

ABSTRACTS OF PAPERS

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Abstracts of Papers for the Eighth Annual Meeting of the
Society of Toxicology, Williamsburg, Virginia
March 10-12, 1969

1. *Rapid Induction of Carcinoma in the Rodent by Polynuclear Hydrocarbons.* B. N. BANERJEE and D. J. HOWARD, Woodward Research Corporation, Herndon, Virginia. (SPONSOR: G. Woodward.)

Mammary tumors were induced in Sprague-Dawley female rats by single intragastric doses of 7,12-dimethylbenz[*a*]anthracene (DMBA). The rats were 55 days old at the time of dosing. Tumors were produced in 60% of 40 rats within 150 days by administration of 20 or 30 mg of DMBA. The histopathologic findings confirmed that these tumors were adenocarcinoma. In contrast, no tumors developed in any of 40 Long-Evans female rats for the same period of time and with the same dose. However, mortality was 20% in both strains of rats given the highest dose. It appears that these tumors were hormone dependent, as no tumors were produced in the bilaterally ovariectomized Sprague-Dawley rats. A few tumors were transplanted in normal 55-day-old female Sprague-Dawley rats, but the results were inconclusive. The importance of the rapid induction of carcinoma for carcinogenic studies will be discussed.

(Supported by a grant from the Damon Runyon Memorial Fund for Cancer Research, Inc.)

2. *Azathioprine and Liver Tumor Induction by N-Hydroxy-N-2-Fluorenylacetamide.* H. H. FRANKEL, R. S. YAMAMOTO, and J. H. WEISBURGER, Biology Branch, National Cancer Institute, Bethesda, Maryland.

Male Sprague-Dawley rats tolerated larger doses of dietary azathioprine (Imuran[®]) than did Fischer 344 rats. Levels of 200 ppm of azathioprine in a semisynthetic diet stunted growth and ultimately led to death in the Fischer group, while Sprague-Dawley rats continued to survive without difficulty. The effect of continuous azathioprine feeding and its influence on liver tumor induction by the carcinogen *N*-hydroxy-*N*-2-fluorenylacetamide (N-OH-FAA) was studied in Fischer rats. One group of 6-week-old Fischer rats was placed on 150 ppm azathioprine in Wayne meal and fed ad libitum. The second group received the above diet plus 160 ppm of N-OH-FAA. The third group received only 160 ppm of N-OH-FAA. Finally, a fourth control group was fed Wayne meal. All animals were weighed and examined at weekly intervals. After 16 weeks on experimental carcinogen diet, the animals were changed to either the azathioprine alone or control diet. Animals were killed at 16, 22, 23, and 24 weeks. Male Fischer rats tolerated 150 ppm azathioprine, but there was a marked fatality rate in the female group. Microscopic examination of liver sections revealed only a few parenchymal cells with mitotic figures and minimal degenerative change. Discrete nodules (trabecular-type hepatocellular carcinoma) and mild cirrhotic changes were observed in the group fed 160 ppm N-OH-FAA. In contrast, the rats fed the combination of 150 ppm azathioprine and 160 ppm N-OH-FAA had firm large livers with multiple nodules. On microscopic examination, many large hyperplastic nodules separated by a large amount of dense fibrous stroma were observed. A number of hyperplastic nodules undergoing malignant change and several atypical types of carcinoma were seen. Thus, azathioprine potentiated the toxic, but not the carcinogenic, effect of N-OH-FAA.

3. *A Carcinogenic Assay Involving 130 Pesticides and Industrial Chemicals.* J. R. M. INNES, A. J. PALLOTTA, B. ULLAND, M. VALERIO, E. R. HART, L. FISHBEIN, L. PETRUCCELLI, J. SWITZER, H. FALK, R. BATES, and M. KLEIN, Bionetics Research Laboratories, Bethesda, Maryland, and the National Cancer Institute, Bethesda, Maryland.

One hundred and thirty compounds selected from pesticides and industrial chemicals of related structure were administered to two hybrid strains of mice (B6C3F1 from

ERRATUM

A CORRECTION should be made in the text of the Abstract No. 3 entitled "A Carcinogenic Assay Involving 130 Pesticides and Industrial Chemicals" by J. R. M. Innes, A. J. Pallotta, B. Ulland, M. Valerio, E. R. Hart, L. Fishbein, L. Petrucelli, J. Switzer, H. Falk, R. Bates and M. Klein. The sentence beginning with the words "All positive controls.....," should be substituted by the following text:

"Uncertainties in the assessment of the results by preliminary statistical analysis preclude adequate evaluation of the results at this time. Performance of extensive multifactorial statistical analyses will be required to assess the compounds studied. Because this could not be accomplished in the available time, the authors elected to withdraw their presentation from the program of this meeting and will report their studies fully through the scientific literature as soon as the analyses will permit evaluation of the findings."

C₃H/Anf♂ × C₅₇B1/6♀ and B6AKF1 from AKR♂ × C₅₇B1/6♀). The majority of compounds were studied in 18 animals of each strain and each sex by both subcutaneous and oral routes. Thus, each compound was tested in 144 mice. A single subcutaneous dose was administered on the seventh day of life, whereas treatment by the oral route was continuous until the end of the experiment. Both positive and negative control groups were placed among the 22,000 mice used in this study. All groups were studied 18 months and then subjected to necropsy with histologic study of all lesions and of the major organs of all mice. Most animals survived the entire observation period. All positive controls were successfully identified as tumorigenic in this bioassay. A statistical analysis was performed on the tumor incidence obtained combining both sexes and both strains for each route of administration; 51 compounds which are not ordinarily considered tumorigenic produced statistically significant increases in the tumor incidence as compared to the controls. These included 33 in the oral study and 31 in the subcutaneous study. Problems in the conduct and interpretation of a study of this magnitude are discussed.

4. *Carcinogenicity of Food Additives, Pesticides, and Drugs after Parenteral Administration in Infant Mice.* K. FUJII and S. S. EPSTEIN, Laboratories of Environmental Pathology and Carcinogenesis Children's Cancer Research Foundation, Inc. and Department of Pathology, Harvard Medical School, Boston, Massachusetts.

