reached criterion, the lamb was advanced to the next problem. The average number of days for lambs within groups to learn all 7 prob-

lems was 42 days for control, 47 days for low-lead, and 74 days for high-lead groups. The high-lead lambs required significantly more days ( $P < 0.05$ ) to learn the problems. The results indicate that subclinical prenatal exposure to lead can retard postnatal learning of a visual discrimination problem.

73. *The Effects of Lead on Rat Liver Cytochrome P-450*. G. F. EGAN and H. H. CORNISH, School of Public Health, The University of Michigan, Ann Arbor, Michigan.

Much of the research concerning the influence of lead on the metabolism of heme has been focused on hemoglobin biosynthesis. Recently, investigations have demonstrated that other heme moieties may also be affected by this metal. Chronic studies (5-9 weeks) conducted in our laboratories involving the po (150 or 300 ppm) or ip (20 mg/kg) administration of lead acetate to rats have revealed that liver cytochrome P-450, a heme containing component of the microsomal enzyme system, became alternately depressed and elevated during the course of treatment. Subsequent acute studies involving the administration of a single dose (100 mg/kg, ip) of lead acetate resulted in a decrease of liver P-450 values to half of the control levels by day 3 post treatment, a return to normal limits by day 7, and a near doubling of the P-450 estimates during the days 10 through 14. Test values then returned to normal by day 17 following treatment. Studies were then conducted in order to gain some insight into the mechanism involved in this phenomenon. Urinary and plasma amino levulinic acid (ALA) levels were found to be fifteen times greater than controls by day 10 while total liver and mitochondrial lead values peaked on days 7 and 10 respectively. The estimated ALA levels were then employed in ALA dehydrase assays in order to determine the influence of excess heme precursors on overall heme synthesis. The rate of P-450 catabolism was also ascertained to determine if any modifications in P-450 degradation may have occurred during the observation period which might account for the observed changes in P-450 values.

74. *Toxicity of  $\Delta$ -Amino Levulinic Acid*, G. L. KENNEDY, JR., and J. C. CALANDRA, Industrial BIO-TEST Laboratories, Inc., Northbrook, Illinois.

In persons exposed to excess amounts of lead the excretion of increased amounts of  $\Delta$ -amino levulinic acid (ALA) in the urine is observed. Inhibition of the enzyme  $\Delta$ -amino levulinic acid dehydrase (ALAD) interferes with the heme biosynthetic chain and is responsible for the rise in urinary ALA. A 90-day subacute toxicity study was conducted treating albino mice with ALA to determine whether pharmacologic signs or pathologic changes could be produced. Groups of 10 animals of each sex were given 3 ip injections of 0, 10, 50, or 100 mg ALA/kg weekly for 13 consecutive weeks. Growth, blood chemistry, and hematologic and urologic parameters were evaluated during the exposure period and at the termination of the experiment. Following 13 weeks of exposure, all animals were sacrificed and complete macro and microscopic examination of major tissues and organs was conducted. Growth, reactivity, and all hematologic, clinical blood chemistry, and urine analyses failed to reveal any ALA induced changes. The urinary excretion of ALA was elevated in the 3 exposed groups, the degree being proportional to the amount of ALA administered. Organ weights of treated animals were not statistically different from those of controls. Gross pathologic and histologic evaluation of tissues from treated animals revealed no differences attributable to treatment with ALA.

75. *Nickel Chloride-Induced Changes in Glucose Metabolism in the Rat*. JOHN J. CLARY and LOUIS VIGNATI, National Institute for Occupational Safety and Health, Cincinnati, Ohio. (H. E. Stokinger.)

Exposure to industrial metals may cause subtle long-term health effects. This study was undertaken to explore a heretofore unreported effect of  $\text{NiCl}_2$  on carbohydrate metabolism and elucidate the mechanisms involved.  $\text{NiCl}_2$  was given by various routes: intraperitoneal (8 mg/kg), intratracheal (0.5 mg), and by long-term ingestion (drinking water—225 ppm). In addition, intragastric glucose load (600 mg) was also given to some of the intratracheally injected animals. The following parameters were measured in the serum: glucose, insulin, total lipids, cholesterol and triglycerides. Liver parameters measured were glycogen and glucose-6-phosphatase.  $\text{Ni}^{63}$  tissue distribution and excretion were also measured. A single ip injection

of Ni to rats caused a rapid, transient fourfold increase in serum glucose and consequent glucosuria. This hyperglycemia was also associated with hyperlipidemia and insulin resistance. Nickel appears to be an antagonist to exogenous insulin. Repeated exposure to ingested Ni (drinking water) also resulted in elevated serum lipids, especially the triglyceride fraction. An increase in pancreatic insulin was also observed. Daily Ni ingestion causes no change in liver glycogen content or in fasting blood glucose values. This study points out the subtle changes in the metabolic pathways which might affect the health of industrial workers exposed to Ni.

76. *An Appraisal of Improved Lung Clearance of Particles in Rats Exposed to Low SO<sub>2</sub> Exposure.* J. FERIN, School of Medicine and Dentistry, University of Rochester, Rochester, New York. (R. A. Scala.)

Our study designed to assess the effect of some air pollutants on lung clearance of particles has revealed stimulation, no effect, as well as a depression of particle clearance, depending on SO<sub>2</sub> exposure characteristics. These results bring additional information to a toxicological discussion on "no-threshold", "threshold" or "several thresholds" of response. In a series of experiments the nontoxic-particle-challenge system was used to measure the integrated alveolar clearance function by serially sacrificing rats after TiO<sub>2</sub> aerosol exposure and by determining the amount of TiO<sub>2</sub> retained in the lung. Prior to TiO<sub>2</sub> exposure, different groups of animals were exposed for various times (70–170 hr, 6 hr a day, 5 days a week) to sulfur dioxide in concentrations of 0.1, 0.5, 1, and 20 ppm, respectively. Normalized retention data, indicative of clearance, were plotted against an empirical SO<sub>2</sub> exposure index (the product of concentration and time to the fourth power). The resulting dose-response line, described by the equation  $Y = 0.051 X + 43.1$ , gave a satisfactory fit to the experimental data. The increased particle clearance by about 30% under certain exposure conditions, which by conventional standards suggest a beneficial effect of SO<sub>2</sub> inhalation, has to be appraised from a general view point of chemical agents. (Supported by a PHS Grant 2 RO1 OH 00334 and by the US AEC, Report No. UR-349-201.)

77. *Sensory Irritation and Desensitization by Sulfur Dioxide.* Y. ALARIE, I. WAKISAKA, and S. OKA, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania.

Sulfur dioxide was shown to initiate sensory irritation of the upper respiratory tract in mice. The reaction was accompanied with desensitization. The degree of sensory irritation was linearly related to the logarithm of the SO<sub>2</sub> concentration while the time for maximum reaction to occur varied inversely with that factor at concentrations ranging from 17 to 298 ppm. The rate of desensitization following maximal response appeared similar to all concentrations tested. The desensitized state was specific for SO<sub>2</sub> since the reaction to other sensory irritants (*o*-chlorobenzilidene malonitrile, capsaicin) was not altered by prior exposure to SO<sub>2</sub>. The desensitized state to SO<sub>2</sub> was short lasting and a return to a normal state of sensitivity to the action of SO<sub>2</sub> was reached within a period of 12 min after desensitization had been accomplished. The sequence of events for simultaneous stimulation and desensitization of the nasal trigeminal nerve endings, from which the sensory irritation sensation originates, is explained by a model based on the reducing properties of sulfite toward disulfide bonds in a receptor protein (stimulation), the formation of an inactive receptor (desensitization) and recovery to an active receptor form once SO<sub>2</sub> is removed. We conclude that SO<sub>2</sub>-induced stimulation and desensitization of trigeminal nerve endings in the nasal mucosa in the same fashion as that demonstrated by Widdicombe (*J. Physiol.* 21, 55–104, 1954) for receptors of the tracheo-bronchial tree in cats. (Supported under Research Grant No. 1-RO1-OH-00367 from NIOSH and under Special Fellowship No. 5 FO3-ES-46, 198 from NIEHS (Y. Alarie) and Visiting Fellowship from the Ministry of Education of Japan (I. Wakisaka).)

78. *Cardiovascular Alterations Resulting from Inhalation of 1,1,1-Trichloroethane.* P. A. HERD, H. F. MARTIN, and M. LIPSKY, Department of Pathology, Rhode Island Hospital, Providence, Rhode Island. (L. Vinson.)

1,1,1-Trichloroethane (TCE) is an organic solvent which has enjoyed increased use in recent years because it is less toxic than several other halogenated hydrocarbons. However, some

clinical toxicity data has suggested that inhalation of TCE is responsible for direct, and possibly long-term alterations in cardiovascular function. To explore this possibility, anesthetized (chloralose) dogs were attached to a bubble vaporizer and controlled dosages of TCE ( $10^4$ – $4 \times 10^4$  ppm/min) were found to produce a biphasic decline in arterial blood pressure. The initial decline in pressure (within 10 to 15 sec after introduction of TCE) was found to be due to a decrease in total peripheral resistance (TPR), since cardiac output increased (40–45%) initially. Infusion of the pure  $\alpha$ -agonist, phenylephrine, reversed these peripheral vascular effects indicating that TCE does not act directly on the vascular musculature. Also, in contrast to other catecholamines (e.g. epinephrine), no arrhythmogenic effects were noted. The second phase of blood pressure decline is associated with a decrease in myocardial contractility, reflected by a decline of both heart rate and stroke volume. In contrast, the change in TPR was greatly diminished during this phase. Exogenous  $\text{Ca}^{++}$  reversed the TCE-induced decline in myocardial contractility but had no effect on the initial phase of peripheral vasodilatation. The data indicate that drug-sensitive cardiovascular components are involved in TCE-induced intoxication. These phenomena cannot be attributed only to generalized central nervous system depression as proposed by others. Further characterization of the nature of these components may enable more effective patient management. (Supported, in part, by Rhode Island Hospital and the Tupper Foundation, New York City.)

79. *The Effects of Bromotrifluoromethane on Myocardial Metabolism.* K. C. BACK and E. W. VAN STEE, 6570 Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio.

The inhalation exposure of dogs to bromotrifluoromethane was reported earlier to cause a reversible, concentration-dependent decrease in mean arterial blood pressure as a result of decreased vasoconstrictor tone and a negative inotropic effect on the left ventricular myocardium. Dogs were acutely instrumented to provide the following information: vasoconstrictor tone, arterial blood pressure, left ventricular pressure,  $dP/dt$ ,  $dP/dt \div P$ , ascending aortic flow, and left coronary circumflex arterial flow. Animals were exposed to 50 and 75%  $\text{CBrF}_3$  for 10-min periods. Recordings were made and arterial and coronary sinus blood samples were obtained before, during, and after the  $\text{CBrF}_3$  exposures. A small decrease in the vigor of myocardial contraction, decreases in vasoconstrictor tone and mean arterial blood pressure, an increase in aortic blood flow, an increase in myocardial lactate utilization, and a decrease in myocardial oxygen consumption were observed as functions of the  $\text{CBrF}_3$  concentration. Conclusions were that under the conditions of this experiment (1) a decrease in pressure-volume work done by the left ventricular myocardium during  $\text{CBrF}_3$  exposure resulted in a decrease in myocardial oxygen consumption; (2) myocardial metabolism was little affected; and (3) decreased peripheral vascular flow resistance was accompanied by increased cardiac output.

80. *The Cardiovascular Effects of Exposure to Bromochlorodifluoromethane.* E. W. VAN STEE and K. C. BACK, 6570 Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio.

Dogs were exposed to 15% bromochlorodifluoromethane using a cross-circulation preparation described earlier by the authors. Donor arterial blood was used to perfuse the hind limb of the recipient. When the flow rate of perfusion was held constant, changes in perfusion pressure provided an index of vascular flow resistance. Donor dogs were treated with reserpine, and the recipient dogs were treated with atropine. The perfused hind limb was treated with propranolol and diphenhydramine. A small decrease in donor arterial blood pressure and no change in recipient hind limb perfusion pressure were seen during exposure of the donor. A decrease in recipient arterial pressure and recipient hind limb perfusion pressure were seen during exposure of the recipient. The decrease in hind limb perfusion pressure was abolished following treatment of the recipient with hexamethonium. Conclusions were: (1) the decrease in peripheral vascular flow resistance during  $\text{CBrClF}_2$  exposure was the result of a decrease in vasoconstrictor tone, and (2) humoral, local metabolic, active innervated vasodilator, and cholinergic, histaminergic, and beta-adrenergic mechanisms, regardless of innervation, were ruled out as participating in the vascular flow resistance change.



81. *Fluoride Ion Excretion After Inhalation of Several Fluoroethylene Derivatives.* J. V. DILLEY, V. L. CARTER, JR., and E. S. HARRIS, Northrop Services, Inc. and NASA, Manned Spacecraft Center, Houston, Texas.

Male rats were exposed to atmospheres of various fluorinated ethylene compounds or hexafluoropropene in sub-lethal concentrations. The increase in urinary fluoride after exposure to these fluorocarbon atmospheres suggested that they were degraded biologically to free fluoride ion. Inhalation of these fluorocarbon-containing atmospheres also produced a moderate to marked diuresis, and an increased potassium and creatinine excretion. The animals also exhibited a severe glucosuria for 24-72 hr after exposures to atmospheres containing hexafluoropropene or tetrafluoroethylene. These studies suggest that simple fluorinated alkenes are biodegradable and that this can be demonstrated by the increased urinary excretion of fluoride ion after exposure. They also exhibit a nephrotoxicity when given in sub-lethal quantities.

- ✓ 82. *Pulmonary Histopathology of Hamsters Exposed to Smoke.* F. HOMBURGER, A. B. RUSSELL, and P. BERNFELD, Bio-Research Consultants, Inc., Cambridge, Massachusetts.

One hundred and two inbred Syrian hamsters of the BIO 87.20 and BIO 15.16 strains were exposed twice daily, 5 days a week, to 8 puffs from Kentucky 1R1 cigarettes of 2-sec duration taken every minute by a Walton-Morrissey reverse smoker. The exposure to 19.3% smoke concentration lasted 15 sec and was followed by 43 sec of laboratory air. Sixty animals of each strain were held in cages without manipulation and 60 additional control animals of each strain were exposed as the experimental animals except that no cigarette was inserted into the machine. In the BIO 87.20 strain animals were killed at 45 weeks, and those of the BIO 15.16 strain at 48 and 78 weeks. Animals dying spontaneously were also studied histologically. In hamsters exposed to smoke, macrophage granulomas were seen in the pulmonary parenchyma, especially frequently and early in 87.20 hamsters. Rare malignant tumors were found in the nasopharynx of smoke-exposed 15.16 animals. Premalignant dysplastic changes were seen in the larynx after approximately 40 weeks of smoke exposure. Invasive neoplastic changes were found in the larynx of smoke-exposed animals of both strains, beginning at about 80 weeks of smoking. This confirms the observations of Dentenwill of laryngeal changes and demonstrates the usefulness of the hamster inhalation model for studies on carcinogenesis of whole smoke.

83. *Response of Rats to Pyrolysis Products of Fluorinated Polymers.* E. S. HARRIS, H. P. WARRINGTON, J. W. BAILEY and J. V. DILLEY, NASA, Manned Spacecraft Center, Houston, Texas.

A previous report from this laboratory presented the LD50 and microscopic pathology resulting from the exposure of rats to the pyrolysis products of copolymers of vinylidene fluoride and hexafluoropropene (I) and (II) plus additives produced by two manufacturers. This report extends that work to examination of physiological responses at sublethal levels. The pyrolysis products of all three materials produced marked diuresis and increased water consumption following exposure. Concomitant with the diuresis was an increased output of fluoride ion, glucosuria and increased urinary potassium. Manometric assay of kidney succinic dehydrogenase shows an apparent inhibition produced by all three materials. This was not found when using a spectrophotometric method. Gross observation of the kidney shows medullary hyperemia. All biochemical parameters returned to normal within 14 days. The overall picture indicates proximal tubule damage following sublethal exposures to the pyrolysis products of all three materials. It is not yet certain that this damage is entirely due to inhalation of hydrolyzable fluoride during the exposure period.

84. *Respiratory Retention of Acetone and Ammonia in the Dog.* JOHN L. EGGLE, JR., Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia. (J. F. Borzelleca.)

Acetone and ammonia are among the many compounds of toxicological interest found in the vapor phase of cigarette smoke. This study deals with the handling of these compounds by

the respiratory tract of the dog. Specific objectives included determination of uptake of each compound by the entire respiratory tract and by upper and lower portions under varying conditions of ventilatory rate, tidal volume and concentration inhaled. Uptake of acetone by the total respiratory tract ranged between 65 and 70% while retention of inhaled ammonia was about 10% higher. In terms of regional uptake, retention of ammonia was higher than that of acetone in both the upper and lower respiratory tract. Uptake of acetone was found to be directly related to the concentration of the vapor inhaled. Retention of ammonia varied only slightly with concentration. Retention of acetone was inversely related to tidal volume, but ammonia uptake was found to be directly related to tidal volume. Experiments were also carried out to determine the effect of simultaneous inhalation of either acetone or ammonia with acetaldehyde on the respiratory uptake of acetaldehyde. The respiratory handling of acetaldehyde vapor alone has been previously studied. It was found that the uptake of acetaldehyde was enhanced by the presence of acetone over the entire range of respiratory rates studied (5-65/min). The presence of ammonia reduced the retention of acetaldehyde at low ventilatory rates (6-12/min) and increased acetaldehyde uptake at high rates (20-40/min). The overall effect of the presence of ammonia in the inspired air was a disappearance of the strong inverse relationship observed between respiratory rate and retention when acetaldehyde vapor was inhaled alone. (Aided by a grant from the American Medical Association-Education and Research Foundation.)

