

ABSTRACTS OF PAPERS

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Abstracts of Papers for the Ninth Annual Meeting of the
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March 15–19, 1970

1. *Effects of Amantadine Hydrochloride on Cleavage and Embryonic Development in the Rat and Rabbit.* J. K. LAMAR, F. J. CALHOUN, and A. G. DARR, Bureau of Medicine Laboratory, Food and Drug Administration, Washington, D.C.

Amantadine HCl (Symmetrel®) has been used as a prophylactic agent against the A₂ influenza virus. Previous studies by other workers have shown inhibition of cleavage and DNA synthesis in amantadine-treated sea urchin eggs. An investigation was undertaken to test the possible interference of amantadine HCl with fertilization, cleavage, implantation, and development of mammalian ova in vivo. Virgin Holtzman rats and New Zealand white rabbits were dosed orally with amantadine HCl (0, 50, 100 mg/kg) from 5 days prior to mating until day 6 of pregnancy. In rats, but not in rabbits, results of autopsies performed on day 14 of gestation showed significant decreases in the number of implantations and increases in the number of resorptions at 100 mg/kg. Teratology studies were performed in rats (0, 37, 50, and 100 mg/kg) and in rabbits (0 and 100 mg/kg) by administering the drug orally on days 7–14 of gestation. Autopsy was just before expected parturition. In rats increases in resorption and decreases in the number of pups per litter were noted at 50 and 100 mg/kg. Gross examination of rat pups at these dose levels revealed no malformations at 37 mg/kg. Malformations at 50 and 100 mg/kg included edema, malrotated hindlimbs, missing tail, stunting, and brachygnathia. Examination of cleared and alizarin stained skeletal preparations of fetuses revealed cases of absent ribs and absence of the lumbar and sacral portions of the spinal column in the 50 and 100 mg/kg groups. No gross malformations were observed in rabbit pups in control or 100 mg/kg groups. Thus, in rats but not in rabbits, amantadine HCl seems to be both embryotoxic and teratogenic. Teratogenicity in rats occurs at 50 mg/kg/day, or about 12 times the usual human dose.

2. *Effect of Antineoplastic Agents on Fertility of Male Mice as Determined by Serial Mating.* ROBERT L. DIXON and INSU P. LEE, National Cancer Institute, Bethesda, Maryland.

These experiments were performed to assess the utility of serial mating of mice, after treatment with anticancer agents, as a predictive model of drug action. Spermatogonia appear in the ejaculate as mature sperm after 35–42 days in mice. The relative durations of the other main spermatogenic stages are as follows: spermatocytes, 22–34 days; spermatids, 8–21 days; and spermatozoa, 1–7 days. Thus, the spermatogenic cell population provides distinct cell populations, DNA synthesizing, proliferating, and nonproliferating, whose function can subsequently be monitored by mating. Serial mating (mating the same treated male with a different untreated female at 5-day intervals) provides a fertility profile when percent conception is correlated with days after drug treatment. It is assumed that drug treatment does not significantly interfere with the timing of spermatogenic events, with sperm transport mechanisms, nor with the physical capacity to copulate. Cell turnover is known to be independent of frequency of pairing. The following prototype drugs were tested after a single treatment and injection ever 3 hr for 24 hr: methotrexate (NSC-740), cyclophosphamide (NSC-26271), cytosine arabinoside (NSC-63878), and vincristine (NSC-67574). A variety of newer anticancer agents have also been studied. Methotrexate and cytosine arabinoside, which inhibit DNA synthesis, resulted in a sharp decrease in conception after 5 weeks of normal reproduction. Cyclophosphamide, an alkylating agent, and vincristine, which blocks cell division, affected cells later in spermatogenesis as reflected by a much earlier loss and recovery of fertility. Serial mating is a useful model for the study of various aspects of cancer chemotherapy.

3. *The Effects of Carbaryl on Reproduction of the Rat and of the Gerbil.* THOMAS F. X. COLLINS, WALTER H. HANSEN, and HELLYN V. KEELER, Bureau of Science, Food and Drug Administration, Washington, D.C. 20204.

The pesticide carbaryl (Sevin) was fed at 0, 2000, 5000, and 10,000 ppm to Osborne-Mendel rats for three generations. At 10,000 ppm no litters were produced after the first litter of the second generation. Decreases were observed in the fertility, viability, survival, and lactation indices in all litters produced at 10,000 ppm. The survival index also showed a decrease at 5000 ppm. Apparently dose-related decreases were observed in the ratio of average number of animals weaned per number of litters at both 5000 and 10,000 ppm. A dose-related decrease was seen in weanling weights at all three dose levels.

In a comparative study three generations of gerbils were fed Sevin at dose levels of 0, 2000, 4000, 6000, and 10,000 ppm. No second litters were produced in the third generation at 10,000 ppm. Decreases were observed at 6000 and 10,000 ppm in the viability index. Dose-related decreases were seen at the same two dose levels in the survival index and in the average number of animals weaned per litter.

Results were similar for both species.

4. *The Failure of Caffeine to Induce Mutations or to Synergize the Mutagenic Effects of X-Rays or of Alkylating Agents in Mice.* SAMUEL S. EPSTEIN, Childrens Cancer Research Foundation, Inc., and Harvard Medical School, Boston, Massachusetts.

Caffeine at high concentrations is mutagenic to bacteria. Additionally, caffeine inhibits DNA repair following shortwave UV damage in bacteria. The presumptive relevance of such microbial data to human mutagenic hazards is, however, questionable in view of the wide range of metabolic and other differences obtaining between microbial and mammalian systems.

The dominant lethal assay is a relatively simple and sensitive method for assessing mutagenic effects of X-rays or of chemicals in mice. Early fetal deaths, in an untreated female mated to a male previously treated with a chemical under test, afford a convenient and precise parameter of mutagenic effects due to dominant lethal mutations. Using this system, caffeine was tested over a wide range of concentrations, acutely and chronically, in mice both by itself and in combination with X-rays and with various aziridine- and alkanesulfonate-alkylating agents. Caffeine alone did not induce dominant lethal mutagenic effects as measured directly by early fetal deaths or indirectly by preimplantation losses. Additionally, caffeine failed to augment or synergize mutagenic effects induced by alkylating agents or by X-rays.

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5. *Studies on the Mechanism of Diphenylhydantoin Teratogenicity.* R. D. HARBISON and B. A. BECKER, Oakdale Toxicology Center, College of Medicine, University of Iowa, Iowa City, Iowa.

Diphenylhydantoin (DPH), 87.5 mg/kg, administered ip to pregnant Swiss-Webster mice on gestational days 11, 12, and 13 produced an 85% incidence of cleft palate in the offspring. The metabolites of DPH are 5-(*p*-hydroxyphenyl)-5-phenylhydantoin (HPPH), diphenylhydantoic acid, and α -aminodiphenylacetic acid. These were similarly administered to pregnant mice at equimolar dosages to DPH, 87.5 mg/kg; the nonsignificant ($P < 0.05$) incidences of cleft palate was 2 ± 2 , 4 ± 2 , and $3 \pm 2\%$, respectively. Dosages 2 to 4 times equimolar also did not induce significant incidences of cleft palate nor affect fetal growth. These results suggest that DPH is the active teratogen. Alternatively, DPH can alter corticosteroid metabolism and stimulate release of teratogenic adrenocortical steroids. Cortisone is teratogenic in the Swiss-Webster strain of mouse. The spectrum of anomalies induced by treatment with cortisone during late embryogenesis is quite similar to that of DPH. If the teratogenic activity of DPH were the result of adrenocortical stimulation, then ablation of the adrenal should abolish DPH teratogenesis. Adrenalectomy (gestational day 10) reduced

DPH-induced (50 mg/kg, days 11, 12, and 13) orofacial anomalies from $28 \pm 10\%$ to $17 \pm 6\%$, a nonsignificant effect. The response to concomitantly administered teratogens was additive: DPH, 50 mg/kg, and cortisone acetate, 0.5 mg, administered on gestational days 11, 12, and 13 produced $28 \pm 10\%$ and $4 \pm 2\%$ cleft palate, respectively; concomitant administration of the two compounds produced $48 \pm 8\%$ cleft palate. These results suggest the teratogenic responses induced by cortisone and DPH are probably the result of different mechanisms of action. The teratogenic action of DPH may be enhanced by corticosteroids, but is apparently not mediated through release of endogenous maternal corticosteroids.

(Supported by NIH grant, No. GM-12,675.)

6. *Chronic Toxicity and Teratology Studies of L-Monosodium Glutamate and Related Compounds.* A. G. EBERT, Growth Sciences Center, International Minerals and Chemical Corporation. (R. J. Weir.)

Two-year feeding studies of L-monosodium glutamate (L-MSG), DL-monosodium glutamate (DL-MSG), and L-glutamic acid (L-GA) were carried out using Sprague-Dawley rats (75 animals of either sex per group + 150 controls) and C-57 black mice (100 per group, all males + 200 controls). Rats received diets containing 0.1 or 0.4% of test compound; mice 1 or 4%. Possible adverse effects on reproduction and the teratogenic potential of L-MSG were measured in rabbits (New Zealand white variety) following 2-3 weeks of feeding at 0.1, 0.825, or 8.25% in the diet. There were 22-24 does per group and 16 males per group, all having sired or produced one litter prior to test compound feeding. Test compound feeding was continued in pregnant rabbits throughout gestation.

In rats, there were no differences between treated and control groups with respect to weight gains, food intake, hematologic values, gross and histopathologic observations at any of the 3 intervals studied (241, 567, and 681 days). Survival rates at 2 years averaged 59% over all groups (range 53-63%). Gross abnormalities seen with increasing age included a white film over the cornea and a high incidence of fibrous tumors. Presumed tumor incidence at completion of the 730-day test averaged 40.1% across all treatments, 42.4% in controls. The most common microscopic lesion, seen in all groups, was a low grade inflammatory response in lungs, kidneys, and spleen.

In mice, however, only a single benign adenoma was found in controls or all L-MSG, DL-MSG, and L-GA treated animals. Gross and histopathologic observations suggested renal effects in DL-MSG treated animals. Other findings were within normal limits with the exception of low 2-year survival rates (20% overall) due to the combatant nature of this strain of mouse.

L-MSG, at all levels studied, was without deleterious effects on reproduction of rabbits with respect to number of fetal resorptions, birth rate, survival rate at weaning, presence of internal and external abnormalities, and skeletal development.

7. *The Teratogenic Effect of Lithium Carbonate Upon the Palate of Rando-bred Mice.* K. T. SZABO, A. M. HAWK, and M. HENRY, Smith Kline & French Laboratories, Philadelphia, Pennsylvania. (Joseph F. Borzelleca.)

Lithium carbonate, the salt of the lightest metal, has been indicated as a therapeutic agent for the manic phase of the manic-depressive psychosis. Determinations of plasma lithium levels were made on female mice (Charles River HaM/ICR strain) after they had been dosed with 100, 400, or 800 mg/kg lithium carbonate for 10 consecutive days. The corresponding plasma lithium concentrations were: 0.45, 1.25, and 4.26 meq/liter, respectively. On the basis of this study, three doses producing plasma lithium levels comparable to those of human therapeutic doses were investigated for embryotoxic and teratogenic effects in the same strain of mice. Lithium carbonate was given orally from day 6 through day 15 of pregnancy. Fetuses were delivered by Caesarean section on gestational day 18, weighed, and examined. Soft tissues were examined following Bouin fixation and freehand cross section. Cleared fetal skeletons were examined in alizarin red-stained preparations.

The incidences of cleft palate at doses of 200, 300, or 465 mg/kg body weight were 0.4, 6, and 30%, respectively; it was 0% for the controls. The incidence of cleft palate was related to both maternal and fetal weights, but not by the uterine position of the fetuses. The incidence of resorbed embryos was also dose-related, and the litter size was significantly reduced ($P < 0.01$) by a dose of 465 mg/kg.

8. *Embryotoxicity and Fetal Malformations of Rats and Mice Due to Maternally Administered Ether.* B. A. SCHWETZ and B. A. BECKER, Oakdale Toxicology Center, University of Iowa College of Medicine, Iowa City, Iowa.

Time-dated (mating equals day 1) pregnant Swiss-Webster mice and Sprague Dawley rats were anesthetized for 1 hr in a 5-liter, closed-circuit vapor exposure chamber (VEC) with diethyl ether during early or late embryogenesis. Preliminary studies indicated the required ether concentrations to maintain 1 hr anesthesia in the VEC were 6.5 volume percent (vol%) for mice and 7.3 vol% for rats. Mice were placed in the VEC initially containing ether, 6.5 vol%, for various lengths of time, 60 to 360 min. Twenty-four hours later, the number of dead per group was counted, and the median time to 50% kill was estimated. Fifty percent of the mice died after 100 (95% confidence limits: 85–125) min anesthesia. For rats, the median anesthesia time to effect 50% kill was 150 (130–175) min. Under these conditions blood-ether concentration during anesthesia for 1 hr was maximal at 45 min in mice (45 ± 2 mg%) and at 60 min in rats (46 ± 2 mg%). In both species, ether was not detectable in the blood 7 hr after anesthesia. Fetuses were delivered by Caesarian section 1 day before normal parturition, examined, measured, and prepared for examination for soft tissue and skeletal anomalies. In mice, ether anesthesia (in VEC at 6.5 vol% for 1 hr) during early organogenesis caused a significant incidence of fetal resorptions (14/56) and hydronephrosis (2/26). Anesthesia during early or late organogenesis caused a significant incidence of generalized edema (19/172), missing sternum (10/172), unossified phalanges (9/72), and missing cervical vertebrae (10/72). Anesthesia at either stage did not alter fetal body weight or crown-rump length. Length of fetal long bones was decreased by treatment during early organogenesis. In rats, ether anesthesia (in VEC at 7.3 vol% for 1 hr) did not increase the incidence of resorptions, or soft-tissue or skeletal anomalies. Anesthesia during early or late organogenesis did significantly decrease fetal body weight and length of long bones. Histologic examination of mouse and rat fetal brain, heart, kidney, liver, and skeletal muscle revealed no changes except hepatic parenchymal cell vacuolation in mouse livers. In summary, ether anesthesia is not highly teratogenic to either mice or rats. Rats are more resistant than mice to the embryotoxic and teratogenic effects of ether anesthesia. (Supported by NIH Grant GM-12675 and NIH Training Grant GM-1308.)

9. *Teratological Evaluation of Metaproterenol in the Rhesus Monkey (Macaca mulatta).* B. N. BANERJEE and G. WOODARD, Woodard Research Corporation, Herndon, Virginia 22070.

In order to further evaluate the teratological potential of metaproterenol (Alupent) an experiment was conducted in timed pregnant rhesus monkeys (*Macaca mulatta*). Pregnancy was established by bioassay of serum for chorionic gonadotropin according to the method of Tullner and Hertz using the uterine weight of immature mice. Metaproterenol as a 1 mg/ml solution in water was administered by stomach tube at a dose of 5 mg/kg (approximately three times the human dose) for 11 consecutive days beginning on days 18 to 23 of gestation in a total of 12 animals. At approximately the 100th day of gestation, the fetus was removed from each monkey by Caesarean section. Each fetus was examined for external abnormalities, the viscera for any deviations from normal, and the skeletons, after clearing and staining with alizarin red S, for any structural deviations. No changes were observed that could be attributed to the administration of metaproterenol. Four concurrent untreated pregnant animals were included.

10. *Dieldrin-DDT Interactions in Guinea Pigs*. D. J. WAGSTAFF and J. C. STREET, Interdepartmental Program in Toxicology and Department of Animal Science, Utah State University, Logan, Utah 84321. (J. L. Shupe).

Dieldrin storage in rats is antagonized by concurrent consumption of DDT. The theorized mechanism is induction of dieldrin-metabolizing microsomal enzymes. For this report, dieldrin-*DDT* interactions were studied in adolescent male guinea pigs on experimental diets for 14 days. Pesticides were determined in body fat by gas-liquid chromatography, and hepatic microsomal enzyme activity was assayed by *in vitro* *O*-demethylation of *p*-nitroanisole. DDT fed at 50 ppm did not antagonize storage of dieldrin concurrently consumed at 1 ppm. Indeed the reverse was suggested, dieldrin antagonized storage of DDT. This was verified by a later experiment, in which DDT storage was reduced 76% in guinea pigs fed 25 ppm DDT when 50 ppm dieldrin was also fed. Dieldrin was likewise a more potent inducer of microsomal enzyme activity in guinea pigs than was DDT.

11. *Cytogenetic Effects of DDT and Derivatives of DDT in a Cultured Mammalian Cell Line*. K. A. PALMER, S. GREEN, and M. S. LEGATOR, Food and Drug Administration, Washington, D.C. (Leonard Friedman.)

Cytogenetic effects of *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDD, *o,p'*-DDD, *p,p'*-DDE, *o,p'*-DDE, and *p,p'*-DDA were investigated in a cultured cell line derived from the kidney of a marsupial (rat kangaroo). In this cell line, *p,p'*-DDA was toxic at a concentration of 200 $\mu\text{g/ml}$. The other compounds prevented cell growth at 50 $\mu\text{g/ml}$. The *p,p'* forms of DDT, DDD, and DDE produced twice as many chromosome breaks as did the corresponding *o,p'* forms. At a concentration of 10 $\mu\text{g/ml}$, *p,p'*-DDT showed the highest number of breaks, 23%, with *p,p'*-DDD showing 16%, and *p,p'*-DDE showing 14%. Of these total breaks, approximately 12% were exchange figures in the case of *p,p'*-DDT and *p,p'*-DDE and 1.2% in the case of *p,p'*-DDD. The *o,p'* forms and the control cells exhibited no exchange figures. The frequency of total chromosome breaks in the controls was 2%.

12. *DDT Concentrations in Blood, Brain, and CSF of Rats After DDT-Induced Convulsions*. GLENN MORRISON, Food and Drug Administration, Perrine Primate Research Branch, Box 490, Perrine, Florida.

This study was undertaken to ascertain the absolute and relative amounts of *p,p'*-DDT in blood, brain, and cerebral spinal fluid (CSF) at a given time after the oral administration of an intoxicating dose of the compound in order to elucidate the role of CSF in the pathophysiology of DDT intoxication. Although there have been previous reports of blood and brain concentrations of DDT, our study appears to represent the first reported data of DDT in CSF.

Adult male and female rats were orally dosed with 200, 400, or 600 mg/kg of *p,p'*-DDT. When convulsions occurred, samples of CSF, blood, and brain were collected and assayed for *p,p'*-DDT. Means and standard deviations of 0.12 ± 0.03 , 3.4 ± 1.1 , and 16 ± 3 ppm, respectively, were found. Neither the sex of the animal nor the dosage given appeared to affect the convulsion-associated concentrations in these three tissues.

Blood and brain have lipid concentrations of approximately 400 and 8000 times that of CSF. However, in this study the blood and brain DDT concentrations were only 30 and 130 times that found in the CSF. Thus there was more DDT in the CSF than would be anticipated from its lipid content alone. The significance of this finding regarding a possible storage and/or transport role for CSF in the dynamics of DDT intoxication remains wholly speculative.

13. *Dynamics of DDT Storage in the Squirrel Monkey*. M. CRANMER, F. COPELAND, and J. CARROLL, Food and Drug Administration, Perrine Primate Research Branch, P.O. Box 490, Perrine, Florida.

It is often desirable to study exogenous chemicals including pesticides and drugs to ascertain a species which stores and metabolizes the material in a manner similar to man. This investiga-

tion was undertaken to study the tissue residues of p,p'-DDT and metabolites in the squirrel monkey.

Thirty squirrel monkeys were divided into five groups of six animals each and were dosed daily with 0.0, 0.05, 0.5, 5.0, and 50 mg p,p'-DDT/kg body weight/day. Two animals of each group were sacrificed after 2, 4, or 6 months of treatment. Blood samples were taken every 2 weeks. The levels of p,p'-DDT, p,p'-DDD, and p,p'-DDE were determined for brain, fat, liver, kidney, adrenal, bone marrow, and blood. The activity of liver microsomal enzymes was determined at the time of sacrifice by studying hydrolysis of paranitroanisole and EPN.

The concentrations of p,p'-DDT and metabolites did not plateau in any of the treatment groups. The levels of p,p'-DDT stored in the group receiving 5.0 mg/kg decreased during the last 2 months of the experiment. Liver microsomal enzymes were stimulated by the 5.0 and 0.5 mg/kg/day doses.

14. *Comparative Toxicologic Studies of Selected Organochlorine Pesticides in Native and Laboratory Mice.* MARK M. LUCKENS, College of Pharmacy, University of Kentucky Medical Center, Lexington, Kentucky 40506.

This report is part of an ongoing study of the toxicity and storage of pesticides in native mice. Since most toxicity data are derived from laboratory animals which are usually inbred and reared under controlled conditions, data from such test animals are not comparable to those derived from animals born and living under natural conditions and whose genetic makeup is much more heterogeneous. The LD50, for various time periods, was determined (in parallel) in Swiss-Webster mice as well as in *M. pennsylvanicus* and *P. leucopus*. The wild species were trapped during the fall and early winter in an area with no history of spraying with insecticides. The latter point was confirmed by analyses of the soil and forage sampled at the time of trapping. In general, the native mice were found to be more resistant to the insecticides studied. The Microtine mice, while usually less resistant than the Peromyscines, were found to be more resistant to toxaphene, than either the latter or Swiss-Webster mice.

It was found that there was an interspecies difference in the response of the Peromyscine animals to endrin. This study documents the response of animals-in-the-wild to insecticides used in agriculture, compares these data with that obtained from the usual laboratory test animal, and emphasizes that species, strain, and environmental history of the test animal used in the bioassay affects the results obtained therefrom.