The carcinogenicity of various food additives, pesticides, and therapeutic drugs was determined by repeated subcutaneous injection of infant Swiss mice, at ages of 1, 17, 14, and 21 days after birth, at dose levels determined by previous acute toxicity tests. Mice were sacrificed at 1 year. A high incidence of hepatomas, based on mice at risk on weaning, were found in males injected with safrole (60%), maleic hydrazide (43%), and griseofulvin (24%) at total doses of 6.6, 55.0, and 3.0 mg, respectively, compared with an incidence of < 5% in groups injected with Tween® 60, alginic acid, RA® 858, EMQ®, Freon® 112, piperonyl butoxide and controls. Pulmonary adenocarcinomas were also found in mice injected with safrole. The incidence of subcutaneous sarcomas was < 1% in all test and control mice. These results confirm the practical utility of neonates for carcinogenicity testing in that remote tumors develop rapidly following restricted parenteral administration of relatively small quantities of test materials.

(These studies were supported by Grants C-6516 and FR-05526 from the N.I.H.)

5. *Toxic Effects of the Intravenous Administration of Neocarzinostatin (NSC-69856) Lot 67L1038 and 67L1048 in Dogs.* U. SCHAEPI, M. M. MASON, A. BOGDEN, P. S. SCHEIN, and R. D. DAVIS, Mason Research Institute, Worcester, Massachusetts, and National Cancer Institute, Bethesda, Maryland. (Sponsor: D. P. Rall.)

Neocarzinostatin, an antibiotic from streptomyces carzinostaticus (a protein, molecular weight 9000, with marked antitumor activity) was tested for preclinical toxicology in 22 beagle dogs. Six dogs treated with a single dose of 10 mg/kg, 28 daily consecutive doses of 1.25 mg/kg, or 3 weekly doses of 5 mg/kg developed precipitating antibodies against neocarzinostatin. Antibodies appeared in the dog serum 29 days or more after onset of treatment, peaked, and regressed thereafter. The peak in antibody formation was followed by a progressive increase in BUN and the animals became critically ill or moribund (58-108 days). The autopsy showed degenerative changes of the small arteries and arterioles in kidneys and heart, severe renal tubular and glomerular damage and myocardial infarctions. The findings indicate that neocarzinostatin produced a foreign-protein nephritis which led to renal failure and death. The dogs treated at lower dose levels survived. Animals which received repeated daily doses of 0.32 and 0.16 mg/kg for 28 days did not react with serious toxic effects, although they developed precipitating antibodies to a minimum of 3 antigenic components of neocarzinostatin. Treatment with weekly doses of 0.625 mg/kg for 6 weeks or with a single dose of 1.25 mg/kg was also tolerated without toxic manifestations.

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

6. *The Toxicity of Escherichia coli L-Asparaginase*. N. RAKIETEN, P. S. SCHEIN, B. M. GORDON, R. D. DAVIS, D. A. COONEY, and D. P. RALL, The South Shore Analytical and Research Laboratory, Inc., Islip, New York, and The Laboratory of Chemical Pharmacology, National Cancer Institute, Bethesda, Maryland.

L-Asparaginase, specific activity 47–363.4 IU/mg protein, when administered iv 350–2000 IU/kg/day \times 5 to rhesus monkeys (*Macaca mulatta*) produced hepatic fatty metamorphosis of the liver. Related biochemical changes include BSP retention and elevations in serum triglycerides and occasionally increases in serum alkaline phosphatase, SGOT, and SGPT. The pathology was not related to specific activity, nor was it prevented by pretreatment with asparagine. Reversibility of the pathology was noted in monkeys sacrificed 11–69 days post treatment with one exception. The major overt signs resulting from treatment included anorexia, weight loss and occasionally blood-streaked stools, diarrhea, and in one instance ulcerative colitis. Control monkeys when starved developed BSP retention suggesting that the liver pathology noted in the enzyme-treated animals was due to nutritional factors. Some monkeys also showed a decrease in leukocytes or a leukopenia and transient elevations in BUN. None of this pathology was seen in the beagle dog despite treatment with higher and more prolonged dosages of the enzyme. The dogs never became anorexic and showed weight gains. Following equivalent doses to the dog and monkey serum levels in the dog were approximately half those of the monkey, but monkeys with lower plasma levels than dogs developed fatty livers. While the enzyme is antigenic and some batches are pyrogenic to rabbits, the implication of endotoxin in the monkey pathology is equivocal.

(This study was carried out under Contract No. ph-43-64-932, National Cancer Institute, USPHS.)

7. *Whole-Body Radioautographic Localization and Semiquantitative Determination of Chemotherapeutic Agents in Normal and Tumor-Bearing Rodents*. R. H. LISS, F. A. COTTON, and C. J. KENSLER, Arthur D. Little, Inc., Cambridge, Massachusetts.

A semiquantitative radioautographic method has been used to study the whole-body distribution of trifluoromethyldeoxyriboside (F_3TdR), 5-fluorouracil (5-FU) and 6-mercaptopurine (6-MP) in normal and solid L1210 or carcinoma 755 tumor-bearing rodents. The animals were frozen in liquid N_2 between 2 and 96 hours after iv dosage with $^{14}C-F_3TdR$ (150 mg/kg, 100 $\mu Ci/kg$), 24, 48, and 96 hours after iv dosage with $^{14}C-5-FU$ (200 mg/kg, 980 $\mu Ci/kg$) and 24 hours after iv dosage with $^{35}S-$ or $^{14}C-6-MP$ (120 mg/kg, 80 $\mu Ci/kg$). X-ray film was developed after exposure to the tissue sections and ^{14}C -standards (dpm range = 5.0×10^3 to 3.9×10^5). A standard curve, radioautogram density *vs.* dpm, was determined by a linear least squares regression model, and densities corresponding to tissues in whole-body sections were converted to dpm. F_3TdR , 5-FU, and 6-MP radioactivity leaves the blood rapidly. Biliary excretion of F_3TdR and 6-MP radioactivity is important in BDF₁ mice, but not significant for 5-FU. The semiquantitative method indicates the tissue:plasma ratio of $^{14}C-F_3TdR$ radioactivity, in tumor-bearing BDF₁ mice, 24 hours after iv dosage, was: tumor, 8.8; thymus, 15.3; bone marrow, 4.7; intestinal contents, 20.0; testes, 15.0; spleen, 3.7; and muscle, 2.0. Time-related differences in tissue:plasma ratios at 2, 4, and 24 hours were: gall bladder, 76.0, 32.0, 4.0, and thymus, 6.9, 11.2, 15.3; $^{14}C-5-FU$, $^{35}S-$, and $^{14}C-6-MP$ radioactivity distribution differs. Drug radioactivity is localized in solid L1210 and carcinoma 755 tumors, but varies from one area of a tumor to another. The relative concentration of drug radioactivity in whole-body and tumor sections can be estimated by the semiquantitative method, for whole-body data are comparable with independent investigations on the physiologic disposition of these drugs by biochemical extraction techniques.