85. *The Influence of p,p'-DDE, A Model Liver Microsomal Enzyme Stimulator, on p,p'-DDT Toxicity in the Bluegill, Lepomis macrochirus (Rafinesque)*. PATRICIA A. SPIEGEL and MORRIS F. CRANMER, Environmental Protection Agency, Perrine Primate Laboratory, Perrine, Florida.

The possible role of liver microsomal enzyme (LME) induction in mechanisms operative in providing fish with resistance to toxic foreign substances, such as insecticides, was investigated. Hatchery-reared populations of the bluegill, *Lepomis macrochirus* (Rafinesque), were pretreated with different water and food concentrations of *p,p'*-DDE prior to acute exposure to *p,p'*-DDT. LME activity was determined by gas-liquid chromatography and liquid scintillation analyses of extracts of parathion-LME incubates. These analyses indicated that LME of the bluegill are unable to metabolize paraoxon. Exposure to DDE in the water produced a toxic effect on the liver and no induction of the LME system. DDE pretreatment resulted in higher susceptibility to DDT toxicity. Both control and treated fish demonstrated a wide range of LME activity. Although statistically significant differences were not demonstrated between the control and treated groups, a few individuals in the treated group showed exceptionally high LME activity. The results of this study support the conclusion that induction of LME does not play a major role in fish resistance to DDT.

- ✓ 86. *The Effect of Age and Long-Term, Low-Level DDT Exposure on the Response to Enzyme Induction in the Rat*. R. CHADWICK, R. LINKO, J. FREAL, and A. ROBBINS, Perrine Primate Laboratory, Perrine, Florida. (R. A. Scala.)

Some earlier studies have indicated there is an age-dependent susceptibility to enzyme induction in animals while other work indicates that the apparent lack of response in older animals may just be the result of an induction lag period. The existence of such an induction lag as well as the effect of long term low level DDT exposure on the response to enzyme induction were investigated in this study. Groups of weanlings and 14-month-old adult female rats received either 2.5 mg/kg of dieldrin in DMSO or just DMSO ip. Half the adults had received 5 ppm DDT in their feed since they were 3 months old. The rats were sacrificed at 24, 48, 72, 96 and 120 hr after the administration of dieldrin. Twenty-four hours before each group was sacrificed 10 mg/kg of lindane in peanut oil was administered po. Within 24 hr after the administration of dieldrin, there were significant increases in the *in vitro* activity of various hepatic microsomal enzymes from the weanling rats. There were no such increases in the activities of these enzymes in the adults. In general, throughout the study, the weanlings had the highest enzyme activity, the DDT-fed adults were next and the control adults had the lowest activity. In the metabolism of lindane to chlorophenols, the weanlings exceeded the adults

within 72 hr after the administration of dieldrin. Similarly the storage of lindane in the body fat of the weanling significantly decreased at 72 hr below that observed in the adults. There was no evidence of an induction time lag in this study. If such a lag exists, it must exceed 120 hr. Data from this experiment support the suggestion of age-dependent susceptibility to enzyme induction.

87. *Some Species Differences in DDT Metabolism and Tissue Distribution.* RALPH GINGELL and LAWRENCE WALLCAVE, Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, Nebraska. (F. Oehme.)

*p,p'*-DDT has been shown to be a weak carcinogen in mice, but is non-tumorigenic in hamsters. The mouse is also more susceptible to the acute and subacute toxic effects of DDT. Tissue levels of DDT and residues were 7-8 times higher in mice than in hamsters maintained on a diet containing 250 ppm DDT for 6 weeks. The levels of DDE in mouse liver were relatively much higher than in the hamster tissue, and DDD levels relatively lower, suggesting that there may be a quantitative species difference in DDT metabolism via DDD to conjugates of bis(*p*-chlorophenyl)-acetic acid, (DDA), which are excreted. When [<sup>14</sup>C]-DDT (25 mg/kg) was administered po to mice and hamsters there was little difference in the amounts of <sup>14</sup>C recovered in the urine. However when [<sup>14</sup>C]-DDT was administered to animals maintained on a diet of 250 ppm DDT for 6 weeks, hamsters excreted 2-3 times more <sup>14</sup>C than mice, suggesting that dietary DDT enhances its own metabolism and excretion in hamsters, but not in mice. This dietary DDT treatment decreases hexobarbital sleeping time in hamsters, but has little effect in mice. Species differences in the toxicity and carcinogenicity of DDT may thus be explained by this species difference in the effect of DDT treatment on DDT metabolism and excretion. (Supported by NIH Contract PH 43-68-959.)

88. *Urinary and Fecal Metabolites of p,p'-DDT in the Syrian Golden Hamster and Swiss Mouse.* L. WALLCAVE, R. GINGELL, and S. BRONCZYK, The Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, Nebraska. (F. Oehme.)

Previous studies have shown that the Syrian hamster is resistant to *p,p'*-DDT in the diet at levels as high as 1000 ppm whereas several strains of mice are profoundly affected with neurological and other toxic signs after being on a diet containing over 250 ppm of *p,p'*-DDT for a few weeks. In order to determine if this species difference is related to metabolic differences, the urine and feces of hamsters and mice fed 250 ppm of *p,p'*-DDT in the diet were examined. Ether extracts of base-hydrolyzed urine were methylated and analyzed by gas chromatography. In both series the principal urinary metabolite was an alkali labile conjugate, probably the glucuronide, of bis(*p*-chlorophenyl)acetic acid (*p,p'*-DDA), but smaller amounts of conjugates of DDA with glycine and alanine were also found. The identities of the conjugates, bis(*p*-chlorophenyl)acetyl-glycine and bis(*p*-chlorophenyl)acetyl-alanine, were proved by mass spectrometry and chromatographic identification with authentic synthetic samples. Neither metabolite has previously been described. Feces were extracted with acetic acid-hexane and the principal neutral metabolite was *p,p'*-DDD. Little unabsorbed DDT was present. It does not appear that qualitative differences in the nature of excreted DDT metabolites of the hamster and mouse can explain the differing toxic effect of that compound in the two species examined. (Supported by NIH Contract PH 43-68-959.)

✓ 89. *DDT, Heptachlor, Chlordane, and Parathion Toxicity in Adult, Newborn, and Phenobarbital-Treated Newborn Rats.* R. D. HARBISON, Department of Pharmacology and Center in Toxicology, Vanderbilt Medical Center, Nashville, Tennessee.

Pesticide lethality and effect of phenobarbital (PB) treatment on pesticide lethality were compared between age groups. DDT, heptachlor (H), chlordane (C), and parathion (P)-induced lethality were determined in adult (A), newborn (NB), and PB-treated NB rats. Adult Sprague-Dawley rats, 100 to 120 g, and 5-day post-partum NB rats, 10 to 12 g, were used in the study. PB, 40 mg/kg, was administered for 4 days and the pesticide administered on day 5. Median lethal dosage (MLD) was calculated from the number of deaths at the end of a 7-day

period. DDT MLD is 224 mg/kg for A and 2356 mg/kg for NB. PB treatment of NB rats enhances lethality of DDT to a MLD of 1344 mg/kg. Heptachlor MLD is 71 mg/kg for A and 531 mg/kg for NB. PB treatment of NB rats enhances lethality of H to a MLD of 133 mg/kg. Chlordane MLD is 344 mg/kg for A and 1121 mg/kg for NB. PB pretreatment of NB rats enhances lethality of C to a MLD of 539 mg/kg. Thus, toxicity of DDT, H, and C is 5 to 10 times less in NB when compared to A, but PB treatment enhances the toxicity of all three pesticides in the NB. Parathion MLD is 8.8 mg/kg in A and 1.9 mg/kg in NB. However, PB treatment of NB rats antagonizes P lethality to a MLD of 4.8 mg/kg. Toxicity of DDT, C, H, and P is age dependent and altered by PB treatment. Although PB treatment antagonizes P toxicity it enhances DDT, C, and H toxicity. (Supported by NIH Grants ES 00267 and ES 00782.)

90. *The Extrahepatic Metabolism of Parathion to Paraoxon.* RAYMOND E. POORE and ROBERT A. NEAL, Center in Environmental Toxicology, Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee.

A semiquantitative method for measuring the *in vivo* metabolism of parathion to paraoxon has been developed. The metabolism that has occurred in individual tissues is determined by the amount of radioactive sulfur that is released and subsequently covalently bound to the tissues in the metabolism of  $S^{35}$  labelled parathion to paraoxon. The *in vivo* metabolism of parathion to paraoxon has been measured in 9 tissues. Pretreatment of rats with SKF 525-A and piperonyl butoxide 1 hr prior to administration of parathion decreased the *in vivo* metabolism of parathion to paraoxon in the liver, lung, and brain. A comparison of the amount of sulfur bound to tissues at three different levels of parathion intake has also been made. At an oral dose of 12/mg/kg of [ $^{35}S$ ]parathion, tissues from weanling male rats showed less metabolism than the same tissues from the adult male rat. Our data show that the *in vivo* metabolism of parathion to paraoxon in individual organs or tissues can be measured semiquantitatively and that the amount of metabolism that occurs in a tissue varies with the tissue examined.

✓ 91. *Species Variations in the Activation and Degradation of Parathion by Mammalian Hepatic Enzymes.* L. WHITEHOUSE and D. J. ECOBICHON, Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada.

The toxicity of phosphorothionate insecticides in animal species is largely dependent upon the rate of "oxon" formation and the rates of hydrolytic and/or oxidative degradation by hepatic enzymes. The *in vitro* activation of parathion (*O,O*-diethyl-*O*-4-nitrophenyl phosphorothionate) was studied using isolated hepatic microsomes from the following species (mouse, hamster, rat, guinea pig, rabbit, cat, dog, hog, cow and human). The incubation mixture (5.0 ml) consisting of microsomes, substrate, a NADPH-generating system, EDTA and Tris-HCl buffer (pH 7.4), was extracted with ethyl acetate and the metabolite paraoxon (*O,O*-diethyl-*O*-4-nitrophenyl phosphate, PO) was quantitatively analyzed by gas-liquid chromatography using an electron capture detector and a column packed with 3% OV-1 on Chromosorb W-HP (80-100 mesh). Using short incubation times (2.5-15 min), the initial rates of activation were estimated graphically. Kinetic constants ( $K_m$ ,  $V_{max}$ ) and average initial rates of PO formation were determined for males and females of each species. Statistically significant strain and sex differences were observed in the laboratory animal species. Few significant differences were observed between males and females of the larger species and humans, the variations in activity being much greater. The rate of PO hydrolysis was investigated using a spectrophotometric technique to determine the formation of 4-nitrophenol by hepatic arylesterases. Activities varied considerably from species to species, no activity being detected in the guinea pig while rat and rabbit liver exhibited the highest rates of hydrolysis.

92. *Comparative Toxicity, Anticholinesterase Action and Metabolism of Methyl Parathion, Parathion and Guthion in Sunfish and Mice.* G. M. BENKE, K. L. CHEEVER, and S. D. MURPHY, Department of Physiology, Harvard School of Public Health, Boston, Massachusetts.

Methyl parathion has been suggested as a substitute for many uses of DDT. The literature indicates that the organophosphorus insecticides methyl parathion, parathion and guthion

are nearly equitoxic to rats by the oral route. However, parathion is 20 times more toxic and guthion is nearly 400 times as toxic as methyl parathion in sunfish when tested by 96-hr LC50 determinations. We found that lethal dose ranges (by ip injection) in pumpkinseed sunfish for methyl parathion, parathion and guthion were >2500, 10-200 and 1-10 mg/kg respectively. In mice, corresponding values were 10-12, 13-15 and 3-4.5 mg/kg. Lethal dose ranges for the corresponding oxygen analogs (methyl paraoxon, paraoxon and gutoxon) in sunfish were 8-20, 5-10 and 0.01-0.05 mg/kg. The greater toxicity of guthion, relative to methyl parathion and parathion in sunfish, could be partly explained on the basis of greater sensitivity of their brain and muscle cholinesterase as indicated by *in vitro* I<sub>50</sub> values of  $4.8 \times 10^{-10}$  M for brain and  $2.4 \times 10^{-10}$  M for muscle. I<sub>50</sub> values for sunfish brain for methyl paraoxon and paraoxon were  $2 \times 10^{-8}$  M and  $2.4 \times 10^{-8}$  M respectively and for muscle were  $4 \times 10^{-8}$  and  $1.8 \times 10^{-7}$  M. Therefore brain cholinesterase sensitivity cannot explain the differences in susceptibility of sunfish to poisoning by methyl parathion and parathion. The nearly equal toxicity of paraoxon and methyl paraoxon suggest that either methyl parathion is detoxified more rapidly than parathion or that parathion is activated to paraoxon at a greater rate in sunfish. However, *in vitro* studies showed that the rates of activation of the two thiono derivatives by fish-liver homogenates did not differ sufficiently to explain the differences in toxicity. In addition, the oxidative cleavage of parathion and methyl parathion to *p*-nitrophenol and the dialkyl phosphates proceeded at approximately the same rates. It appears that alternate detoxication pathways in liver or extrahepatic tissues must be sought to provide a metabolic explanation for differences in the toxicity of parathion and methyl parathion in sunfish. (Supported by Research and Training grants ES 00084 and ES 00045 from the NIEHS, US DHEW.)

93. *Cholinesterase Inhibition and Mortality of Japanese Quail Following Dietary Exposure to Parathion (0,0-Diethyl-0-p-nitrophenyl phosphorothionate.)* J. LARRY LUDKE, ELWOOD F. HILL, and MICHAEL P. DIETER, Patuxent Wildlife Research Center, Bureau of Sport Fisheries and Wildlife, Laurel, Maryland. (Lucille F. Stickel.)

Plasma and red blood cell cholinesterase (ChE) activity has been used extensively as a monitoring method for exposure of human beings to organophosphorus (O-P) insecticides. Brain ChE activity has been helpful on a limited basis in monitoring exposure of wildlife to O-P compounds. The dietary LC50 and brain and plasma ChE activities were studied in Japanese quail of different ages and sex exposed to parathion in an effort to correlate ChE activity and mortality with exposure time and concentration. There were no marked differences in brain ChE activity attributable to age or sex. Plasma ChE in sexually mature females averaged 82.5% that of males. There was a relationship between the degree of parathion exposure and brain ChE levels; no birds that died had a brain ChE activity exceeding 55% of normal and enzyme activity lower than 80% of normal was considered indicative of parathion intoxication. The dietary LC50 values were 150, 197, 295, and 1,739 ppm for 1, 2, 4 and 8-week old birds respectively.

94. *Inorganic Tin Metabolism in Rats.* RICHARD A. HILES, Professional and Regulatory Services Division, Miami Valley Laboratories, The Proctor & Gamble Co., Cincinnati, Ohio. (W. R. Michael.)

<sup>113</sup>Sn(II) and <sup>113</sup>Sn(IV) metabolism was studied in rats. From a single ig dose (4 mg Sn), 40 μg and 12 μg of Sn from <sup>113</sup>Sn(II) and <sup>113</sup>Sn(IV) respectively were excreted in the urine while > 98% of the dosed Sn was in the feces. More than 70% of the urinary <sup>113</sup>Sn was excreted in the first 10 hr. After 48 hr the retained <sup>113</sup>Sn was distributed mainly between liver and kidneys [4 μg from Sn(II) and 0.8 μg from Sn(IV)] and bone [8 μg/g from Sn(II) and 2 μg/g from Sn(IV)]. None was detected in the lungs or brain. Changing the anion between F<sup>-</sup> and citrate did not affect the metabolism of <sup>113</sup>Sn(II) or <sup>113</sup>Sn(IV). From an iv dose, 11% from <sup>113</sup>Sn(II) and 0.1% from <sup>113</sup>Sn(IV) was excreted in the bile. Groups of young rats were dosed ig over 27 days with <sup>113</sup>SnF<sub>2</sub> or <sup>113</sup>SnF<sub>4</sub> (20 mg Sn/kg/dose, 59 mg total). This prolonged exposure to Sn had no effect on the absorption of the Sn from the gut. Bone contained 75 and 14 μg Sn/g from the dosed <sup>113</sup>Sn(II) and <sup>113</sup>Sn(IV) respectively. These levels were decreased by >75% within 40 days postdosing. Fetuses taken at 10 and 21 days from rats dosed ig with 20 mg Sn/kg/day as <sup>113</sup>SnF<sub>2</sub> or <sup>113</sup>SnF<sub>4</sub> from conception contained no significant

$^{113}\text{Sn}$ . These studies show that inorganic Sn is poorly absorbed from the gut, rapidly excreted and poorly retained by the rat; that a significant amount of absorbed Sn(II) is eliminated in the bile; and that the placenta does not transport Sn.

95. *Thirty-Day Toxicity of Chronically Administered Sodium Pentafluorostannite in Male and Female Rats.* D. L. CONINE, M. N. YUM, R. C. MARTZ, G. L. RAY, G. K. STOOKEY, and R. B. FORNEY, Indiana University School of Medicine, Indianapolis, Indiana.