(Supported by Grant No. FD-00254.)

15. *Organochlorine Insecticides in Normal and Carcinogenic Human Lung Tissues.* JACK C. DACRE and RALPH W. JENNINGS, Food and Drug Administration, U.S. Department of Health, Education and Welfare, Atlanta, Georgia 30333.

Human lung tissues from persons in New Zealand who had no known occupational exposure to pesticides and who died during 1967-68 were analysed for residual organochlorine insecticides. The extraction and cleanup was carried out according to the method of Jennings. The extracts were analysed by electron-capture, gas-liquid chromatography using glass columns packed with 3% QF-1 on 60/80 mesh, acid-washed, DMCS-treated Chromosorb G.

The following values (ppb, \bar{x} + SE) were found: dieldrin, 5.79 + 1.18; p,p'-DDT, 7.69 + 1.13; p,p'-DDE, 14.24 + 3.05; p,p'-DDD, 5.90 + 1.17; o,p'-DDT, 2.29 + 0.61; o,p'-DDE, 2.29 + 0.54; o,p'-DDD, not found; total as DDT, 34.39 + 5.19; α -BHC, 0.39 + 0.09; β -BHC, 1.99 + 0.39; γ -BHC, 1.64 + 0.23; δ -BHC, present in two samples only. There were no significant differences between the 13 males and the 13 females. When the values for persons known to have died of lung cancer were compared to those without lung cancer, statistically significantly higher concentrations of DDT and dieldrin were found in those with lung cancer.

16. *Dieldrin Induced Hyperglycemia in Rats.* J. W. HIDDEMEN and H. H. CORNISH, School of Public Health, The University of Michigan, Ann Arbor, Michigan.

The biochemical and pharmacological effects of the chlorinated hydrocarbon pesticide, dieldrin, have been studied by many investigators; however, some effects have never been

fully elucidated. Adult male albino Sprague-Dawley rats weighing 250–300 g were dosed ip with technical dieldrin (95+%) at 25 mg/kg in corn oil for 1, 2, 3, or 4 days, respectively. Control rats were dosed for the same number of days with 0.25 ml of corn oil per rat. After the last injection, each group was deprived of food for 21 hr, and blood samples were obtained at the end of this period and subsequently analyzed for glucose. Those groups of rats receiving the daily dieldrin injections for 1, 2, 3, or 4 days had fasting blood sugar levels 18, 24, 47, and 60% higher than those of the controls. In these same rats, corticosterone levels in the plasma were determined and found to be 45, 102, 97, and 98% higher than the control values in each respective group. Adrenalectomized rats dosed with similar levels of dieldrin failed to show a hyperglycemic response. Two groups of rats were placed on a 10-day subacute study in which the experimental group received a daily ip injection of 5 mg/kg of dieldrin, and the control group received an injection of corn oil. The fasting blood glucose levels in the rats receiving dieldrin were 25% higher than in the control group. It was concluded from the data obtained that dieldrin had an effect on the normal utilization and metabolism of endogenous glucose, and an effect on the adrenal cortex resulting in prolonged elevated corticosterone levels.

(Supported in part by N.I.H. Grant GM-15269 and 8-TOL EC 00037-06.)

17. *Some Observations on the Comparative Toxicity of Four Organochlorine Pesticides After Short and Prolonged Oral Administration in Rats.* L. R. WEISS, R. BRODIE, J. F. REILLY, and S. KROP, Bureau of Science, Food and Drug Administration Washington, D.C. 20204.

Dieldrin (10 and 50 ppm), chlordane (10 and 50 ppm), DDT (50 and 250 ppm), and methoxychlor (500 and 2500 ppm) in the diet were given to adult male rats for short or prolonged periods to correlate certain functional effects with morphologic changes. Weekly food intakes and weight changes were measured for 4 weeks after which hexobarbital sleep times (HST), pentylenetetrazol (PTZ), and hexafluorodiethyl ether (HFE) convulsive thresholds and spontaneous motor activity (SMA) were determined in half the animals. After 6 months, cardiovascular effects (arterial pressure, heart rates, EKG, and pressure responses to acetylcholine, epinephrine, and norepinephrine) were assessed in the "high-dose" rats. The surviving "low-dose" groups were tested at 12 months for differences in HST, PTZ, HFE, weight gain, brain and liver body weight ratios, and pathologic lesions.

The results showed weight loss, decreased food intakes, increased HST, and tendencies for elevated PTZ and HFE thresholds with methoxychlor at 1 and 12 months. Hepatomegaly was seen with high and low DDT and high dieldrin intakes at 1 month. Significantly decreased HST were noted with low and high doses of dieldrin, DDT, and chlordane at 1 month and to a lesser degree with low doses at 12 months. Survival rates were unaffected by these doses of the pesticides. All of the pesticides appeared to increase SMA to a statistically nonsignificant extent. Decreases in various PTZ threshold values were found with "high-dose" dieldrin and DDT after 1 month feeding; after 12 months feeding, "low-dose" dieldrin, DDT, and chlordane showed a trend towards decreased PTZ and HFE thresholds. Cardiovascular effects at 6 months did not differ from controls. These findings on the CNS, hepatic, and cardiovascular systems are consistent with the suggestion that liver enzyme induction is one of the more sensitive measurements of low level exposure to the organochlorine pesticides.

18. *Subacute Toxicity of Photodieldrin 3,4,5,6,6,7-hexachloro-12-oxahexacyclo-(6,5,0,0²,10,0³,7,05,9,0¹¹,13) tridecane, a Photodecomposition Product of Dieldrin.* MAE S. WALTON, VIVIAN BECK, and RONALD L. BARON, Bureau of Science, Food and Drug Administration, Washington, D.C. 20204. (Clara H. Williams.)

The toxicological hazard of photodieldrin, a photodecomposition product of dieldrin, which has been found as a residue on certain agricultural commodities treated with the dieldrin pesticide has not been fully evaluated. A 90-day subacute rat feeding study with dieldrin and photodieldrin was performed using concentrations of 1, 5, and 25 ppm in the diet. In addition to gross evidence of toxicity, several biochemical parameters and spontaneous motor activity were examined.

Diet concentrations of photodieldrin were lowered from 25 to 12.5 ppm within the first week because of toxicity. At the end of 90 days no significant differences were observed in growth, food consumption, or gross evidence of toxicity. Enzyme induction was apparently dose-dependent as evidenced by activity or concentration increases in liver microsomal aniline hydroxylase and microsomal cytochrome P450. Liver aliesterase activity was slightly decreased, but the effect was not dose-dependent. Total protein content of the liver was not affected by the pesticide.

While photodieldrin was more acutely toxic than dieldrin, it appeared that subacutely the two compounds exerted a similar effect at the levels studied. These findings indicate that a "no-effect" level for photodieldrin is probably below 1 ppm although the toxicological significance of a slight increase in the mixed function oxidase system is not fully apparent.

19. *Oncogenic Bioassay of N-Nitrosodiethylamine in Newborn Rodents.* JAMES L. GARGUS and LEONARD D. SHOTT, Hazleton Laboratories, Inc., Falls Church, Virginia. (D. C. Jessup.)

The objective of these studies was the bioassay of *N*-nitrosodiethylamine (DNA) as an oncogenic compound in newborn rodents. ICR Swiss mice, 24 hr of age, received a single (maximum tolerated) sc dose of 0.25 mg per animal (50 mg/kg). At 6 months the lung adenoma incidence was 80% with a mean number per animal of 2.96; control values were 16% and 0.16. In another experiment, mice were given DNA sc at the age of 24 hr, followed by an ip dose at 7 days of age, and oral doses at 14 and 21 days (total dose 1.3 mg). At 6 months the lung adenoma incidence was 92% with a mean of 7.2; control values were 17% and 0.19. Sprague-Dawley rats received one sc dose and four oral doses during the neonatal period (total dose 5.1 mg). Lung, liver, and kidney were examined for pathologic effects.

In both mouse experiments the lung adenomas in the control and treated animals were similar histologically. No other abnormalities attributable to DNA were observed in the lungs. Nearly all mice exhibited hepatocytic carcinoma. Neoplasia was not observed in kidney sections from the mice. Lung adenomas were not observed in test or control rats. Hepatic cell carcinoma was observed in test rats. Renal neoplasms observed in DNA-treated rats were either papilliferous or sclerosing carcinomas.

In neonatal mice, a limited number of exposures to DNA increased the incidence of lung adenoma and induced hepatic neoplasia. In rats neoplasia was induced in the liver and kidney. These effects, observed within 6 months of compound administration, confirm the value of newborn rodents in the bioassay of this chemical carcinogen.

20. *Influence of Dosage Scheduling on Toxicity and Effectiveness of Antitumor Chemotherapeutic Agents in Mice.* ELTON R. HOMAN, National Cancer Institute, Bethesda, Maryland; ROBERT P. ZENDZIAN, Hazleton Laboratories, Falls Church, Virginia; and GARY L. NEIL, The Upjohn Co., Kalamazoo, Michigan.

Many cancer chemotherapeutic agents act selectively on a single phase of the cell cycle. A simple model of cell kill kinetics predicts maximal toxicity at the cellular level from divided doses of cell cycle phase specific drugs when the interval is half the cell cycle time. We undertook experimental studies to determine whether when 1- β -D-arabinosyl-cytosine (Ara-C) is administered to mice as divided doses, there is a dosage interval at which the net effects on the whole animal are maximal. Ara-C was consistently more toxic at intervals near 10.5 hr as reflected in net lethality and mean survival time.

The effect of dosage interval on therapeutic effectiveness in L1210 leukemic mice was also studied. Both efficacy and toxicity, as measured by increase in survival time and weight loss, respectively, are sensitive to the interval employed. Although we achieved maximum therapeutic effectiveness with intervals of 9 to 10 hr, which also produced the greatest weight loss, we obtained excellent results at other intervals without weight loss.

21. *The Variable Susceptibility of Inbred Strains of Syrian Hamsters to Subcutaneous Injection of 9,10-Dimethyl-1,2-benzanthracene (DMBA)*. F. HOMBURGER and S.-S HSUEH, Bio-Research Institute, Cambridge, Massachusetts.

Syrian hamsters are being used increasingly in studies on chemical carcinogenesis. Until recently most of this work has been done in random bred hamsters of unknown genetic background. At present 27 inbred lines of Syrian hamsters developed by brother × sister matings for up to 50 generations are available at this laboratory and are designated as BIO[®] hamsters. It is important to know whether genetic factors determine the susceptibility of this species to chemical carcinogens. The response of ten of these inbred lines of BIO[®] hamsters to a single sc injection of 0.5 mg of DMBA has been studied. In nine of these lines, the time elapsing between carcinogen injection and appearance of a palpable fibrosarcoma was 13 to 16 weeks with no significant differences between lines at $p = 0.05$. In the BIO[®] 15.16 line, on the contrary, this time of average latency was 10 weeks, the difference being highly significant at $p = 0.001$. The malignant nature of the induced tumors was confirmed by transplantation studies. Genetic differences clearly influence the neoplastic response of Syrian hamster sc tissue to the injection of DMBA. It may be concluded that studies on chemical sc carcinogenesis in Syrian hamsters will not be reproducible unless inbred lines or their hybrids are used.

(Supported by USPHS Research Grant No. CA 10101.)

22. *Phenester (NSC-116 785): Convulsive Properties of an Alkylating Agent with Antitumor Activity*. U. SCHAEPPI, G. R. THOMPSON, S. STADNICKI, R. PHELAN, D. A. COONEY, and R. D. DAVIS, Mason Research Institute, Worcester, Massachusetts, and National Cancer Institute, Bethesda, Maryland. (M. M. Mason.)

Phenester, acetic acid [*p*-(bis(2-chlorethyl)amino)phenyl]ethyl ester] is known to possess marked oncolytic activity against the mammary adenocarcinoma 13,762 in rats. For evaluation of toxicity 12 dogs were treated with daily doses of 80, 28, 14, 7, or 3.5 mg/kg/day for 5 consecutive days.

The essential toxicity consisted of centrally induced vomiting, muscle twitches, tremors, and finally clonic and tonic convulsions. EEG recordings (2 dogs) from thalamus and cerebral cortex demonstrated subcortical and cortical spikes and hypersynchronous slow waves which, during tonic convulsions, progressed into cortical and subcortical seizures followed by postictal silence. The spikes appeared first in the subcortex and later in both subcortex and cortex. Six dogs developed tonic convulsions and five of them died or became moribund on treatment days or soon thereafter. Mortality resulted from treatment with 28 mg/kg (2/2), 14 mg/kg (1/2), or 7 mg/kg (2/5). The other dogs, including those treated with 80 mg/kg (1 dog) or with 3.5 mg/kg (2 dogs), survived. Evidently mortality and convulsions were not strictly dose related; however, mortality depended upon the appearance of neurotoxicity. Survivors exhibited dose related inanition. Several dogs reacted with marked lymphopenia, moderate neutropenia, thrombopenia, anemia, hepatic, renal or intestinal toxicity. At autopsy there occurred hypoplasia of the lymphoid tissues and the bone marrow, and fatty changes of the myocardium, and hepatocytes. One dog showed degeneration of the spermatogenic cells.

Treatment with 3.5 mg/kg/day for 5 consecutive days elicited vomiting but was otherwise tolerated without adverse side effects (MTD). A single dose of 5 mg/kg produced no toxic manifestations.

Therefore Phenester produces the same overall toxic effects as other alkylating agents (e.g., Phenestrin), yet with Phenester convulsive properties predominate.

(Supported by Contract PH 43-65-51 with the Chemotherapy Program, National Cancer Institute, USPHS.)

23. *The Chronic Effect of Dietary 8-Hydroxyquinoline Alone and with the Carcinogen N-2-Fluorenylacetamide in Rats*. R. S. YAMAMOTO, H. H. FRANKEL, and J. H. WEISBURGER, National Cancer Institute, Bethesda, Maryland 20014.

The reported carcinogenicity of 8-hydroxyquinoline (HOQ) was reinvestigated, and the possible effect of HOQ on the carcinogenicity of *N*-2-fluorenylacetamide (FAA), similar

to the inhibiting action of acetanilide, was also examined. Male Fischer rats were fed diets with 0.8% HOQ for 16 weeks (followed by control diet for an additional 10 weeks), for 52, and for 78 weeks; other groups were given HOQ + 0.02% FAA, or FAA alone, for 16 weeks and control diet for 10 weeks, or control diet alone throughout; also studied were rats on HOQ + FAA for 52 weeks, and appropriate HOQ, FAA, and control groups. In the 16 + 10-weeks test HOQ reduced the toxicity and the carcinogenicity to the liver of FAA. In the longer experiment also all rats on FAA alone were dead with hepatoma in about 34 weeks, but rats on HOQ + FAA survived and had hepatoma at 52 weeks. Rats on HOQ alone for 26, 52, or 78 weeks had no cancer ascribable to HOQ. These animals, however, had enlarged spleens and livers with excessive iron deposition which increased in severity with time on HOQ. Thus, 0.8% dietary HOQ led to hemochromatosis but was not carcinogenic. HOQ reduced the toxicity and carcinogenicity of FAA to the liver of rats.

24. *Effect of Dipyridamole on the Toxicity of Various Antineoplastic Agents.* INSU P. LEE and ROBERT L. DIXON, National Cancer Institute, Bethesda, Maryland.

Dipyridamole (Persantin) is a structural analog of the purine and pyrimidine nucleosides and is used clinically as a vasodilator. It is pharmacologically a smooth muscle relaxant with properties very similar to those of papaverine. Dipyridamole has been reported to decrease nucleotide breakdown and also to inhibit the transport of adenosine by erythrocytes and nucleosides by chick fibroblasts. We have investigated the effect of dipyridamole on the toxicity, experimental therapeutic efficacy, and uptake of various purine and pyrimidine antimetabolites by tumor cells.

Dipyridamole, 1 mg/kg administered ip to mice 20 min prior to the administration of antineoplastic agents, significantly reduced the lethality of 6-mercaptopurine (NSC-755), fluorouracil (NSC-19893), and cytosine arabinoside (NSC-63878). Dipyridamole also decreased the relative effectiveness of these drugs in prolonging the survival times of mice inoculated with L1210 leukemia.

Preliminary in vitro studies indicated that dipyridamole at a concentration of 10^{-5} M was capable of decreasing the uptake of various purine and pyrimidines and their antimetabolites by L1210 leukemia and 6C3HED lymphosarcoma cells.

25. *Toxicity and Carcinogenesis from Various Chemicals Used in the Preparation of Vaccines.* M. M. MASON and C. C. CATE, Mason Research Institute, Worcester, Massachusetts.

Since many chemical compounds are used in the manufacture of vaccines, it was decided to determine the toxic and carcinogenic potential of seven representative ones: benzethonium chloride, ethylene chlorohydrin, ethylene glycol, thimerosal (Merthiolate), methylparaben, phenol red and pyridine. Fisher rats were used in both the toxicity as well as the carcinogenicity trials. The single LD50 and the maximum tolerated dose for 30 daily doses were determined for each compound so that the maximum tolerated dose could be estimated for twice weekly administration for 1 year.

The toxicity of the compounds given over a period of 1 year did not exceed the estimates based on the preliminary toxicity trials. At the end of 1 year, the mortality of the treated groups (1.85%) did not exceed that of the untreated and vehicle treated controls (2.0%), and after 18 months the treated groups had a mortality of 6.5% while the average of the controls was 7.05%. After 18 months, benzethonium chloride and Merthiolate at the highest doses caused decreased weight gains, as compared with the controls of 12 and 22%, respectively. At the lower doses all compounds allowed weight gains similar to the controls.

Benzethonium chloride gave rise to 26 injection site-related tumors in the 200 treated animals; all the other test groups had from two to four such tumors, while the controls had none. The high incidence of injection site tumors was correlated with a high incidence of induration and granulomas caused presumably by the irritating activity of the subcutaneously injected compounds. The tumors were fibrosarcomas of which none metastasized. Merthiolate had numerous injection site indurations and was second highest with fibromas. Many tumors were observed that had no relation to the injection site. Mammary fibroadenomas were

common to all groups, and the incidence varied from 2 to 5%. The methylparaben group showed an incidence of 8%. Testicular tumors were found in most of the males that lived to 18 months. These are interstitial cell tumors peculiar to the Fisher rat. It is noteworthy that Merthiolate caused a dose-related inhibition of these tumors. Uterine polyps were seen commonly in all groups, and the test groups had an incidence range of 4 to 11% as compared to 10% in the controls.

Eighteen pituitary adenomas were found in the 1800 rats. Fifteen of these were in females. The ethylene chlorohydrin group had 7 in the 100 females with none in the males. Of the eight adrenal tumors seen, six occurred in males, but there was no significance in the test group incidence. Seventeen leukemias were found distributed throughout all the groups. The only noteworthy finding was that 15 of the 17 occurred in females.

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26. *Fate of Sodium Cyclamate-¹⁴C in the Rhesus Monkey (M. mulatta)*. C. PAREKH, E. K. GOLDBERG, and L. GOLDBERG, Institute of Experimental Pathology and Toxicology, Albany Medical College, Albany, New York.

Golberg *et al.* reported the conversion in man of cyclamate to cyclohexylamine (CHA), *N*-hydroxycyclohexylamine (NHC), cyclohexanone, and cyclohexanol. In mongrel dogs CHA was the only transformation product observed. The regularity of urinary excretion of CHA was greater in rhesus monkeys than in dogs, although the extent of conversion was still small. In order to check whether the monkey could serve as an animal model for human cyclamate degradation, cyclamate-¹⁴C was given as a single oral dose (200 mg/kg) to three untreated monkeys and to five which had received the same dose of cyclamate daily 5 days a week for 56 weeks. Previously untreated control monkeys excreted 65.6% of the radioactivity in urine and 29.9% in feces; thus urinary excretion exceeded that observed in man and dog. Animals previously treated with cyclamate excreted 61.8% in urine and 34.8% in feces, results not significantly different from controls. Tissue radioactivity at 9 days totalled less than 0.2% of the dose.

Identifiable degradation products in methylene chloride extracts of urine and feces represented only 0.1–0.2% of the dose of cyclamate-¹⁴C. As in man, CHA was the main product and NHC, cyclohexanone, and cyclohexanol were present in urine but not in feces. In the monkey, as in man and dog, intestinal bacteria are mainly if not entirely responsible for CHA formation. Although it is likely that NHC is formed from CHA endogenously, no tissue site has yet been identified for this reaction.

27. *Myocardial Lesions Induced by Calcium Cyclamate in Syrian Hamsters*. E. BAJUSZ, Bio-Research Institute, Cambridge, Massachusetts. (F. Homburger.)

Histological and histochemical reassessments of the toxicology of Ca-cyclamate in hamsters revealed myocardial lesions accompanied by coronary sclerosis as well as soft tissue calcification in other organs. Groups of 20 virgin female BIO hamsters of the London School of Hygiene line with average initial weight of 86 g were used. Ca-cyclamate (0.2 g in 2.0 ml of dist water) or an equimolecular amount of Ca in form of CaCl₂, Ca acetate, Ca aspartate, or Ca ascorbate, respectively, was administered by stomach tube twice daily during the first 2 days and 3 times every day thereafter until the termination of the experiment on the morning of the 6th day. Ca-cyclamate—unlike the other Ca salts—regularly induced calcified, focal myocardial degeneration; Mönckeberg type of coronary sclerosis, skeletal muscle lesions, and cortical nephrocalcinosis were also evident in the majority of these animals. Among the various Ca salts tried, only Ca-cyclamate elevated significantly the concentration of myocardial Ca, prior to the occurrence of cardiac pathology, from a normal value of 11.9 ± 0.47 to 318.2 ± 41.8 (mg/100 g dry weight) in another experiment of 3 days' duration. Additional studies indicated that: alloxan-diabetic hamsters were much more sensitive than healthy ones to the adverse effect of Ca-cyclamate; vitamin D derivatives (e.g., dihydro-tachysterol) greatly sensitized the cardiovascular system to the toxic actions of Ca-cyclamate;

Na-cyclamate when given to hamsters with hereditary cardiomyopathy more rapidly elicits cardiocirculatory congestion including edema formation than equimolecular amounts of Na given in the form of NaCl. It seems that cyclamate enhances the toxicity of Ca as well as of Na with either of which it forms a stable, water-soluble salt.