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

8. *Effects of Phenobarbital and Dexamethasone on the Adrenocortical Response of Rats to Toxic Chemicals and Other Stresses.* R. J. SZOT and S. D. MURPHY, Department of Physiology, Harvard School of Public Health, Boston, Massachusetts.

Changes in rat adrenocortical activity, manifested by increased plasma corticosterone levels and increased activity of glucocorticoid-inducible liver enzymes after various toxic chemicals and acute cold exposure have been reported previously. Phenobarbital (PB) and dexamethasone (DM) inhibition of response to stressful toxic and physical stimuli were studied in this investigation. Adrenocortical response in male rats following DDT (200 mg/kg, po), parathion (3 mg/kg, ip), acrolein (3 mg/kg, ip), acute cold exposure (6°) and hind-leg ligation was determined by measuring plasma-free corticosterone (PC) levels, and in some experiments, adrenal corticosterone (AC). Other groups of rats were pretreated with PB (75 mg/kg, ip) or DM (0.25 mg/kg, sc) 15 min or 4 hours, respectively, before all stresses except DDT. Phenobarbital or DM was given 3 hours after or 3 hours before DDT, respectively. The animals were sacrificed 5 hours after DDT and 1 hour after administration of the other stresses. Phenobarbital inhibited elevations in PC caused by DDT and parathion but not those caused by the other stimuli. Phenobarbital pretreatment did not inhibit the adrenal response to ACTH, this suggests that PB inhibited ACTH release after DDT and parathion. Dexamethasone inhibited elevations in PC after all stimuli except for acrolein. The increases in PC after parathion or acrolein in unpretreated rats were approximately equal. Inhibition by PB and DM did not appear, therefore, to be totally dependent on the degree of PC elevation. In experiments where AC was measured, the effects of stresses with and without drug treatments were similar to those observed for PC. Although adrenocortical stimulation is a response common to many stressful stimuli, the results of these experiments suggest that the pathways or mechanisms differ among different toxic chemicals or other stressors.

(Supported by Training and Research Grants TO1-ES-00045 and RO1-UI-00424 from the Division of Environmental Health Sciences, NIH, and National Center Urban and Industrial Health.)

9. *The Toxicology of Modified Polyacrylamide Resin.* E. E. CHRISTOFANO, J. P. FRAWLEY, O. E. FANCHER, and M. L. KEPLINGER, Hercules Incorporated, Wilmington, Delaware and Industrial Bio-Test Laboratories, Inc., Northbrook, Illinois.

To improve settling of solids in liquid suspensions, coagulant aids are being introduced into various operations, possibly including food processing. A series of flocculating agents have been developed as strongly cationic, high molecular weight, water-soluble polymers. The toxicity of these modified polyacrylamide resins has been investigated in acute, sub-acute, and chronic feeding studies with dogs and rats and in a reproduction study in rats. Water solutions are very high viscosity liquids. The maximum single dose administered to rats (464 mg/kg) was accepted by the animals without toxic manifestations. Rats were fed for 90 days at 500, 2000, 10,000 and 50,000 ppm without effects upon growth rate, mortality, urinalysis, hematologic studies, gross pathology, or microscopic pathology. At the two higher levels, liver weight was altered in female rats. Dogs fed for 90 days at dietary levels of 10,000 ppm exhibited depressed weight gain, increased organ weight, and abnormal stomach contents. At 500 or 2000 ppm all findings were normal.

Chronic studies in which rats were fed 500, 2000, and 10,000 ppm in their total diet for two years showed no significant adverse effects upon any observed reaction including growth, food consumption, hematology, clinical chemistry, cholinesterase activity, organ weight, and pathology. A similar study on dogs also fed 500, 2000 and 10,000 ppm of their total diet demonstrated no effects at the two lower levels of feeding and only questionable findings at the 1% level. The first and second generations of a continuing three-generation reproduction study in rats fed 500 and 2000 ppm exhibited normal findings in all reproduction indices measured including: mating index, incidence of pregnancy and parturition, gestation time, and lactation index. Other observations including body weight, growth rate, behavioral reactions, skeletal abnormalities, mortality, clinical and pathological findings were normal.

among parents and progeny. It appears at this time that 2000 ppm in the total diet of dogs and rats results in normal findings.

10. *Tobacco Smoke; Comparison of Chamber and Face Mask Exposure Systems in Guinea Pigs.* R. E. GOLDHAMER, S. CARSON and E. E. VOGIN, Food and Drug Research Laboratories, Inc., Maspeth, New York.

A comparison was made of face mask and chamber exposure techniques in unanesthetized guinea pigs exposed to combustion products of tobacco. Respiratory and cardiovascular parameters were employed to evaluate the physiologic consequences of acute and chronic inhalation. Full body plethysmographic procedures were utilized for simultaneous measurements of pulmonary ventilation, total pulmonary resistance, and electrocardiography. Ventilatory and lung diffusing capacity were also determined by gas-liquid chromatography. Direct blood pressure recordings were made by right ventricular catheterization of animals from the group exposed in the chamber and by carotid catheterization of those exposed by face mask. Guinea pigs exposed to a continuous flow of tobacco smoke in a chamber at maximum tolerated doses for 5 days per week over a 72-week period showed no significant changes in blood pressure. Significant hypertension of 9-17% was seen following a puffed-smoke face mask exposure regimen. Chronic hypoxia with accommodation characterized the responses. Respiratory rate and minute volume changes were significant. No differences were found in total respiratory resistance, lung diffusing capacity, and EKG.

11. *The Recovery of Particle Transport Activity and Cellular Replacement after Mechanical Abrasion of the Trachea of Intact Chickens.* S. P. BATTISTA, E. P. DENINE, and C. J. KENSLER, Arthur D. Little, Inc., Cambridge, Massachusetts.