A 30-day subacute toxicity study of sodium pentafluorostannite ( $\text{NaSn}_2\text{F}_5$ ) was performed in male and female Harlan/Wistar rats. The rats were dosed orally each day for the duration of the study. Rats were dosed for 6 succeeding days with distilled water. On day 7 each sex was divided into 4 groups of 12 to 19 animals each. Rats in each group were then dosed daily beginning on days 7 through 36 with one of the following regimens: distilled water, 20, 100, or 175 mg  $\text{NaSn}_2\text{F}_5/\text{kg}$ . The weight of each rat was recorded daily. On days 21 and 36 whole blood was collected from 5 rats in each group and hemoglobin concentration and hematocrit determined. On days 22 and 37, the same rats were sacrificed and serum BUN, direct and total bilirubin, SGOT, inorganic phosphorus, calcium, and glucose concentrations determined. Each rat was autopsied and examined grossly. Organs were weighed and sections prepared for microscopic examination. Growth and serum glucose concentrations were found to be depressed in a dose related manner on days 22 and 37. Other biological parameters were not consistently altered. Few gross pathologic changes were observed in animals sacrificed on days 22 or 37. Microscopic examination of organs taken from the 175 mg  $\text{NaSn}_2\text{F}_5/\text{kg}$  groups on days 22 or 37 were generally unremarkable except for the appearance of renal tubular changes. Approximately 15 to 20% of the rats showed early degenerative changes of the proximal epithelium. Eleven animals (8 from the 175 mg  $\text{NaSn}_2\text{F}_5/\text{kg}$  groups) died spontaneously during the study. Many of these animals, both grossly and microscopically, displayed congestion of the liver, spleen, lungs, and kidneys. In addition most displayed moderate to severe renal tubular necrosis.

✓ 96. *Biliary Excretion of Arsenic.* CURTIS D. KLAASSEN, University of Kansas Medical Center, Kansas City, Kansas.

The disappearance of arsenic-74 from the blood and plasma of rats and its excretion into the bile was measured for 2 hr after the iv administration of 0.01, 0.46, 1.0, 2.1, and 4.6 mg/kg of arsenic given as the trichloride. Arsenic disappearance from the plasma was biphasic; the half-life during the late phase was greater than 2 hr. Even though the arsenic was injected iv, the concentration in the blood increased throughout the first 2 hr. Arsenic was rapidly excreted into the bile, reaching its highest rate of excretion 6 min after administration; the rate then rapidly decreased. This rapid decrease in excretion is due to redistribution of arsenic from the liver to the blood. Arsenic enters bile against a bile/plasma concentration gradient of 630, 8 min after a 1 mg/kg dose of arsenic. At this time the liver/plasma gradient is 17 and the liver/bile gradient is 37. Twenty-five percent of the arsenic administered to bile duct-cannulated rats is excreted into the bile within 2 hr after administration. However, less than 10% of the administered dose is excreted into the feces of intact rats over a 7-day period. These data demonstrate that arsenic is excreted into the bile of rats. Further, these data suggest that arsenic is transported into bile by an active transport system, and then enters an enterohepatic circulation. (Supported by USPHS Grant GM15956.)

97. *Behavioral Changes to Define Threshold Concentrations of Metal Intoxication in Fish.* W. S. McCLAIN, S. J. EILTS, P. A. WEIR, and F. W. WEIR, The Ohio State University, Department of Preventive Medicine, Columbus, Ohio.

This study was undertaken to determine the degree of impairment on conditioned responses of fish exposed to copper and other metals. Concentrations which adversely affect fish are compared with levels found by other test types, such as Laboratory Fish Production (LFPI). Fathead minnows were chosen as a representative freshwater species. Correspondence between test results should add support for the conditioned response technique as a method for evaluating lesser studied toxicants and mixed agents. Our efforts compliment EPA investigations of

Application Factors for these toxicants. Fish were exposed to the toxicant when they had achieved a 5-day stable performance ( $\pm 5\%$ ). Copper concentrations investigated were: 2, 5, 10, 12 and 37  $\mu\text{g/liter}$  in hard water (300  $\mu\text{g/liter}$  as  $\text{CaCO}_3$ ). Exposure time varied between 12 and 14 days, and was determined by a new 5-day stable performance. In all cases, this stable level was significantly lower than pre-exposure. The severity of impairment appeared to be related to the copper concentration. Seven and 30-day post-exposure trials showed only limited recovery. Tests with other metal toxicants have shown comparable alteration in the conditioned response test. The short test time, and statistical reliability of the conditioned response technique, make it a useful method for quantification of intoxication. (This work was supported in part by EPA Project Number 18050-FBK.)

98. *Cytotoxic Effects of Vanadium and Other Metals In Vitro*. M. D. WATERS, D. E. GARDNER, and D. L. COFFIN, Pathobiology Research Branch, EBL, NERC-RTP, Environmental Protection Agency, Research Triangle Park, North Carolina.

Studies have been initiated to assess the relative cytotoxic effects of several metallic environmental pollutants to rabbit pulmonary alveolar macrophages (PAM's) and human lung fibroblasts (strain WI-38) *in vitro*. Rabbit PAM's obtained by lung lavage were exposed to soluble salts of Cd, Cr, Fe, Mn, Ni or V in supplemented tissue culture medium 199. Cell viability determinations after 20 hr indicated that Cd and V were of comparable toxicity to rabbit PAM's. Ni was somewhat less toxic than Cd or V, and the other metals tested appeared relatively non-toxic at the highest screening concentration employed (100  $\mu\text{g}$  of metal/ml). Since V occurs as an air pollutant commonly in the form of particulate oxides, additional experiments were performed using  $\text{V}_2\text{O}_5$ ,  $\text{V}_2\text{O}_3$  and  $\text{VO}_2$ . In the PAM test system, toxicity was determined to be directly related to solubility in the order  $\text{V}_2\text{O}_5 > \text{V}_2\text{O}_3 > \text{VO}_2$ . Studies using  $\text{V}_2\text{O}_5$  dissolved in media prior to exposure of cells indicated that PAM phagocytic activity decreased in direct proportion to decreases in cell viability. The concentration of soluble  $\text{V}_2\text{O}_3$  which reduced PAM viability by 50% in 20 hr was equivalent to approximately 6  $\mu\text{g V/ml}$ . In PAM cell sonicates, acid phosphatase was strongly inhibited at 1  $\mu\text{g V/ml}$ ; lysozyme was not inhibited by 100  $\mu\text{g V/ml}$  and  $\beta$ -glucuronidase was only moderately inhibited at 100  $\mu\text{g V/ml}$ . Preliminary studies with strain WI-38 lung fibroblasts showed significant inhibition of [ $^3\text{H}$ ]thymidine and [ $^{14}\text{C}$ ]leucine uptake following exposure to V at 1  $\mu\text{g V/ml}$  for 3 to 6 days. The results suggest the need for continued research into toxic effects of V compounds, especially as they may relate to pulmonary defense.

99. *Suppression of Delayed Hypersensitivity to Beryllium in Guinea Pigs by Cyclophosphamide*. NEIL D. KRIVANEK and ANDREW L. REEVES, Wayne State University, School of Medicine, Detroit, Michigan.

Ten female guinea pigs were administered cyclophosphamide (CPA), ip (20 mg/kg) for 9 consecutive days. On day 7 of CPA treatment, the animals received 0.1 ml of a solution containing 5.0  $\mu\text{g Be}$  (as  $\text{SO}_4$ ) intradermally in an attempt to induce sensitization to the beryllium. Fourteen days after the sensitizing challenge the animals were skin tested for beryllium hypersensitivity. These skin reactions were compared statistically to skin reactions of guinea pigs which had received identical sensitization injections but did not receive CPA. The results of this comparison indicated that the CPA prevented the development of delayed cutaneous hypersensitivity to beryllium. In a group of previously beryllium-sensitized guinea pigs, CPA was given for 3 days (20 mg/kg) and on day 3 the animals were skin tested for beryllium hypersensitivity. The results of the skin testing indicated that CPA was not effective in suppressing previously established beryllium hypersensitivity in guinea pigs.

100. *Tissue Levels of Cadmium in Different Disease States*. C. H. HINE, J. WRIGHT, and D. GOODMAN, Toxicology Activity, Department of Pharmacology and Experimental Therapeutics, School of Medicine, University of California, San Francisco, California.

Cadmium was determined in the blood, bone, hair and kidney of persons dying with various diseases. Ages of the decedent ranged from less than 1 year to the 9th decade. The highest cadmium levels were found in the kidney cortex. No appreciable cadmium deposit occurred in the

other tissues. Cadmium was present in the kidneys in greater amounts than lead or copper but less than zinc or calcium. No correlation was noted between cadmium tissue content and any disease state, including hypertensive heart disease.

101. *The Lack of Effects of Phenobarbital, Diphenylhydantoin and Chloramphenicol on the Biologic Activity of Vitamin D<sub>3</sub> in Rachitic Rats.* T. BALAZS and W. M. HOOPER, Food and Drug Administration, Washington, D.C.

Anticonvulsant drugs such as phenobarbital and diphenylhydantoin have been reported to cause rickets or osteomalacia in epileptics. This was attributed to the drug-induced increase in the rate of metabolism of 25-hydroxycholecalciferol to inactive substances; a phenomenon which has been demonstrated in man as well as in rats dosed with phenobarbital (Hahn *et al.*, *J. Clin. Invest.* **51**, 741-748, 1972). We studied the effects of these drugs on the biological activity of Vitamin D<sub>3</sub> in rachitic rats as measured by the line test (AOAC Official Methods of Analysis, 1970). Forty to 60 mg/kg phenobarbital sodium or 20 to 30 mg/kg diphenylhydantoin sodium were injected ip daily for 9 to 10 days to 7- to 8-week old rats kept on a rachitogenic diet. Vitamin D<sub>3</sub> (3.75 and 1.87 U) was given orally on each of the 1st and 3rd or 4th days of the 2nd week of dosing to these and to control groups. Pentobarbital sodium (25 mg/kg, ip) sleep time measured on the last day of the test was significantly shorter in the phenobarbital-treated groups than in the controls. The healing grades of rickets were comparable in each Vitamin D<sub>3</sub>-treated group consisting of at least 10 rats. In similar experiments, chloramphenicol, an inhibitor of hepatic microsomal enzymes, was fed at levels of 0.1 and 0.2% in the diet. Sleep time became significantly prolonged, but the healing of the rickets was not affected. Results of this study indicate that these compounds, which serve as models for inducers and inhibitors of hepatic microsomal enzymes, do not alter the biological activity of Vitamin D<sub>3</sub> in the rat. Thus it is unlikely that the alteration of Vitamin D<sub>3</sub> metabolism rate would be responsible for the alleged effects of anti-epileptic drugs.

102. *The Effects of Pretreatment by Phenobarbital and Chlorpromazine on the Acute Toxicity of Benzene.* ROBERT T. DREW and JAMES R. FOUTS, Pharmacology and Toxicology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

The object of these experiments was to determine if the acute toxicity of benzene was altered by pretreatment with either phenobarbital or chlorpromazine. Female CD rats used in this study were injected ip daily for 3 days with either phenobarbital (75 mg/kg) or chlorpromazine (15 mg/kg). On the 4th morning the animals were either subjected to a 4-hr inhalation exposure of benzene or given an ip injection of 50% (v/v) of benzene and mineral oil. Injected animals were given doses of 1, 2, 3, and 4 g/kg. Animals exposed via inhalation were exposed to 6 levels of benzene ranging from 11,500 to 15,500 ppm. The LD<sub>50</sub> for animals injected with benzene and the LC<sub>50</sub> for animals inhaling benzene were determined for control groups and those pretreated with either phenobarbital or chlorpromazine. There were no significant differences in the LD<sub>50</sub> or the LC<sub>50</sub> for any of the groups. In order to assure that the pretreatment was stimulating benzene metabolism, a method of measuring benzene metabolism has been developed using <sup>14</sup>C-tagged benzene. These studies have shown that phenobarbital does induce benzene metabolism in the liver, that chlorpromazine does induce slightly benzene metabolism in the lung and that pretreatment by either compound does not effect the acute inhalation toxicity or the ip toxicity of benzene.

103. *Cyanate and Thiocyanate: Acute Toxicity.* ROGER P. SMITH, Dartmouth Medical School, Hanover, New Hampshire.

Cyanate salts are of current interest as possible therapeutic agents in the management of sickle cell anemia. When thiocyanate salts were used as anti-hypertensive drugs, they had unpredictable toxicity even when the dosage was adjusted on the basis of periodic determinations of the blood level. It has long been suggested that cyanide contributes to the toxic syndrome elicited by thiocyanate. Recently (*J. Biol. Chem.* **246**, 555, 1971), the reaction between



human oxyhemoglobin and thiocyanate *in vitro* was found to result in the formation of sulfate, cyanide and methemoglobin. Cyanide was further converted to cyanate and ammonium ion. When given to mice in lethal doses here, sodium thiocyanate significantly elevated circulation methemoglobin levels ( $15 \pm 4\%$  after 2 hr). An additional 10% of the total pigment could not be accounted for as either oxyhemoglobin or methemoglobin. Even a 25% compromise of the oxygen transport capability of mouse blood, however, cannot account for death in these animals. A role for cyanide in thiocyanate poisoning appears unlikely because, (1) nitrite-induced methemoglobinemia does not protect animals against death by thiocyanate, (2) thiosulfate does not protect against thiocyanate poisoning, and (3) thiocyanate itself generates low levels of methemoglobin. Similar signs (tremor, hyper-reactivity to tactile and auditory stimuli, and tonic-clonic convulsions) in poisoned animals and a common pattern of interaction with central nervous system drugs (non-sedative doses of phenobarbital protect and non-depressant doses of morphine increase mortality) suggest that cyanate and thiocyanate have similarities in their mechanism of action. Mortality after thiocyanate (but not after cyanate) was so unpredictable that an LD50 could not be defined. Both cyanate and thiocyanate appear to be directly acting neurotoxins. (This work was supported in part by Grant HL 14127 from the National and Heart and Lung Institute.)

104. *Comparison of the Acute Toxicity, Distribution, Fate and Some Pharmacologic Properties of the Non-benzenoid Aromatic Compound Azuloic Acid with those of Benzoic and Naphthoic Acids.* G. C. McCORMICK and T. J. SPEAKER, School of Pharmacy, Temple University, Philadelphia, Pennsylvania. (J. F. Borzelleca.)

This report compares some of the toxicologic and biologic properties of the non-benzenoid aromatic compound azuloic acid with the more familiar benzenoid aromatic substances, benzoic and naphthoic acid. In mice, azuloic acid shows greater acute oral toxicity (LD50 = 800 mg/kg) than do benzoic (LD50 = 2370 mg/kg) or naphthoic (LD50 = 1620 mg/kg) acids. Three metabolites of azuloic acid have been recognized, a glycine conjugate, an azuloyl ester of glucuronic acid, and an as yet incompletely characterized acidic product which appears in urine only after continued dosage. The elimination rates for azuloic acid and its metabolites and the rates of conversion of azuloic acid to its metabolites have been determined; azuloic acid is proportionately much less converted to its glycine conjugate than are either benzoic or naphthoic acids. Azuloic acid appears to be a potent inducer of microsomal drug metabolizing enzymes, reducing hexobarbital sleeping time by as much as a quarter and zoxazolamine paralysis time by over half.

105. *Inhalation of a Powdered Aerosol Medication by Non-Human Primates in Individual Space-Type Exposure Helmets.* J. SCHEIMBERG, O. P. McSHANE, S. CARSON, H. E. SWANN, JR., and A. M. J. N. BLAIR, Food and Drug Research Laboratories, Inc., Maspeth, New York and Fisons Limited, Pharmaceutical Division, Loughborough, England.

The objectives of this study were to explore a method of administration of a powdered drug by inhalation and to make observations for adverse effects. Three groups of 9 stumptailed monkeys were exposed to the contents of 4, 16, and 72 capsules/day of a prophylactic asthma drug (Intal) 5 days/week for 4 months. Another group was exposed to 72 capsules of lactose and another group to empty capsules. All exposures were made using clear acrylic plastic helmets of approximately 2.5 liters capacity into which Intal or lactose was introduced directly via pulsed air flow through a Spinhaler® containing a capsule of powder. Capsules were placed into the Spinhaler at regular intervals during an 8-hr day. Monkeys were seated in chairs with restraint at the neck and waist but with arms and legs free. Helmets, containing a port for the Spinhaler and two valved exhaust ports, were placed over the heads of the monkeys and fastened to the top of the chairs. A solenoid allowed 2-sec bursts of ultra-filtered compressed air through the Spinhaler into the helmet and kept the particles in motion in the helmet and moisture from collecting on the walls. Periodic sampling in the helmet was made each minute during a 5-min period to determine the number and size of particles that would remain suspended. Analysis was made with a Baush and Lomb 40-1A counter. Criteria of response included ophthalmic examinations, neurologic examinations, cardiopulmonary measurements, hematology, blood

chemistry, urine analysis, renal function and hepatic function. The results show that large numbers of particles below 5 microns remained suspended in the helmet for the entire 5 min of each capsule exposure. There were no visible signs of irritation such as sneezing or coughing. Ophthalmic examination indicated no abnormalities and neurologic reflexes were normal. There were no indications of any adverse drug-related effects by the physiological measurements or pathological observations.

106. *A Study of the Chronic Toxicity of Inhaled Disodium Cromoglycate in the Squirrel Monkey.* B. G. PROCTER, R. G. BURFORD, A. M. J. N. BLAIR, and H. MACFARLAND, Bio-Research Laboratories Ltd., Pointe Claire, Quebec, Canada, and Fisons Limited, Loughborough, England.