(Supported by Bio-Research Consultants, Inc.)

28. *The Comparative Toxicity and Biotransformation of Phenol.* FREDERICK W. OEHME and LLOYD E. DAVIS, Kansas State University, Manhattan, Kansas, and Ohio State University, Columbus, Ohio.

Phenol toxicity and biotransformation were investigated in several animal species in order to examine the reported sensitivity of cats to phenol and to clarify species differences in detoxication pathways. ^{14}C -Phenol (P) was administered iv to dogs, cats, pigs, and goats at three dose levels. Clinical response, urinary changes, microscopic lesions, and plasma and urinary kinetic data were determined. Death occurred consistently in cats given 50 mg/kg, while only severe toxicity resulted in dogs, pigs, and goats given 100 mg/kg. Neuromuscular irritability, coma, and convulsions were seen. Intravascular hemolysis and green-brown urine containing protein, hemoglobin, and bilirubin occurred commonly. Microscopic lesions were found primarily in the kidney. Plasma disappearance of injected P occurred most rapidly in goats, followed in order of decreasing rate by pigs, dogs, and cats. Fat sequestration was suggested in all species but cats. Urinary metabolites were free P, phenylsulfate (PS), and phenylglucuronide (PG). Dogs excreted the injected P as PG, PS, and free P; Cats excreted primarily PS and smaller quantities of PG; pigs excreted large quantities of PG, with smaller amounts of free P and PS; goats excreted large quantities of PS, moderate amounts of PG, and minor portions of free P. The proportion excreted as PG increased as the dosage was increased in all species but cats. Highest rates of PS excretion were found in goats, while pigs had the highest rate of PG excretion. Cats had the lowest rate of PG excretion. Rate of P metabolite excretion increased with dose. Except for cats, the rate of PG excretion progressively increased with dose, thus supporting reports of a quantitative deficiency in ability to form glucuronides. At high doses of P the rate of PG excretion predominated over the rate of PS excretion and suggested early saturation of the PS pathway. Cats were shown to be more susceptible to P and to have more limited detoxication schemes for P than did the other species examined.

(Supported by Grants 5-FO3-GM36359 and GM12386 from USPHS and a grant from the Morris Animal Foundation.)

29. *Factors Modifying Excretion of Salicylate by the Dog, Comparison of Urinary and Salivary Routes.* J. W. PUTNEY, JR. and J. F. BORZELLECA, Medical College of Virginia, Richmond, Virginia.

Although the level of drugs and toxic agents in urine is used as an index of the level in blood and tissue, saliva is perhaps a more reliable monitor. In order to determine whether salivary levels were dose related in a manner similar to those in urine, mongrel dogs, anesthetized with sodium pentobarbital, received 0.5 mg/kg pilocarpine and 25, 50, or 75 mg/kg sodium salicylate (Na Sal) via the femoral vein. Over a period of 6 hr, salicylate levels in plasma, saliva, and urine were found to be dose related over the experimental range.

In order to determine the effect of parameters other than dose on salivary levels of the drug, dogs were infused, via the femoral vein, with one of the following at 0.15 ml/kg/min for 3 hr: NaHCO_3 (80 mg/ml), HCl (9.8 mg/ml), saline (9.0 mg/ml), glucose (55 mg/ml). At the end of 1 hr, 75 mg/kg Na Sal was administered iv. Blood, urine, and parotid saliva were sampled every 30 min throughout the experimental period. Control dogs received 75 mg/kg Na Sal. The infusion of saline and glucose did not alter the pH of blood, saliva, or urine. The infusion of NaHCO_3 and HCl raised and lowered, respectively, the pH of both blood and urine but did not markedly affect the pH of saliva. The only effect observed was a 16-fold increase in the total urinary salicylate output following the infusion of NaHCO_3 . Saline and glucose

infusion increased salivary salicylate levels during the periods of infusion, and thereafter levels fell to those of control. Salivary salicylate levels in HCl- as well as NaHCO₃-infused dogs increased in a similar manner, but were not noticeably different from one another or from levels in dogs infused with saline or glucose. These data suggest that increased hydration of the animal may result in an increase in the salivary elimination of salicylate by affecting the rates of filtration, secretion and/or reabsorption. Elevation or depression of blood pH appears to have no effect on salivary levels of the drug.

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30. *Preclinical Toxicology of NSC 68075 (Thalicarpine) in Mice, Hamsters, and Dogs, Including Physiological Disposition Studies.* P. E. PALM, M. S. NICK, D. W. YESAIR, M. M. CALLAHAN, and C. J. KENSLE, Life Sciences Division, Arthur D. Little, Inc., Cambridge, Massachusetts.

The dimeric isoquinoline alkaloid thalicarpine is of interest as a cancer chemotherapeutic agent. The single-dose ip and po LD₅₀ values for this drug in mice are 247.2 and 1542.5 mg/kg, respectively. Solutions of thalicarpine applied topically to the hamster cheek pouch produced severe vasoconstriction accompanied by a marked decrease in blood flow with hemostasis at concentrations of > 1.0 mg/ml.

A dose of 15.6 mg/kg × 1 administered iv to a dog produced immediate polypnea, slight to moderate bilateral râles, and mydriasis, with increases in heart and respiratory rates noted days 2-4. At 31.3 and 62.6 mg/kg × 1, mydriasis and severe dose-related cardiopulmonary effects were noted with death occurring at the higher dose. The maximum tolerated repeated dose in dogs appeared to be ~ 5.2 mg/kg, following either a 5-day or 5-day-repeated course regimen; however, slight transitory cardiopulmonary effects and mydriasis were detected with this dose. Transient elevations in SGPT and/or SGOT were observed in 2/4, 4/4, and 4/4 dogs surviving repeated doses at levels of 5.2, 10.4, and 20.8 mg/kg, respectively. Acute death, presumably of cardiovascular origin, occurred in 3/3 female dogs after 2 doses of 41.6 mg/kg, compared to 0/2 male dogs administered 5 doses of 41.6 mg/kg. Micropathologic observations in dogs which survived repeated doses ≥ 10.4 mg/kg included: focal cloudy swelling of kidney proximal tubules; liver degeneration shown by reduced glycogen content, central congestion, and cloudy swelling of parenchymal cells; and, in the heart, a reduction in ventricular ATPase activity. Tissues from dogs which died also showed pulmonary edema and hemorrhage; congestion of kidneys and liver; and myocardial edema and swelling (1/4). The compound was cleared from the plasma and deposited in tissues within 10 min after injection. About 1% of the drug appeared in the urine. In dogs sacrificed 24 hr after the last dose, the highest concentrations of drug were found in the liver, lung, kidney, pancreas, spleen, and heart. No drug could be detected in tissues of dogs sacrificed 30-47 days after the last dose.

(Supported by Contract No. PH-43-65-61 with Chemotherapy, National Cancer Institute.)

31. *Metabolism of Labeled Dypnone Guanyldiazone Hydrochloride (WR-5677) in Monkeys and Rats.* CARL C. SMITH, University of Cincinnati College of Medicine, Cincinnati, Ohio.

Dypnone guanyldiazone hydrochloride, WR-5677, a new antimalarial compound, labeled with ¹⁴C in the dypnone chain has been investigated for blood concentration, excretion, and tissue distribution in rhesus monkeys and albino rats following single oral doses of 5 mg/kg. The blood level curves showed peaks at 8-12 and 24 hr, and levels were still just detectable at 6 days. Concentrations of ¹⁴C activity were always higher in the red cells than in the plasma, the RBC/plasma ratios varying from 2 to > 20. Excretion occurred both in the urine and in the feces with the latter route being more prominent. Excretion was negligible by either pathway after 144 hr. Although high concentrations of drug appeared in the bile and in the liver, the ¹⁴C activity in other organs never exceeded about 1% of the dose and generally was below 0.1%. Preliminary studies in two monkeys with exteriorized biliary cannulae indicated rapid and extensive enterohepatic circulation of the compound, the biliary ¹⁴C activity being equivalent over a 24-hr period to the total ¹⁴C administered.

In rats, fecal excretion far exceeded that measured in the urine. In view of the evidence obtained in the monkey for extensive enterohepatic circulation of the drug and/or its metabolites, one would expect the compound to undergo the same pattern of transport in rats. Extraction and chromatography of simian urine samples indicated the presence of the parent compound and about equal amounts of a more polar metabolite. Similar studies on biliary radioactive materials suggested the presence of more than one labeled substance. Some suggestions of possible metabolites, including a three-ring asymmetrical triazine, are considered. (Support in part by Army Contract DADA17-67-C-7065.)

32. *Metabolism of ^{14}C -Metolazone, a New Diuretic, In Naive and Chronically Treated Dogs and Monkeys.* A. I. COHEN, O. N. HINSVARK, and D. J. SULLIVAN, Pennwalt Corporation, Pharmaceutical Division, Rochester, New York. (Z. Hadidian.)

The physiologic distribution and excretion pattern of this drug has been studied with dogs and monkeys which were part of a 12-month oral toxicity program. Each animal received a dose of 2, 10, or 50 mg/kg daily for a year prior to receiving a single oral dose of the radioactive compound. Peak blood levels, with urinary excretion correlating closely, were obtained within a few hours after dosing in both naive and chronically treated animals. In both, the peak blood and 24-hr urine levels seemed to bear an exponential relationship to dose. The distribution of the urinary and fecal metabolites indicated that in almost all instances chronically treated animals handled the drug similarly to the singly dosed naive animals and that the major portion of the drug was excreted within 24 hr. The previous long-term administration of the drug did not seem to affect significantly the physiologic distribution and excretion patterns of the drug. The total recovery data indicated little possibility for drug accumulation after chronic administration of metolazone.

33. *Gas Chromatographic Determination of Ethanol, Acetaldehyde and Acetone in Microvolumes of Biofluids.* B. B. COLDWELL, G. SOLOMONRAJ, H. L. TRENHOLM, and G. S. WIBERG, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Canada.

In conjunction with a study under way in our laboratory on the mechanism(s) of the interaction of ethanol with drugs, an analytical method was required for the simultaneous estimation of minute concentrations of ethanol, acetaldehyde, and acetone in microliter samples of biological fluids obtained at frequent intervals from small laboratory animals. The gas chromatographic methods of Duritz and Truitt, and Boiteau and Mousson were selected for intensive study. The latter was the most sensitive, permitting the sample volume to be reduced 25 times. Reproducibility was similar in both methods.

Of five support and four stationary phase materials investigated, the combination of Gas-Chrom T or Haloport 60F coated with 5 to 8% of Carbowax 1500 proved superior, both in respect to sensitivity and retention time for all three components. The method has been used to compare the decay profiles of ethanol, acetaldehyde, and acetone in individual rats and in man utilizing tail blood and fingertip blood, respectively.

34. *Procedures for Evaluating the Nonabsorption of a Polymer from the Gastrointestinal Tract.* R. C. REYNOLDS, B. D. ASTILL, and D. W. FASSETT, Laboratory of Industrial Medicine, Eastman Kodak Company, Rochester, New York.

The absence of significant levels of absorption is important in evaluating safety for food use of polymers. Procedures developed for two partly oxidized polyethylenes of approx. MW 2500 (Wax I) and 2100 (Wax II), were: (1) recoveries from feces by extraction and precipitation after feeding polymer to rats in standard or low residue diets (Wax I), (2) disposition and elimination of radioactivity by rats after being fed tritiated polymer (Wax I), and (3) assay for polymer of lymph from the cannulated thoracic duct of a dog after oral injection (mixed Waxes I and II).

Procedure 1 showed extensive nonabsorption of Wax I (97-103% in feces) but was less successful in establishing absorption limits (< 5%) because of variable normal extractives

and experimental error. Procedure 2 gave ambiguous results because of incompletely removable ^3H from polymer; 10–12% of ingested radioactivity occurred in urine while procedure 1 showed 96% of tritiated polymer present in feces. Residual body radioactivity suggested < 1% of absorption. With procedure 3 a 4-hr periodic lymph collection contained < 0.15% of polymer intake from a dog given po 0.066 g/kg of each wax, based on net wt and comparative infrared spectra of predose and experimental extractives. Taken together, procedures 1 and 3 demonstrate almost no absorption of Wax I. The same is suggested for Wax II.

Thoracic duct lymph assays are of value in establishing limits to absorption of polymers, particularly where recoveries from feces are inaccurate and labelling is impracticable.

35. *In Vitro and In Vivo Concentrations of Thiopental, Hexobarbital and Pentobarbital in Rat Lungs.* W. L. MARCUS and F. SPERLING, Howard University Medical School, Washington, D.C. 20001.

Barbiturates were found in rat lung at high concentration for relatively long periods of time, after single ip anesthetic doses of 125 mg/kg of hexobarbital (I), 40 mg/kg of thiopental (II), or 40 mg/kg of pentobarbital (III). The concentrations per g dry wt of lung were compared for rats sacrificed after loss of righting reflex. From peak values at 15 min, the concentration of I dropped 52%, of II 85%, and III 0% by 240 min. The $t_{1/2}$ s of I and II, from the peak values, were 160 min and 10 min, respectively. The concentration of III began to fall after 6 hr, and measured from then its $t_{1/2}$ was 53 min.

The 30-min blood concentration of I was 19% less than at 10 min and in the following 30 min rose to 65% higher than the initial value; at 4 hr, it had dropped to 60% of the initial value. Blood concentration of II dropped progressively for the first 2 hr to 72% of its 15-min value and during the next 2 hr to 31% of that value. Blood concentration of III during 6 hr dropped to 94% of the 15-min value. The blood $t_{1/2}$ s were 78, 54, and 86 min after the respective peaks.

In vitro, 338, 100, or 50 μg of I, II, or III were injected into the pulmonary arteries of respiring lungs excised from electroshocked animals. Five minutes later I, II, and III were 647, 225, and 208 μg per g dry lung wt. At 60 min, 21, 33, and 100% of the 5-min value remained. By 90 min III fell to 63%. In the in vitro lung the $t_{1/2}$ of I was 36 min, of II 47. The $t_{1/2}$ of III, after peak values found at 60–90 min, was 94 min.

Lung and blood concentrations appear to be unrelated. Lung concentrations in vitro and in vivo followed similar patterns. In vivo and in vitro, 100% of III was retained for long times vs. progressive decrease of I and II. These data support the postulate previously proposed that these barbiturates have peripheral effects on the lung.

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36. *Increased Toxicity of Carbon Tetrachloride in Phenobarbital Treated Animals.* T. M. FARBER, A. HEIDER, E. L. PETERS, D. L. RITTER, M. DISRAELY, and E. J. VAN LOON, Division of Pharmacology and Toxicology, Food and Drug Administration, Washington, D.C.

A highly significant increase in the toxicity and lethality of carbon tetrachloride has been observed when this chemical was administered to rats, dogs, and pigs which were pretreated with phenobarbital. The LD₁₀₀ for carbon tetrachloride administered orally to phenobarbital-treated dogs and miniature swine was approximately 0.1 ml/kg. Death occurred within 1 to 2 days after dosing. Normal, untreated dogs and swine receiving 6 ml/kg and 4 ml/kg, respectively, of carbon tetrachloride survived with few or no overt signs of toxicity. The LD₅₀ of carbon tetrachloride administered to phenobarbital-treated dogs and swine was estimated to be approximately 0.05 ml/kg. With the LD₅₀ being greater than 5 ml/kg for untreated dogs and swine, an increase of 100-fold or more must have occurred in the lethality of carbon tetrachloride for the phenobarbital-treated animals.

Liver function tests performed on control and phenobarbital-treated dogs and pigs given 0.01 ml/kg and 0.05 ml/kg of carbon tetrachloride orally revealed marked elevations in SGOT, SGPT, and LDH.

This work, although preliminary, indicates that the possibility exists that increased toxicity from carbon tetrachloride exposure may occur in humans exposed to this substance while on medication containing phenobarbital or possibly other known microsomal inducers.

37. *Lindane Toxicity, A Comparative Study in Dogs and Miniature Swine with and without Phenobarbital.* F. L. EARL, E. J. VAN LOON, B. E. MELVEGER, J. E. REINWALL, G. W. BIERBOWER, R. KASS, and W. MIGUEZ, Division of Pharmacology and Toxicology, Food and Drug Administration, Washington, D.C. 20204.

Paired groups of six dogs were fed lindane in the diet at levels of 0, 7.5, 15.0, and 22.5 mg/kg/day for 24 weeks. Another paired group, previously treated with phenobarbital, was fed 35 mg/kg/day of phenobarbital and 30 mg/kg/day of lindane until death. Four paired groups of four miniature swine each were fed lindane in the diet at levels from 0 to 80 mg/kg/day for 14 weeks.

Hematological findings showed decreased numbers of reticulocytes and platelets, and frequent crenated, burred, bizarre-type red blood cells in the higher dose levels in both species. A myeloid: erythroid ratio in excess of 60:1 was found in swine and 750:1 in dogs. Biochemical findings showed only an increase in alkaline phosphatase levels in dogs. Histopathological findings were nonspecific in swine, and only slight changes were noted in liver of the treated dogs.

Although phenobarbital, added to the diet, decreased the early toxic manifestation of lindane, it did not ameliorate the lethal effects of the pesticide when compared to nonpretreated dogs. A lethal chronic dose of lindane is 22.5 mg/kg/day for dogs, but swine survived doses three times as great for a longer period of time. Swine have tolerated up to 125 mg/kg/day of lindane for short periods.

38. *Inhibition of Hepatic Drug Metabolism by Amphetamine, Methamphetamine, Hydroxyamphetamine, and Chloramphetamine.* ROBERT LOUIS-FERDINAND, SURENDRA PURI, FRANK KOWAL, GEORGE FULLER, and HARBANS LAL, University of Rhode Island, Kingston, Rhode Island 02881.

Addition of amphetamine in incubation mixture or its ip injection inhibited in vitro metabolism of hexobarbital in a previous study. The present experiment was undertaken to determine the mechanism of inhibition of hepatic drug metabolism by amphetamine and its clinically important analogues.

Demethylation of *p*-chloro-*N*-methylaniline (PCMA) by rat liver 9000 *g* supernatant obtained from male and female Sprague-Dawley rats, was determined in the presence of 3×10^{-3} M *d*-amphetamine sulfate. Kinetic constants obtained from Lineweaver-Burke plots suggested that amphetamine was a competitive inhibitor of PCMA demethylation. Apparent amphetamine-inhibition constants for the liver preparations from male rats (3.25×10^{-3} M) differed from those of female rats (2.74×10^{-3}) suggesting a sex difference. Hydroxyamphetamine, *p*-chloroamphetamine and methamphetamine studied in male rats were also inhibitors of PCMA demethylation. Amphetamine inhibition of demethylation was not prevented by substitution of NADPH-generating system with NADPH. These data suggest that inhibition of hepatic drug metabolism by amphetamines was due to an interaction with a component of the drug metabolizing system other than the NADPH-generating system.

(Supported in part by NSF Grant GY 5902 and U.S. Department of Interior under Public Law 88-397).

39. *Inhibition of Microsomal Enzyme Induction by Piperonyl Butoxide.* M. A. FRIEDMAN and S. S. EPSTEIN, Children's Cancer Research Foundation, Inc., and Harvard Medical School, Boston, Massachusetts.

Piperonyl butoxide (PB) is a synthetic methylenedioxyphenyl compound which synergizes the insecticidal effects of pyrethrins, putatively, acting by inhibiting microsomal enzyme

function in insects. PB also produces *immediate* but transient inhibition of microsomal enzyme activity in mammals. We report here the *delayed* effects of PB on microsomal enzyme function in mice.

Mice were injected ip with a single dose of PB, 2.5 mg/kg or 10 mg/kg, and liver aminopyrene demethylase activity was assayed 48 hr later. This resulted in a 1.4-fold enzyme induction. However, following higher doses of PB, 160 mg/kg or 640 mg/kg, no induction of aminopyrene demethylase was observed. When mice injected with a single dose of PB were also given two successive daily injections of benzo[α]pyrene, 50 mg/kg, and then killed, no difference in enzyme activity from mice receiving PB alone was observed. Mice injected with benzo[α]pyrene alone, on the other hand, had a 1.7-fold induction of enzyme activity.

These data suggest that the metabolism of benzo[α]pyrene by microsomal enzymes may be intrinsically involved in its ability to induce microsomal enzymes. Thus, when PB inhibits microsomal function and benzo[α]pyrene metabolism as an *immediate* effect, it may also inhibit enzyme induction as a *delayed* effect.

(These studies were supported by Grants C-6516 and FR-05526 from the National Institutes of Health.)

40. *Quantitative Measurement of Hepatic Microsomal Enzyme Induction After Dietary Intake of Chlorinated Hydrocarbon Insecticides.* F. K. KINOSHITA and C. K. KEMPF, University of Chicago, Chicago, Illinois. (K. P. DuBois.)