A new technique has been developed for denuding the epithelium of chicken tracheas *in situ* as a means of studying regeneration of epithelium of the respiratory tract along with the restoration of ciliary particle transport activity (CPTA). Denuding the upper half of the tracheal epithelium was accomplished by introducing and withdrawing a fine bristled nylon brush in the exteriorized trachea of anesthetized chickens. Particle transport activity was measured on both an abraded and unabraded section by the *in vitro* method of Kensler and Battista. Additional pieces from both top and bottom parts of the trachea were studied histologically to determine the proportion of cells bearing cilia and the mucous cells (those containing PAS-positive material). Preliminary results show that immediately and 24 hours after abrasion, there is no measurable CPTA, whereas after 2-4 days, a measurable level of activity was observed. The major portion of the CPTA is restored by 7 days and appears to be complete after 14 days. The return of ciliary function roughly paralleled the morphologic regeneration of ciliated cells, except that at 4 days, the restoration of function was disproportionately greater than that suggested by the proportion of these cells. Although more than 90% of the epithelial cells were destroyed as a result of the abrasion, the major portion of the regeneration of the epithelium of the chicken trachea, both functionally and morphologically, occurs by 14 days. This method should permit the study of the effects of potentially harmful or beneficial agents on respiratory epithelium during the active phase of cellular replacement.

12. *Comparative Cytopathologic Alterations Induced by Alkylnitrosamines in Nasal Epithelium of the Syrian Hamster.* M. GREENBLATT, Eppley Institute for Research in Cancer, University of Nebraska College of Medicine, Omaha, Nebraska. (Sponsor: P. Shubik.)

Despite the similarity in chemical structure of dimethylnitrosamine (DMN) and diethylnitrosamine (DEN), their biologic activity differs. In the Syrian golden hamster DEN produces nasal tumors while DMN is an ineffective nasal carcinogen. This study describes the acute pathologic effect of DMN and DEN over a wide range of doses in the hamster, emphasizing changes in nasal epithelium. Cytopathologic changes were found especially in the olfactory

sensory epithelium with both drugs. The response was dose related. Progressive degenerative and necrotizing changes were seen in the olfactory epithelium. Changes in respiratory epithelium were slight or absent at the same dose levels. The coincidence of these acute data with those of tumor incidence at this site in this species suggests a relationship between the acute effects produced by these nitrosamines and the carcinogenic activity. The mechanism of action of these nitrosamines is discussed.

13. *Psychopharmacology of Carbon Monoxide under Ambient and Altitude Conditions*. K. C. BACK and A. M. DOMINGUEZ, Toxicology Branch (MRBTT), 6570th Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio and Toxicology Branch, Armed Forces Institute of Pathology, Washington, D.C.

The Air Force has become very interested in the short- and long-term effects of carbon monoxide (CO) since studies of gas-off products of space cabin materials and processes (including man's contribution) reveals that the compound is likely to be found in space cabins if not properly scrubbed. Recent investigations have indicated that very low concentrations of CO can cause subtle decrement in high level performance. In order to test performance changes, two continuous 100-day CO exposure studies to 55 mg/m³ in ambient air, and in a space cabin atmosphere of 5 psia total pressure (27,000 ft simulated altitude), mixed gas environment consisting of 68% oxygen and 32% nitrogen (pO₂ = 160 mm Hg) were performed. At the end of the second experiment, and without stopping exposures, CO concentrations were increased to 110, 220, and 440 mg/m³, respectively, at 7-day intervals. The experiments were performed on 12 trained monkeys in which operant behavior was conditioned to both continuous and discrete avoidance tasks by both audio and visual cues. The animals performed 15 min/hour, 8 hours/day and 5 days/week, during the continuous exposure period. Extensive clinical laboratory determinations were performed, including blood carboxyhemoglobin levels, throughout the test periods. In the two experiments conducted at 55 mg/m³, carboxyhemoglobin per cent saturation plateaued after the first 48 hours' exposure at 3.7 and 4.7, respectively. There was no observable decrement in performance under either ambient or altitude conditions, nor were there changes in other clinical parameters. Exposure to 110, 220, and 440 mg/m³ caused mean CO saturations of 8.3, 19.5, and 30.1%, respectively. These levels produced performance changes in two of the 12 monkeys; the 440 mg/m³ level also produced changes in appetite and outward appearance in 6 of the 12 animals.

14. *The in Vitro Effect of Some Commonly Prescribed Drugs on Routine Laboratory Tests*. A. S. TEGERIS, J. C. COTTRELL, and E. A. CRUZ, Group Health Association, Washington, D.C.

Drugs are known to interfere with the assay of various laboratory tests. It is known, for instance, that the urine of patients on the antibiotic oleandomycin gives falsely elevated steroid results, while the serum of patients on aspirin or diphenylhydantoin gives falsely depressed PBI values. The effect of 44 commonly prescribed drugs, available as intravenous preparations, was investigated on 20 routine laboratory procedures. The drugs were diluted in serum from patients receiving no medication of any kind. Two concentrations were used, 1.0 mg/ml and 100.0 µg/ml. Eighteen drugs interfered with one or more tests at the high concentration, the interference ranging from an increase of 16% in blood glucose for quinidine to an increase of 1924% in serum phosphorus for prochlorperazine. Sixteen of these 18 drugs interfered with one or more tests at the lower concentration, the interference ranging from an increase of 16% in blood glucose for tetracycline to an increase of 152% in PBI for diazepam.

15. *A Gas Chromatographic Assay for Ethyleneglycol Monomethyl Ether*. ESAM Z. DAJANI and JOHN H. MENNEAR, Department of Pharmacology and Toxicology, Purdue University, Lafayette, Indiana.

The tissue distribution of ethyleneglycol monomethyl ether (Methyl Cellosolve) was studied in male albino rats. Methyl Cellosolve was administered by either the intraperitoneal or

inhalation route. Concentrations in plasma, brain, liver, lung, and perirenal fat were determined gas chromatographically using a 6 foot \times 1/8 inch o.d. Porapak P column (80-100 mesh). A specially constructed, heated injector trap permitted the direct injection of plasma or tissue homogenate supernatants into the column with only minimal column contamination. Plasma samples were prepared by centrifuging whole blood at 3000 rpm for 15 min. All other tissue samples were prepared by homogenization in 2 volumes of distilled water and centrifugation at 3000 rpm for 15 min. Methyl Cellosolve was found to be evenly distributed in all tissues studied except fat, where concentrations were only 10% of those in other tissues. The time decay curves for Methyl Cellosolve in plasma, liver, and brain were found to be parallel. The slopes indicate a biologic half-life of approximately 1 hour.