A chronic inhalation toxicological study of Intal® (FPL-670, disodium cromoglycate, cromolyn sodium) was performed in the Squirrel monkey, *Saimiri sciureus*. Each of 5 experimental groups consisted of 3 male and 3 female monkeys. Groups I and II were exposed 6 hr/day, 7 days/week, for 1 year to aerosols containing Intal® blend in approximate concentrations of 0.5 and 0.05 mg/liter of air, respectively. Group III animals were similarly exposed to an aerosol containing 0.01 of lactose/liter of air. Group IV subjects served as chamber controls and the room controls (Group V) were maintained in the animal holding room throughout the study. A comprehensive toxicological evaluation of the monkeys was carried out prior to and throughout the study. At appropriate intervals, observations were made of physical appearance and behavior, weight gain and ophthalmoscopic appearance; electrocardiograms, systolic blood pressure measurements, hemograms, blood chemical profiles and urinalyses were obtained. At termination necropsies were conducted and organ weights were determined. A variety of staining techniques was employed in the histopathological examination of tissues. Special attention was given to heart, kidney and lung structures and to vascular and nerve tissues. Bone marrow smears and the distribution and morphology of mast cells in the lung were studied. Ultrastructural studies, using the electron microscope, were made of the lungs and kidneys. No changes that could be attributed to the action of Intal® were seen in any variable. A special absorption study in 5 monkeys showed that the high-dose animals absorbed about 60 times the recommended human dose. (Supported by Fisons Limited, Pharmaceutical Division, Loughborough, England.)

107. *Testicular Atrophy from Inhalation of Ethylene Oxide Cyclic Tetramer.* BASIL K. J. LEONG, TIMOTHY O. T. TS'O, and MAYNARD B. CHENOWETH, Chemical Biology Research, The Dow Chemical Company, Midland, Michigan.

The toxicological property of ethylene oxide cyclic tetramer, a new synthetic compound which may be used for forming soluble complexes with metal ions, has been evaluated. Rats were exposed to 0.5 and 1.0 ppm ethylene oxide cyclic tetramer vapors for 7 hr/day, 5 days/week for 3 weeks. Both concentrations produced marked testicular atrophy which was associated with degeneration of the germinal epithelium. This effect appears to be long lasting since testicular atrophy persisted as long as 4 months post-exposure. Even though the rats exhibited normal sexual activity, they remained sterile. Atrophy of the prostate gland and seminal vesicles was also noted immediately following exposure. However, within 2 to 3 weeks, these organs returned toward normal appearance and size. Administration of testosterone, 10 mg/kg, prevented atrophy of the prostate and seminal vesicles but not that of the testes. Exposure to 1.0 ppm produced a prominent degradation of conditioned behavioral performances, depression of food and water intake, retardation of growth and body tremors. These effects were reversible. At 0.5 ppm, these latter effects were much less severe. This data indicates that extreme caution should be exercised in the handling and manufacturing of this compound.

108. *Sensitization of Guinea Pigs by Inhalation of Aerosolized Proteins.* O. P. MC SHANE, J. SCHEIMBERG, S. CARSON, and H. E. SWANN, Jr., Food and Drug Research Laboratories, Inc., Maspeth, New York.

Previous experiments by these authors have shown that an anaphylactic reaction could be elicited in guinea pigs by the inhalation of sensitizing and challenging doses of purified egg

albumen. This study was designed to explore the possibility that the inhalation of other aerosolized proteins might produce sensitization which could lead to anaphylactic type reactions upon subsequent challenges. Two hair spray preparations with protein and a protein concentrate were used in this study and the protein content determined. Ten guinea pigs with equal numbers of each sex were used in each test. Eight animals were placed in individual wire cages within a dynamic 125-liter exposure chamber. Two animals were placed in body plethysmographs in each side of the chamber with heads protruding into the chamber. All animals were at the same level within the chamber. Tidal volume, respiratory rate, depth and configuration were measured on the 2 animals during exposure. When a challenge exposure produced changes in any parameters, pulmonary flow resistance measurements were made on 2 animals. For the hair spray preparations, the contents of 1 can was used for the sensitizing dose and each challenging dose. The animals were exposed every 5 days until 5 challenges were given. The concentration of protein was 1 to 3 mg/ml in the hairsprays. Chamber concentrations of the protein were approximately 1 to 2 mg/liter. No anaphylactic reactions were produced from the hair spray preparations used. A protein concentrate (animal source) with concentration (6 mg/ml) was dispersed into the chamber by DeVilbiss Nebulizer No. 40. Chamber concentrations obtained were 0.2 to 0.3 mg/liter. Anaphylactic reactions occurred in one-half of the animals. Sensitization may occur but not necessarily in every animal. These data and previous data seem to indicate that sensitization may be more dependent upon the type (simple or derived) and/or the source (animal or vegetable) of the protein than the concentration. In the hairspray preparations, the type or source of the protein was not known.

109. *Comparative Cataractogenic Studies of Dibenzoxazepines in Laboratory Animals.* P. J. DEBAECKE, J. S. KULESZA, and J. W. POUTSIKA, Squibb Institute for Medical Research, New Brunswick, New Jersey.

Studies were conducted to investigate susceptibility of Charles River rats and mice, Golden Syrian hamsters, beagles, and rhesus monkeys to the cataractogenic effects of orally administered SQ 11,290, (4-[3-(7-chloro-5,11-dihydrodibenz[b,e][1,4]-oxazepin-5-yl)propyl]-1-piperazineethanol dihydrochloride, a psychopharmacologically active compound in laboratory animals. Lenticular changes were observed after 4 weeks in rats (30 or 65 mg/kg daily), after 14 weeks in dogs (65 mg/kg daily), and after 26 weeks in mice (100 mg/kg daily). Cataracts were not observed in hamsters (100 mg/kg daily) or in monkeys (25 mg/kg daily) after 34 weeks. Cataracts in the three susceptible species differed in location, density, and progression. In rats, progression of the lenticular changes to hypermature white cataracts was generally correlated with lower-than-control amounts of reduced glutathione in the lens. Supplementing the diet containing SQ 11,290 with 0.5% DL-methionine and 0.3% L-cysteine had no inhibitory effect on the morphology or progression of cataracts in rats. Blood level studies of SQ 11,290-<sup>14</sup>C and its metabolites in dogs and monkeys, as well as studies of the distribution of radioactivity in the lens and other ocular tissues of rats, dogs, and monkeys did not reveal any species specificity to account for the susceptibility of rats and dogs to cataractogenesis. Seven other dibenzoxazepines, structurally related to SQ 11,290, were also tested in rats. Two of the 7 compounds produced lenticular changes, and the data suggest that certain side-chain substituents attached to the nitrogen atom of the dibenzoxazepine nucleus contributed to the cataractogenesis.

110. *Influence of 2,6-Dichloro-4-nitroaniline (DCNA) on the Hepatic Lysosomal Response to Iron Dextran in the Rat.* M. A. GALLO, R. ABRAHAM, and L. GOLBERG, Albany Medical College, Albany, New York.

Liver enlargement may be brought about by administration and reticulo-endothelial storage of macromolecular materials, associated with increased activity of lysosomal enzymes, or by the action of agents causing induction of mixed function oxidases and/or liver damage. Parenteral iron dextran fulfills the first of these functions, resulting in intense Gomori's acid phosphatase staining accompanying the deposition of iron, while 2,6-dichloro-4-nitroaniline (DCNA) has been found to cause hepatomegaly associated with effects on endoplasmic reticulum, mitochondria and lysosomes. In order to investigate the influence of DCNA on the lysosomal response to, and hepatic distribution of iron dextran I group of adult female

Sprague-Dawley rats was given iron dextran (50 mg Fe/kg, ip) daily for up to 4 weeks, while a second group was pretreated with DCNA (500 mg/kg, po) daily for 8 weeks before iron dextran was administered. Animals were sacrificed after 1, 2 and 4 weeks of iron dextran treatment and the hepatic lysosomal response was examined using biochemical and histochemical techniques. In rats given iron dextran only, a striking rise in lysosomal acid phosphatase activity occurred within organelles having an increased sedimentation coefficient. Treatment with DCNA alone did not affect the specific activity of acid phosphatase nor the sedimentation characteristics of lysosomes. In rats pretreated with DCNA, iron dextran brought about an additional increment in relative liver weight, but no increase in acid phosphatase activity and little change in lysosomal characteristics. The basis for the difference in response brought about by DCNA was seen histochemically as a limitation in the amounts of iron deposited in Kupffer cells and hepatocytes as well as a reduction in the extent and intensity of Gomori-positive staining. In short-term studies of rats and rhesus monkeys given DCNA an effect on hepatic lysosomes has been observed, possibly mediated by oxidized quinoid or quinoneimine forms of DCNA and its metabolites. In the present instance, initial iron deposition in the liver would be expected to enhance the rate and degree of oxidative change. Accumulation of such products of oxidation in protein bound form could stabilize lysosomal membranes and thus account for the observed decrease in secondary lysosomes following the combined treatments with DCNA and iron dextran. Irrespective of the mechanisms involved in such interactions between agents inducing hepatomegaly, the capacity to influence the end result constitutes a problem worthy of further exploration. (Supported by Research Grant 2P01-ES00226-06 from the National Institute of Environmental Health Sciences, NIH, and by National Institutes of Health Training Grant 2T01-ES00103-06.)

111. *Vacuologenic Activity of Nicotine in Macrophages.* SORELL L. SCHWARTZ and JANE E. LUNDIN, Department of Pharmacology, Georgetown University Schools of Medicine and Dentistry, Washington, D.C.

Induction of cytoplasmic vacuolation by nicotine is known to occur in fibrocytes and cultured heart cells. Prior studies from our laboratory indicated that nicotine both inhibits endocytosis and increases exocytosis by mouse peritoneal macrophages. As part of a study of the mechanisms responsible for this activity of nicotine we investigated the possible vacuologenic effect of nicotine (0.29–14.35 mM) on these macrophages. Exposure of coverslip monolayers of peritoneal macrophages to nicotine tartrate resulted in a contraction and pinocytotic-like vacuolation of the cells. Both effects were dose and time-dependent. Time-lapse cinematography of monolayer cultures in Sykes-Moore chambers indicated that loss of vacuolation and respreading began occurring immediately after the removal of nicotine and that cells had regained normal histologic appearance within the subsequent 15-min period. Indeed, the reversal of effect was so rapid that we were led to postulate that the vacuoles, rather than being pinocytotic, might have been a result of a form of spontaneous emulsification caused by nicotine where the continued presence of the alkaloid as a surfactant is required to maintain vacuolation. Two observations lend support to this possibility. One is that isotopic data showed that nicotine is, in fact, taken up by the cells and that it leaves the cell rapidly (80% within 15 min) upon removal of the alkaloid from the media. The second is that we measured the surfactant activity of nicotine and found it to lower the surface tension of water in concentrations as low as 0.013% and the interfacial tension between water and carbon tetrachloride in concentrations at least as low as 0.10%. (Supported by a grant from the American Medical Association, Education and Research Foundation.)

12. *Histochemical Quantitation of Lytic Enzyme Activity Associated with Plastic Implants.* T. N. SALTHOUSE, BARBARA F. MATLAGA, and R. K. O'LEARY, Ethicon Research Foundation, Somerville, New Jersey.

*In vivo* tissue reaction to implants is usually assessed by a histologic and morphologic approach. Although some parameters of such evaluations can be semi-quantitated, they remain largely subjective. The aim of the present studies was to develop quantitative procedures for measuring the lysosomal enzyme activity of the cellular response evoked in tissue

by plastic materials. The data obtained could be useful in evaluating candidate materials for tissue compatibility. Leucine aminopeptidase and acid phosphatase activities were measured in tissue sections of the implant sites. Such activities are lysosomal and largely contributed by the macrophage cells present at these sites. Polyvinyl chloride (PVC) rods 15 mm long and 1 mm in diameter were prepared. These rods contained from 0.05 to 1.00% dibutyltin diisooctyl mercaptoacetate, a known tissue irritant. These samples, and in addition, polypropylene strands (suture material) and high density polyethylene rods (USP standard) of similar dimensions were implanted in the gluteal muscles of Long Evans rats, 150–200 g. After 14 days, the rats were sacrificed and the muscles excised and quick-frozen. Sections of the implant sites, 8- $\mu$  thick, were cut in a cryostat microtome. After attaching to cover glasses, the sections were placed in acetone ( $-30^{\circ}\text{C}$ ) for 15 min, rinsed in saline, and placed on well slides containing the substrate medium (substrate, dye coupler, saline,  $\text{MgCl}_2$ , agarose). The slide with section and medium was incubated ( $37^{\circ}\text{C}$ ) on the microscope stage. The dye formed by enzyme activity was measured at 530 nm, as increase in optical density ( $\Delta$  OD/hr) employing a Zeiss microscope photometer. The rate of increase in accumulated dye product was linear between 10 and 60 min of incubation. The polypropylene and polyethylene samples induced the least activity. The enzyme activity of the cellular reaction zones of the PVC-organotin implants increased to a maximum proportionately with the level of organotin in the sample, up to concentrations of 0.6%. Higher concentrations of organotin in the samples induced less lysosomal activity. This loss of activity is attributed to increased macrophage mortality. It is proposed that this type of approach has certain advantages over reliance solely upon microscopic observations for judging tissue compatibility. The results are not subjective, and are based upon function and behavior of the cells affected by the implant. The level of macrophage lysosomal enzyme response adjacent to the implant site appears to be related to the toxicity of the implant components.

- ✓ 113. *Cytogenetic Effects of the Polychlorinated Biphenyls (Aroclor 1242) on Rat Bone Marrow and Spermatogonial Cells.* S. GREEN, K. A. PALMER, and E. J. OSWALD, Food and Drug Administration, Washington, D.C. (Leonard Friedman.)

In a continuing program by the FDA to evaluate the mutagenicity of the PCB's, a cytogenetic investigation of Aroclor 1242 was conducted in rats. In this investigation, the effects of the environmental contaminant on bone marrow and spermatogonial cells were ascertained. Randomly-bred albino Osborne-mendel rats weighing 180–250 g were used. Aroclor 1242 was given as an acute dosage (po) at 5000, 2500 and 1250 mg/kg or as a subacute regimen at 500 mg/kg/day for 4 days. It was administered as an undiluted solution at 5000 mg/kg and as a solution in corn oil at the other levels. Controls received corn oil. Twenty-four hours after the first or last treatment, animals were administered colcemide at 4 mg/kg and killed after an additional 4 hr. Bone marrow nad spermatogonial preparations were made according to the usual cytogenetic procedures. The results from the bone marrow study showed no significant increases in chromosomal abnormalities or inhibition of cellular division. The study of spermatogonial cells, showed no significant increase in abnormalities but statistically significant decreases in the number of dividing spermatogonial cells ( $P < 0.05$ ). This effect was noted at  $500 \times 4$  and  $5000 \times 1$  dosages. It is concluded that Aroclor 1242 does not produce chromosomal abnormalities in rat bone marrow or spermatogonia but does cause, at relatively high dosages, a decrease in the number of dividing spermatogonial cells.

114. *Cytotoxic Effects Induced by Various Agents in Rat Thymus Lymphocytes.* NATHALIE M. FEDYNSKYJ, Department of Pharmacology, Medical Sciences Program, Indiana University, Bloomington, Indiana. (Roger P. Maickel.)

While the thymus gland is required for the development and control of the immune response, it has been postulated that thymus cells do not form antibodies *in situ* since there is no penetration of systemically administered antigens into the thymus. Rats have been given ip doses of various combinations of Freund's adjuvant, guinea pig spinal cord, and *B. pertussis* vaccine; in some cases followed by reserpine (100  $\mu\text{g}/\text{kg}$ ) or LSD (50  $\mu\text{g}/\text{kg}$ ). Histological examinations were performed at 1, 3, 7, 10 or 14 days. In addition, DNA synthesis was measured by incorporation of [MH]thymidine administered 1 hr prior to sacrifice. A highly significant effect was

seen only after dosage of spinal cord plus *B. pertussis* vaccine. The large lymphocytes were increased in number; this effect was potentiated by both reserpine and LSD. DNA synthesis was also increased under these conditions. In contrast, the small lymphocytes became hyperchromatic and enlarged 3 days after antigen administration, and pycnotic in 7 days, an effect also potentiated by reserpine and LSD. Complete regeneration was seen by 14 days. These data support the hypothesis that some antigens can penetrate thymic tissue, causing differential lymphocytosis and lymphocytolysis, actions probably involved in the immunological response. (Supported by an Indiana University Faculty Research Grant-in-Aid.)

115. *Subacute Toxicity Studies of a New Differential Immunosuppressant, Oxisuran, in Rats and Dogs.* D. ABRUTYN, G. BRIZIARELLI, J. TORNABEN, and E. SCHWARTZ, Warner-Lambert Research Institute, Morris Plains, New Jersey.

Subacute toxicity studies of Oxisuran, a new differential immunosuppressant, were performed in rats and dogs. The compound was given orally for 90 days at doses of 125, 500 or 2000 mg/kg/day to dogs and 100, 300 or 1000 mg/kg day to rats. Control animals were utilized for each species. Unlike general immunosuppressants no evidence of hematopoietic, gastrointestinal or lymphoid activity was noted nor were signs of toxicity found in most other organ systems studied. Slight histologic alterations consisting of follicular hyperplasia and colloid depletion in the thyroid glands of the treated animals were seen. Additional studies were undertaken to determine their functional significance and the results indicated that the effects on thyroid function were minimal, if at all present. Two to 4 weeks after the discontinuation of drug administration, the histological appearance was within normal limits in both species. It was concluded that under the conditions of our studies, Oxisuran resulted in a low order of toxicity in rats and dogs.