Previous investigations in this laboratory demonstrated that low dietary levels of DDT and toxaphene caused dose-related increases in the activities of three hepatic microsomal enzymes (phosphorothioate detoxification, *O*-demethylase, and *N*-demethylase). The greatest increases in enzyme activities were observed after 1 week of dietary intake followed by decreases during 13 weeks of continuous pesticide intake. The no-effect dietary levels for enzyme induction were near 1 ppm for DDT and 5 ppm for toxaphene. The present investigation was conducted to determine the potency of enzyme induction with respect to the time of maximal induction and the persistence of its effect. Various levels of chlordane, gamma-chlordane, heptachlor, heptachlor epoxide, aldrin, and dieldrin were fed to male and female rats for 13 weeks. All three hepatic microsomal enzymes were induced in a dose-related manner. The greatest amount of increase for a particular dietary level of a compound occurred by the end of the first or third week. Elevated enzyme activities were maintained throughout the feeding period, although in most instances there was a gradual decrease. The most persistent induction occurred with heptachlor and heptachlor epoxide. Male rats were more sensitive to the enzyme-inducing effects of chlordane, gamma-chlordane, heptachlor, and aldrin, while females were more sensitive to heptachlor epoxide and dieldrin. The no-effect dietary levels for enzyme induction by the pesticides used in this study were approximately 1 ppm. In descending order of potency as hepatic microsomal enzyme inducers these chlorinated hydrocarbons may be ranked as follows: aldrin, dieldrin, heptachlor epoxide, heptachlor, chlordane, and gamma-chlordane.

41. *Influence of Metiapine on the Drug Metabolizing Systems of the Rat Liver.* G. A. LEESON, A. KANDEL, W. L. KUHN, and G. J. WRIGHT, Wm. S. Merrell Company, Cincinnati, Ohio.

The influence of metiapine, 2-methyl-11-(4-methyl-1-piperazinyldibenzo [*b,f*][1,4]-thiazepine, on enzyme systems of the rat liver was investigated by pharmacologic and biochemical techniques. Male Sprague-Dawley rats weighing 180–200 g were used. The pharmacological test parameters were the duration of hexobarbital hypnosis and zoxazolamine-induced paralysis; the biochemical test systems were aminopyrine demethylase, hexobarbital oxidase, and zoxazolamine hydroxylase. Metiapine was administered by gavage as a single dose of 15 mg/kg; or 15 mg/kg per day for 14 days; or in incremental doses: 15 mg/kg on days 1–2, 30 mg/kg on days 3–5, 60 mg/kg on days 6–9, 120 mg/kg on days 10–12, and 240 mg/kg on days 12–14 to three separate groups of rats. Pharmacologic and biochemical determinations were carried out 24 hr following the final dose of metiapine for each group. There

were no significant differences from controls elicited by the single dose of metiapine. Administration of 15 mg/kg per day for 14 days resulted in a significant reduction in zoxazolamine-induced paralysis but not in the duration of hexobarbital hypnosis. The activities of the enzymes, hexobarbital oxidase, zoxazolamine hydroxylase, and aminopyrine demethylase, were not, under these conditions, comparably increased. The regimen of incremental doses of metiapine resulted in significant reductions in the duration of hexobarbital hypnosis and in zoxazolamine-induced paralysis. The activities of aminopyrine demethylase and zoxazolamine hydroxylase, under these conditions, were increased, while that of hexobarbital oxidase remained unchanged. No increase in liver weight was noted in any group. Though the influence exerted by metiapine on the specific liver enzyme systems was apparent, the results of the pharmacologic challenges were not, however, always consistent with the biochemical evaluations.

42. *Comparative Stimulation of γ HCH Metabolism by Pretreatment of Rats with γ HCH, DDT, and DDT + γ HCH.* R. W. CHADWICK, M. F. CRANMER, A. J. PEOPLES, Food and Drug Administration, Perrine Primate Research Branch, P.O. Box 490, Perrine, Florida.

Pretreatment of rats with γ HCH, DDT, and DDT + γ HCH accelerates the metabolism of γ HCH. A single oral dose of ^{14}C - γ HCH was administered to all animals after a 2-week pretreatment period. The treated rats excreted significantly more radioactivity and stored significantly less ^{14}C - γ HCH than the control animals. In addition to significantly larger livers, the treated rats exhibited more liver protein, higher cytochrome P450 content and greater in vitro enzyme activity than the control animals. The self-induction of γ HCH metabolism has not previously been reported and could be of major importance in establishing a steady state relationship between exposure and storage of such pesticides by mammals. Both quantitative and qualitative differences in γ HCH metabolism were observed between the treated groups of rats.

Results of this study suggest that the mechanism by which γ HCH induces its own degradation differs from the mechanism through which DDT stimulates γ HCH metabolism.

43. *Effect of Methylchloroform Inhalation on the Hepatic Microsomal Electron Transport System Related to Drug Metabolism.* ARNOLD OLSHAN, GEORGE C. FULLER, and HARBANS LAL, University of Rhode Island, Kingston, Rhode Island 02881.

Inhalation of methylchloroform (1,1,1-trichloroethane) alters drug responsiveness and hepatic drug metabolism in mice. The present study was conducted to determine the effect of methylchloroform inhalation on components of the hepatic drug-metabolizing systems in rats. Blood and liver methylchloroform levels, measured at the end of a 24-hr inhalation (2500–3000 ppm) indicated hepatic accumulation of this compound. No methylchloroform was detected 24 hr after the termination of inhalation. At this time, duration of loss of righting reflex due to hexobarbital (120 mg/kg ip), meprobamate (300 mg/kg ip), or zoxazolamine (80 mg/kg ip) was reduced significantly. Significant increases in microsomal *N*-demethylation of aminopyrine were accompanied by significant increase in CO-binding pigment (P-450) and NADPH-cytochrome *c* reductase. The spectral properties of CO-binding pigment did not change. Cyclohexamide (2.5 mg/kg sc) given 30 min before exposure to methylchloroform inhalation blocked the decrease in hexobarbital hypnosis and increase in aminopyrine demethylation. Our data suggest that the enhanced hepatic drug metabolism after methylchloroform inhalation is due to accelerated synthesis of drug-metabolizing enzyme systems. (Supported by Office of Naval Research Contract NOOO14-68-A-0215.)

44. *Toxicosis in Mice Induced by Corn Cultures of Penicillium Cyclopium and Penicillium frequentans.* WILLIAM W. CARLTON and JOHN TUITE, Departments of Veterinary Physiology and Pharmacology and Botany and Plant Pathology, Purdue University, Lafayette, Indiana.

Mycotoxicosis was induced in male Swiss mice by feeding of corn cultures of *P. cyclopium* and *P. frequentans*. Cultures of the fungi were prepared by the inoculation of moistened corn

with spores and incubation at 54°C for 2 weeks. The cultures were covered with chloroform for 72 hr, and after chloroform evaporation, the cultures were dried at 100°F for 5 days and ground for mixing. The ground corn cultures were mixed with a purified diet at a 50% concentration. Weanling mice were fed the diet ad libitum, were weighed at weekly intervals, and were necrotized when groups were terminated. Of the three isolates of *P. cyclopium* studied, two produced lesions in the liver and kidneys. Hepatic alterations included hydropic degeneration, hypertrophy of hepatocytes and nuclei, and focal necrosis. In the kidneys, numerous giant epithelial cells with hypertrophied nuclei were observed in the cortical tubules near the corticomedullary junction. Isolates of *P. frequentans* reduced weight gains and induced hepatic lesions. The severity of the lesions varied among different livers as among different treatments, but the characteristics of the changes were similar. Focally, or in a diffuse pattern, enlarged hepatocytes had ballooned, vacuolated cytoplasm. Cytoplasm varied from finely granular eosinophilic material to that composed of numerous small vacuoles or a large clear vacuole. The material in the vacuole did not stain by the alcian blue-PAS technique nor with oil red O and was presumed to be water. Reactive cellular infiltration and bile ductule cell hyperplasia were not observed in tissues of mice fed either of the fungi.

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45. *Effects of Nutrition on Response to Liver Toxins*. PAUL M. NEWBERNE and ADRIANNE E. ROGERS, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.

Studies have continued in our laboratory on the interaction of nutrition and liver toxins and carcinogens. We have demonstrated a protective effect of a diet low in lipotropes against the acute toxic action of lasiocarpine and monocrotaline. Compared to animals fed control diets, the LD50 of lasiocarpine was raised from 110 to 150 mg/kg and that of monocrotaline from 75 to 110 mg/kg body weight in male Sprague-Dawley rats fed a low lipotrope diet. Furthermore, rats fed a low lipotrope diet failed to respond to the toxins by a dissociation of the liver polysome profile while the profile of rats fed a normal diet dissociated within 1 hour of dosing. Drug-metabolizing enzymes were decreased in the lipotrope-deficient rats which may, therefore, have been protected because of failure to form an active metabolite from the alkaloids. Protection was afforded also by mercaptoethylamine but not by other antioxidants, e.g., α -tocopherol and ubiquinone. These and other acute responses will be discussed along with results of more chronic studies.

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46. *Induction of Delayed Hypersensitivity to Aflatoxins in Guinea Pigs and Their Cross-Reactivity to Related Compounds*. C. W. CHUNG, T. R. CARSON, and A. L. GILES, JR., Food and Drug Administration, Washington, D.C. (F. N. Marzulli.)

Aflatoxins and other mycotoxins, frequent food contaminants with varying degrees of toxicity, contain the coumarin or furan moiety or both. Knowledge of their immunogenicity and immunological cross-reactivity is important because coumarins, furans and furocoumarins are found in drugs, foods, and cosmetics. Guinea pigs were administered an intradermal injection of 25 μ g of aflatoxins B₁ and G₁, sterigmatocystin, coumarin, furazolum chloride, or 8-methoxypsoralen, accompanied by a foot-pad injection of adjuvant. After a resting period of 2-4 weeks animals were challenged with 2 μ g of the test substance. Almost all animals were found to be sensitized. Two weeks later these animals were tested for cross-reactivity to 10-19 structurally related chemicals (including the original sensitizer) by a 2- μ g intradermal injection.

On second challenge aflatoxin B₁ was found to be less reactive than aflatoxin G₁ or sterigmatocystin, in roughly inverse proportion to their toxicity. The smaller molecules (coumarin, furazolum, and 8-methoxypsoralen) were stronger sensitizers than aflatoxin G₁ or sterigmatocystin. All sensitized animals cross-reacted to other structurally related compounds. The degree of activity was related to the structural composition of the substances and the degree

of sensitization of the animals. Guinea pigs sensitized to aflatoxins or sterigmatocystin showed stronger cross-reactions to coumarins than to aflatoxins or sterigmatocystins; weaker reactions to aversin, versicolorin A, and furans. Guinea pigs sensitized to coumarin, psoralen, or furazolum showed stronger reaction to coumarin, psoralen, furans, aversin, and versicolorin A, but they were slightly less reactive to aflatoxins. The results implicate the role of intact furofurocoumarin of aflatoxins in skin sensitization. The coumarin moiety appears to play a greater role than the furofuran moiety.

47. *The Analysis of Feed Efficiency Data as Related to Various Degrees of Aflatoxicosis in Growing Swine.* B. H. ARMBRECHT, H. G. WISEMAN, W. T. SHALKOP, and J. N. GELETA, Food and Drug Administration, Beltsville, Maryland 20705.

The order of appearance for various effects among pigs fed diets containing 0.2 or 0.7 mg aflatoxin ($G_1/B_1 = 3$)/kg during a 16-week growing period was: (1) impaired feed efficiency (FE); (2) stunted growth; and (3) organ enlargement. Among pigs fed diets containing 2.0 or 4.0 mg aflatoxin/kg, the following additional signs were observed: inappetance; liver injury resulting in icterus, abnormal serum chemical and histopathological deviations; a morbid condition with altered hematologic parameters; and mortality.

FE is a sensitive parameter for the identification of subclinical effects in growing swine exposed to aflatoxin. A plot of FE versus time exhibits a significantly abrupt change after a period of feeding. This period, the induction time in days (t), is related to the daily dose rate (DR) by the regression $\log t = 3.048 - 0.907 \log DR$.

A trend to impaired growth was noted with groups fed diets containing 0.2 or 0.7 mg/kg, but a significant change was found in groups fed 1.0, 2.0 or 4.0 mg/kg. This less sensitive parameter is described by the regression $\log t = -2.308 - 0.379 \log DR$.

48. *The Development of a Systematic Method for Toxicological Research Using an Integrated Multidisciplinary Approach.* G. S. WIBERG, B. B. COLDWELL, and H. L. TRENHOLM, Food and Drug Directorate, Ottawa, Canada.

Toxicology has been called a "conglomerate" or an "eclectic" science drawing upon such diverse disciplines as analytical, physical, and organic chemistry; cellular, subcellular and clinical biochemistry, physiology, pathology, electron microscopy, pharmacology, physiology, enzymology, nutrition, etc. Since it is difficult for the toxicologist to have expertise in all these areas, a multidisciplinary approach is essential. The authors have integrated the disciplines of analytical chemistry (forensic toxicology), cellular biochemistry and enzyme chemistry to gain greater understanding of drug interactions. This permitted detailed study of the ethanol-barbiturate interaction in the whole animal, at the cellular level and in isolated enzyme systems. Studies in systemic toxicity also lend themselves to integrated multidisciplinary methods. An investigation would start with the intact animal and proceed in sequence to studies of the physiological systems, isolated organs, metabolic processes using tissue slices, homogenates, subcellular organelles, and finally to the molecular level with target enzymes. This comprehensive evaluation would benefit greatly from the inclusion of scientists of several disciplines. The particular advantages which may be derived from some of these specialized skills will be discussed.

49. *A Versatile Data Processing System Developed for Animal Reproduction and Teratology Data.* H. HARTMAN, R. HRAB, and P. CARTY, Sandoz Pharmaceuticals, Hanover, N. J. (R. E. Bagdon.)

A group of five interchangeable programs have been developed using the IBM 360 Model 30 computer. They provide convenient in-laboratory use for review of experimental data, minimum input effort and maximum but concise disclosure of large volumes of output. These data are generated during the conduct of various segments of animal reproduction and teratology studies currently prescribed by various government agencies.

The benefits realized are those of reduced manpower requirement, efficiency of data handling, and most meaningful, the additional time available for evaluation and interpretation of experimental data.

In a routine evaluation of one chemical substance seven studies are performed. These produce a data input from approximately 9000 sires, dams, embryos, fetuses, and neonates concerning such parameters as relative weight gain, food consumption, numerous characteristics of pregnancy, fetal dimensions and weight, survival rates, and sex ratio. These data are handwritten once prior to review and submission to the data processing facility. Only four standard input forms are necessary for the five programs.

During these seven studies, over 100,000 input entries are processed to produce organized outputs of 90,000 entries of raw data, calculations of 4000 means, 2000 standard deviations, and numerous data segregations. In addition the output is formatted with appropriate titles, etc., permitting immediate insertion into report appendices.

At present we have found it more effective to handle morphological observations manually because of the variety to be anticipated and the low frequency of occurrence.

Programs to test statistical significance can be included in the above described system on line or performed by using an in-laboratory computer terminal to which we are subscribers.

The system has worked at near 100% efficiency for more than 2 years on a variety of studies. Its success is due primarily to the preplanned flexibility to allow change in experimental design and the freedom to change parameters.

50. *A Small Real-Time Data Logger to Analyze Spontaneous Animal Activity.* B. L. MYERS and A. A. THOMAS, Technology Incorporated, Dayton, Ohio, and 6570th Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio.

Early experiments at the Aerospace Medical Research Laboratory revealed that activity cage and light beam interruption techniques did not adequately reflect the spontaneous activity of large laboratory animals. Time-lapse photography was then successfully explored to overcome this deficit. To facilitate automatic processing of the photographic data and to experiment with real-time evaluation, the Spontaneous Activity Analyzer was developed.

The Spontaneous Activity Analyzer monitors, categorizes, and displays the changes in the spontaneous activity of dogs presented either photographically, live, or via remote television. The operating principle is based on the optical comparison of one reference and one activity area in the experimental environment, each of which is sensed by light sensitive devices yielding an electrical output proportional to the average light intensity. As the successive electrical analogs are sampled, subtracted, amplified, and retained, the system compares them to find differences. When a difference exists between two consecutive samples indicating a change in spontaneous activity, it is categorized into each level that the spontaneous activity surpasses. After the highest level is selected, its counter is incremented in order to count the occurrence of this level of activity.

Using ambient light, the system is capable of different sampling rates (1, 2, 5, or 10 secs or 1 min apart) or automatically in the time-lapse film or remote television modes. Each counter for the nine levels and the total activity counter can register up to 99,999 activities. The accumulated data may be printed out by activity levels and total activity and is also traced by an analog plotter to show graphically the distribution of activity levels and a weighted average of the nine activity levels.

51. *Acute Cardiovascular Responses to Cadmium.* GENE J. YOUKILIS, RICHARD J. MORROW, and ROBERT B. FORNEY, Indiana University Medical Center, Indianapolis, Indiana.

Previous investigations have demonstrated a pressor effect following iv injection of 50 μg cadmium ion/100 g body weight in rats. In other studies larger intra-arterial quantities (80-320 μg /100 g body weight) were initially depressor and subsequently pressor. In order to further investigate these macrocirculatory responses, cadmium ion (as the chloride) was

administered to groups of anesthetized rats at doses of 0.5, 1.0, or 2.0 mg/kg. The mean arterial pressure (MAP), ECG, and cardiac output (in some animals) responses were observed over control (10 min) and postinjection (30 min) periods. In all cases, both MAP and heart rate increased in relation to the increase in dose. At 0.5 and 1.0 mg/kg the MAP tended to return to baseline values toward the end of the postinjection period while the MAP for the 2.0-mg/kg group remained significantly increased at 30 min postinjection. Heart rates in all but the lowest dose group remained elevated at the termination of the experiment. Several pharmacologic agents including phenoxybenzamine, hexamethonium, MJ-1999, propranolol, atropine, and reserpine were administered to individual groups of rats followed by an iv dose of cadmium ion (2.0 mg/kg) as the chloride. Various blood pressure and heart rate responses to the combined drug administrations were observed. These included potentiated pressor and positive chronotropic effects following the hexamethonium and cadmium combination as well as complete inhibition of the cadmium responses in the MJ-1999 pretreated group.

Isolated perfused rat hearts were subjected to doses of 0.1, 0.2, 0.4, 0.8, or 3.2 $\mu\text{g}/\text{min}$ over a 10-min period. There was no effect on the spontaneous heart rate at any dose. Coronary vascular resistance increased and myocardial contractal force decreased in a dose dependent manner. These responses were partially reversible following the infusion period. The data to date suggest the possibility of several autonomic responses to the cadmium ion including the release of endogenous catecholamines.

52. *Lead and Lindane Interactions on Heme Synthesis in the Mouse.* C. R. CRESS and R. E. LARSON, Oregon State University, Corvallis, Oregon 97331.

Lead depresses concentrations of hemoglobin in the blood by interference with heme synthesis. The pesticide lindane (1,2,3,4,5,6-hexachlorocyclohexane) is also capable of eliciting anemia. Because both agents depress the hematopoietic system and because both lead and lindane are environmental contaminants to which the entire human population is exposed, studies were undertaken to determine the effects of combinations of lead and lindane on the synthesis of hemoglobin and another important heme protein, cytochrome P450. CF No. 1 male mice were given 0, 75, 150, or 300 ppm lead in the form of the acetate in drinking water for 8 weeks. During the final 2 weeks of lead administration, they were also given 0 or 40 mg/kg lindane in corn oil by daily oral intubation. The concentration of hemoglobin in the blood was decreased by lead and was not affected by lindane given alone. Given with lead, lindane prevented the decrease. Lead also depressed levels of hepatic microsomal cytochrome P450 by as much as 50%; lindane had the opposite effect and completely abolished the effect of lead. Both lead and lindane elicited slight increases in urinary excretion of coproporphyrin III; the effect of coadministration was additive. Lead brought about an increase in urinary excretion of delta-aminolevulinic acid; lindane had no effect on this parameter when given alone but diminished the increase produced by lead. It was concluded that lindane is capable of abolishing the effects of lead with regard to overall heme synthesis, and that lead and lindane cause the buildup of coproporphyrin III by different mechanisms.

53. *Influence of Age, Sex, and Hormones on Trialkyl Phosphate-Induced Narcosis in Rats.* D. R. BROWN and S. D. MURPHY, Harvard School of Public Health, Boston, Massachusetts.

Dimethoate [*O,O*-dimethyl, *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate] and triethylphosphate (TEP) rapidly produce loss of righting reflex in rats which has been reported as a deep narcosis or anesthesia. This action of trialkyl phosphates has received relatively little attention, and the present investigation was undertaken to further characterize this effect. Dose- and time-response relationships for production and duration of narcosis (loss of righting reflex) were compared with brain cholinesterase inhibition in male and female rats given dimethoate ip. Onset of narcosis preceded inhibition of cholinesterase, and time of recovery from narcosis did not appear to be dependent upon the degree of brain cholinesterase inhibition. The ED₅₀ dose for dimethoate-induced narcosis was greater for adult male rats (453 mg/kg) than for females (245 mg/kg). The ip ED₅₀ for narcosis with TEP was

412 and 330 mg/kg for adult (> 10-week-old) male and female rats, respectively. Four-week-old rats were less sensitive than adults to the narcosis produced by TEP with ED₅₀ of 495 and 412 mg/kg for males and females, respectively. The duration of the loss of righting reflex after 500 mg/kg of TEP was about equal (25–35 min) in 5-day-old and adult rats of either sex, but rats between 16 and 42 days of age slept only 5 to 10 min. The sex and age differences suggested a possible hormonal influence on the narcotic action of TEP. Orchidectomy at either 4 or 12 weeks of age did not significantly affect the duration of narcosis in adult males. Ovariectomy, however, prevented the normal development of increased sensitivity of adult females. The results indicate that there are age and sex differences in the susceptibility of rats to trialkyl phosphate-induced narcosis. The age differences in females, but not males, appear to be under gonadal influence.