(Supported in part by GM-15005 and Purdue Research Foundation.)

16. *Isoenzyme Profiles and Specific Organ Damage.* H. H. CORNISH, M. L. BARTH, and V. N. DODSON, The University of Michigan Department of Industrial Health, School of Public Health, Ann Arbor, Michigan.

Serum enzyme levels of a variety of enzymes have been shown to be elevated in cases of specific organ damage. However, the increase in a particular enzyme activity may result from injury to one of a number of organs and thus is not necessarily organ specific. The measurement of a number of serum enzymes and the use of enzyme ratios has been used as a means of increasing the likelihood of identifying the damaged organ. Serum electrophoretic patterns, stained for enzyme activity, often show the presence of a number of molecular species all exhibiting the same enzyme activity (isoenzymes). Since the isoenzymes may vary in number and activity from tissue to tissue, the release of these enzymes from damaged organs into the serum provides an additional means of detecting organ damage. Isoenzyme patterns, which demonstrate the differences found when specific organs are damaged (liver, kidney, heart), will be presented. Patterns produced when liver is damaged by a variety of hepatotoxins will be compared. In addition, isoenzyme patterns showing the time sequence in the development of organ damage have been obtained. The problems inherent in the technique and difficulties in interpretation of such isoenzyme patterns are discussed.

17. *Vaginal Cytology in the Rhesus Monkey.* JACQUELINE MAURO, ARTHUR STEIN, and P. SOMSIN, Institute of Toxicology and Experimental Pathology, Albany Medical College, and Microscopy for Biological Research, Albany, New York.

Vaginal cytology has become an inexpensive, accurate method for determining ovulation and suggesting abnormal hormonal states in the human female. The use of the rhesus monkey in drug teratology, reproductive studies, and assessment of the hormonal actions of a compound necessitates some accurate but economical method of determining the presence and time of ovulation and hormonal abnormality. This study of vaginal cytology was undertaken to establish baselines in mature, regularly menstruating rhesus monkeys, with special attempts to evaluate the presence of ovulation. The hormonal indices in use for human females and the adaptation to the rhesus female are discussed. The cyclic pattern of the rhesus monkey is described. Alterations of these patterns in varying physiologic states and test situations are reported.

18. *Isolation of Nalorphine Ethereal Sulfate and Nalorphine Glucuronide.* J. M. FUJIMOTO and W. M. WATROUS, Department of Pharmacology, Marquette School of Medicine, Milwaukee, Wisconsin.

Two metabolites of nalorphine, one from the urine of the cat and another from the urine of rabbit, have been isolated. The primary procedure is the same as that described for the isolation of metabolites of morphine (*J. Pharmacol. Exptl. Therap.*, in press). Doses of 580 and 630 mg of nalorphine-HCl were given to two cats for 3 days, and the urine was collected. The pooled urine was filtered, and 4 portions of 100-150 ml each were processed through

Amberlite XAD-2 resin. The main methanol eluate fractions were pooled. Free nalorphine was removed by extraction twice with chloroform-isoamyl alcohol (100:5) at pH 8.5. The aqueous phase was evaporated; the resulting residue was dissolved in water and methanol and 0.5 ml acetic acid was added. The precipitate (I) which formed weighed 35 mg. The filtrate was evaporated to dryness; this residue dissolved in hot methanol yielded on cooling 55 mg of precipitate. The latter was dissolved in 4 ml 1 N NaOH; 0.7 ml of acetic acid was added, and crystals formed gradually (II), 31 mg. A male rabbit was given 1.6 g of nalorphine-HCl during 12 hours, and the urine was collected for 4 days. The urine was filtered through diatomaceous earth and processed on two Amberlite XAD-2 columns. Crystals formed in the methanol eluate over 5 days to yield 31 mg (III). The filtrate was reduced *in vacuo* to 100 ml. Yield of powdery precipitate was 36 mg (IV). The infrared spectrum of (II) was identical to that of chemically synthesized nalorphine-3-ethereal sulfate. Elemental analysis of the latter was satisfactory. Conjugation of H₂SO₄ occurs at 3-OH of nalorphine since no bathochromic shift occurs in UV spectra. Fractions (III) and (IV) are nalorphine-3-glucuronide. The infrared and UV spectra, hydrolysis by β -glucuronidase, and content of nalorphine support this conclusion. Possibly (I) is also the same. Nalorphine ethereal sulfate and glucuronide are internal salts.

(Supported by USPHS grants GM 16503 and 370).

19. *Toxicity and Anticholinesterase Action of O-Ethyl O-2,4,5-Trichlorophenyl Ethylphosphonothioate*. M. ROOT, F. K. KINOSHITA, M. FLYNN, and K. P. DuBOIS, Toxicity Laboratory, University of Chicago, Chicago, Illinois.

The present investigation was conducted to measure the mammalian toxicity and anticholinesterase action of *O*-ethyl *O*-2,4,5-trichlorophenyl ethylphosphonothioate (Bay 37289). The acute intraperitoneal LD₅₀ values in mg/kg were as follows: female rats 11, male rats 10, female mice 33, male mice 35, and male guinea pigs 26. By the oral route the LD₅₀ values were 16, 16, and 40 mg/kg for female and male rats and male guinea pigs, respectively. Atropine given to female rats 10 mins before Bay 37289 only increased the LD₅₀ from 11 to 15 mg/kg and a single dose of 2-PAM was ineffective as an antidote. Simultaneous administration of Bay 37289 and malathion resulted in 2-fold potentiation of the toxicity of malathion. In subacute toxicity tests the highest daily intraperitoneal dose that could be tolerated for 60 days without mortality was 2 mg/kg, and this dose caused about 75% inhibition of tissue cholinesterase activity. Bay 37289 was ineffective as a cholinesterase inhibitor *in vitro*, but a strong anticholinesterase action was demonstrated *in vivo* after intraperitoneal administration of a sublethal dose (7 mg/kg) to adult female rats. Maximum inhibition of cholinesterase activity in brain, serum, and submaxillary glands occurred in 1-6 hours. Slow reversal of the inhibition was indicated by a return of enzyme activity to a level of only 60-75% of normal in 7 days. Subchronic feeding studies conducted on dogs and rats for 12 and 16 weeks, respectively, indicated that the no-effect level is 5 ppm for cholinesterase inhibition.