✓ 116. *Metabolism and Elimination of Tri-o-Tolyl Phosphate (TOTP), a Neurotoxic Organophosphate, in Chicken.* R. P. SHARMA and P. G. WATANABE, Toxicology Curriculum, Utah State University, Logan, Utah.

Tri-o-tolyl phosphate (TOTP) is present in a widely used commercial compound, tricresyl phosphate (TCP), used as flame retardant, plasticizer, lubricant, gasoline additive, etc. TOTP has been responsible for causing a delayed paralysis in a large number of people at various times. Chickens resemble man in their response to TOTP, both in the clinical signs and the time lapse before paralysis appears. Little is known about the metabolism and elimination of TOTP in chickens. Adult White Leghorn hens were given 1.16 g (1 ml) of [<sup>32</sup>P]TOTP (243 μCi) po. The relative distribution of the radioactivity, in terms of TOTP and its metabolites was followed at different time intervals up to 72 hr in plasma, liver and excretory products. Radioactivity was concentrated in liver; the concentration at 72 hr was 25 times greater than in plasma. TOTP and three metabolites were separated from the liver using adsorption column chromatography; these metabolites showed similar chromatographic characteristics to those reported in rats. Metabolite I, presumably the cyclic saligenin, was the major metabolite appearing in the liver; the amount of TOTP and its major metabolite increased throughout the 73-hr period. The liver concentration of metabolite I increased from 8.5 μg/g at 1 hr to 377 μg/g at 72 hr (expressed as TOTP). A fraction of the administered TOTP appeared to be excreted unabsorbed during the first 24 hr. The total rate of excretion increased during the first 12 hr and then declined. The results indicated that TOTP is slowly metabolized and excreted; the compound and its metabolites are retained in the visceral organs for several days after a single oral dose.

117. *A Simplified Bioassay for Predicting Organophosphate Potentiation.* S. D. COHEN and S. D. MURPHY. University of Connecticut School of Pharmacy, Storrs, Connecticut and Harvard School of Public Health, Boston, Massachusetts.

Predictive tests for detecting malathion (MAL) synergists measure the effect of organophosphates on the detoxification of MAL or malaaxon (MX). Previous studies of MX detoxification required incubation of MX with tissue for a specified time after which an aliquot was removed and assayed in an anticholinesterase (antiCHE) test system to determine the

amount of MX remaining. The simplified bioassay incorporates the initial MX-tissue incubation step directly into the antiCHE assay system. Fifty mg of mouse brain was added to Ringer bicarbonate buffer in Warburg flasks containing selected amounts of control or treated liver. At the start of the assay 0.5 nmol MX was added to the incubate and 25 min later acetylcholine (ACh) was added and brain CHE activity was measured manometrically. Liver activity, expressed as  $\mu\text{l}$  equivalents of MX inactivated, was calculated as the difference in  $\text{CO}_2$  production between incubates containing brain, ACh and MX with and without liver. Mean  $\pm$  SE activities of 10 mg of tissue from 5 or more control mice were  $143 \pm 9$ ,  $77 \pm 13$ ,  $67 \pm 8$  and  $56 \pm 5$   $\mu\text{l}$  equivalents of MX inactivated by liver, lung, kidney and plasma, respectively. Ultracentrifugation studies with liver indicated that microsomes contributed 61% of the total activity. Liver inactivation of MX was 40, 79 and 100% inhibited 18 hr after 0.5, 10, and 125 mg/kg (ip) of triorthotolylphosphate (TOTP), respectively. Other organophosphate potentiators of MAL proved to be potent inhibitors of MX detoxification as measured in the simplified bioassay system. A similar assay system was developed for measuring liver inactivation of paraoxon (PX). An 18-hr pretreatment with 125 mg TOTP/kg inhibited PX inactivation by 50% and caused a 4-5 fold increase in PX toxicity. Aldrin (16 mg/kg, po), given 4 days before sacrifice increased liver and plasma inactivation of MX and PX to 125 to 183% of control activity and decreased the acute toxicity of MX and PX. The simplified bioassay for determining tissue inactivation of MX or PX may be useful in predictive tests for detecting both synergists and antagonists of these organophosphates. (Supported by Research Grants ES00084 and ES00002 from the National Institute of Environmental Health Sciences, USDHEW.)

- ✓ 118. *Effect of Chronic Dieldrin Exposure on the Hepatic Microsomal Mixed Function Oxidase System from Male and Female rats.* JAMES T. STEVENS, STEVEN R. WAGNER, MICHAEL A. ZEMAITIS, and FRANK E. GREENE, Department of Pharmacology, The M.S. Hershey Medical Center, The Pennsylvania State University, Hershey, Pennsylvania.

Previous results from this laboratory have shown that certain cyclodiene insecticides interact with components of the hepatic microsomal mixed function oxidase enzymes that metabolize foreign compounds and that sex differences exist for these interactions (*Tox. Appl. Pharmacol.* **22**, 309, 1972). The purpose of this study was to investigate the effects of feeding a diet containing 50 ppm dieldrin for 8 weeks on the activity of this enzyme system. In most instances maximal induction was achieved during the first week of feeding, although random fluctuations in some parameters were seen during the 8-week period. The level of NADPH cytochrome c reductase, cytochrome P-450 and NADPH oxidase remained significantly above control levels throughout the experiment, but no consistent sex differences were noted. The maximal spectral change ( $A_{\text{max}}$ )/mg protein induced by addition of ethylmorphine (EM), aniline (AN), and aldrin (AL) to microsomal suspensions was consistently higher in dieldrin-fed rats. However when  $A_{\text{max}}$  was calculated in terms of cytochrome P-450 content, there was a 30-60% decrease for EM and AN. In contrast to these results, AL binding was increased in most instances regardless of the way the data were expressed. Determinations of the spectral binding constant ( $K_s$ ) indicated that the apparent affinity of EM and AN for cytochrome P-450 was decreased by dieldrin feeding in female but not in male rats. The affinity of AL, however, was increased in both sexes. The induction effect of both EM and AN metabolism on female rats was maintained during the course of the experiment. However, AM and EM induction in male rats tended to decline over the 8-week period. Results from these experiments suggest that there may be sex differences in the effect of chronic dieldrin feeding on the metabolism of certain compounds, but that these differences may not be predictable by changes in content and activity of components of the mixed function oxidase system. (Supported by NIEHS Research Grant 9-R01-ES00638-02.)

- ✓ 119. *Distribution and Penetration of Lindane into Brains of Normal and Phenobarbital Pretreated Dogs.* C. L. LITTERST, E. MILLER, T. MICHEL, V. OLIVITO, and E. J. VAN LOON, Division of Toxicology, Bureau of Foods, Food and Drug Administration, and Laboratory of Toxicology, National Cancer Institute, Bethesda, Maryland.

In earlier studies Koransky and Portig (*Arch. Ex. Path. und Pharmak.* **244**, 564, 1963) investigated distribution of lindane (gamma BHC) in the rat. The purpose of the present

investigation is to map out the levels and the degree of penetration of lindane in discrete areas of the central nervous system of the normal and phenobarbital-pretreated beagle dog. An iv infusion of lindane emulsion (15 mg/ml) was given to dogs that had been treated with phenobarbital in the diet, or to untreated dogs. In untreated dogs full convulsive seizure with loss of posture occurred within 20–35 min after the infusion began, at which time administration of lindane was terminated. Phenobarbital-pretreated dogs were infused for 60 min, at which time no signs of central nervous system excitability, with the exception of an occasional jerk, were evident. At the onset of the convulsions or after 60 min dogs were killed and the brains excised and dissected into 12 discrete structures. A section of the spinal cord also was obtained. The content of lindane in each structure was determined by gas-liquid chromatography. Lindane was recovered from all structures studied. Distribution of lindane was approximately equal in the brains of treated and non-treated dogs. The proportion of the administered dose of lindane was significantly smaller in the brains of phenobarbital-treated as compared to non-treated dogs. There appeared to be wide spread localization in both white and grey matter. It was concluded that phenobarbital, in addition to acting as a physiological antagonist, alters the proportion of the administered dose of lindane in the central nervous system of the beagle dog, probably due to the increased rate of lindane metabolism.

- ✓ 120. *Delayed Acquisition of a Successive Reversal Behavioral Task in Dieldrin-Dosed Squirrel Monkeys.* GARY A. VAN GELDER and RICHARD M. SMITH, Behavioral Toxicology Laboratory, Veterinary Diagnostic Laboratory, Iowa State University, Ames, Iowa, and Department of Psychology, Alverno College, Milwaukee, Wisconsin.

Dieldrin is a persistent chlorinated hydrocarbon insecticide. Clinical dieldrin toxicosis is manifested by neurological involvement. The present study investigated effect of subclinical exposure on acquisition of a non-spatial successive discrimination reversal task in adult male squirrel monkeys. Monkeys were required to meet a reversal criterion of 10 consecutive correct response in a 30-min daily session before exposure began. On the day following the first reversal, the criterion was increased to 15 consecutive correct responses and the 55-day exposure period was started. The 0.1 mg/kg group achieved significantly fewer reversals than the low and control groups. There was no effect on acquisition in the 0.01 mg/kg exposed group. At the end of 55 days the average number of reversals/subject/day was 12 for the controls (N = 3), 17 for the 0.01 mg/kg group (N = 4), and 2 for the 0.1 mg/kg group (N = 3). During the following 54 days, exposure was discontinued in the 0.1 mg/kg group and increased to 0.1 mg/kg in the original 0.01 mg/kg group. The increased exposure level did not impair performance in the original 0.01 mg/kg group. The average number of reversals/subject/day at the end of the 54-day period was 11 for the controls, 18 for the original 0.01 mg/kg group, and 4 for the original 0.1 mg/kg group. The results showed that a continuous subclinical exposure to 0.1 mg/kg of dieldrin markedly impaired acquisition, while the same exposure had no effect on the maintaining of a high level of performance.

121. *Lesions of Pesticide Poisoning in Fish.* A. H. WALSH and WM. E. RIBELIN, The Chas. Pfizer Co., Groton, Connecticut, and Department of Veterinary Science and Center for Environmental Toxicology, University of Wisconsin, Madison, Wisconsin.

Two species of fish, coho salmon and lake trout, were chronically exposed to pesticides in order to determine whether characteristic lesions might be detected and found useful for the diagnosis of fish kills. Of the pesticides studied (carbaryl, malathion, DDT, thiodan, dieldrin, atrazine and 2,4-D) none produced pathognomonic lesions and only carbaryl and atrazine produced lesions suggestive as to their cause. No changes characteristic of pesticides as a class were identified. The primary role of the pathologist in the diagnosis of pesticide-suspected fish kills would appear to be the elimination of the possible role of other factors such as infectious or nutritional disease.

- ✓ 122. *The Biochemical Pathology of Rat Lung After Acute Paraquat Poisoning.* HANSPETER WIRSCHI, Department of Pharmacology, Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada.

The pathological biochemistry of the lung has so far received little attention, although biosynthesis of pulmonary macromolecules, activities of pulmonary enzymes and their response



to metabolic inhibitors can be evaluated in ways quite similar to the approaches used for studying liver metabolism. In order to test how a specific lung poison would affect pulmonary biochemistry, we studied some acute effects of paraquat (1,1'-dimethyl-4,4'-bipyridylum dichloride). Given parenterally to rats, paraquat is known to produce within 12 to 24 hr such histopathological changes in the lung as acute edema, congestion and widespread necrosis of pulmonary cells. The following enzyme activities were determined in lungs of male Sprague-Dawley rats (180-200 g) given from 20 to 80 mg/kg of paraquat ip: aryl hydrocarbon hydroxylase (benzpyrene hydroxylase), uridine kinase, ATPase and isocitric dehydrogenase. Only aryl hydrocarbon hydroxylase was inhibited in a dose-dependent manner 24 hr after paraquat administration; with doses of 20, 30, 40, 50 and 60 mg/kg ip, enzyme activities were 81, 70, 52, 55 and 27% of control levels. In a time-response study it was found that aryl hydrocarbon hydroxylase activities were somewhat lowered 12 hr after 60 or 80 mg/kg of paraquat and significantly depressed 18 hr after these doses. Forty and 30 mg/kg, however, failed to lower enzyme activities significantly at 12 and 18 hr and did so after 24 hr only. Paraquat also depressed pulmonary aryl hydrocarbon hydroxylase in rats treated with methylcholanthrene. In no instance was there any evidence that paraquat would depress the activity of hepatic aryl hydrocarbon hydroxylase. Activities of pulmonary uridine kinase, ATPase and isocitric dehydrogenase were never significantly affected by paraquat. The incorporation of orotic acid into total cellular RNA and of leucine into total protein was also not changed after a pretreatment of the animals with 20-60 mg/kg of paraquat for 24hr. The interaction of paraquat with pulmonary microsomal enzymes might be of importance in the mechanism of the toxic action paraquat displays in lung. Measuring the activity of aryl hydrocarbon hydroxylase could be also used as a biochemical parameter for evaluating damage to pulmonary tissue. (Supported by grants from the National Cancer Institute of Canada and the Canadian Medical Research Council, MRC Group in Drug Toxicology.)

- ✓ 123. *Factors Influencing Renal Excretion of Paraquat*. D. M. FERGUSON, Imperial Chemical Industries Ltd., Alderley Park, Macclesfield, Cheshire, United Kingdom. (A. A. B. Swan.)

Paraquat (Pq; 1,1'-dimethyl-4,4'-dipyridyl) is the most important of a series of bipyridylum herbicides with unusual toxicological properties. The mechanism by which paraquat is excreted in the kidney has been studied using anesthetized adult male beagle dogs. In clearance experiments, paraquat within the kidney, the fraction of the filtered paraquat reabsorbed varied from 0.25 to 0.75. That clearance was independent of plasma paraquat concentration over a wide range (10-150  $\mu\text{g/ml}$ ) and urine-to-plasma concentration ratios always exceeded unity, though approaching it at high urine flow rates, suggests reabsorption is passive. The effect of urine flow rate on clearance was examined using mannitol (osmotic diuresis) or specific diuretics (mersalyl, ethacrynic acid and furosemide). A direct relationship between the two parameters was seen in most experiments. Substitution of iso-osmotic sodium chloride solutions for hypertonic mannitol infusions reduced urine flow, but unexpectedly increased the percentage excretion of paraquat. Urine pH was altered by ammonium chloride or sodium bicarbonate po or by iv infusion of 0.1 N hydrochloric acid or sodium bicarbonate. Over the range pH 5-8, clearance remained constant. This is consistent with the view that paraquat exists as a cation at physiological pH. Initial stopflow experiments indicated that paraquat was reabsorbed in the proximal half of the nephron; there was no indication of a secretory component. Subsequent experiments have been more equivocal. In summary, paraquat is reabsorbed in the dog kidney, probably in the proximal tubules. Most results can be explained by passive diffusion although the presence of an active component has not been completely excluded.

124. *The Significance of Human Metabolism of Cocaine, Dextropropoxyphene and Methadone in Routine Urine Drug Screening*. D. A. KNOWLTON, S. MARENBERG, and M. SHAWKY, Toxicology Laboratory, Department of Pharmacology, College of Medicine, The Ohio State University, Columbus, Ohio. (D. Couri.)

Cocaine, dextropropoxyphene and methadone have similar TLC  $R_f$ 's in solvent systems commonly used for urine drug screening. Similar colors are produced with the above compounds when the chromatogram sheets are sprayed with commonly used chromogenic reagents (e.g. iodoplatinate). It has been our experience that these drugs can be readily differentiated and

presumptively identified from TLC of urinary extracts by consideration of the compounds produced by their *in vivo* metabolism. Cocaine is converted by ester hydrolysis to benzoylecgonine and ecgonine. Parent cocaine is found only if the urine is processed within a short time after voiding. Ecgonine is not recovered from urine by routine extraction processes. Dextropropoxyphene is excreted largely as the demethylated metabolite, norpropoxyphene. Methadone is excreted largely as the parent compound and also as a cyclized derivative which forms spontaneously following demethylation. Consideration of our data indicates that if an individual has ingested cocaine, benzoylecgonine will be found by the drug screen; if dextropropoxyphene has been ingested, norpropoxyphene will be found; and if methadone is ingested, the parent compound will be found. Benzoylecgonine, norpropoxyphene and methadone have different TLC *R<sub>f</sub>*'s and chromogenic reactions and this information can readily be used to differentiate these three compounds. These results were confirmed with authentic norpropoxyphene, and the cyclized metabolite of methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, which were kindly supplied by Dr R. E. McMahon of the Lilly Research Laboratories and authentic ecgonine and benzoylecgonine which were synthesized in our laboratories. Confirmation was by TLC, gas chromatography and mass spectroscopy. Mass spectroscopy was performed by Dr Rodger Foltz of the Battelle Memorial Institute.

- ✓ 125. *Replicate Control Values in Large-Scale Mouse Dermal Bioassays of Tobacco Smoke Condensates*. JAMES L. GARGUS, JOHN J. GART, ROBERT P. KWAPIEN, and GIO B. GORI, Hazleton Laboratories, Inc., Vienna, Virginia, and National Cancer Institute, Bethesda, Maryland.