(Supported by Training and Research Grants TO 1-ES-00045 and RO 1-ES-00084 from the National Institute of Environmental Health Sciences.)

54. *Fission Products: Uptake and Retention of Cesium-137, Rubidium-86, and Potassium-42 by Isolated Rat Organs.* B. V. RAMA SASTRY, RALPH H. GEORG, and ALLAN D. BASS, Vanderbilt University School of Medicine, Nashville, Tennessee 37203.

Ingested ¹³⁷Cs is localized intracellularly in the rat and other mammals. The tissue/plasma concentration ratios for ¹³⁷Cs peak to higher values than those for potassium. We have studied the uptake and retention of ¹³⁷Cs, ⁸⁶Rb and ⁴²K by the isolated perfused rat liver and the rat hemidiaphragm to comprehend the accumulation or retention of ¹³⁷Cs by these tissues. When the concentrations of the radionuclides in the plasma and the liver tissue were equal, the initial relative rates of their uptake had the following order: ⁴²K 1.00, ⁸⁶Rb 0.93, ¹³⁷Cs 0.39. The initial relative rates of the release of ⁴²K, ⁸⁶Rb, and ¹³⁷Cs into the perfusate containing no radionuclides had the following order: ⁴²K 1.00, ⁸⁶Rb 0.54, ¹³⁷Cs 0.22. These observations suggest the relative influx/efflux ratios of 1.00, 1.74, and 1.82 for ⁴²K, ⁸⁶Rb, and ¹³⁷Cs, respectively, in rat liver. The relative rates of uptake (⁸⁶Rb > ¹³⁷Cs) and release (⁴²K > ⁸⁶Rb > ¹³⁷Cs) of these radionuclides by the rat hemidiaphragm were similar to those obtained with rat liver; therefore, the slow efflux of ¹³⁷Cs was the major contributing factor for its buildup of high concentrations in rat tissues.

(Supported by U.S. PHS Grant No. RO1 EC 00128 and AEC Contract AT-(40-1)-3066.)

55. *Comparison of Serum Lithium Concentrations After LiCl and Li₂CO₃ Administration by Different Routes in Rats.* J. MICHAEL MORRISON, JR., WILLIAM D'AGUANNO, HAROLD D. PRITCHARD, and MONIQUE C. BRAUDE, Bureau of Medicine, Food and Drug Administration, Washington, D.C.

Lithium is currently undergoing investigation for its use in the therapy of manic-depressive psychoses. Lithium is usually administered orally as the carbonate in clinical trials, but most absorption, distribution, and pharmacological studies have been done with LiCl iv or ip. A study of the absorption of Li⁺ after ip or po administration of both compounds has been done to obtain data for correlation with preclinical and clinical studies as well as with future drug interaction studies. Male Wistar rats (200–250 g) were given LiCl dissolved in 0.9% NaCl or Li₂CO₃ suspended in 0.5% CMC, at doses of 0, 2.5, 5.0, 10.0, and 20 meq/kg and were sacrificed $\frac{1}{2}$, 1, 2, 4, 8, 12, 24, or 48 hr after drug administration. The serum Li⁺ levels were determined by the automated flame photometric method of Nevius and Lanchantin. Following ip administration, peak serum Li⁺ was reached faster (15 min vs. 1 hr) and was significantly higher after LiCl than after Li₂CO₃. Serum Li⁺ following LiCl dosing decreased rapidly between 15 min and 1 hr while after Li₂CO₃ the levels showed a slower rate of decline. At 8 hr, however, serum Li⁺ levels were similar for both compounds. After po administration, serum Li⁺ levels showed little change between 2 and 12 hr, with Li₂CO₃ showing slightly higher levels than LiCl. After 8 hr, serum Li⁺ levels following Li₂CO₃ po were similar to those following ip administration. Peak blood levels of 3.8 and 2.75 meq/l reached in this acute study after 20 meq/kg ip LiCl and Li₂CO₃, respectively, did not produce signs of toxicity

although reported to do so in man following repeated administration of the drug. Spontaneous motor activity, as measured in the actophotometer, failed to reveal significant dose-related changes after ip treatment. The data show that the blood levels produced are markedly different for LiCl and Li₂CO₃.

56. *Acute Effects of Chloroacetophenone and o-Chlorobenzylidenemalononitrile in Rats and Rabbits.* J. R. GASKINS, R. M. HEHIR, D. F. MCCULLLEY, E. W. LIGON, JR., Food and Drug Administration, Office of Product Safety, Washington, D.C. 20204.

Chloroacetophenone (CN) and *o*-chlorobenzylidenemalononitrile (CS), commonly referred to as tear gases, have recently enjoyed wide usage in solution in pressurized devices for personal protection against assailants. Solutions and suspensions in organic solvents were tested in unanesthetized rats and rabbits.

In acute oral studies in rats, 1-4% CN and CS produced severe gastroenteritis and deaths, occurring generally within 2 days after a single dose. The LD₅₀ values, depending on vehicle and concentration employed, were within the range of 50 to 225 mg/kg for CN and 175 to 360 mg/kg for CS.

Preparations containing 1% CN or CS were instilled directly (0.1 ml) and were sprayed directly (1 sec at 4 inches) into the eyes of rabbits with lids held open, conditions simulating maximum anticipated field exposure. Only brief and mild irritation was apparent, CS being less injurious than CN. Under exaggerated test conditions, the compounds produced moderate to severe conjunctival irritation with facial depilation, with complete recovery in 3 to 30 days. Some preparations containing more than 3% CN produced permanent corneal injury, while one containing 10% CS produced no corneal injury.

Solutions containing 1-4% CN or CS were applied to rabbit skin; 0.5 ml under an occluded patch for 24 hr produced redness of several days' duration. CN also usually produced a slowly developing superficial purpura and necrotic eschar. CS sometimes produced an eschar without purpura. Early washing with water or with soap and water provided little benefit. Recovery was substantially complete at 5 weeks.

All these data indicate CS is less injurious than CN.

57. *Comparison of Gastric Toxicity of Acetylsalicylic Acid with Route of Administration in the Rat.* C. J. PFEIFFER and L. GROGAN, Pennsylvania Medical Center, Philadelphia, Pennsylvania.

Gastric hemorrhage is frequently observed following salicylate administration to man and animals. The present study was undertaken to study comparatively the influence of dose and route of administration, plasma level of salicylate, and gastric acidity in a standardized preparation on salicylate-induced gastric hemorrhage. Animals were pylorus-ligated 4 hr prior to salicylate injection and sacrificed 1 hr after drug administration. It was found that acetylsalicylic acid was maximally ulcerogenic (U.I. = 14.1 ± 1.79 , max. poss. = 20) when given po, where a log dose response relationship was observed. Aspirin induced slight gastric hemorrhage in sc injected rats (U.I. = 6.2 ± 1.49), but not in ip or intrarectally (ir) injected rats at 31.2, 65.5, or 125 mg/kg doses. Plasma salicylate levels peaked 37-45 min after injection by all routes except sc administration, where only slight elevation occurred by 1 hr. Plasma concentration-time curves for salicylate provided the following relative areas: po, 1.00; sc, 0.29; ip, 0.86; and ir, 0.68. Changes in gastric secretion were slight or nonexistent in rats treated by po, sc or ir aspirin, but volume and concentration were decreased in rats treated by ip salicylate. The present study provides additional evidence of the systemic ulcerogenicity of acetylsalicylic acid but suggests that the direct effect on the mucosal surface contributes significantly to the pathogenesis. The gastric erosion does not correlate well with plasma concentration-time relationships nor with the level of gastric acidity. Depression of gastric secretion (or resorption of water and acid) was inversely correlated with dose of ip salicylate.

(Supported by the William H. Rorer Gastroenterology Research Fund.)

58. *Biochemical and Morphological Parameters of Tauroolithocholate-Induced Cholestasis.* B. G. PRIESTLY, M. G. COTE, and G. L. PLAA, Faculty of Medicine, University of Montreal, Montreal, Canada.

Lithocholates can produce cholestasis in several animal species; this seems to be related to the insolubility of the salts. Intravenous administration of sodium tauroolithocholate (TL) in an albumin/dextrose/saline vehicle produces an immediate cessation of bile flow in rats and mice. A dose of 100 mg/kg was chosen to study the temporal effects on bile flow (BF), serum bilirubin (SB), serum transaminase (SGPT), and serum alkaline phosphatase (SAP). In rats, BF recovered slowly, reaching normal values after 24–30 hr; SB was elevated at 1 hr; peak levels (3.3 mg%) occurring after 12–18 hr; SB was normal after 24 hr; SGPT activity rose slowly to a peak (350 U/ml) at 18 hr; SAP activity varied only slightly. In mice, BF, and SAP changes were similar to those of the rat; SGPT peak activity was higher (1600 U/ml) and occurred after 1 hr; SB elevation was less marked (1.4 mg%); all parameters were normal after 24 hr. After bile duct ligation in both species, SGPT was the only parameter which did not mimic the changes seen in TL-treated animals. Simultaneous injection of taurochloic acid (200 mg/kg) and TL (100 mg/kg) reduced the effects on BF, SAP, and SB but not SGPT. In TL-treated rats after 1 hr, the most marked changes in hepatic ultrastructure were dilatation of the bile canaliculi and endoplasmic reticulum. After 12 hr the changes were more marked; the canaliculi contained plugs; the sinusoids and space of Disse contained cell debris; some hepatocytes were degenerated. After 24 hr, the ultrastructure was essentially normal. The above changes are consistent with mechanical obstruction of the biliary tree by TL precipitation, but evidence of cellular damage suggests involvement of the hepatocyte as well.

(Supported by the Medical Research Council and the Pharmaceutical Manufacturers Association Foundation.)

59. *Enzymatic Activity Patterns in the Rabbit After Caffeine-Ethanol Administration.* R. L. ALSTOTT, D. J. BROWN, and R. B. FORNEY, Indiana University Medical Center, Indianapolis, Indiana.

Caffeine has been found to be an antagonist of the depressant effects of ethanol. However, those findings have not coincided with the findings of Forney and Hughes who found that caffeine, after ethanol administration did not improve the performance on a test designed to measure psychomotor impairment. Furthermore, enhancement of depression and toxicity has been shown in the rat after caffeine-ethanol administration. In view of these findings, we decided to study the interaction of the two compounds.

Depression was measured using a shock avoidance box designed by Hughes *et al.* Urea nitrogen, glucose, and drug levels were measured in blood samples drawn at intervals during a 5-hr test period. Blood was withdrawn by a single cardiac puncture 24 hr after the administration of either or both drugs for the determination of serum GOT, GPT, LDH, and isozymes, amylase, alkaline phosphatase, and urea nitrogen. Baseline activity ranges for these enzymes and baseline values for the urea nitrogen and glucose have been established for our experimental animal, the Dutch rabbit. The administration of 100 mg/kg of caffeine and/or 1.0 gm/kg of ethanol has been shown to produce changes from control values in certain of the previously named tests.

The results of the various enzyme studies and the urea nitrogen determinations were evaluated in an attempt to locate organ system changes. The other parameters were studied to obtain information on possible metabolic alterations.

60. *Effects of NO₂ on Enzyme Activity in Rat Tissues.* R. L. KILLENS, F. DOMBKOSKI, M. F. HENDERSON, and K. I. CAMPBELL, Division of Health Effects Research, Department of Health, Education and Welfare, Cincinnati, Ohio 45237.

Previous studies have shown that moderate to high concentrations of nitrogen dioxide cause enzyme alterations in animals. Our investigation confirms and extends these previous studies in rats. Time-related patterns of metabolic effects of nitrogen dioxide were measured

for lung and other body tissues. Rats were subjected to 25 ppm of nitrogen dioxide. Enzyme activities (aldolase, lactic dehydrogenase, and alkaline phosphatase) from homogenates of lung, liver, and kidney were examined in rats sacrificed after 0, 24, 72, 168, 240, 312, and 360 hr of continuous exposure. Significant differences were observed between enzyme activity levels of control and NO₂-exposed rats.

61. *Liver and Retinal Alcohol Dehydrogenases*. W. D. WATKINS and T. R. TEPHLY, University of Michigan, Ann Arbor, Michigan.

Studies have been performed in this laboratory with respect to the role of liver alcohol dehydrogenase (LADH) in the metabolism and toxicity of methanol. Recently, retinal alcohol dehydrogenase (RADH) has been investigated in order to assess its activity toward various alcohol substrates and its sensitivity toward inhibitors of LADH.

LADH and RADH activities were found in precipitates obtained after 33–50 and 50–70% saturation of the cytosol fraction with ammonium sulfate, but the majority of the activity was in the 50–70% fraction. RADH obtained after DEAE-cellulose chromatography yielded a Michaelis constant of about 0.2 M with ethanol as substrate. This value was about 100-fold higher than that observed for LADH. Pyrazole exerted profound inhibition of both LADH and RADH. At very high concentrations of methanol (2 M), RADH activity was observed in the 33–50% ammonium sulfate fraction of the cytosol. This activity was retained after DEAE-cellulose chromatography. Liver acetaldehyde dehydrogenase activity was not separable from LADH activity on DEAE-cellulose. However, acetaldehyde dehydrogenase activity obtained from rat retina was separated from RADH on DEAE-cellulose. These and other studies indicated that retinal enzymes concerned with alcohol oxidation had different characteristics than those which were studied in liver.

(Supported by USPHS Grant GM-14209 and PHS Training Grant No. TO ES00106 from the National Institute of Environmental Health Sciences.)

62. *Enzyme Correlations in Intramuscular Testing in the Dog, Miniature Pig, and Man*. J. E. GRAY, R. WEAVER, M. PRESTRUD, and R. DEHAAN, The Upjohn Company, Kalamazoo, Michigan.

An im formulation (100 mg/ml) of an experimental antibiotic, U-21,251F, was injected daily for 30 days in nine beagles and nine Labco miniature pigs. Twenty-five human volunteers were injected once. Characteristically, serum glutamic oxaloacetic transaminase (SGOT) values elevated faster and rose higher than those of serum glutamic pyruvic transaminase (SGPT). Normal indications of function and appearance of the liver in the dogs and pigs suggested that the elevations were related to the injection sites. In addition, in man, a marked jump in creatine phosphokinase (CPK) was observed 24 hr postinjection.

In dogs, 8 to 10 injections in one site produced early induration, local edema, and swelling of the regional lymph node. Microscopic examination revealed chiefly a rather slowly advancing fibroblastic replacement within the perimysial stroma. Upon necropsy of the pigs, multiple pale tracts (1–2 cm in cross-section to 12 cm in length) were present in the ham. The microscopic appearance resembled that of a typical tract observed in musculorritancy testing in the rabbit which is an inflammation bounded area of coagulation necrosis with peripheral mineralization.

A subsequent single dose study (20 mg/kg) in six dogs confirmed the predictiveness of a reversed pattern of transaminase elevations (SGOT preceding SGPT) coupled with an increased CPK value in distinguishing injection produced muscle damage from drug-related hepatic injury.

63. *Mitochondrial Effects of Ethylene Glycol in Vitro and in Vivo*. E. BACHMANN, L. GOLBERG, and P. DIGIACOMO, Institute of Experimental Pathology and Toxicology, Albany Medical College, Albany, New York.

Reappraisal of the toxicology of ethylene glycol (EG) has necessitated a better understanding of the effects of this compound, as well as some of its metabolites (glycollate, glyoxylate, and

oxalate) on the metabolic activities of mitochondria. The sources of the mitochondria studied were: liver, kidney, and cerebral cortex of rats (Sprague-Dawley), dogs (pure-bred beagles), and monkeys (*Macaca mulatta*) of both sexes. Electron transfer, oxidative phosphorylation, citric cycle activities, and substrate-level phosphorylation were measured.

Regardless of the source of mitochondria, the only activity affected by EG was substrate-level phosphorylation in concentrations of 5 mM and higher. Glyoxylate was effective in concentrations as low as 0.5 mM. Glycollate had no effect on the mitochondrial activities measured. Glyoxylate and oxalate inhibited most of the mitochondrial activities studied at concentrations of 0.5 mM or higher. They decreased respiration rates whatever the substrate but uncoupled oxidative phosphorylation only when α -ketoglutarate was the substrate. Mitochondria isolated from monkey tissue were a striking exception. Glyoxylate uncoupled oxidative phosphorylation in brain mitochondria when either succinate or α -ketoglutarate was the substrate. Liver mitochondria were not affected by glyoxylate, even with α -ketoglutarate as substrate.

These in vitro experiments clarify to some extent the findings with mitochondria isolated from liver tissue of monkeys inhaling EG mist at an average concentration of 0.5 mg per l of air for 5-7 months. A reduction in mitochondrial respiration rates was observed with either succinate or α -ketoglutarate as substrate. Oxidative phosphorylation was uncoupled with α -ketoglutarate as substrate but not with succinate or β -hydroxybutyrate.

We conclude from these observations that changes in mitochondrial metabolism brought about in vivo by the administration of EG are likely to be attributable to the effect of glyoxylate or some of its metabolites rather than to EG itself.

64. *Hematological and Serum Enzyme Determinations in Cynomolgus Monkeys.* Y. MADDOX, A. WOLF, E. F. DAVIS, and Y. ALARIE, Hazleton Laboratories, Inc., Falls Church, Virginia. (L. W. Hazleton.)

Measurements were made in male and female cynomolgus monkeys (*Macaca irus*) of 3.04 ± 0.5 kg body weight to delineate various parameters of arterial blood in this species. Blood samples were taken after an 8-week quarantine period during which the monkeys were tested for tuberculosis and after a minimum 10-week adaptation period in our laboratory during which they were fed a standard diet consisting of Wayne monkey biscuits and apples. The parameters measured were hematocrit, hemoglobin, rbc, wbc, BUN, SGOT, SGPT, LDH, alkaline phosphatase, and body weight. Data obtained on a group of nine animals over a period of 78 weeks indicated a general trend toward lower values in serum enzymes.

(Sponsored by the Air Pollution Research Program of the Electric Research Council.)

65. *Retinal Lysosomal Changes in Pigmented and Albino Rats Treated with Chloroquine.* R. ABRAHAM and R. J. HENDY, British Industrial Biological Research Association, Carshalton, Surrey, England, and Institute of Experimental Pathology and Toxicology, Albany Medical College, Albany, New York. (L. Goldberg.)

Any explanation of human chloroquine retinopathy should take into account the known action of this drug on lysosomes and the likelihood that such action will be reflected in hepatic, cardiac, and retinal lysosomes alike. Chronic administration of chloroquine to rats produces a sequence of changes in hepatic and cardiac lysosomes which proceeds through the formation of autophagic vacuoles and ends with membranous whorls (myeloid bodies). A histochemical and ultrastructural study of the distribution and properties of retinal lysosomes of albino and pigmented rats revealed that the characteristics of these organelles are similar to those of lysosomes in other organs.

In albino and pigmented rats given 40 mg/kg chloroquine orally daily for 6 months, numerous myeloid bodies were found in the cytoplasm of retinal ganglion and bipolar cells. Three months after cessation of drug treatment myeloid bodies were still present in the retina, whereas they had disappeared from liver and heart of the same animals. Thus chloroquine produces striking and persistent lysosomal changes in the sensory cells of the retina in both

albino and pigmented rats, suggesting that alteration of these structures may be of significance in the development of chloroquine retinopathy.

66. *The Role of Electron Microscopy in the Study of Hepatocarcinogens.* JACQUELINE A. MAURO and ARTHUR A. STEIN, Microscopy for Biological Research, Ltd., Albany, New York 12210.

There have been many studies of sequential changes in hepatocytes following the administration of a number of carcinogens. In some of these studies, changes in the hyperplastic nodule of liver have been compared with findings in the hepatoma. These studies have been reviewed in conjunction with our own studies on the carcinogenic effect of 2-7FAA in rats. The role of electron microscopy in the prediction of the hepatocarcinogenicity of a particular compound will be discussed. In addition, the sequential pattern of alteration of specific subcellular organelles may provide direction to biochemical search for mechanisms involved.

67. *Ultrastructural Histochemical Changes in Purebred Canine Hepatic Cells Following Acute Feedings with DDD.* J. M. POWERS, G. R. HENNIGAR, JR., W. J. DOUGHERTY, and J. NICHOLS, Medical University of South Carolina, Charleston, South Carolina, and University of Kansas Medical Center, Kansas City, Kansas.

In an attempt to clarify the potentiation of pentobarbital anesthesia by DDD in dogs, seven purebred dogs were given certain chlorinated insecticides (DDD, DDT, and Perthane) and some of their breakdown products. Four doses (200 mg/kg) of these compounds (DDD, Perthane, and DDT) produced an increase in smooth endoplasmic reticulum (SER). Five more purebred dogs were given a single dose (200 mg/kg) of commercial DDD to delineate the course of this SER proliferation. From 6 hours to 10 days, an increase in dilated SER was noted. At 14 days and 21 days a prominent SER was still present but had decreased and reverted to a normal pattern. Histochemical increases in ATPase in TPNH diaphorase tended to parallel the changes in SER. The solubility of DDD and its ability to induce enzymes could explain the potentiation of pentobarbital anesthesia.

68. *Light and Electron Microscopic Studies of Beryllium Hydroxide-Induced Rat Pulmonary Tumors.* G. R. MACKAY, D. H. GROTH, and M. L. MEAD, Bureau of Occupational Safety and Health, Cincinnati, Ohio 45202.

Rat pulmonary tumors were encountered in recent experiments in this laboratory for the first time after intratracheal instillation of commercial beryllium hydroxide. Difficulties with the classification of early tumor types and with the evaluation of epithelial metaplasia as a preneoplastic response prompted the present morphological characterization of the mature tumors.