20. *Relationship between Blood Concentration and Signs of Acute Poisoning by DDT in the Dog*. M. C. BRAUDE, R. BRAUNBERG, and S. KROP, Food and Drug Administration, Washington, D.C.

Central nervous system (CNS) effects of DDT in acute poisoning were first described by Phillips and Gilman [J. Pharmacol. Exptl. Therap. 86, 213 (1946)]. However, due to the lack, until recently, of reliable methods to analyze DDT in blood and tissues, correlation between behavioral signs, convulsive threshold changes, and blood levels of DDT were not previously investigated. DDT, suspended in emulsion or dissolved in dimethyl sulfoxide (DMSO) was administered iv to 12 male and 12 female Beagle dogs. The effects of DDT on threshold of convulsion for hexafluorodiethyl ether (HFE) was then determined by slow intravenous infusion of a 10% HFE solution, the end point being a full tonic seizure with loss of posture. The blood samples were extracted with mixed alcohols and benzene. After chromatographic cleanup, the chlorinated hydrocarbons were determined by gas-liquid chromatography

according to the method of Klein *et al.* [*J. Assoc. Official Agr. Chemists* **46**, 164 (1963)]. It was found that DDT blood levels, CNS effects, and decreases in HFE seizure thresholds were closely correlated and that DDT was much more potent in producing its effects when administered in DMSO rather than in emulsion.

21. *Biochemical Effects in the Rabbit of Repeated Administration of DDT, Carbaryl, and Parathion.* A. HASSAN and C. CUETO, JR., Pharmacology Section, Pesticides Research Laboratory, Pesticides Program, Food and Drug Administration, Perrine, Florida.

Some biochemical effects of the repeated administration of DDT (5 mg/kg), carbaryl (5 mg/kg) and parathion (0.5 mg/kg) to rabbits were studied during 222 days. No significant changes were found in the blood or urine levels of amino acids. The tubular phosphorus reabsorption remained unaltered. The blood coagulation time was considerably shortened in the treated animals. The urine of treated animals as compared to controls contained high amounts of 5-hydroxy-3-indoleacetic acid (5-HIAA) and 4-hydroxy-3-methoxymandelic acid (VMA). The higher levels of these urinary metabolites are an indication of an increased rate of serotonin and the catecholamines, respectively. This increased metabolism is in turn believed to be a consequence of an increased biosynthesis and release of biogenic amines caused by a "stress mechanism" induced by the administered pesticides. The amounts of 5-HIAA and VMA returned to normal 45 days after cessation of treatment.

22. *Lack of Correspondence at High Acute Doses of DDT between the Levels Found in Blood and Brain.* J. SANTOLUCITO, C. CUETO, JR., E. WHITCOMB, and J. OWENS, Pharmacology Section, Pesticides Research Laboratory, Pesticides Program, Food and Drug Administration, Perrine, Florida.

It is generally acknowledged that the concentration of DDT in the fat does not reflect the amount in brain tissue and therefore does not correlate with acute toxic symptoms. While some investigators have reported that plasma levels tend to correlate with brain levels, others report that no correlation exists. The present study is an attempt to elucidate this question.

In the first experiment 18 mature, male, Sprague-Dawley rats were orally dosed with 200, 400, or 800 mg/kg *p,p'*-DDT. Three animals were treated as a set each day, each dose being represented in every set. All animals were sacrificed at the time of severe tremor and/or convulsion. In the second experiment, another group of 16 rats was orally dosed with 800 mg/kg *p,p'*-DDT, and two animals were sacrificed every 30 min. At the time of sacrifice, blood, cerebral hemispheres, cerebellum, and brain stem were recovered for analysis of DDT by gas chromatography. The results of the first experiment revealed no differences in blood and brain content of DDT due to treatment. On the other hand, replication mean squares were consistently larger than treatment mean squares. The F ratio for replications was highly significant for the three brain regions. Cerebral hemispheres contained less DDT at the time of convulsion than did the other two regions at all dose levels studied. The time to convulsion varied inversely with dose level. In the second experiment, the regression of DDT concentration against time showed similar rates of accumulation for all three brain regions. Correlation coefficients were 0.75, 0.80, and 0.74 for cerebellum, cerebral hemispheres, and brain stem, respectively. The blood concentration, on the other hand, reached a plateau between 30 min and 1 hour and remained constant for the 4 hours of the study.

23. *Effects of Organochlorine Pesticides on Avoidance Behavior in the Rat.* L. R. WEISS and R. BRODIE, Division of Pharmacology and Toxicology, Food and Drug Administration Washington, D.C. 20204.

Earlier studies [Weiss and Brodie, *Toxicol. Appl. Pharmacol.* **12**, 288 (1968)] showed that single doses (1/2 LD50) of dieldrin or chlordane but not methoxychlor or DDT, depressed spontaneous motor activity (SMA), increased amphetamine toxicity, and sensitized rats to pentylenetetrazole convulsions, 24-48 hours after treatment. Chlorpromazine depression of

SMA was reduced by dieldrin pretreatment. This paper reports the effects on discriminated (lever pressing) and nondiscriminated (Sidman) avoidance following single or prolonged (30 days) oral treatment with these organochlorines.

Administration of (1/2 LD50) the organochlorines generally induced shifts from avoidance to escape behavior. An increase in response rates was noted for periods up to 72 hours after treatment. Prolonged exposure to dieldrin or chlordane (1/16 LD50) and methoxychlor or DDT (1/8 LD50) produced shifts in discriminated avoidance which appeared to be related to the onset of toxic cumulative effects. With DDT, the presence of tremors in some rats did not influence avoidance behavior. Single doses of dieldrin, chlordane, or DDT produced a change in responding rate in Sidman avoidance while prolonged exposure produced no change. The depressant effects of chlorpromazine on avoidance were blocked by dieldrin pretreatment indicating a behavioral interaction, but stimulating effects of amphetamine on response rates were not appreciably altered by dieldrin pretreatment. The results suggest a low level of behavioral toxicity at high doses of these organochlorines. Since these pesticides caused pharmacologic and toxic manifestations of CNS actions at doses that caused only marginal changes in avoidance, it appears that the organochlorines lack a specific effect on this type of behavior.