Mouse dermal bioassays were performed in 7600 ICR Swiss female mice random assigned to 76 groups of 100 mice each. Control groups from this study included 5 groups which were untreated, 8 vehicle controls, 3 positive controls, 5 low-level and 5 high-level standard tobacco condensate groups. Treatments in a volume of 0.1 ml acetone were made daily, 6 days each week. The whole smoke tobacco condensates were applied at 25 mg daily (low dose) and 50 mg daily (high dose) for a duration of 546 days. Individual animal data on tumor latent period, number, and size of tumors, body weight, total dose of chemical, and survival were stored in an IBM 360 computer. Using life table analyses we compute the distributions of tumor latent periods after adjustment for deaths without tumor and compare them using the censored rank test of Breslow (*Biometrika* 57, 579-594, 1970). Significant variation among a group's cages is found for two positive control groups, but none is detected elsewhere. Thus the experimental unit for the positive controls is taken to be the cages and, for the condensate, the unit is taken to be the individual mouse. The average median latent periods for the positive controls (3,4-benzo(a)pyrene) are 213, 155, and 135 days for doses of 5, 10, and 15  $\mu\text{g}/\text{day}$  respectively, a relationship well fitted by a semi-logarithmic plot. The median latent periods for the 5 low-dose standards are 493, 523, 481, 474, and 488; while for the high dose they are 493, 449, 425, 418, and 432. The variation of the replicate groups within doses is not significant. However, there is a significant average dose effect for the standard of 46 days (495 vs. 449). A second similar experiment has a single high dose of the University of Kentucky reference blend with median of 481 as opposed to 500 in the first experiment, a difference approaching significance. Only 7 tumors are found among 800 acetone-treated controls in the first experiment and none among 100 in the second experiment. The results indicate a high reproducibility of condensates within an experiment, but caution against comparison of condensates between experiments. The inclusion of both control and standard groups in all such experiments is certainly warranted.

- ✓ 126. *An Assessment of the Delaney Clause After 15 Years*. BERNARD L. OSER, Food and Drug Research Laboratories, Inc., Maspeth, New York.

Amendment of the Delaney Clause to permit reasonable latitude in the exercise of scientific judgement has been increasingly advocated by experts in the safety evaluation of food chemicals. Cumulating experience refutes the premise that a dose-response relationship does not obtain in respect to carcinogenic agents. The natural or unavoidable ubiquity of trace amounts of known carcinogens in foods supports the view that these levels can be safely tolerated and are "socially acceptable risks". In the absence of sound epidemiological evidence this conclusion is as valid as the hypothesis that human cancer is causally related to the presence of chemical

contaminants in foods. The highly exaggerated conditions of testing for carcinogenic potential, which entail dosing at maximum levels compatible with normal longevity, are such as to support the probability that many common food constituents can be shown to initiate or promote cancer in some species of animal. The transition of safe ("no adverse effect") dosage levels in animals to safe ("non-hazardous") dietary levels in man is feasible in the light of present knowledge. The regulatory concept of "zero tolerance" is an illusion since it depends on the finite limits of sensitivity of analytical procedures. Safety evaluations of food components are value judgements which take into account many factors including the conditions of animal tests, the incidence and nature of test responses, natural presence in foods or body constituents, epidemiological evidence, and other factors relevant to human exposure. Scientists should not be hampered by inflexible legal mandates in arriving at such judgements.

127. *Further Studies on the effect of Starvation and Microsomal Enzyme Induction on the Mobilization of DDT Residues in Rats.* JULES BRODEUR and GÉRARD LAMBERT, Département de pharmacologie, Faculté de médecine, Université de Montréal, Montréal, Canada.

The results of previous studies have shown that under certain experimental conditions the combination of starvation and enzyme induction has little advantage over starvation alone in mobilizing the residues of DDT from various tissues in rats. The present investigation was undertaken to study whether or not the experimental conditions could be optimized in such a way as to justify the combination of enzyme induction and starvation as a means of accelerating the decontamination of organisms. Adult female rats were contaminated with 100 mg/kg DDT po (10 mg/kg/day  $\times$  10 days). Then, in a first series of experiments, various groups of animals were treated orally during 14 days with phenobarbital (PB, 50 mg/kg/day), pregnenolone-16 $\alpha$ -carbonitrile (PCN, 30 mg/kg/day), the potassium salt of 9 $\alpha$ -fluoro-11 $\beta$ , 17-dihydroxy-3-oxo-4-androstene-17 $\alpha$ -propionic acid (CS-1, 40 mg/kg/day), and dexamethasone acetate (2 mg/kg/day). On day 15, *p,p'*-DDT, *p,p'*-DDD and *p,p'*-DDE were measured in blood, brain and abdominal fat tissue by GLC. The results show that PB was by far more effective than the other inducers in reducing significantly the concentrations of DDT residues in all three tissues. In a second series of experiments, groups of rats previously exposed to DDT as described above were submitted to periods of starvation of varying duration: 3 consecutive days (days 1, 2 and 3), 5 consecutive days (days 1, 2, 3, 4 and 5), 3 nonconsecutive days (days 1, 3 and 5) and 5 nonconsecutive days (days 1, 3, 5, 7 and 9). Again, on day 15, the residues were measured in blood, brain and fat tissue. The results show that a significant reduction of the residues was obtained in all tissues only in animals starved for a period of 5 consecutive days. In a third series, rats were given PB (50 mg/kg/day, 14 days), while being also submitted to a 5-day period of starvation (days 1, 2, 3, 4 and 5). On sacrifice, day 15, it was seen that the combination of both treatments was more effective in reducing the concentration of the residues in all tissues than was each treatment used alone, although the combination did not yield strictly additive effects. *In vitro* studies conducted on liver microsomal enzyme preparations indicated that a 5-day period of starvation markedly potentiates the inducing effect of a simultaneous pretreatment with phenobarbital (50 mg/kg/day, 5 days) on the amount of cytochrome P-450, as well as on the activity of aniline hydroxylase and aminopyrine-*N*-demethylase. These results show that under certain experimental conditions the combination of starvation and enzyme induction could lead to a greater depletion of pesticide residues from contaminated tissues than the use of either treatment alone. (Supported by the MRC of Canada.)

128. *Influence of Starvation on the Early Phase of Barbital Disposition in Mice.* JULES BRODEUR, JACQUES LEROUX, and SERGE LALONDE, Département de pharmacologie, Faculté de médecine, Université de Montréal, Montréal, Canada.

The influence of starvation on the early phase of drug disposition was studied in adult male Swiss mice (25–30 g). The animals were starved by withholding solid food, but not water, for 48 hr. They were then given sodium barbital (250, 500 or 750 mg/kg) by the ip or sc route and were sacrificed 2.5, 5 or 10 min later for measurement of barbital concentrations in the blood. Higher concentrations of barbital were present in the blood of starved animals by comparison with controls. This effect was abolished by treatment of starved animals with glucose (400 mg/kg, iv) given 60 min prior to barbital. Starvation shortens the onset of sleep, but increases the

duration of sleep to barbital, as shown by other workers. Glucose (400 mg/kg) dose not affect the onset nor the duration of sleep. Starved and control animals with or without glucose awake at similar concentrations of barbital in the brain, thus suggesting that starvation does not alter the sensitivity of brain tissue to barbital. The blood volume of starved animals expressed as a percentage of the body weight is slightly increased, thus eliminating the possibility that hemoconcentration might have a direct influence on the concentration of barbital in blood. It is concluded that starvation, through a yet unknown mechanism, alters the early phase of drug disposition of barbital in mice. (Supported by MRC of Canada.)

129. *The Effect of Spironolactone Treatment on Microsomal Enzymes in the Rat Testis.* R. H. MENARD, B. STRIPP and J. R. GILLETTE, National Heart and Lung Institute, Bethesda, Maryland.

Although a number of studies have revealed that spironolactone treatment in rats may either enhance or decrease the activity of hepatic microsomal enzymes associated with drug metabolism, no studies have been reported on the effect of spironolactone administration on microsomal enzyme activities in extrahepatic tissues, especially in endocrine tissues. The daily administration of 200 mg/kg of spironolactone caused an 80–90% decrease in the microsomal level of cytochrome P-450 in rat testis, but no reduction was detected in the amount of cytochrome *b<sub>5</sub>*. The loss of microsomal cytochrome P-450 was concomitant with an 80–90% loss of both the microsomal heme associated with cytochrome P-450 and the microsomal activity of progesterone 17 $\alpha$ -hydroxylase; whereas, only a 40–50% decrease occurred in 3,4-benzpyrene hydroxylation. The extent of the destruction of cytochrome P-450 by spironolactone was contingent upon the dose given and upon the duration of the treatment. Following a single administration of 100 mg/kg of spironolactone, the level of cytochrome P-450 decreased by 20–25% within one-half hour, reached a maximal disappearance of 75–90% after 16–24 hrs, and returned to control values after 3–4 days. When various doses of spironolactone ranging from 1 to 100 mg/kg were administered, the amount of cytochrome P-450 destroyed after 16 hr ranged 5–75%, with a 50% destruction occurring at a dose of 40 mg/kg. The destructive effect of spironolactone on the testicular mixed function oxidase system was not due to necrosis of testicular cells, since histological studies on testes from rats treated with spironolactone showed no abnormalities either in the interstitial cells or in the seminiferous tubules. Moreover, spironolactone did not decrease the microsomal enzyme activity of testosterone 17 $\beta$ -dehydrogenase, which is a noncytochrome P-450 enzyme.

130. *Effect of Substrates on Carbon Tetrachloride Stimulated Malonaldehyde Formation by Induced Hepatic Microsomes.* G. C. FULLER, B. I. SCHULTZ, and G. P. CARLSON, Department of Pharmacology and Toxicology, College of Pharmacy, University of Rhode Island, Kingston, Rhode Island.

Studies on the alteration of carbon tetrachloride hepatotoxicity by treatment of rats with inducers of microsomal enzymes have established that phenobarbital (PB) induction potentiates the hepatotoxicity while induction with 3-methylcholanthrene (3MC) protects (*Toxicol. Appl. Pharmacol.* **19**, 385, 1971). In this investigation malonaldehyde (MA) formation *in vitro* was measured as an index of the rate of lipid peroxidation in the livers of rats treated with inducers of microsomal enzymes. Male adult rats were treated with PB (50 mg/kg daily for 4 days, killed on day 5) or 3MC in corn oil (40 mg/kg daily for 2 days, killed on day 4), and the microsomal fraction was prepared from the perfused liver. Controls received saline or corn oil. MA was measured after a 15-min incubation of microsomes in the presence of NADPH and methyl arachidonate by the thiobarbiturate method using malonaldehyde diacetyl as a standard. CCl<sub>4</sub> was added to a center well in one of the duplicate flasks prepared for each sample, and the difference was taken as CCl<sub>4</sub>-stimulated MA formation. CCl<sub>4</sub>-stimulated MA formation was increased in microsomes from PB rats with no apparent change in the basal level (no CCl<sub>4</sub>) when compared to saline controls. The CCl<sub>4</sub>-stimulated MA formation and the basal MA formation were decreased in microsomes from 3MC rats. PB (10<sup>-3</sup> M) or 3MC (10<sup>-5</sup> M) had no effect on CCl<sub>4</sub>-stimulated MA formation when added *in vitro* to the microsomal fraction of control rats. However, 3MC (10<sup>-3</sup> M) added *in vitro* prevented the CCl<sub>4</sub> stimulation of MA

formation. These data are consistent with our previous observation that PB induction is associated with potentiated CCl<sub>4</sub> hepatotoxicity while 3MC induction protects rats against CCl<sub>4</sub> hepatotoxicity. (Supported by NIH Grant ES-00596.)

131. *A Study of Some Possible Effects of Chronic Murine Pneumonia on certain Hepatic Microsomal Drug Metabolizing Enzymes.* FRANK E. GREENE and CURT H. BARTHEL, Departments of Pharmacology and Comparative Medicine, The M.S. Hershey Medical Center, The Pennsylvania State University, Hershey, Pennsylvania.

Experiments have been conducted to determine if chronic murine pneumonia (CMP) which is present in many rodent colonies has an effect on the hepatic microsomal enzyme system that metabolizes foreign compounds. To evaluate this possibility, age matched adult male Sprague-Dawley axenic (CMP-free) and conventional rats were obtained and immediately transferred to a conventional environment. Groups of rats were sacrificed on the day of arrival and 2 and 4 weeks later. Complete necropsies were performed and microsomal ethylmorphine *N*-demethylase, NADPH oxidase, NADPH cytochrome *c* reductase and cytochrome P-450 were measured. Intergroup comparisons of CMP infected rats indicated no significant correlation between severity of pathology and enzyme activity. However, ethylmorphine *N*-demethylase, NADPH oxidase and cytochrome *c* reductase activities were higher in CMP-free rats throughout the course of the experiment. In order to determine if these differences were due to an effect of CMP on these enzymes that was not related to severity of lung pathology, a second series of experiments was performed using Fisher F344 axenic (CMP-free) and conventional rats that were raised in our own animal facilities. Groups of male and female rats were sacrificed at 4 and 6 weeks of age with a complete necropsy as in the previous experiments. Evidence of mild CMP was present in all conventional rats but in none of the axenics. In contrast to the previous study, however, no differences were found in intra- or inter-group comparisons of any of the parameters measured, suggesting that the differences observed between the Sprague-Dawley CMP-free and conventional rats were not related to CMP infections. Results from these experiments indicate that neither the presence nor severity of CMP affects the ability of the liver enzymes to metabolize certain drugs *in vitro*. (Supported in part by NIEHS Research Grant 9-R01-ES00638-03.)

132. *Effects of Chlorinated Naphthalenes on Liver Levels of Detoxication Enzymes and Vitamin A.* D. J. WAGSTAFF, Department of Veterinary Physiology and Pharmacology, University of Missouri, Columbia, Missouri.

Chlorinated naphthalenes cause bovine hyperkeratosis, a disease in which hypovitaminosis A is a consistent lesion. This report is on the capacity of chlorinated naphthalenes to stimulate liver detoxication enzymes and association of this stimulated activity with vitamin A deficiency. Female Holtzman rats weighing 100 g were fed various chlorinated naphthalenes added to a vitamin A deficient diet. Hexobarbital sleep time was determined at 10 days. At 15 days, *O*-demethylation of *p*-nitroanisole and oxidative cleavage of EPN were measured in 10,000 × g supernatant fraction of liver. Vitamin A was determined in liver by a Carr-Price method. For organochlorine pesticides and PCB the threshold for liver enzyme stimulation is reported to be 1–10 ppm but when the alphachloro, betachloro, 1,2-dichloro, 1,4-dichloro, or 1,2,3,4-tetrachloro derivatives of naphthalene were fed at 50 and 100 ppm there were no changes in the liver levels of enzyme activity or vitamin A. When 1,4-dichloronaphthalene was fed at 1000 ppm, *O*-demethylase and sleep time were stimulated. RPN detoxication was not stimulated until the feeding level was increased to 5000 ppm at which point body weight gains were impaired. Hepatic vitamin A concentrations were unchanged at all feeding levels. When a series of commercial formulations (Halowaxes) was fed at 3000 ppm, liver weights increased and body growth decreased. Enzyme activities increased as the percent chlorination of the product fed increased, but only up to a point above which activities started to decline from peak values. Again the pattern of responses for *O*-demethylase and EPN detoxication were different. Hepatic vitamin A concentrations were inconsistently decreased in some groups. It is concluded that dietary chlorinated naphthalenes stimulate liver detoxication enzymes, but at a higher threshold and with a different pattern than that reported for organochlorine pesticides

and PCB. No consistent relationship between hepatic enzyme activities and levels of vitamin A was observed.

133. *The Interaction of Adrenalectomy, Partial Adrenal Replacement Therapy, and Starvation with Hepatotoxicity and Lethality after 1,1-Dichloroethylene Intoxication.* R. J. JAEGER, M. J. TRABULUS, and S. D. MURPHY, Harvard School of Public Health, Boston, Massachusetts.

We have observed inconsistent results in dose-mortality studies with 1,1-dichloroethylene (1,1-DCE). The present study was undertaken to determine if several stress-related factors were responsible for the modifications of acute lethality and hepatotoxicity that we observed. Rats received 1,1-DCE orally or 1,1-DCE vapor (4 hr exposure) and were sacrificed at 4 hr, 24 hr or at death. Following a single oral dose (400 mg/kg) of 1,1-DCE, both adrenalectomized (ADX) and sham rats had similar 24-hr mortalities. At this dose ADX resulted in an apparent decrease in hepatic injury as measured by serum alanine- $\alpha$ -ketoglutarate transaminase (AKT) elevation. This decreased hepatotoxic response was due in part to a decreased hepatic activity of this enzyme in ADX rats. The degree of bloody ascites and hemorrhagic congestion of the liver was also reduced. Corticosterone (CS) treatment resulted in a slight decrease of lethal effects of 1,1-DCE in both ADX and sham rats. Serum AKT increases and liver AKT activity in ADX rats were significantly elevated by this treatment. Prolonged (45 hr) fasting also potentiated hepatic injury as measured by the 4-hr elevation of serum AKT. Glucose (25% *ad libitum*) was administered to prevent the hypoglycemia seen in sham and ADX rats at death (serum glucose > 25 mg/100 ml). While some protection against lethality was noted in ADX rats they were still hypoglycemic at death. Sham rats given glucose were hyperglycemic (> 300 mg/100 ml). The estimated 4-hr inhalation LC50 of 1,1-DCE in fed rats was between 10,000 and 15,000 ppm, while the approximate LC50 in fasted rats was between 500 and 2500 ppm. At death fasted rats were hypoglycemic while fed rats were hyperglycemic, and both had bloody ascites. Signs of cardiac failure were not present. In all animals given 1,1-DCE (po or inhalation) recoverable blood volumes at death were reduced. Our results suggest that 1,1-DEC toxicity may be markedly affected by the nutritional state and death appears due to vascular collapse and shock. (Supported by research grant OH00315 from the National Inst. Occ. Safety and Health.)