Bronchiolar alveolar cell tumors (BAT) and mixed adenocarcinoma-bronchiolar alveolar cell tumors were observed in rat lungs 12 months after a single intratracheal injection of 0.25 mg of beryllium hydroxide. By electron microscopy the predominant cell types found in BAT were cuboidal or low columnar cells arranged along both sides of the alveolar wall. The majority of these cells contained numerous osmophilic lamellar bodies, generally larger but similar to those seen in granular pneumocytes of normal lung tissue. Occasionally this pattern was interrupted by individual or small groups of ciliated columnar cells. Mixed tumors contained the cell types described above but in an acinar pattern and with a greater abundance of ciliated columnar cells. In addition, large groups of polygonal cells, filled with dense homogeneous granules 0.21–2.15 μ in diameter, and smaller aggregates of undifferentiated cells lay interposed between the acini.

69. *Two-Year Chronic Toxicity Studies with Moieties of Nicarbazin.* E. E. VOGIN, S. CARSON, K. MORGAREIDGE, and H. M. PECK, Food and Drug Research Laboratories, Inc., Maspeth, New York 11378, and Merck Institute for Therapeutic Research, West Point, Pennsylvania 19486.

Nicarbazin, a coccidiostat, is a complex of 4,4'-dinitrocarbanilide (DNC) and 2-hydroxy-4-6-dimethyl pyrimidine (HDP) which dissociates into the component moieties *in vivo*.

HDP is eliminated rapidly. There is no DNC residue in chicken tissues 3 to 4 days after withholding drug. Residue studies indicated that maximum muscle concentrations of the moieties were about 6 and 2 ppm for DNC and HDP, respectively, in short-term studies while the chickens were receiving nicarbazin. No toxicity was found in dogs given up to 5 g per kg per day of nicarbazin except for slight anemia. Precipitates of the acetylated metabolite of DNC were found in the kidneys of rats that received nicarbazin at a dosage of 500 mg per kg per day. The doses used in the 2-year study were selected on the bases of the subacute toxicity studies and of tissue residues to provide a wide margin of safety. Thus, the dogs were given up to 600 + 200 mg per kg per day and the rats up to 300 + 100 mg per kg per day of DNC + HDP, respectively.

The results showed no adverse effects related to treatment of rats. No acetylated DNC crystals were found in renal tissues at autopsy. In dogs, transitory and inconsistent elevations of SGPT occurred in several animals from the 12th to the 24th month. Since other liver function tests showed no abnormality characteristic of hepatic dysfunction and no changes were seen histologically, the SGPT changes were not related to toxicity.

The data clearly demonstrate a wide margin of safety with respect to residue levels of DNC and HDP in poultry tissue, thus indicating no hazard to man.

70. *100-Day LD50 Index of Chronic Toxicity*. ELTON M. BOYD, Queen's University, Kingston, Ontario, Canada.

The purpose of this communication is to review and assess the need for studies on the 100-Day LD50 Index as an estimate of chronic toxicity. The 100-Day LD50 Index is the quotient obtained by expressing the LD50 (100 days) as a percentage of the acute LD50 (1 dose) and is, therefore an estimate of chronic toxicity in one figure. Most reported studies have been based upon oral toxicity in rats to which the drug or other agent is given by intragastric cannula once following an overnight fast to determine the LD50 (1 dose) and daily without food restriction to determine the LD50 (100 days) or daily dose which kills 50% of animals within 100 days of administration. The LD100 (100 days) of 11 materials averaged 20 to 68% higher than the LD50 (100 days). The LD0 (100 days) was 33 to 77% of the LD50 (100 days) for 10 agents and less than 1% for the eleventh (pilocarpine). The 100-Day LD50 Index was between 12 and 27 for drugs such as atropine, pilocarpine, aspirin, phenacetin, paracetamol and dicophane and between 72 and 82 for sodium chloride, caffeine, and sucrose which are normally part of the human diet. To determine the correct number of days that a drug should be given to estimate chronic toxicity, the dose which kills 50% of animals was calculated after each week of administration, expressed as percent of the LD50 (100 days) and the results plotted against duration of administration. For some drugs such as caffeine, the regression was almost horizontal. For others such as dicophane, the regression line rose steeply during the first 3 or 4 weeks and then became almost horizontal. For both of these types of drugs, the LD50 (100 days) could have been reasonably approximated by daily administration for about 50 days. The regression in a third group of drugs, which included agents such as pilocarpine and aspirin, was almost linear and still rising at 100 days indicating that administration should have been continued beyond 100 days. It is concluded that the LD50 should be calculated at weekly intervals and daily dosing continued until values for the LD50 stabilize.

71. *Chronic Oral Toxicity of Erythrosine (FD&C Red No. 3) in Rats and Gerbils*. W. H. HANSEN, T. F. X. COLLINS, S. L. GRAHAM, Bureau of Science, Food and Drug Administration, Washington, D.C. 20204, and K. H. JACOBSON, Tulane University School of Medicine, New Orleans, Louisiana.

Groups of rats and gerbils were administered erythrosine either in the diet or by stomach tube. Treatment continued for approximately 85 weeks for rats and 2 years for gerbils. Dietary levels for rats were 0.0, 0.5, 1.0, 2.0, and 4.0%; for gerbils 0.0, 1.0, 2.0, and 4.0%. Intubation doses for rats were 0, 100, 235, 750, and 1500 mg/kg twice a week; for gerbils 0, 200, 750, and 900 mg/kg twice a week.

Growth inhibition was observed in the male and female rats fed 4.0% drug-diet and also in females given 2.0%. Female gerbils at all dietary levels and male gerbils at 2.0 and 4.0% exhibited growth depression. No effect on growth was observed on either the rats or gerbils administered erythrosine by stomach tube.

Thyroid function in these rats was unaffected by administration of the dye. Protein bound iodine (PBI) levels were elevated in rats treated with the dye, but this increase is attributed to analysis of serum erythrosine as PBI. Thyroxine (T_4) values were the same in control and test rats. No PBI or T_4 analyses were performed on gerbils. No gross pathological changes attributable to erythrosine were noted.

72. *Some Comparative Aspects of Methemoglobinemia, Heinz Body Formation and Anemia.* J. P. GIBSON, W. J. HUDAK, J. W. NEWBERNE, The Wm. S. Merrell Company, Cincinnati, Ohio.

There is considerable controversy concerning the relationship of methemoglobinemia to Heinz body formation and anemia. Much of this stems from differences in the response of the red cell to different types of chemical compounds, and also to species and strain differences in constitution and metabolism. In order to clarify some of the differences, several types of oxidant drugs were administered to several different species of animals by both po and iv routes. The response of each species varied somewhat depending upon the oxidant chemical to which it was exposed, although the relative sensitivity of the species was consistent. This suggested that in spite of the numerous factors responsible for these species differences, their responses could be used for predicting the hemolytic effect of oxidant drugs in man.

73. *Residual Lesions of Spontaneous Disease Observed in Rats Utilized in Drug Safety Evaluation.* G. H. HOTTENDORF and R. S. HIRTH, Bristol Laboratories, Syracuse, New York. (H. Madisoo.)

Recognition of the residual lesions of spontaneous disease in rats utilized in drug safety evaluations and the differentiation of these lesions from lesions that may be drug-induced is of obvious importance. A definitive pathologic profile consisting of the residual lesions observed in control rats is essential to this recognition and differentiation. The profile should include those sporadic lesions observed in treated rats which have well established etiologies or display an etiologic agent. Such a profile, developed as the result of over 7800 rat necropsies, will be presented. Lesions will be illustrated and the incidence, etiology, significance, and interpretation briefly discussed.

74. *Toxicity of Abate, a Mosquito Larvicide, and its Sulfoxide.* G. J. LEVINSKAS and C. B. SHAFFER, Central Medical Department, American Cyanamid Company, Princeton, New Jersey 08540.

Abate, *O,O,O',O'*-tetramethyl *O,O'*-thiodi-*p*-phenylene phosphorothioate (registered trademark of American Cyanamid Company), is a potent mosquito larvicide with low mammalian toxicity. In studies with tritium-labeled material, unchanged Abate was the principal constituent recovered, and its sulfoxide (*O,O,O',O'*-tetramethyl *O,O'*-sulfinyldi-*p*-phenylene phosphorothioate) was the primary metabolite in bean leaves and in the rat. To evaluate potential hazards to health associated with the use of Abate on edible crops, extensive toxicity tests were conducted with technical materials.

The oral and dermal LD50 of Abate larvicide are about 2 g/kg for rats and rabbits, respectively. The product is not irritating to the eye or skin of rabbits, and rats survived an 8-hr exposure to air near saturation with vapor of the product. The conjoint administration of "equitoxic" mixtures of Abate larvicide with 23 other organophosphates did not show potentiation of acute toxicity. No myelin loss occurred from nerve tissue of adult hens fed 920 ppm of Abate for 30 days. Rats given 15 dermal doses at 60 mg/kg over a 21-day period did not show signs of systemic toxicity or gross or microscopic lesions, but weight gains were depressed and females had increased liver weights. A dose of 12 mg/kg was without effect. Rats tolerated 90 days on a diet containing 350 ppm of Abate larvicide with no untoward

signs and no gross or microscopic evidence of tissue injury. Cholinesterase activity (ChA) in red blood cells (RBC), plasma, and brain was markedly inhibited. A diet containing 6 ppm was without effect on ChA. A diet containing 700 ppm of Abate larvicide produced severe signs of cholinergic stimulation in dogs. Reduction of 500 ppm in the diet markedly decreased cholinergic signs during the rest of the 90-day test period, but ChA of RBC, plasma, and brain was drastically reduced. No gross or microscopic lesions referable to Abate were noted. ChA of dogs fed 18 ppm of Abate was not affected. Abate fed at 125 ppm to rats from weaning through reproductive age for three successive generations had no adverse effect.

Over a 90-day period, rats tolerated a diet containing 9 ppm of the sulfoxide with no apparent effect for inhibition of RBC ChA. No inhibition of ChA occurred at the 3-ppm diet level.

75. *Neuromuscular Block by a New Organophosphorus Pesticide.* J. H. WILLS, J. CHOLAKIS, and F. COULSTON, New York State Department of Health and Institute of Experimental Pathology and Toxicology of Albany Medical College, Albany, N.Y.

Dimethyl-*S*-(2-methoxy,5-*H*,4-oxo-1,3,4-thiadiazole-(4)-methyl) dithiophosphate produces rapid decrement in response to the gastrocnemius-soleus muscle group of the anesthetized (Na pentobarbital), atropinized (2 mg/kg iv) cat to electrical excitation of its motor nerve at 50/sec. Instead of a tetanus maintained during a 15-sec period of stimulation, there is a brief summated response followed by failure of response. The entire response may resemble a large muscle twitch. To test the usual theory of the causation of the failure of response, we have injected acetylcholine (ACh) intraarterially into the muscle at various times after initiation of the normally tetanizing excitation of the sciatic nerve and after the response to this excitation of the motor nerve had failed. When ACh was injected about 5 sec after the initiation of stimulation of the nerve, the injection invariably caused the muscle to contract even though it did not maintain tension in response to stimulation of the nerve. When the same dose of ACh was injected about 10-12 sec after the start of stimulation of the nerve, the injection either did nothing or if the muscle was able to maintain a slight tension removed entirely the ability of the muscle to contract. These findings suggest that something more than simple pile-up of acetylcholine is involved in the production of failure of neuromuscular transmission after a large dose of this organophosphorus inhibitor of cholinesterases.

76. *In Vivo Rabbit Blood Cholinesterase Inhibition and Reactivation Following Organophosphate Infusion.* B. J. GOUGH and T. E. SHELLENBERGER, Gulf South Research Institute, New Iberia, Louisiana 70560.

The present study was undertaken to determine the response of rabbit whole blood cholinesterase during and following the intravenous infusion of a series of dimethyl organophosphate enzyme inhibitors. Inhibitors were infused for 30 or 60 min at two concentrations into the marginal ear vein of adult New Zealand white rabbits. Heparinized whole blood samples were obtained at periodic intervals, from a cannula implanted in the external jugular vein, for measurement of enzyme activity using acetylcholine bromide substrate in an electrometric constant-pH titration assay.

Bidrin, phosphoric acid dimethyl ester, ester with *cis*-3-hydroxy-*N,N*-dimethylcrotonamide; C-2307, 3-(dimethoxyphosphinyloxy)-*N*-methyl-*N*-methoxy-*cis*-crotonamide; DDVP; Gutoxon, the oxygen analog of Guthion, *O,O*-dimethyl-*s*-(4-oxobenzotriazino-3-methyl)-phosphorodithioate; and the oxygen analog of C-9491, *O,O*-dimethyl-*O*-2,5-Dichloro-4-iodophenyl phosphonothioate, produced dose- and time-related inhibition of cholinesterase during infusion; inhibited enzyme exhibited rapid spontaneous recovery of activity to 60 to 80% of normal within 60 to 90 min after infusion. Azodrin produced dose and time related enzyme inhibition during infusion, but spontaneous recovery of inhibited enzyme was negligible during the immediate postinfusion period.

Results suggest a different mechanism of enzyme inhibition by Azodrin compared to the other chemicals which is possibly related to differences in reversibility of the initial enzyme-inhibitor complex.

(Supported by Public Health Service Research Grant ES-00297).

77. *In Vivo Effects of Oxime Reactivators on Organophosphate Inhibited Blood Cholinesterase.*
B. J. GOUGH and T. E. SHELLENBERGER, Gulf South Research Institute, New Iberia,
Louisiana 70560.

Effects of oxime reactivators on organophosphate-inhibited rabbit whole blood cholinesterase were determined to investigate the mechanism of enzyme inhibition and spontaneous reactivation and to compare the activity of various reactivators. Cholinesterase was inhibited by infusing the organophosphates into the marginal ear vein for 30 to 60 min. The oxime reactivators were injected slowly into the ear vein immediately after and at periodic intervals following the organophosphate infusion. Heparinized whole blood samples were obtained periodically, from a cannula implanted in the external jugular vein, for measurement of enzyme activity using acetylcholine bromide substrate in an electrometric constant-pH titration assay.

Blood cholinesterase was inhibited by infusion of Azodrin, Bidrin, the oxygen analog of C-9491 (*O,O*-dimethyl-*O*-2,5-Dichloro-4-iodophenyl phosphonothioate), DDVP, and SD 1652 (*O,O*-dimethyl *O*-2,2-Dichlorovinyl phosphate). Injection of 2-PAM (50 mg/kg) immediately following inhibitor infusion produced rapid reactivation of inhibited enzyme. Progressively less reactivation of inhibited enzyme was obtained by 2-PAM injections 1, 2, or 3 hr following infusion of the dimethyl phosphates; enzyme inhibited with SD 1652, the diethyl phosphate, was reactivated by 2-PAM for at least 48 hr following inhibitor infusion. With other reactivators, TMB-4 and P-2-S at 50 mg/kg produced marked enzyme reactivation when injected immediately following infusion of each inhibitor; MINA and DAM were not effective at dose levels as high as 100 mg/kg.

(Supported by Public Health Service Research Grant ES-00297.)

78. *Some Aspects of the Blockade of Neuromuscular Transmission by Iodo-, Bromo-, Chloro-, and Fluoroacetates and Their Choline Esters in the Rat Phrenic Nerve-Diaphragm Preparation.* B. V. RAMA SASTRY and C. Y. CHIOU, Vanderbilt University School of Medicine, Nashville, Tennessee 37203.

Iodoacetate induces blockade of chemical transmission and causes contracture of the muscle in the nerve-muscle preparations. To understand its mode of action, we have investigated the effects of all sodium monohalogenoacetates (XAc, where X = halogen) and choline esters on the rat phrenic nerve-diaphragm preparation. All halogenoacetates induced full neuromuscular blockade with different minimal doses (IAc 5, BrAc 30, ClAc 200, FAc 200 mM). With choline (25 mM) in the bath, lower doses (IAc 1.6, BrAc 6.4, ClAc 51.2, FAc 51.2 mM) of halogenoacetates produced the full neuromuscular blockade. Iodoacetate and bromoacetate induced irreversible neuromuscular blockade and contracture of the muscle. The neuro-muscular blockade caused by fluoroacetate and bromoacetate was reversible, and no contracture of the muscle was observed. Similar results were obtained with the corresponding halogenoacetylcholines (XAch, where X = halogen). The minimal doses of halogenoacetylcholines (IACh 0.8, BrAch 3.2, ClAch 25.6, FAch 12.8 mM) for inducing the full neuromuscular blockade were lower than those of the corresponding halogenoacetates. The neuromuscular blockades caused by fluoro- and chloroacetylcholines were intensified by physostigmine and were partially reversed by *d*-tubocurarine. They appeared to be of depolarizing type. The irreversible nature of the neuro-muscular blockade caused by iodo- or bromoacetylcholines was possibly related to the lability of the halogen atom.

79. *Dose and Time Relationships for Carboxylesterase Inhibition and Malathion Potentiation by EPN, Abate, and Parathion.* S. D. COHEN and S. D. MURPHY, Harvard School of Public Health, Boston, Massachusetts.

Previous studies indicated that potentiation of malathion toxicity by triorthotolyl phosphate (TOTP) was more closely associated with TOTP inhibition of liver hydrolysis of triacetin (TA) than with inhibition of hydrolysis of either diethyl succinate (DES) or methyl butyrate

(MB). To determine if these findings were applicable to malathion potentiation and carboxylesterase inhibition by other organophosphates, we compared the dose- and time-response relationships for inhibition of liver carboxylesterase activity with potentiation of malathion toxicity by the insecticides EPN, Abate, and parathion. Groups of 8-10 mice were injected ip with various doses of EPN, Abate, or parathion. Half of the mice in each group were sacrificed at various times after pretreatment and hydrolysis of DES, TA, and MB by their livers were determined. The remaining mice in each group were challenged with malathion and sacrificed 2 hr later for brain cholinesterase assays. At 18 hr after 5 mg/kg of parathion, liver hydrolysis of DES, TA, and MB was 70 to 90% inhibited, but the anticholinesterase action of 400 mg/kg of malathion was no greater than in unpretreated controls. As had been observed with TOTP, potentiation of malathion anticholinesterase action by EPN was more closely associated with inhibition of TA hydrolysis than with its inhibition of either DES or MB hydrolysis. Dose- and time-response curves for potentiation of malathion by Abate were not clearly associated with inhibition of hydrolysis of any single ester substrate. The degree of potentiation of malathion anticholinesterase action at approximately equiactive carboxylesterase-inhibiting doses of TOTP, EPN, and Abate were 28.6-, 4.9-, and 2.3-fold, respectively. The results indicate that the degree of inhibition of carboxylesterase activities of the liver by various compounds is not, by itself, sufficient to predict the relative capacities to potentiate malathion.

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80. *Metabolism of Carbamate Insecticides Utilizing Cell Culture Techniques.* RAYMOND K. LOCKE, VIVIAN BECK, and RONALD L. BARON, Bureau of Science, Food and Drug Administration, Washington, D.C. 20204. (Clara H. Williams.)

Establishment of tolerance levels for pesticide residues on crops requires metabolism studies to ascertain the identity of metabolites produced. The use of cell culture techniques was examined to evaluate feasibility for elucidating a preliminary metabolism scheme. Two commercial and one experimental carbamate insecticides [carbaryl, Banol, and UC 34096 (*N'*-(4-*N*-methylcarbamoyloxy-*o*-tolyl)-*N,N*-dimethylformamidine)] labelled with ^{14}C in various positions were introduced into culture media containing the L-132 strain of human embryonic lung cells. The radiolabel distribution after incubation was determined by use of solvent extraction. The identity of organoextractable components was determined by thin-layer co-chromatography with known standards. The water-soluble conjugated components were identified by hydrolysis followed by solvent extraction and co-chromatography of the aglycones, or by ion-exchange column chromatography. The L-132 strain of human embryonic lung metabolized ^{14}C -carbaryl to $^{14}\text{CO}_2$, naphthalene-1,4-diol and the *N*-glucuronides of 4-hydroxycarbaryl and 5,6-dihydro-5,6-dihydroxycarbaryl. Banol was degraded by hydrolysis and conjugation to the *O*-glucuronide of the phenol moiety. UC 34096 was not metabolized but spontaneously decomposed to 4-hydroxy-*N*-formyl-*o*-toluidine.

Metabolites produced with this system have been observed in both in vivo and in vitro plant and animal systems. The use of cell cultures, therefore, has shown itself, with this limited series of compounds, to be a useful tool for preliminary investigation of pesticide degradation.

81. *Human Toxicity Studies on Polyvinyl Chloride Formulation of Dichlorvos.* C. H. HINE and M. B. SLOMKA, Hine Laboratories, Inc., San Francisco, California, and Shell Chemical Company, Princeton, New Jersey.

Phase I studies were conducted on a candidate antihelminth containing an active cholinesterase inhibitor in slow release formulation. The dose range in single administrations to 91 subjects was 1-32 mg/kg and on repeated administration to 10 subjects 1-16 mg/kg. Single doses above 4 mg/kg produced measureable RBC cholinesterase depression with a maximum of 46% at the highest dose. Plasma cholinesterase was affected at lower doses with 50%

depression at 1 mg/kg and 74% at 32 mg/kg. Symptoms elicited following single doses of active drug or placebo were principally related to the gastrointestinal tract and were not dose related. Those following chronic administration occurred after doses of 8 mg/kg or more and were also chiefly referable to the gastrointestinal tract but included giddiness, unusual salivation, and shortness of breath which were inconsistent in their occurrence. The largest total dose ingested was 8,318 mg (cumulative dose of 117.6 mg/kg). The other nine subjects had cumulative doses ranging from 55.1 to 105.6 mg/kg. All subjects were able to perform their normal work during and following the testing period. At 16 mg/kg/day depression of both RBC and plasma cholinesterase reached 90%. There were no significant findings on repeated examination of subjects with tests measuring changes in function due to cholinesterase inhibition. Monitoring clinical laboratory tests indicated that neither single nor repeated administration affected the blood count, urine, liver function, prothrombin time, or blood urea nitrogen. Cholinesterase activity values returned to baseline levels in proportions to rates of plasma protein and erythrocyte turnover.