24. *Changes in LD50 of Parathion and Heptachlor after Turpentine Pretreatment.* FREDERICK SPERLING and HELENAH EWINIKE, Department of Pharmacology, Howard University Medical School, Washington, D.C.

Oral pretreatment of adult male rats with 1.8 g/kg/day turpentine for 3 days changed the oral LD50 of parathion (*O,O*-diethyl *O-p*-nitrophenyl phosphorothioate) from 13 mg/kg (slope 1.80) to 100 (slope 1.27) and of heptachlor (1,2,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene) from 112 mg/kg (slope 1.58) to 70 (slope 1.52). The LD50 of heptachlor epoxide, 62 mg/kg (slope 1.59), was unchanged. Sleeping time after hexobarbital (125 mg/kg ip) was reduced from 54 min to 20. Similar changes occurred in rats pretreated by exposure to 3-4 mg/l turpentine vapors for three daily 6-hour periods, in the inhalation system described by Sperling *et al.* [*Toxicol. Appl. Pharmacol.* **10**, 8 (1967)]. Controls were similarly exposed to only air or not exposed to the inhalation system. Parathion at 50 mg/kg killed all rats, but death times after turpentine inhalation were prolonged to 10-100 times over control. Heptachlor at 80 mg/kg killed 5/5, 0/5, and 2/10, respectively. Hexobarbital sleeping times were 21, 25, and 54 min, respectively. Stimulation of hepatic microsomal enzymes has been reported to result in decrease in hexobarbital sleeping time, decrease in parathion toxicity, and increase in heptachlor toxicity. Similar effects resulting from pretreatment with turpentine orally or by vapor inhalation indicates possible hepatic microsomal enzyme stimulation. Parathion was supplied by courtesy of the American Cyanamide Co., heptachlor and heptachlor epoxide, by courtesy of the Velsicol Chemical Co.

(Supported by USPHS Grant UI 00488, Urban and Industrial Health.)

25. *Binding of Paraoxon by Plasma of Aldrin-Treated Mice.* A. J. TRIOLO and J. M. COON, Department of Pharmacology, Jefferson Medical College, Philadelphia, Pennsylvania.

Previous studies [Triolo and Coon, *J. Agr. Food Chem.* **14**, 549 (1966)] demonstrated that pretreatment with the organochlorine insecticide aldrin protected mice against the toxicity of the organophosphate paraoxon. Lauwerys and Murphy [*Toxicol. Appl. Pharmacol.* **12**, 306 (1968)] suggested that liver or plasma incubated with low concentrations of paraoxon (10^{-7} M to 10^{-6} M) may detoxify this organophosphate by a nonenzymatic tissue binding process. We have investigated this apparent plasma binding of paraoxon as a possible basis for the protection in aldrin-treated mice. Diluted plasma from mice was incubated with paraoxon (1.2×10^{-6} M) and the free paraoxon levels in aliquots of the reaction mixture were measured by determining the inhibition of bovine red blood cell cholinesterase. Paraoxon binding to normal plasma appeared to occur rapidly since there was no significant difference in the anticholinesterase activity after 5, 15, 30, or 60 min of incubation time. Both *in vivo* toxicity and *in vitro* level of paraoxon decreased with increasing doses of aldrin. After 2-16

mg/kg of aldrin orally, the mortality of mice decreased from 60 to 15% after 2 mg/kg of paraoxon ip, as compared with the 90% control mortality. Similarly, as the dose of aldrin increased from 2 to 16 mg/kg, the *free* paraoxon level decreased from 11.9 to 0.6% as compared with a 14.6% control level. Plasma from animals treated with 16 mg/kg of aldrin increased the binding of paraoxon 16.4%, resulting in about a 96% decrease in *free* paraoxon. Lower levels of *free* paraoxon due to aldrin treatment may be an important factor in the protection against paraoxon toxicity.

26. *Effects of Acute Administration of Carbaryl on Cholinesterase Levels in the Central Nervous System of Swine.* E. MILLER, J. REINWALL, J. BROUWER, F. L. EARL, and E. J. VAN LOON, Special Pharmacological Animal Laboratory, Division of Pharmacology and Toxicology, Food and Drug Administration, U.S. Department of Health, Education, and Welfare, Washington, D.C.

Two minutes after the iv administration of 20 mg/kg of carbaryl to miniature swine, marked ataxia develops and rapidly progresses to a paraplegic syndrome which lasts for approximately 30 min. Red blood cell cholinesterase levels were determined during the paraplegic syndrome, and the animal appeared to recover when the values returned to 63% of the original value. Similarly treated animals were sacrificed 20 min after the carbaryl administration, and selected discrete areas of the central nervous system were rapidly removed and cholinesterase levels determined. The results reveal a diffused inhibition of cholinesterase ranging from 44% in the cortex to 75% in the medulla and spinal cord. Lumbar spinal section of swine, with intact blood supply, was followed by administration of a convulsive dose (30 mg/kg) of carbaryl. Direct action of the pesticide on the sectioned portion of the spinal cord and muscle of the hind legs was not observed.

27. *Cholinesterase Inhibition in Macaca mulatta and the Effect of 2-PAM.* D. M. SERRONE, A. STEIN, and F. COULSTON, Institute of Experimental Pathology and Toxicology, Albany Medical College, Albany, New York.

The purpose of these investigations was to correlate observations in intact rhesus monkeys (*Macaca mulatta*) with alterations in cholinesterase levels (blood and brain). Cholinesterase levels were reduced by the oral administration of parathion, and attempts were made in some cases to reverse the depression by administration of 2-PAM (2-formyl-1 methylpyridinium chloride oxime).

Oral administration of a single sublethal dose of parathion (1.6 mg/kg) produced a slow fall in both plasma and red blood cell cholinesterase levels without any signs of toxicity. Maximum inhibition of plasma cholinesterase was observed at 24 hours whereas maximum depression of red blood cell levels occurred at 72 hours, indicating that absorption and conversion to paraoxon occurred at a slow but continuous rate. Both plasma and red cell cholinesterase levels returned to normal in 7 days. Whole-blood cholinesterase levels fell rapidly after oral administration of lethal doses of parathion, and signs of toxicity were observed only after blood cholinesterase levels reached very low values (50% of baseline). Intravenous administration of 2-PAM produced rapid reactivation of blood cholinesterase but did not overcome the depression of brain cholinesterase, nor did it prevent death in the case of a lethal dose.