134. *N-Demethylation of Aminopyrene and p-Chloro-N-methylaniline by Isolated Perfused Rabbit Lung.* V. L. ARMSTRONG, R. W. NIEMEIER, and E. BINGHAM, University of Cincinnati College of Medicine, Cincinnati, Ohio. (A. Wolven.)

The role of the lung in the metabolism of foreign compounds is largely unexplored. An isolated perfused lung was developed in our laboratory (Niemeier and Bingham, 1972) for use in metabolic studies. In addition it is desirable to measure certain physiological changes that may occur in the lung as a result of exposure to pharmacological or environmental agents. The isolated perfused rabbit lung was active in the N-demethylation of aminopyrene (AP) for up to 4 hr of perfusion. *p*-Chloro-*N*-methylaniline (PCMA) was more rapidly *N*-demethylated than AP. It was apparent during the perfusion with the latter compound that methemoglobin formation was occurring. The rate of formation of methemoglobin was followed in this system. The *N*-demethylation of AP and PCMA was measured also after pretreatment with phenobarbital.

135. *Effect of Phenacetin on Salicylate Absorption and Excretion in the Rat.* B. B. COLDWELL, B. H. THOMAS, and G. SOLOMONRAJ, Research Laboratories, Health Protection Branch, Department of National Health and Welfare, Ottawa, Canada. (C. Chappel.)

Recently, we showed that in man the excretion of the toxic metabolite, 2-hydroxyphenetidine, is increased when phenacetin (P) is ingested in combination with acetylsalicylic acid (ASA), caffeine and codeine. A detailed pharmacokinetic study of this interaction is now underway. The effect of P on ASA was investigated in adult male Wistar rats treated orally with ASA (100 mg/kg) alone and together with P (71.4 mg/kg). The dose of ASA per rat included about 5  $\mu$ Ci of ASA-carboxyl-14C. The radioactivity in the blood, urine, stomach and small intestine was measured at specified time intervals after drug administration. Phenacetin

treatment resulted in (1) a decrease ( $P < 0.01$ ) in blood radioactivity from 15–150 min, (2) a decrease ( $P < 0.05$ ) in the amount of radioactivity excreted in the urine during the first 4 hr, and (3) retention ( $P < 0.01$ ) of radioactivity in the stomach 1 hr following drug ingestion. The recovery of radioactivity and the proportions of ASA metabolites in the urine, and the amount of radioactivity in the small intestine were not altered by phenacetin. It is concluded that phenacetin interferes with the absorption of ASA from the stomach of the rat.

136. *Subacute Toxicity Studies with Prostaglandin E<sub>1</sub> (PGE<sub>1</sub> in Laboratory Animal Species.* K. S. RAO, F. E. RENO, and R. G. MCCONNELL, Searle Laboratories, Chicago, Illinois and Hazleton Laboratories, Vienna, Virginia. (V. A. Drill.)

Prostaglandins are a group of modified hydroxy fatty acids with a wide distribution in mammalian tissues. They possess a wide range of potent biological activities and promise to be clinically useful in areas as diverse as the treatment of asthma and termination of pregnancy. In spite of the voluminous literature available on prostaglandins, it is ironic that there is very little information on the safety and toxicity of prostaglandins in animals. In the present study, acute and subacute effects of prostaglandin E<sub>1</sub> (PGE<sub>1</sub>), a representative of the series, was studied in albino rats and Beagle dogs. The compound dissolved in phosphate buffer was administered daily to groups of animals by continuous (3–6 hr) iv infusion at dose levels up to 2.0  $\mu\text{g}/\text{kg}/\text{min}$  for 14 consecutive days. Conventional physical, cardiovascular, hematology, clinical chemical and postmortem examinations were performed. Dogs exhibited catatonia and emesis. In rats no compound-related physical signs were observed. Cardiovascular parameters (blood pressure, ECG and respiratory rate) and hematology findings were unremarkable. Among the clinical chemistry parameters, there was a tendency for a decrease in blood glucose in some of the treated animals. Postmortem findings, gross and microscopic, were unremarkable. It is concluded that daily iv infusion of PGE<sub>1</sub> up to 2.0  $\mu\text{g}/\text{kg}/\text{min}$  for 14 consecutive days to rats and dogs, causes no biologically meaningful detrimental effects.

137. *Repeated-Dose Toxicity of a New Antimetabolite, S-Triazin-2(1H)-1,4-Amino-1- $\beta$ -D-Ribofuranosyl-5-Azacytidine in the Rhesus Monkey.* P. E. PALM, E. P. ARNOLD, P. C. RACHWALL, and M. S. NICK, Arthur D. Little, Inc., Cambridge, Massachusetts.

Eight rhesus monkeys (1 male, 1 female/level) were administered the cancer chemotherapeutic agent, s-triazin-2(1H)-1,4-amino-1- $\beta$ -ribofuranosyl-5-azacytidine by the iv route at levels of 0.28, 0.55, 1.1 or 2.2 mg/kg  $\times$  14. Four control animals received only the diluent PVP or water. Death occurred after 8 and 14 doses in the female and male monkeys, respectively, at the 2.2 mg/kg (32 mg/m<sup>2</sup>) level only, with kidney and liver damage indicated by marked elevations in BUN, SGOT and SGPT values. Micropathology observed in these animals included cloudy swelling and focal necrosis of kidney tubules, hepatic fatty metamorphosis, bone marrow erythroid and myeloid hypoplasia, and lymphoid hypoplasia in the spleen and lymph nodes. Monkeys dosed at levels  $\leq$  1.1 mg/kg (16 mg/m<sup>2</sup>) showed minimal clinical signs. Hematological and blood chemistry changes noted were slight and appeared to be readily reversible. The highest non-toxic dose in the monkey was about 0.28 mg/kg (4 mg/m<sup>2</sup>) on the 14-day repeated-dose regimen. A comparison with data reported earlier in the dog showed the monkey to be about comparable or slightly less sensitive than the dog to the toxic effects of the drug. (Supported by Contract No. PH. 43-65-61 with Chemotherapy, National Cancer Institutes of Health.)

138. *The Parenteral Toxicity of Clindamycin-2-Phosphate in Laboratory Animals.* J. E. GRAY, R. N. WEAVER, J. MORAN, and E. S. FEENSTRA, Research Division, The Upjohn Company, Kalamazoo, Michigan.

The ip and iv LD<sub>50</sub> values of 1145 and 855 mg/kg, respectively, obtained for clindamycin-2-phosphate in the Swiss white mouse were approximately 3 times higher than those of clindamycin hydrochloride. The lesion produced by single injection of 50 or 100 mg/ml in the loin muscles of the New Zealand white rabbit was graded as slight. The 24-hr serum creatine phosphokinase value was 1500 IU/ml which was less than one-half that of the parent antibiotic.

Body weight gains and food conversion ratios in groups of 10 Sprague-Dawley rats injected sc with 120 mg/kg of clindamycin-2-phosphate for 6 days were comparable to those of the control group; 90 mg/kg was tolerated in these terms nearly as well for 30 days as no treatment or doses of 30 and 60 mg/kg. From 22 to 33 injections, each equivalent to 30, 60 or 90 mg/kg, were made bilaterally in the posterior thigh muscles of groups of 3 beagles. The terminal elevations of serum glutamic oxaloacetic transaminase varied from 54 to 400 RF units. The characteristic pathologic change resulting from the superimposed injections was a dose-related progressive scarring of the muscle bundles. Intravenous administration of 60 and 120 mg/kg daily in divided doses in 2 groups of 4 dogs each for 30 days produced no detectable irritation in the peripheral veins or drug-related hemolysis. Tests for drug-induced hemolysis and changes in erythrocyte fragility by *in vitro* methods were negative. In dogs treated iv with 120 mg/kg for 1 week, a slight increase in neutral lipid droplets was present in hepatocytes from 3 hr to 3 days when examined by electron microscopy. This transient change was not observed in dogs injected im with 90 mg/kg for the same period.

139. *Renal Toxicity Produced by  $\alpha$ -Methyl-4-phenylmandelic Acid in the Rat.* D. G. HOFFMAN, G. C. TODD, N. R. BERNHARD, and G. F. KIPLINGER, Toxicology Division, Eli Lilly and Company, Greenfield, Indiana.

$\alpha$ -Methyl-4-phenylmandelic acid (MPMA) is a major, nephrotoxic metabolite of 4-isopropylbiphenyl in the rat. After ip administration, the LD<sub>50</sub> was 450 mg/kg and the plasma half-life was 3.3 hr. Papillary necrosis was apparent after a single 250 mg/kg dose. MPMA produced increases in kidney weight, urine volume, UGOT, serum creatinine, and BUN and a decrease in urine specific gravity. The increase in urine volume, first detected 8 hr after dosing, was statistically significant 5 days after dosing. Three daily 250 mg/kg doses produced progressively greater increases in kidney weight, serum creatinine, and BUN. The data suggest that MPMA is responsible for the nephrotoxicity noted after oral administration of high doses of 4-isopropylbiphenyl to rats.

140. *In Vivo and In Vitro Metabolic Studies of  $^{14}\text{C}$ -Labeled Propham and Chloroprotham in the Rat and Rat Tissues.* S. C. FANG, ELIZABETH FALLIN, and V. H. FREED, Department of Agricultural Chemistry, Oregon State University, Corvallis, Oregon.

The excretion and tissue distribution of [2- $^{14}\text{C}$ ]isopropyl-m-chlorocarbanilate ([chain- $^{14}\text{C}$ ]chloroprotham), [isopropyl-ring- $^{14}\text{C}$ ]m-chlorocarbanilate ([ring- $^{14}\text{C}$ ]chloroprotham), [2- $^{14}\text{C}$ ]isopropyl-carbanilate ([chain- $^{14}\text{C}$ ]protham), and [isopropyl-ring- $^{14}\text{C}$ ]carbanilate ([ring- $^{14}\text{C}$ ]protham) was investigated in the adult female rat after a single oral dosage. The average 3-day urinary excretions were 55.9, 82.6, 79.5 and 85.4% of the oral dose for [chain- $^{14}\text{C}$ ]chloroprotham, [ring- $^{14}\text{C}$ ]chloroprotham, [chain- $^{14}\text{C}$ ]protham and [ring- $^{14}\text{C}$ ]protham, respectively. With (chain- $^{14}\text{C}$ )chloroprotham 35.4  $\pm$  7.5% of the administered radioactivity appeared in the respired air, whereas only 5.0  $\pm$  0.8% was found in CO<sub>2</sub> from [chain- $^{14}\text{C}$ ]protham. There was no significant difference in the rate of excretion or the route of elimination among rats receiving different oral dosages, ranging from less than 4 to 200 mg/kg. The radioactivity was distributed in all tissues; the highest concentration was found in the kidney. The average biological half-life of chloroprotham and protham in most organs was short, ranging between 3 to 8 hr; however, it was about twice longer in the brain, fat and muscle. Both compounds were metabolized by hydrolytic and oxidative mechanisms and the resulting metabolites were excreted either as free forms or as conjugates. Paper chromatographic and TLC separation of urine samples revealed the presence of 2 major and at least 3 minor metabolites; the relative ratio of the labeled urinary metabolites was dependent on the dosage used, the  $^{14}\text{C}$ -labeled position, and the time of sampling. Subcellular distribution of  $^{14}\text{C}$  in the rat liver and kidney following an oral administration of chloroprotham and protham or *in vitro* incubation of the tissue slices with the labeled herbicides was investigated. The percentage distribution of  $^{14}\text{C}$  in the particulate and soluble fractions was dependent on several factors, particularly the compound used and the time of reaction. The binding of chloroprotham to mitochondrial and microsomal fractions was always greater than that of protham. *In vitro* incubation studies



of kidney or liver slices with labeled herbicides revealed that the chain moiety of these herbicides was not hydrolyzed and degraded to CO<sub>2</sub> as found in the *in vivo* experiment. Only liver slices can convert the herbicides to their metabolites suggesting that the liver is a possible site for degradation of these compounds *in vivo*.

141. *Studies of the Metabolism of Saccharin in Man.* E. W. MCCHESENEY and L. GOLBERG, Institute of Experimental Pathology and Toxicology, Albany Medical College, Albany, New York.

Doses of saccharin (SA) ranging from 0.5 to 1.0 g have been given to 7 male volunteers, on 10 occasions, and the urinary excretion has been followed for the ensuing 72 hr, using a gas chromatographic method of analysis for SA and for *o*-sulfamoylbenzoic acid. In a preliminary experiment involving 3 subjects, 2 appeared to excrete the dose quantitatively within 48 hr as SA, but in the third subject only about  $\frac{1}{3}$  of the dose was recovered. In a second more detailed experiment, involving the administration of 7 doses to 6 subjects, the mean per cent recovery as SA ( $\pm$  SE) was as follows: 0-8 hr, 61.8  $\pm$  4.1; 0-24 hr, 82.0  $\pm$  2.9; 0-48 hr, 88.8  $\pm$  4.0; 0-72 hr, 88.8  $\pm$  2.4. On alkaline hydrolysis of the urine samples and determination of the excretory products as *o*-sulfamoylbenzoic acid, the mean percent recovery increased to the following values: 0-8 hr, 64.6  $\pm$  3.6; 0-24 hr, 86.2  $\pm$  1.8; 0-48 hr, 95.2  $\pm$  1.1; 0-72 hr, 96.1  $\pm$  1.4. Neither excretion rate nor percent recovery was affected by the size of the dose administered. The nature of the substance present in urine, from which the extra *o*-sulfamoylbenzoic acid was being formed by alkaline hydrolysis, has not been definitely established. One possibility was the presence of *o*-sulfamoylhippuric acid, and another was that the extra SA was merely loosely bound to a normal urinary constituent so that its extraction characteristics were changed. In any case, no free *o*-sulfamoylbenzoic acid was detected in the urine of any of the 7 subjects. The general conclusion is that saccharin is excreted by man essentially in unchanged form and at the same rate as that observed in laboratory animals. (Supported by FDA Contract 69-7, by Research Grant 2P01-ES00226-06 from the National Institute of Environmental Health Sciences, NIH, and by National Institutes of Health Training Grant 2T01-ES00103-06.)

142. *Drug Mediated Displacement of Protein Bound Bilirubin.* ROGER A. YEARY and DAVID R. DAVIS, Department of Veterinary Physiology and Pharmacology, The Ohio State University, Columbus, Ohio.

Examination of several procedures was made to evaluate the most meaningful model for determining the capacity of drugs to displace bilirubin from serum albumin, a hazard in the perinatal period. *In vitro* studies using Sephadex Gel filtration coupled with *in vivo* studies in the hyperbilirubinemic Gunn rat provided both qualitative and quantitative data for evaluating the potential for bilirubin displacement at therapeutic dosages of drugs. Using sulfadimethoxine (SDM) and sodium salicylate (NaSal) as prototype drugs the per cent of bilirubin displaced was found to be independent of the bilirubin content of the system using bilirubin-albumin molar ratios less than 1:1. Relationship of per cent bilirubin displaced to dosage *in vitro* was log-linear. Lineweaver-Burke plots were made to calculate the maximum displacement and kinetic constant for displacement (K<sub>d</sub>). The maximum displacement for SDM and NaSal were 26.6 and 8.8% respectively. The respective K<sub>d</sub> values were 1.92  $\times$  10<sup>-3</sup>M and 4.96  $\times$  10<sup>-3</sup>M. Extrapolation of the log-dose response curves predicted that serum concentrations at which the drugs would begin to displace bilirubin were 6.7 mg/100 ml for SDM and 9.9 mg/100 ml for NaSal. These predictions proved to be valid when tested in the hyperbilirubinemic Gunn rat. The use of both Sephadex Gel filtration and the Gunn rat overcame most of the deficiencies of using only Sephadex Gel filtration for studying the drug-mediated displacement of protein bound bilirubin. (Supported by NIH Grant HD 03867.)

143. *Perinatal Development of Biliary Excretion.* S. W. HWANG and R. L. DIXON, Pathologic Physiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

Environmental chemicals (including drugs) are eliminated from their biological sites of action by metabolism, storage, or excretion. None of these mechanisms appears to be completely

developed at birth. This condition may contribute to the greater toxicity observed in young animals for many exogenous chemicals. We have studied the postnatal development of biliary excretion of indocyanine green (ICG) in guinea pigs. Animals ranging in age from 1 day old to adult were anesthetized, and the bile ducts cannulated. ICG was either injected or infused into a femoral or jugular vein, and bile was collected at predetermined periods. ICG was determined in bile, plasma, and liver. There was no difference in the rate of bile flow ( $\mu$  l/20 min/g) when animals 1, 2, 5, and 10 days old were compared to adults. The mean bile/plasma ratio in adults was approximately 50 while the newborn mean ratio was 7; guinea pigs 10 days old had a bile/plasma ratio less than half that of the adult. Clearance from plasma to bile was greater than 50 ml/20 min for the adult while less than 2 ml/20 min for the newborn. Maximum biliary transport capacity ( $T_m$ ) was also determined. The adult mean  $T_m$  value was more than 3 times that for the newborn (275 and 85  $\mu$ g/min/kg, respectively). Renal excretion did not appear to play an important role in these studies. Urine contained no ICG, and similar results were obtained in animals after renal ligation. Significant differences do exist in the plasma disappearance curves for newborn and adult animals, and aspects of plasma and tissue protein binding are being further investigated.