82. *Comparative Toxicity of the Herbicide Paraquat in Laboratory and Farm Animals.* H. E. SMALLEY and R. D. RADELEFF, Veterinary Toxicology and Entomology Research Laboratory, College Station, Texas.

World literature reflects the lethality of paraquat in man. A latent fibrosing pneumonitis, progressive and remorseless, was the cause of death.

In the present study, paraquat was given by various routes to guinea pigs, hamsters, sheep, and swine. Lethal doses were established, development of toxicity was observed, and lesions were studied microscopically.

In guinea pigs 3 mg/kg paraquat ip caused a fatal pulmonary congestion within 3 days. Doses of 1.5 mg/kg ip killed some 5–11 days later; there was fibrosing pneumonitis. Doses of 6 mg/kg ip killed hamsters in 5–7 days; lesions included pulmonary congestion, edema, and chronic nephritis. Lower doses of paraquat (1.5–3.0 mg/kg) killed in 14–15 days with the same lesions as the higher doses; there was no evidence of fibrosing pneumonitis. Doses of 10 mg/kg ip killed sheep within 18 hr with pulmonary congestion and edema. Intraruminal injections above 45 mg/kg proved lethal in 2–5 days, but 45 mg/kg was not uniformly lethal. Doses of 10 mg/kg iv or 20 mg/kg ip killed swine within 24 hr. Oral administration of 50 mg/kg paraquat was an apparent median lethal dose. Local reaction to paraquat were extensive: mucosal erosions and ulceration from buccal cavity through the stomach. Pigs dying 9 days after administration had extensive fibrosing pneumonitis, bronchitis, bronchiolitis, congestion, and edema of the lungs.

Paraquat toxicity seemed to be a double-jeopardy situation in guinea pigs and swine—high doses caused death relatively quickly; if the animals survived the initial toxicity, a second hazard (fibrosing pneumonitis) might develop after several more days and was invariably fatal.

83. *Experimental Human Exposure to Carbon Monoxide.* RICHARD D. STEWART, JACK E. PETERSON, and EDWARD D. BARETTA, Marquette School of Medicine, Milwaukee, Wisconsin.

Human volunteers were exposed to carbon monoxide at concentrations of < 1, 25, 50, 100, 200, 500, and 1000 ppm for periods of one-half to 24 hr. Blood samples for carboxyhemoglobin (COHb) were obtained during the exposures and for up to 24 hr into the postexposure period. Postexposure treatment with oxygen at 1.0 and 3.0 atmospheres was included. One theoretical equation was derived which accurately predicted COHb levels resulting from continuous and discontinuous exposures to unvarying concentrations and from continuous exposure to a steadily rising concentration.

No untoward effects were observed in sedentary males exposed to 100 ppm for 8 hr. Exposures producing COHb saturations > 15–20% resulted in delayed headaches, changes in the visual evoked response, and impairment of manual coordination.

84. *Effects of Hypoxia or Sodium Nitrite Injection on Operant Behavior.* ROBERT SCHATZ, IRWIN BAUMEL, ARTHUR PITTERMAN, JOHN DEFEO, and HARBANS LAL, University of Rhode Island, Kingston, Rhode Island 02881.

Mice were trained to free-operant, shock-avoidance responses in a LHV skinner box enclosed in Plexiglas environmental chamber. Each response postponed the paw shock for 20 sec. No responding for 20 sec resulted in continuous shock to be terminated by a response. Exposure of mice to hypobaric hypoxia [10% (364 mm Hg), 8% (289 mm Hg), 7% (235 mm Hg) oxygen] or sc injection of sodium nitrite (100–120 mg/kg) depressed free operant behavior and induced reversible hypothermia. Behavioral depression induced by sodium nitrite coincided with methemoglobinemia produced by this compound. Both effects were reversed by treatment with methylene blue.

Young chicks were tested for memory consolidation task through one-trial avoidance conditioning which utilized inhibition of pecking 24, 48, and 72 hr after one peck on a methylanthranilate-coated object. Sodium nitrite treatment (acute or chronic) blocked the inhibition of pecking after methylanthranilate.

Sodium nitrite-induced depression of behavior appears to be due to functional hypoxia produced by this agent.

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85. *Human Motor and Mental Performance Under the Influence of Alcohol and/or Marihuana.* JOSEPH E. MANNO, GLENN R. KIPLINGER, IVAN BENNETT, and ROBERT B. FORNEY, Indiana University School of Medicine, Indianapolis, Indiana 46202.

Previous work in our laboratories has indicated that smoking a marihuana cigarette can produce decrements in human mental and motor performance.

The present investigations were undertaken to establish (1) a dose-response relationship for human motor and mental performance after smoking marihuana, and (2) the combined effect of marihuana with sufficient alcohol to produce a blood alcohol level of 0.05%. Motor and mental performance was tested according to the techniques described by Hughes and Forney.

Twelve paid male volunteers were each administered on six different occasions a marihuana cigarette calibrated to deliver in the smoke approximately 0, 2.5, or 5 mg of delta-9-tetrahydrocannabinol. In addition, the subjects consumed a flavored beverage with or without alcohol. The drug combinations were administered in a randomized double-blind manner. Pulse rates and alveolar breath alcohols were determined periodically throughout the experimental period. The subjects consumed the beverage over a 30-min period, after which they smoked the cigarette. The performance testing was begun 30 min after the start of the smoking period.

Marihuana components in the cigarettes and cigarette remains were analyzed by gas chromatography. The gas chromatograph was equipped with a flame ionization detector and a 9-ft, ¼-inch od glass column packed with 3 ft of 10% QF-1 and 6 ft of a mixture of 1% OV-1 and 1% OV-17, all on Chromasorb G/AW-DMCS, 100–120 mesh.

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86. *The Effects of Various Psychotropic Agents on Behavioral Multiple Schedule Experimentation in Monkeys.* S. FIELDING, A. CUTI, M. L. MILLER, C. KOSCH, and G. VARADI, Ciba Pharmaceutical Co., Summit, New Jersey. (H. Lal.)

The various components of a behavioral multiple schedule of reinforcement were used to determine specificity of drug action as well as important side effects. Four squirrel monkeys were trained to operate a behavioral multiple schedule of reinforcement consisting of an avoidance RS-20, SS-20 schedule, a positively rewarding fixed ratio 15:1 schedule, and a conflicting positively and negatively (foot shock) rewarding fixed ratio 15:1 schedule.

The results indicated that Valium and Librium at oral doses which alleviated conflict behavior showed little or no change in avoidance rate, while fixed ratio responding to the

neutral stimulus was slightly disrupted, indicating specific behavioral depression. Imipramine and amitryptiline at doses of 5–20 mg/kg which produced moderately high rates in the avoidance, markedly disrupted fixed ratio responding without affecting conflict behavior. A sub-hypnotic dose of Doriden (25 mg/kg) alleviated the disruptive effects induced by the probability of punishment while producing rate-increasing effects in avoidance and marked disruption in fixed ratio responding to the neutral stimulus. Doriden at 50 mg/kg po produced a dose-related response increase in avoidance followed by depression in this and other components. At higher doses all drugs showed behavioral toxicity. This schedule has shown differential sensitivity to several drugs administered orally. The gross differences in contingencies in the various schedule components offer a wide field for the observation of specific effects.

87. *The Effect of Bromotrifluoromethane on Operant Behavior in Monkeys.* V. L. CARTER, JR., D. N. FARRER, and K. C. BACK, 6570th Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio, and 6571st Aeromedical Research Laboratory, Holloman Air Force Base, New Mexico.

Bromotrifluoromethane (CBrF₃) is a very effective fire suppressing agent for use in oxygen rich atmospheres. The chemical similarity between this compound and other known central nervous system depressants prompted this investigation to quantify the performance decrement following exposure to various concentrations of CBrF₃. Seven monkeys trained on continuous and discrete avoidance performance tasks were exposed to concentrations of CBrF₃ ranging from 10.5 to 42.0%. Significant performance decrements were observed in all monkeys during exposures to 20–25% CBrF₃. Higher concentrations resulted in impaired performance to the point of complete disruption of operant behavior in some monkeys. No visible signs of central nervous system depression or analgesia accompanied this loss of ability to perform conditioned performance tasks. These results suggest that the mechanism by which CBrF₃ causes impaired performance differs from the central nervous system depression and analgesia produced by halogenated anesthetics.

88. *Central Nervous System Supersensitivity to Chlorinated Pesticides and Pentylene-tetrazole after Chronic Barbiturate Administration.* E. MILLER, T. C. MICHEL, V. L. OLIVITO, and E. J. VAN LOON, Division of Pharmacology and Toxicology, Food and Drug Administration, Washington, D.C. 20204.

Development of supersensitivity of the dog CNS to chlorinated pesticides and pentylene-tetrazole following chronic administration of phenobarbital and subsequent withdrawal has been investigated. Changes in convulsive threshold served as the quantitative index of the increase in seizure susceptibility. Convulsive thresholds for dieldrin, lindane, and pentylene-tetrazole were determined by iv infusion. Pretreatment with phenobarbital reduced the convulsive thresholds for dieldrin, lindane, and pentylene-tetrazole by 33, 50, and 23%, respectively. At the same time, effects of acute iv injection of lindane on the convulsive thresholds for pentylene-tetrazole in normal and phenobarbital pretreated animals were studied. In each case a single injection of lindane significantly increased the convulsive threshold of the dog for pentylene-tetrazole. The results are in agreement with those reported by Coper *et al.*

89. *Effect of Atropine on Pesticide, Carbaryl (1-naphthyl N-methyl carbamate) Induced Behavioral Changes in Rats.* JASBIR M. SINGH and SISTER LEE FRAZOLD, Xavier University, College of Pharmacy, New Orleans, Louisiana 70125.

Carbaryl has been shown to decrease caffeine-induced increased performance behavior of female rats in activity wheel cages (PBAWC). To test our hypothesis, i.e., the effect of carbaryl on the PBAWC in normal and atropine sulfate-treated (0.2 mg/kg) female rats, the following experiments were performed. (1) Control, polyethylene glycol 400, (2) Atropine

sulfate, 0.2 mg/kg, (3) Carbaryl, 0.56 mg/kg, (4) Carbaryl, 2.24 mg/kg, (5) Atropine, 0.2 mg/kg plus carbaryl, 0.56 mg/kg, and (6) Atropine, 0.2 mg/kg plus carbaryl, 2.24 mg/kg. There were eight animals per group. After determining the initial PBAWC (0-30, 0-60 min) of each female rat, the animals were allowed to rest for 1 hr. Then the animals were injected ip with atropine and carbaryl. In groups 5 and 6 atropine was injected first, and 30 min later carbaryl was injected. Carbaryl in the doses 0.56 and 2.24 mg/kg and atropine, 0.2 mg/kg, significantly ($P < 0.05$) decreased PBAWC. When carbaryl was administered to atropine-treated rats, neither of the drugs produced additive or interaction in decreasing the PBAWC. The PBAWC was not restored to normal values by atropine. It is suggested from our data that PBAWC is significantly ($P < 0.05$) affected by carbaryl and atropine treatment.

90. *Acute and Chronic Toxicity of Diethanolamine*. R. HARTUNG, L. K. RIGAS, and H. H. CORNISH, The University of Michigan, Department of Industrial Health, School of Public Health, Ann Arbor, Michigan.

Diethanolamine (DEA) is one of the most frequently employed gas absorbants for CO_2 and H_2S in industrial processes. Diethanolamine salts are widely used as emulsifiers, surfactants and pesticides. Our studies have confirmed that the acute toxicity of DEA is low. Repeated ip DEA (250 mg/kg) resulted in increased liver weight accompanied by decreased total liver lipids. Short-term inhalation of high concentrations of vapor (200 ppm) or aerosols (1400 ppm) resulted in respiratory difficulties and some deaths. Inhalation of 25 ppm DEA for 216 hr continuously resulted in increased liver weight, elevated SGOT, increased kidney weight, and elevated BUN. Inhalation of 6 ppm DEA on a workday schedule for 13 weeks resulted in depression of growth rate, increased lung and kidney weights, and some deaths among male rats. Neutralized DEA in drinking water produced many deaths at 4 mg/ml when fed for 7 weeks. There were liver and kidney damage. The most obvious finding was a pronounced normocytic anemia without bone-marrow depletion, and without obvious increase in the number of reticulocytes. The findings indicate that DEA has an appreciable chronic toxicity.

91. *Acute Toxicity and Proposed Emergency Exposure Limits of Nitrogen Trifluoride*. C. C. HAUN, J. D. MAC EWEN, E. H. VERNOT, G. F. EGAN, SysteMed Corporation, Wright-Patterson Air Force Base, Ohio.

The consideration of nitrogen trifluoride as a rocket propellant necessitated a series of inhalation toxicity investigations which would reveal its relative hazard to manufacturing and handling personnel. Mice, rats, dogs and monkeys were exposed separately to NF_3 for 15-, 30-, or 60-min periods of time. Signs were noted during and following all tests. Gross and histopathologic evidence was gathered on animals succumbing to the effects of the insult, or at the time of sacrifice 14 days postexposure. Blood samples were collected from dogs and monkeys at varying periods of time postexposure. Collective evidence indicated that death was the result of methemoglobin formation exceeding approximately 80%. Tissue pathologic findings, blood data and signs from these acute inhalation experiments were employed in subsequent tests in an effort to reevaluate the current Emergency Exposure Limits for NF_3 . Rats, mice, dogs and monkeys were exposed separately to 500, 1000 or 2000 ppm of NF_3 for 60-, 30- or 15-min periods, respectively. Evidence from tissue examination, blood data and signs demonstrated no effect from the contaminant at these concentration-time levels.

92. *Lung Clearance of TiO_2 Particles in Rats*. J. FERIN, School of Medicine and Dentistry, University of Rochester, Rochester, New York.

Clearance of particles deposited in the lung is an important defense function against inhaled noxious particulate matter. The mechanism of lung clearance is not fully understood. In model experiments nontoxic and insoluble particles of TiO_2 have been used to expose rats in a dusting chamber. By producing different lung burdens and using different time intervals, it was possible to find and describe different clearance rates and their relation to the localization of the particles in the lung tissue in normal rats as well as rats with pathologically changed lungs.

93. *Measurements of Respiratory System Flow Resistance in Cynomolgus Monkeys.* C. E. ULRICH, R. H. HADDOCK, H. J. JENNINGS, and Y. ALARIE, Hazleton Laboratories, Inc., Falls Church, Virginia. (O. E. Paynter.)

The technique for measurement of respiratory system flow resistance (Rrs) described by Mead provides a simple method to investigate the effects of airborne materials on the respiratory system. This technique was adapted for measurement of respiratory system flow resistance during inspiration [Rrs(i)] and during expiration [Rrs(e)] in unanesthetized cynomolgus monkeys (*Macaca irus*). Also, a digital computer system was developed for real-time data evaluation.

Since no measurements of Rrs(i) or Rrs(e) have been published previously, the first objective was to evaluate a large population of animals over a short period of time. The second objective was to measure a group of nine animals over a period of 78 weeks to delineate the trends and variation in Rrs(i) and Rrs(e). A total of 297 measurements was made. The results indicated that Rrs(i) and Rrs(e) were decreasing with time and growth of the animals, and a regression equation was fitted to the data. Taking into account the statistical parameters of the regression analysis, it was determined that a minimum slope increase of 14.5% was needed to accept that a significant change ($P < 0.05$) had occurred in an experimental group of comparable size.

(Sponsored by the Air Pollution Research Program of the Electric Research Council.)

94. *Effects of Long-Term Continuous Exposure to Sulfur Dioxide in Cynomolgus Monkeys.* Y. ALARIE, C. E. ULRICH, A. KRUMM, E. F. DAVIS, and H. N. MACFARLAND, Hazleton Laboratories, Inc., Falls Church, Virginia.

Groups of nine cynomolgus monkeys were exposed to 0.14, 0.64, 1.28, and 4.69 ppm of sulfur dioxide for a period of 78 weeks, while a similar group was used as a control. The exposure was 24 hr a day except for a 20-min period twice a day to clean the chambers and an interruption in the exposure when animals were removed for physiological measurements. Measurements were made prior to and during the exposure for growth and survival, and pulmonary function tests were conducted to evaluate the mechanical properties of the lung, ventilation, distribution of inspired air, diffusion, and arterial blood gas tension. Also, hematology and clinical biochemical measurements were performed. No significant detrimental changes related to sulfur dioxide were detected in the groups exposed to 0.14, 0.64, or 1.28 ppm during or after 78 weeks of exposure or in the group exposed to 4.69 ppm during 30 weeks of exposure. In this last group, a 90-min overexposure to 200–1000 ppm of sulfur dioxide occurred after 30 weeks of exposure to 4.69 ppm. The animals were then removed from this atmosphere, and no further exposure to sulfur dioxide was conducted. However, this group remained in the exposure chamber in similar condition to the control group for a period of 48 weeks following this accident. During this period, pulmonary parameters were measured as originally scheduled, and the results indicated a significant deterioration in pulmonary function immediately after the overexposure and during the entire 48-week period.

We, therefore, conclude that continuous long-term exposure to levels of sulfur dioxide up to 1.28 ppm for a period of 78 weeks and 4.69 ppm for a period of 30 weeks did not result in detrimental effects on the pulmonary system of the animals as monitored by the physiological parameters chosen. Histopathological evaluation of various organs will be presented separately at this meeting.

(Sponsored by the Air Pollution Research Program of the Electric Research Council.)

95. *Effects of Long-Term Continuous Exposure to Sulfur Dioxide in Guinea Pigs.* H. E. SWANN, JR., C. E. ULRICH, H. N. MACFARLAND, and Y. ALARIE, Hazleton Laboratories, Inc., Falls Church, Virginia.

Groups of 60 male and 60 female Hartley strain guinea pigs were exposed to 0.13, 1.01, and 5.72 ppm of sulfur dioxide for a period of 1 year, while another similar group of animals

was used as a control. The exposure was 24 hr a day except for a period of 20 min twice a day to clean the chambers and for an interruption in the exposure when animals were removed for physiological measurements. Measurements were made of growth, survival, pulmonary function including tidal volume, respiratory rate, total respiratory system flow resistance, pulmonary flow resistance, dynamic compliance, work of breathing, and carbon monoxide uptake as a diffusion index. Also, hematology and clinical biochemistry measurements were performed. No significant detrimental changes related to sulfur dioxide were detected among the exposed groups or the control group with the exception of carbon monoxide uptake and survival. The diffusion index was significantly higher in the group exposed to 5.72 ppm, and the survival rate was also significantly higher in this group than for the control group. We, therefore, conclude that continuous long-term exposure to levels of sulfur dioxide up to 5.72 ppm did not result in detrimental effects on the pulmonary system of the animals as monitored by the physiological parameters chosen. Histopathological evaluation of various organs will be presented separately at this meeting.

(Sponsored by the Air Pollution Research Program of the Electric Research Council.)

96. *Histopathological Effects of Long-Term Continuous Exposure to Sulfur Dioxide in Guinea Pigs and Cynomolgus Monkeys.* W. M. BUSEY, H. E. SWANN, JR., and Y. ALARIE, Hazleton Laboratories, Inc., Falls Church, Virginia. (J. W. Clayton, Jr.)

Groups of 60 male and 60 female Hartley strain guinea pigs were exposed to 0.13, 1.01, or 5.72 ppm of sulfur dioxide for a period of 1 year, while a similar group of animals was used as a control. Groups of 9 cynomolgus monkeys were exposed to 0.14, 0.64, 1.28, and 4.96 ppm of sulfur dioxide for 78 weeks, while a similar group of animals was used as a control. In both species of animals, the exposure was for 24 hr except for two 20-min periods in which the animals were fed and the cages cleaned. Six male and 6 female guinea pigs were sacrificed following 3 months of exposure, and the remaining animals in each group were sacrificed following 12 month of exposure. A definite reduction in the incidence and severity of pulmonary disease was present in the guinea pigs exposed to 5.72 ppm of sulfur dioxide for 3 or 12 months. Hepatocyte swelling and vacuolation were present to a slight degree in the guinea pigs exposed to 5.72 ppm of sulfur dioxide. All surviving cynomolgus monkeys were sacrificed following 78 weeks exposure to sulfur dioxide. No histopathological alterations related to sulfur dioxide were seen in the animals exposed to 0.14, 0.64, or 1.28 ppm. In the group initially exposed to 4.64 ppm, a 60-min overexposure of 200-1000 ppm occurred at 30 weeks. These animals were removed from this atmosphere and held under control conditions for the remainder of the 78 weeks. Severe microscopic alterations were seen focally distributed throughout all lobes of the lung in this group. These alterations involved the respiratory bronchioles, alveolar ducts, and alveoli.

(Sponsored by the Air Pollution Research Program of the Electric Research Council.)

97. *Preparation and Preservation of Δ^9 -THC.* ROBERT F. TURK, RICHARD N. PHILLIPS, JOSEPH E. MANNO, NARESH C. JAIN, and ROBERT B. FORNEY, Indiana University School of Medicine, Indianapolis, Indiana.