144. *Correlation Between Biliary Flow and Hepatic Excretion of Intravenous Cholangiographic Agents.* D. R. VANDERIFE and A. VALENTI, Mallinckrodt Pharmaceuticals, St. Louis, Missouri. (T. W. Tusing.)

Two iodinated contrast media, MP 271 and MP 117, demonstrated improved efficacy and safety over iodipamide in preclinical testing programs. Their iv LD50 values in mice were: MP 271, 13.1 g/kg; MP 117, 4.8 g/kg; iodipamide, 3.6 g/kg. Their efficacies, as judged by biliary opacification in cats, ranked them as MP 117 > MP 271 = iodipamide. Clinically, however, both MP compounds failed to opacify bile ducts as well or as early as iodipamide. Therefore, a program was undertaken to determine the comparative tissue distribution and excretion patterns of these three agents. X-Radiographic studies were carried out in unanesthetized cats and dogs and radioactive tracer studies were conducted in both anesthetized and unanesthetized cats and dogs. Biliary excretion in cats ranked the agents as MP 117 > MP 271 = iodipamide. In dogs, as in patients, the agents ranked iodipamide > MP 271 > MP 117. In general the three agents demonstrated similar biliary concentrations and flow rates in cats but, compared to iodipamide, MP 271 and MP 117 showed anticholeresis and hepatic retention in dogs. In dogs, cholereitics increased the total bile flow and excretion of MP 271 and MP 117, but at a sacrifice of the iodine concentration in the bile. The mechanism of the delayed hepatic excretion of MP 271 and MP 117 in dogs and humans is not known at this time. The differences in early hepatic excretion of the three agents in dogs and cats was not due to differences in urinary excretion.

145. *An On-line Computer System for Chronic Toxicology Studies.* Y. SHIRASU, T. USUI, K. W. TAKAHASHI, H. INOMATA, and H. OHASHI, The Institute of Environmental Toxicology, Tokyo, and Naka Works, Hitachi Co., Ibaraki-ken, Japan.

In chronic toxicity studies many parameters on numerous animals have to be collected and handled statistically. In order to save labor cost and to increase the accuracy of data handling an on-line computer system, IET system, has been developed in collaboration with toxicologists and computer scientists. This system consists of an Hitachi Hitac 10 computer and its related instruments connected on-line to Mettler electronic balances, Hitachi biochemical auto-analyzer M-400, Technicon hematological auto-analyzer SMA 4A. Food intake and body weight of rats or mice are regularly recorded by electronic balances and sent to the computer to be stored. The computer can calculate exact body weight in 2 sec even though an animal moves on the balance. At autopsy organ weights also can be handled by this system. Blood biochemical values are calculated from pre-input standard curves by the computer. White and red blood cell numbers, Hb and CCV values are also stored in the computer. Besides individual data mean values with SD of these parameters for each group are printed out in tables by a teletypewriter. The data can be stored in the memory of the computer up to 2 years and are readily available when needed.

146. *Dibutyryl Cyclic AMP—an Antidote to Hypnotic, Sedative, and Tranquilizer Overdosage in the Rat.* M. L. COHN, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania. (M. Eisler.)

We have previously reported that  $N^6, O^2'$ -dibutyryl adenosine 3':5'-cyclic monophosphate (db cyclic AMP) administered intracerebroventricularly (icv) in the rat, shortened amobarbital-induced sleeping time in a dose-related manner. Five neuropharmacologic agents, which included the aliphatic hypnotics chloral hydrate and paraldehyde, the tranquilizer diazepam, the phencyclidine derivative ketamine, and the inhalation agent halothane, were also tested. Db cyclic AMP shortened, in a dose-related manner, the duration of narcosis of each of these structurally unrelated central nervous system depressants. The purpose of the present research was to determine if db cyclic AMP administered icv protected rats treated with a toxic dose of each of these drugs. Male Sprague-Dawley rats weighing 80–100 g were weighed and one of the central nervous system depressants was injected ip according to the following dose schedule: amobarbital, 175 mg/kg; chloral hydrate (Noctec), 500 mg/kg (diluted 1:1 in sterile saline), diazepam, 70 mg/kg, and paraldehyde, 1.5 ml/kg. Immediately after the loss of righting reflex, 225  $\mu$ g of cyclic AMP in 15  $\mu$ l of 0.001 M potassium phosphate buffer solution, pH 7.5, was administered icv. Our data showed that db cyclic AMP significantly reduced the mortality of amobarbital from LD50 to LD3, chloral hydrate from LD50 to LD17, and paraldehyde from LD67 to LD17. Repetitive icv doses of db cyclic AMP were required to decrease the mortality due to ketamine and diazepam. Whereas higher doses of db cyclic AMP (300  $\mu$ g) given to rats treated with toxic doses of diazepam and ketamine resulted in shortening of the sleeping time, no reduction of the LD50 was observed. Analeptic drugs (methamphetamine, picrotoxin, doxapram, caffeine, theophylline, and strychnine) administered icv did not shorten the sleeping time or reduce the mortality of any of the central nervous system depressants used in this study.

147. *Spontaneous Tumours of the CNS in the Rat.* LIONEL E. MAWDESLEY-THOMAS and A. JOHN NEWMAN, Huntingdon Research Centre, Huntingdon, England.

Spontaneous tumours of the CNS in the rat are rare and poorly documented. During the past 3 years detailed studies involving the central nervous system have been performed. This paper describes over 30 spontaneous tumours found in the brain and spinal cord of the rat. These tumours were found in a Sprague-Dawley derived population of rats and some 35,000 animals were examined. The tumours varied considerably in size from those which were obvious on macroscopical examination to those which were detected only at microscopy. Various cell types were seen which included the astrocytoma, glioblastoma, oligodendroglioma, meningioma and reticulosarcoma. Varying degrees of malignancy were seen in the various cell types. In addition several tumours of the spinal cord were noted. The difficulty of tumour classification is discussed together with the diagnosis and technical problems associated with CNS tumours in the rat brain. The classification of spontaneous tumours sheds some light on the possible histogenesis of induced tumours.

148. *Effect of Succinic Acid, Pargyline or L-Ascorbic Acid on Brain Ammonia and Glutamine: A Mechanism of Protection Against Hyperbaric Oxygen (OHP) Toxicity.* ROBERT SCHATZ and HARBANS LAL, Mental Health Research Institute, University of Michigan, Ann Arbor, Michigan, and Department of Pharmacology and Toxicology, College of Pharmacy, University of Rhode Island, Kingston, Rhode Island.

Since increased levels of brain ammonia and decreased levels of glutamine have been implicated in OHP toxicity, we investigated the effects of pargyline, succinic acid and ascorbic acid on brain ammonia and glutamine in relation to OHP toxicity. Intraperitoneal administration of pargyline (100 mg/kg), succinic acid or ascorbic acid (12 mmol/kg) provided protection against OHP toxicity (convulsions, pulmonary edema and hemorrhage, post-exposure mortality). All three agents prevented the OHP-induced elevation in brain ammonia levels and the OHP-induced decrease in brain glutamine. Further, there was a significant correlation between seizure susceptibility and brain ammonia and glutamine levels in pargyline, succinic acid or ascorbic acid-treated mice. It is suggested that the protective effects of these three agents is mediated via effects on ammonia metabolism.

149. *The Toxicity of Hyperosmolar Solutions of Sorbitol, Mannitol, and Dextrose in Dogs.*

S. R. BOWERS, P. J. GARVIN, and R. K. AUSMAN, Travenol Laboratories, Inc., Morton Grove, Illinois.

Intravenous infusions of hyperosmolar solutions of sorbitol, a hexitol sugar used in parenteral nutrition, caused toxic reactions in dogs during preliminary studies. To determine if the cause of this toxicity was hyperosmolarity or the sorbitol itself, hyperosmolar sorbitol solutions were infused into normal and surgically prepared renally deficient dogs weighing 6.5 to 21.5 kg, in doses of 300, 500, 750, and 1000 mOsm/hr over a 2-hr period. Each dose was administered alternatively with and without concomitant iv injection of 0.45% saline to replace water lost in the urine during infusion. A recovery period of 7 days was allowed between each infusion. Hyperosmolar mannitol and dextrose were similarly infused into other groups of normal dogs at the same doses, and mannitol was likewise infused into renally deficient dogs. Mannitol served as a non-metabolized control and indicated only osmotic effects, while dextrose was included to determine toxicity, if any, of a readily metabolized sugar, infused at hyperosmolar concentrations. Urinary flow and carbohydrate levels and osmolarity of blood and urine were determined before, during, and after each infusion. The experiment was thus designed to study the toxicity of the three carbohydrates in terms of the effects of osmolar load, water replacement, and reduced kidney function. The responses following the infusion of hyperosmolar solutions into normal dogs were quantitatively similar: plasma osmolarity and serum and urine concentrations of the infused carbohydrates were elevated during and returned to normal following infusion. The degree of increase was dose-related. Urine flow was markedly increased and osmolarity of urine markedly decreased during infusion. Serum carbohydrate concentrations and plasma osmolarity were not as greatly increased at the lower doses in normal dogs when excreted water was replaced. Renally deficient dogs also had elevated blood carbohydrate concentrations and osmolarities at the lowest dose but could not tolerate doses over 300 mOsm/hr. All of the dogs in the study died showing signs of CNS disturbances. These results support the hypothesis that the toxicity of hyperosmolar sorbitol is due to osmotic effects.

150. *The Use of Females in Clinical (Human) Bioavailability Studies: The Effect of Menstruation.* ANDREW S. TEGERIS and PETER N. PANTELEAKIS, Pharmacopathics Research Laboratories, Inc., Laurel, Maryland.

In some forms of clinical bioavailability cross-over studies in the human, governmental authorities usually advise that a small group (about 20) of "standard" healthy adult subjects be used. In these studies no more than 20% variation is allowed between the reference standard and the new product. In our experience when females are used, in some cases, there is a wide discrepancy between the blood concentration during the menstruation and during the non-menstruating phase of the same woman's cycle. In the first phase of this experiment 6 adult healthy females of normal weight and height received orally one dose of 250 mg suspension of ampicillin trihydrate and a standard 6-hr blood clearance assay was performed, using the Code of Federal Regulations approved technique for blood and tissue antibiotic assays. The results were expressed in  $\mu\text{g}/\text{ml}$ . The blood ampicillin concentration of D.N. at 30 min, non-menstruating, was 6.5  $\mu\text{g}$  as opposed to 1.8  $\mu\text{g}$  while menstruating; the corresponding values for J.P. were 2.5 and 1.2  $\mu\text{g}$ , respectively. For subject H.B. at 2 hr the values were 3.5  $\mu\text{g}$ , non-menstruating, and 0.75  $\mu\text{g}$ , menstruating. The study is continuing. In view of these findings, when women are proposed to be in such studies, the phase of their menstrual cycle must be rigidly controlled.

151. *The Detection of Thiazide Diuretics in Urine: Column Extraction and Thin-Layer Chromatography.* D. SOHN, J. SIMON, M. A. HANNA, G. GHALI, and R. TOLBA, The Laboratory for Chromatography, Bayside, New York. (H. Birnbaum.)

Thiazide diuretics have been detected in urine by hydrolysis, diazotisation, and coupling to form an azo-dye; by ultraviolet spectrophotometry between 264 to 300 nm; by paper chromatography; and by thin-layer chromatography (TLC), either one or two dimensional,

with or without hydrolysis. These techniques all employ some form of solvent extraction. In some methods, urine is deproteinized before extraction. A method is presented employing resin columns (Drug Extraction Columns, Bio-Rad Laboratories, Richmond, California) for extraction and concentration of these drugs from urine. Ten ml of urine buffered to pH5 with acetate buffer is poured through the column. This column is washed with distilled water, eluted with methanol, evaporated, then redissolved and applied to a silica gel G plate. The plate is developed in Davidow's developer and sprayed with ammoniated dimethylamino-benzaldehyde. Good separations and delineations of thiazide diuretics are obtained. The column extraction and the spray reagent used have not been previously reported. The screening for these drugs is of toxicologic importance since they may be used to increase barbiturate excretion in cases of overdose and may have to be distinguished during screening methods for poisons.

152. *Studies of Tumorigenic and diabetogenic potential of Certain Oral Contraceptive Steroids in Female Dogs and Monkeys.* F. X. WAZETER, R. G. GEIL, V. R. BERLINER and J. K. LAMAR, International Research and Development Corporation, Mattawan, Michigan, and Food and Drug Administration, Rockville, Maryland.

The purpose was to study the long-term effects of certain steroids proposed or used in oral contraceptives, by a regimen comparable to human use, in female beagle dogs and rhesus monkeys. The drugs are: ethynone plus mestranol, Wy-4355 plus mestranol, anagestone acetate plus mestranol, mestranol, and ethynone. The dose levels are 2 $\times$ , 10 $\times$ , and 25 $\times$  the human dose, for dogs, and 2 $\times$ , 10 $\times$ , and 50 $\times$  the human dose, for monkeys. The dosing is oral, cyclic, 21 days on drug, 7 days off. The animals, 16 per group, have been on test 42-56 months. In dogs the 3 progestogens plus mestranol have produced the greatest dose-related tumorigenic effect, as measured by total mammary nodules, number of dogs with nodules, or maximum size of nodules. Ethynone alone has produced significant nodules, but mestranol-treated dogs show no more nodules than controls. Nodules by biopsy or autopsy were diagnosed as: cystic lobular hyperplasia, papillary adenoma, mixed mammary tumour (both benign and malignant), and adenocarcinoma. One dog on anagestone acetate plus mestranol died of multiple metastases to the lung from an adenocarcinoma. Several dogs given Wy-4355 plus mestranol or anagestone acetate plus mestranol have developed diabetes mellitus. Monkeys have not shown tumors or diabetes to date. Tumorigenic and diabetogenic potential differ with compound, and with species. (Supported by Contract FDA 68-45.)

153. *Effect of Phenobarbital on Production of Bladder Cancer (Dog) by 4-Aminobiphenyl.* W. E. MACDONALD, W. A. D. ANDERSON, M. COPLAN, F. WOODS, and WM. B. DEICHMANN, University of Miami, School of Medicine, Coral Gables, Florida.

Since it has been demonstrated that phenobarbital increases significantly the rate of metabolism of 2-naphthylamine to 2-nitrosonaphthalene, studies were initiated to determine the possible effect of phenobarbital in reducing the latent period for the production of bladder cancer in the dog by 4-aminobiphenyl, the most potent presently known bladder carcinogen in man and dog. Six pure-bred beagle dogs, approximately 6 months old, were given, by capsule, 1 mg/kg of 4-aminobiphenyl as a 1.5% w/v solution in corn oil on each of 5 days of the week until bladder tumors were observed on cystoscopy. The dogs were then killed for gross and histopathological examination. Three of these dogs received, in addition, a daily dose (capsule) of 20 mg/kg of phenobarbital on 5 days of the week. The 3 dogs given 4-aminobiphenyl developed multiple bladder tumors after 36 months (9.07 g/dog; 0.74 g/kg), 40 months (7.99 g/dog; 0.86 g/kg), and 40 months (5.75 g/dog; 0.80 g/kg), respectively. The dogs treated with phenobarbital plus 4-aminobiphenyl developed tumors after 23 months (4.38 g 4-aminobiphenyl per dog; 0.50 g/kg), 23 months (4.39 g/dog; 0.51 g/kg), and after 36 months (2.83 g/dog; 0.51 g/kg). While the number of dogs per group was small, the data are significant. Concomitant administration of phenobarbital and 4-aminobiphenyl reduced the dose producing bladder tumors from a mean of 0.80 g/kg/dog over 38 months to 0.51 g/kg/dog over a period of 27 months. The tumors were diagnosed as papillary transitional cell carcinomas of low grade malignancy (infiltrative bladder cancer).

- ✓ 154. *A Comparison of the Tumorigenicity of Cyclophosphamide and Urethan in Newborn Mice.*  
W. A. KELLY, L. W. NELSON, H. C. HAWKINS, and J. H. WEIKEL, JR., Mead Johnson Research Center, Evansville, Indiana.

The tumorigenic potential of cyclophosphamide was compared with the known tumorigen urethan by ip administration of normal saline or 0.8, 4.0, or 20.0 mg/kg of cyclophosphamide or 700.0 mg/kg of urethan to newborn offspring of Charles River CD<sup>®</sup>-1 strain mice at 0, 3 and 6 days of age. Tissues and organs from mice dying during the study or sacrificed at 79 weeks were examined grossly and microscopically. Cyclophosphamide was not leukemogenic. The incidence of malignant lymphoma was comparable for the control, low and intermediate dose levels and histologic evidence of this neoplastic disease was totally absent from the high-dose cyclophosphamide-treated mice. Liver neoplasms did not occur in the low and high-dose cyclophosphamide groups and the frequency in the intermediate-dose group was similar to that of the controls. The frequency of pulmonary adenoma development was slightly increased in the low and intermediate-dose cyclophosphamide-treated males and females and high-dose cyclophosphamide-treated females, but proliferative lesions were totally absent from the lungs of the high-dose cyclophosphamide-treated males. Urethan was leukemogenic since malignant lymphoma developed in a high percentage of the urethan-treated male and female mice. The urethan-treated males also had an increased incidence of lung and liver tumors and hyperplastic nodules.

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