Isolation and purification of *l*- Δ^9 -tetrahydrocannabinol (THC) was accomplished in sufficient quantities for pharmacologic and toxicologic studies. Flowering tops of marihuana were extracted in a soxhlet extractor with petroleum ether. The petroleum ether was then extracted with 5% NaOH in 50% ethanol. The basic ethanolic extract was then acidified with 6 N HCl and re-extracted with petroleum ether. Following the removal of the petroleum ether the extract was taken up in benzene and chromatographed on a silica gel-silver nitrate CaSO₄ column (3:1:0.5). The resin was eluted with benzene, and 20-ml fractions were collected. Each fraction was analyzed by gas-liquid chromatography and all like fractions pooled. All fractions containing 99% *l*- Δ^9 -THC were washed with 0.1 N HCl to remove silver nitrate and stored under nitrogen at 0°C. Analysis of purity was made by rechromatographing on GLC and by NMR and mass spectroscopy. Stability of the purified THC was determined

for six different storage conditions by analyzing the THC periodically by GLC. It was concluded from this study that the best method of THC preservation was storage under nitrogen at 0°C.

98. *Response of the Isolated, Perfused Rat Heart to Δ^9 -Tetrahydrocannabinol (THC).*

BARBARA R. MANNO, JOSEPH E. MANNO, GRACE S. KILSHEIMER, and ROBERT B. FORNEY, Indiana University School of Medicine, Indianapolis, Indiana.

Manno *et al.* observed a tachycardia in humans who had smoked marihuana. In order to elucidate a mechanism for this action, hearts from decapitated male Cox Holtzman rats were perfused by a modified Langendorf technique described by Paradise and Griffith. A modified Krebs-Henseleit bicarbonate medium, pH 7.4, was perfused at a rate of 10 ml/min. A 10-g diastolic tension was maintained on the heart throughout the experimental period.

THC prepared according to the method of Turk and Forney was suspended in Krebs-Henseleit medium without the sodium bicarbonate. The resulting suspension was then filtered through a Millipore filter (0.22 μ pore) and assayed in a gas chromatograph. Dilutions of this suspension were prepared for infusion.

The hearts were allowed either to beat spontaneously or were electrically driven at 420 beat/minute. Infusions of 0.1 to 0.4 μ g of THC/3-min period produced a slight decrease in the force of contraction of the heart. This force of contraction returned to control values within several minutes of the cessation of THC infusion. Two μ g of THC produced an initial increase followed by a decrease in the force of contraction which ultimately stabilized at a lower level than control values. Further infusion of THC produced greater decreases in the force of contraction which did not return to control level.

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99. *Toxicity of Δ^9 -Tetrahydrocannabinol in Rats and Mice.* R. N. PHILLIPS, R. F. TURK, and B. B. FORNEY, Indiana University Medical Center, Indianapolis, Indiana.

Crude Thailand marihuana was extracted by the method of Turk. Initial purity of the Δ^9 -THC after extraction was 99% as determined by nuclear magnetic resonance, mass spectroscopy, and gas-liquid chromatography. Purity of the compound prior to administration was unchanged as determined by gas-liquid chromatography. Using either Tween 20 or Tween 80 as suspension vehicles, LD50 values were determined in rats and mice utilizing iv, ip, or oral administration. Behavioral observations of the test animals were made.

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100. *Antidoting of Dimethyl-d-Tubocurarine in the Mouse.* MARTIN W. WILLIAMS, C. S. WILLIAMS, and LAURENCE A. MEEKS, Laboratory of Toxicology, Veterans Administration Hospital, Tucson, Arizona 85713.

Because of the continuing need for the development of drugs capable of reversible immobilization of wild animals and because of the use of curare related agents in human medicine, the quantitation of antidoting drugs and procedures for one of these materials has been studied. The drugs used were dimethyl-d-tubocurarine iodide (DMTC), neostigmine methyl sulfate, and atropine sulfate. Doses were by weight of the above salts and were, in all cases, by the ip route. Acute mortality data indicated the LD50 for DMTC in the mouse to be 465 μ g/kg. When neostigmine was given in equal dosage and by the same route (dose ratio 1/1) 3 min after the DMTC, the LD50 was raised to 630 μ g/kg. When DMTC was given simultaneously with atropine at half the dosage (2/1), the LD50 was 491 μ g/kg. When DMTC was given with half the dosage of atropine simultaneously, followed in 3 min by equal dosage of neostigmine, the ip LD50 was raised to 725 μ g/kg (dose ratio 2:1:2). Use of the dose of 2:1:1 DMTC, atropine and, 3 min later, neostigmine, respectively, raised the LD50 to 900 μ g/kg.

101. *Some Toxicological Aspects of the Chronic Administration of Phenothiazine Tranquilizers.* ROGER P. MAICKEL, MARIANNE MCGLYNN, and WAYNE R. SNODGRASS, Departments of Pharmacology and Psychology, Indiana University, Bloomington, Indiana.

Various derivatives of phenothiazine, differing in the nature of the substituents in the 2- and 10-positions on the tricyclic structure, are in common clinical use as antipsychotic agents. These compounds have received wide acceptance, despite a high incidence of side effects, including pseudoparkinsonism, neuroendocrine abnormalities and hepatic dysfunction. We have examined various aspects of the chronic toxicity of several of the compounds, attempting to correlate structural characteristics with degree of accumulation in tissues and with specific undesirable effects. Daily doses of chlorpromazine (4 mg/kg), promazine (10 mg/kg), or triflupromazine (2 mg/kg) to rats for 30 days produced evidence of fatty infiltration of the liver, hyperactivity of the pituitary-adrenocortical system, and (except for promazine) visible tremors. A comparison of gross motor activity prior to and following each daily dose demonstrated an adaptation to the drugs, most pronounced for chlorpromazine. Accumulation of the drugs in brain and plasma was greatest for promazine and least for triflupromazine.

102. *Effect of Repeated Administration of Thiopental on the Development of Tolerance and Mortality in Female Rats.* JASBIR M. SINGH, Xavier University College of Pharmacy, New Orleans, Louisiana 70125.

Tolerance has been developed when after repeated administration, a given dose of a drug produces a decreased effect. Tolerance to pentobarbital and thiopental can be induced. Animals for the present study were divided into four groups. *GP 1*: Different doses of thiopental (25, 30, 37.5, and 50 mg/kg) were injected at intervals of 24 hr for 96 hr. *GP 2*: The second injection of thiopental was introduced at intervals of 2, 4, 5, 6, 8, 24, 26, 28, or 30 hr. *GP 3*: At different time intervals, injections of thiopental, 25 mg/kg (0, 2, 21, 23, and 0, 4, 25 hr) or 37.5 mg/kg (0, 4, 24, 28, and 0, 6, 24, and 30 hr) were administered. *GP 4*: The clinical signs were observed in the animals that had developed tolerance to thiopental, 37.5 mg/kg. Percentage Tolerance Index (PTI) was calculated as follows: Hypnotic effect of the first injection/Hypnotic effect of the second injection multiplied by 100. If PTI is unity, i.e., 100%, it is indicative of no tolerance. PTI greater or less than unity indicates tolerance or cumulative effect. In this study, a cumulative response approach was applied, along with decrease in the sleeping time. Significantly ($P < 0.05$) decreased cumulative response from the initial response was an indication of developed tolerance. A minimum of 4 hr was necessary for the tolerance to thiopental to be developed in female albino rats. Peak tolerance effect was exhibited at 8 hr after the initial administration and then declined between 9 and 24 hr. When four injections were given within the span of 30 hr, a significantly ($P < 0.05$) greater degree of tolerance was shown at the fourth injection, as compared to the second injection. If the drug was administered at an interval of 24 hr, the developed tolerance increased on subsequent administrations, and this was true for at least five administrations. While studying tolerance to thiopental, the prostration effect should be taken into consideration. Thiopental at 37.5 or 50 mg/kg exerted a prostration effect whereas 25 mg/kg did not. When these doses were administered daily, the LD50 occurred on the fifth day. Urine output increased significantly ($P < 0.05$) during and after the developed tolerance. Water consumption increased also once the tolerance had developed.

103. *Dialysis of Drugs Against Activated Charcoal.* W. J. DECKER, H. F. COMBS, and J. J. TREUTING, William Beaumont General Hospital, El Paso, Texas 79920. (J. F. Borzelleca.)

Orally administered activated charcoal has been shown to reduce systemic absorption of many drugs and household poisons, but once a toxic dose is absorbed, such therapy is useless. Hemodialysis has been extensively used to combat systemic drug toxicity, but is often ineffective, particularly when the drug is only slightly soluble or has a high oil/water partition

coefficient. Addition of activated charcoal to the dialysis bath theoretically should improve the efficiency of this procedure. A 2-compartment, 4-pool in vitro model (plasma water, plasma protein: dialysis solution water, activated charcoal) was constructed and tested using various drugs in a plastic dialysis cell with a cellophane membrane. The significant decrease in plasma drug concentration obtained when activated charcoal was added to the dialysis solution indicates that this agent may greatly improve the efficiency of hemodialysis in the treatment of acute drug intoxication.

104. *Separation of Marihuana Components by Gas-Liquid Chromatography.* ROBERT F. TURK, RICHARD N. PHILLIPS, JOSEPH E. MANNO, NARESH C. JAIN, and ROBERT B. FORNEY, Indiana University School of Medicine, Indianapolis, Indiana.

A search for an ideal column which would afford excellent component separation and peak height for quantitative analysis of marihuana components resulted in the following:

A four foot salinized glass column (6 mm OD and 3 mm ID) packed first with 1.5 ft of 10% QF-1, then with 1.5 ft of a mixture of 1% OV-17-1% OV-1 and finally with 10 inches of 2% OV-1, all on Chromosorb G 100/120 mesh. The 2% OV-1 phase was positioned towards the flame detector.

This column was used in conjunction with an F & M (Hewlett-Packard) Scientific 402 high efficiency gas chromatograph, equipped with a flame ionization detector and operated under the following conditions: Injection port temperature 285 °C; oven temp. 255 °C; flame detector temp. 285 °C; carrier gas (Helium) flow 60 ml/min; hydrogen and oxygen flow rates 35 and 260 ml/min, respectively.

This chromatographic system was employed to analyze: (1) the components present in marihuana, (2) the best method of storage of the plant material, and (3) the rate of decomposition of purified Δ^9 -tetrahydrocannabinol (Δ^9 -THC). This study resulted in the finding that the components of marihuana can be separated and quantitated, and decomposition of marihuana and/or Δ^9 -THC is minimal if stored under nitrogen at 0 °C.

105. *Effects of Alcohol, Atropine, and PAM on the LD50 Values of Ibogaine in Rats.* H. I. DHAHIR, N. C. JAIN, A. B. RICHARDS, and R. B. FORNEY, School of Medicine, Indiana University Medical Center, Indianapolis, Indiana.

The alkaloid ibogaine, obtained from a West African shrub called *Tebernanthe iboga* (Fam. *Apocynaceae*), is classified as a psychotropic drug which belongs to the indole group. Neuropharmacological studies indicate that ibogaine hydrochloride has distinct central nervous system stimulating properties and shows an inhibitory effect on serum cholinesterase in rats. Atropine was found to abolish the altering response produced by ibogaine. In this study the ip LD50 of ibogaine HCl was determined in white male Holtzman rats and was compared to those LD50 values obtained with ibogaine + alcohol, ibogaine + atropine, ibogaine + PAM, and ibogaine + atropine + PAM to see if any significant change in the degree of toxicity would occur.

106. *Toxicological Comparison of 1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) and 1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) in Rats and Mice.* G. R. THOMPSON and R. E. LARSON, School of Pharmacy, Oregon State University, Corvallis, Oregon.

BCNU and CCNU have been reported to exert equal carcinostatic activity in screening studies, but CCNU has been said to appear to be less toxic of the two. No critical comparisons of their toxicities have appeared, however. In this study the toxicities resulting from the administration of single oral doses of BCNU and CCNU have been compared in both rats and mice. Determination of 30-day LD50s revealed that BCNU was twice as toxic as CCNU when doses were expressed either as mg/kg or as mm/kg. Deaths never occurred earlier than 4 days posttreatment for either compound even at doses that were 5 times the respective

LD50. Comparison of median lethal times at the LD99 doses revealed identical time courses in the distribution of deaths. Indeed, equitoxic doses of the two agents produced similar patterns of toxicity which included weight loss, anorexia, diarrhea, jaundice, and bone softening. Liver, lung, kidney, and brain masses did not decrease proportionally to body weight losses which resulted in apparent increases in their fractional contribution to body weight. For both agents, the rate of development of the toxic pattern was reduced as the dose range was descended. Ultimately, the same magnitude of effect was attained, however, at all except the lowest dose employed (\approx n LD1). No significant species, sex, route, or solvent differences were observed. These data suggest that low doses of BCNU and CCNU induced subtle but persistent changes in systems which were affected more acutely by large doses.

107. *An Evaluation of the Effects of Monosodium Glutamate in Man and Animals.*

I. ROSENBLUM, D. M. SERRONE, J. C. KILLEEN, JR., J. BRADLEY, J. H. WILLS, and F. COULSTON, Institute of Experimental Pathology and Toxicology, Albany Medical College, New York State Department of Health, Albany, New York, and Medical Department of Clinton Prison, Dannemora, New York.

Three aspects of the effects of monosodium glutamate (MSG) have been studied. The ability of MSG to sensitize or desensitize receptors to endogenous mediators was investigated in isolated tissues of several species. Results indicate that MSG sensitizes the frog rectus abdominis to potassium and acetylcholine but desensitizes the rabbit aorta strip to histamine.

Acute oral administration of 5 or 8 g of MSG to monkeys and intravenous infusions of lesser amounts to rabbits and monkeys did not affect the general behavior pattern, gross appearance, or electrocardiogram.

Three groups of rats were given 1, 2, or 4 gm/kg of MSG as a single daily oral dose, and one group received an iso-osmotic amount of NaCl. This experiment was carried out for 90 days. Body weight gain, hematologic values, blood chemical determinations, and urinalysis did not differ significantly between groups. A histologic appraisal of tissues taken at sacrifice showed no significant pathologic changes.

Ninety-eight male volunteers of varying age and background were fed a single 5-g dose of MSG dissolved in either tap water or diluted chicken soup stock. Each volunteer filled out a questionnaire consisting of 14 symptoms; they were also closely observed. Approximately one-half of the subjects affirmed one or more symptoms, but only one of these appeared to have "reacted" to MSG.

108. *Comparative Acute Ulcerogenicity of Nonsteroid Antiinflammatory Agents in Rats.*

J. E. WALTER, R. M. DIENER, and A. E. EARL, Ciba Pharmaceutical Company, Summit, New Jersey.

Nonsteroid antiinflammatory agents administered iv were assayed for gastrointestinal ulcers and mortality in Wistar rats. Multiple intestinal ulcers were induced within 4-8 hr after injection of any compound tested. Threshold doses for ulcerogenicity varied with each drug and ranged from 10.0 mg/kg for indomethacin to 125.0 mg/kg for acetylsalicylic acid. Mature male rats were significantly more susceptible to ulcers and mortality than females with some antiinflammatory phenylacetic acid derivatives. This difference in susceptibility was eliminated in castrated males but was readily reversed with testosterone replacement. Ovariectomized females were somewhat less susceptible to treatment than mature females, and estradiol replacement did not alter the toxicity. The comparative toxicologic results in sexually immature males and females were identical. Therefore, the increased susceptibility of male rats to some phenylacetic acid derivatives appears to be due to testosterone. The iv administration of nonsteroid antiinflammatory agents to rats offers a simple, rapid assay system for ulcerogenicity, which appears to be directly proportional to the antiinflammatory activity of these compounds.

109. *Some Factors Affecting Intracutaneous Irritation Tests in Rabbits.* WALLACE L. GUESS and DAVID J. CALLEY, College of Pharmacy, University of Texas, Austin, Texas 78712.

The United States Pharmacopeia XVII requires an erythema test in rabbits for intracutaneous irritation liability for extracts of plastics intended for use as containers for parenteral drugs. Many research laboratories use the technique for control procedures. There are inherent disadvantages to this technique, and the trypan blue extravasation method has been used to supplement the erythema test. During the course of some intracutaneous irritation experiments in rabbits, it became apparent that factors other than inherent toxicity of the test material were influencing the results. Two variables in the injected solutions were evaluated, namely pH and tonicity. It was decided to evaluate the site of injection in relation to shoulder, middle, or pelvic area and the age of the rabbit as animal variables. The results showed that intracutaneous irritation from pH alone could be observed below pH 4.0 or above pH 9.0. Salt concentrations above 3.5% elicited irritation of sc tissues, but concentrations down to 0.1% sodium chloride caused no response. The age of the rabbit (reflected as thicker skin) influenced the results of selected pH and tonicity values. The data suggest that solutions for intracutaneous irritation studies should be buffered and maintained at a reasonable tonicity value in order to evaluate inherent toxicity of the test material.

110. *Effect of Diphenylhydantoin on Storage of DDT in the Rat.* M. F. CRANMER, Food and Drug Administration, Perrine Primate Research Branch, P.O. Box 490, Perrine, Florida.

Dilantin is a widely used anticonvulsant drug which has been shown to stimulate liver microsomal enzymes and reduce DDT and DDE residues in man. Our investigation was undertaken to study the effect of Dilantin on the storage of DDT and DDE in the rat.

One hundred and forty-four female rats were divided into three groups and received 5, 10, or 15 ppm p,p'-DDT in their diet for 3 weeks. One-half of each group received 250 ppm Dilantin plus their respective doses of p,p'-DDT for the second 3 weeks of treatment. Four rats of each treatment group were sacrificed at the end of weeks 3, 4, 5, and 6. At the beginning of week 7 p,p'-DDT was removed from the diet of all rats, and four animals of each group were sacrificed at the end of week 7, 8, and 9. Levels of p,p'-DDT and DDE in fat were determined for each sacrificed animal. Liver microsomal enzyme activity was measured for those animals sacrificed at the end of the sixth and ninth weeks of treatment.

A second group of 24 rats was fed 10 ppm p,p'-DDT for 14 months. Half the group was sacrificed, and the remaining 12 animals were fed 10 ppm p,p'-DDT and 250 ppm Dilantin for 6 weeks prior to sacrifice. Levels of p,p'-DDT and p,p'-DDE in fat and liver microsomal enzyme activity were measured for all 24 animals.

Dilantin was found to reduce the levels of p,p'-DDT and p,p'-DDE in the fat and to increase liver microsomal enzyme activity in all animals except those receiving 10 ppm p,p'-DDT for 14 months.

111. *Comparative Studies of the Hepatic Effects of the Hypolipidemic Agents SaH 42-348(1-methyl-4 piperdyl bis(p-chlorophenoxy) acetate) and Clofibrate(ethyl chlorophenoxyisobutyrate) in Rats.* H. A. HARTMAN, R. E. BAGDON, R. J. VAN RYZIN, and A. J. TOUSIMIS, Sandoz Pharmaceuticals, Hanover, N. J., and Biodynamics Research Corp., Rockville, Maryland.

Rats were administered 0.120% SaH 42-348 and 0.300% clofibrate for 6 and 12 weeks; additional groups were continued on nonmedicated diet for 3 and 6 weeks after cessation of the initial 6-week administration of the drugs. For both agents, food intake, weight gain, and serum cholesterol levels were comparable to controls. Also, serum triglycerides were decreased but returned to control levels 3 and 6 weeks after withdrawal of treatment. Relative and absolute liver weights were increased after 6 and 12 weeks but reverted to normal 3 and 6 weeks after the agents were withdrawn. Serum bilirubin, SGPT, and serum ornithine carbamyl transferase were normal. Hepatic hexobarbital metabolizing activity was greater than in controls after 6 weeks but similar to controls at 12 weeks in response to both agents.

For both agents, light microscopic findings showed increased cytoplasmic granularity and cell diameter and decrease of perinuclear open spaces of hepatocytes; both were reversible. Electron microscopy showed an increase in microbody mitochondria ratio (Mb:M) and decreased glycogen pools; lysosomes were increased but mitochondria and rough endoplasmic reticulum were unchanged. At 6 weeks in the SaH 42-348 group, prominent swirls of smooth endoplasmic reticulum were noted which were not apparent at 12 weeks or 3 or 6 weeks following medication. After 12 weeks, the Mb:M ratio was also increased, the RER was disorganized and evenly distributed. Upon cessation of treatment, ultrastructural changes largely reverted to normal in both treatment groups, with only minor residual effects present in the clofibrate group.

Thus, hepatotoxic effects were not observed based upon measurements of liver function, and light and electron microscopy. The hepatomegaly was readily reversible and may represent an adaptive response to administration of these hypolipidemic agents. It would appear that by all parameters studied, the responses of the rat liver to SaH 42-348 or clofibrate administration were essentially identical.

112. *The Effects of Short-Term Administration of Ethylenethiourea upon Thyroid Function in the Rat.* STUART L. GRAHAM and WALTER H. HANSEN, Bureau of Science, Food and Drug Administration, Washington, D.C. 20204.

Thyroid changes have been reported in rats fed the ethylenebisdithiocarbamate fungicides throughout the life span. Some of these fungicides are unstable and break down rapidly in animal feed supplemented with them. One of the decomposition products is ethylenethiourea (ETU), and a short-term study of the effects of ETU on thyroid function was conducted. Osborne-Mendel rats were fed ETU for 30, 60, 90, or 120 days at dietary concentrations of 0, 50, 100, 500, or 750 ppm. At the end of each feeding period, animals on each diet were injected with ^{131}I ip and divided into two groups; the radioactivities of the weighed thyroid pairs of individual animals were determined at 4 hr (Group 1) and 24 hr (Group 2) post-injection.

The results show that there was decreased growth at all levels of ETU and at all testing periods. A dose-related increase in thyroid weight and a decline in the uptake of ^{131}I was found at all levels and after all testing periods.

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