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28. *Interaction of Lindane and Phenobarbital on the Induction of Microsomal Enzymes in Dogs.* E. J. SMITH, F. L. EARL, and E. J. VAN LOON, Special Pharmacological Animal Laboratory, Division of Pharmacology and Toxicology, Food and Drug Administration, U.S. Department of Health, Education, and Welfare, Washington, D.C.

Original baseline data for the *in vitro* activity of microsomal enzymes in normal dog liver homogenates are presented. With the substrates tested, there were no significant sex differences

in activity of the demethylases, hydroxylases, or nitro reductase. However, homogenates obtained from dogs fed 7.5–15 mg/kg lindane in the diet exhibited a significant loss in codeine demethylase activity whereas nitroreductase activity doubled. In a second experiment, phenobarbital dietary supplementation stimulated all microsomal enzyme systems tested; however, subsequent addition of lindane to this diet caused a significant decline in microsomal activity after only two feedings. Moreover, the loss in demethylase activity was significantly greater than the diminution in aromatic hydroxylase or nitrobenzoate reductase activities. Possible factors responsible for the antagonistic effect of lindane on phenobarbital induction are discussed.

29. *Paradoxical Stimulation of Hepatic Tyrosine Aminotransferase (TAT) of Rat Liver by CCl₄.*
RAYMOND D. MAGUS, Center for Research in Pharmacology and Toxicology University of North Carolina School of Medicine, Chapel Hill, North Carolina. (Sponsor: Herbert E. Christensen.)

A previous observation [*Mol. Pharmacol.* **4**, 465 (1968)] that CCl₄ pretreatment hindered the inducing action of hydrocortisone (HC) on hepatic tryptophan oxygenase (TPO) in the rat prompted a study of this effect on TAT, another glucocorticoid-inducible enzyme system.

Intact rats receiving only CCl₄ (0.1–1.0 ml/kg, ip, in corn oil) demonstrated a marked dose- and time-dependent increase in hepatic TAT activity. Adrenalectomy and hypophysectomy markedly reduced, but did not abolish, this action of CCl₄. Pretreatment of intact rats with inhibitors (i.e., actinomycin D, cycloheximide, 5-fluorouracil) of glucocorticoid induction of hepatic enzymes failed to abolish the TAT increase following CCl₄ treatment; cycloheximide itself increased TAT activity comparably to CCl₄, whereas 5-FU markedly enhanced the action of CCl₄. In contrast with the impairment of HC induction of TPO by CCl₄ pretreatment, the response of TAT to HC was exaggerated, with a pronounced leftward shift in the HC:TAT dose-response curve. The following components of CCl₄ action on TAT are being considered and/or investigated: (1) involvement of the pituitary-adrenal system both directly in response to CCl₄ and in a quasi-permissive manner; (2) inhibition of TAT degradation by CCl₄, such as has been observed with cycloheximide [*Science* **156**, 525 (1967)], with a concomitant increase in immunologically reactive enzyme protein; (3) inhibition of hydrocortisone metabolism by CCl₄ treatment.

(Supported by Research Grant GM 13606 from the NIGMS, USPHS.)

30. *Modification of Microsomal Enzyme Activity by Methylenedioxyphenyl Insecticidal Synergists and Related Compounds.* H. JAFFE, K. FUJII, and S. S. EPSTEIN, Laboratories of Environmental Pathology and Carcinogenesis Children's Cancer Research Foundation, Inc., and Department of Pathology, Harvard Medical School, Boston, Massachusetts.

Certain methylenedioxyphenyl [MDO] compounds are widely used as insecticidal synergists and their efficacy appears to depend on blockage of insect microsomal systems similar to those well characterized in mammalian liver. As recently indicated [Epstein *et al.*, *Toxicol. Appl. Pharmacol.* **11**, 442 (1967)], these compounds pose potential toxicologic hazards to man by producing synergistic toxicity and carcinogenicity with certain pollutants and drugs. Accordingly, we have studied the mode of action of MDO compounds on drug detoxification by hepatic microsomal enzymes. In general, the structure-activity correlations for *in vivo* inhibition (by MDO derivatives and related compounds) of microsomal hydroxylation of hexobarbital and aminopyrine were shown to correspond closely to the relationships reported for insecticidal synergism. However, with biphenyl as a substrate, potent MDO synergists, such as piperonyl butoxide and Sesamex[®], produced early and transient dose-dependent bimodal stimulation of *o*-hydroxylation and concomitant suppression of *p*-hydroxylation. This bimodal effect also occurs *in vitro* and appears to be due to a shift in equilibrium between the two microsomal activities, presumably through isozymic transformation.

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31. *Stimulation of Hepatic Drug-Metabolizing Enzymes by Chlorphenothane (DDT) and its Relationship to Liver Enlargement and Hepatotoxicity in the Rat.* D. G. HOFFMAN, H. M. WORTH, J. L. EMMERSON, and R. C. ANDERSON, The Lilly Toxicology Laboratories, Eli Lilly and Company, Greenfield, Indiana.

To study the relationship between induction of drug-metabolizing enzymes, liver enlargement, and hepatotoxicity, we have administered DDT [1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane] to weanling male rats for 14 days. While administration of DDT at dietary levels of 0.5 or 2 ppm had no effect on the rate of *p*-nitroanisole (*p*-NA) *O*-demethylation, levels of 4–750 ppm produced an increase in the rate of metabolism proportional to the log dose. Extrapolation of this portion of the dose-response curve to the abscissa provided a calculated no-effect level of 3.27 ± 1.02 ppm. At dietary concentrations greater than 750 ppm there was no further increase in *p*-NA metabolism. Liver weight increased in proportion to the dose when rats were maintained on diets containing 128–512 ppm DDT. Levels less than 128 ppm had no effect, and concentrations greater than 512 ppm produced a submaximal increase in liver weight. Although the total homogenate protein content (mg/g liver) was not affected by DDT administration, the microsomal protein content was increased at dietary levels above 16 ppm. Only at dietary levels above 750 ppm, where *p*-NA reached a maximum, were signs of neurotoxicity apparent. These results suggest that stimulation of drug-metabolizing enzymes, and the accompanying increases in liver weight and microsomal protein, are beneficial processes.

32. *Enhancement of Hepatic Drug Metabolism in Rats after Methyl-Chloroform (MC) inhalation.* H. LAL, A. OLSHAN, S. PURI, H. C. SHAH, and G. C. FULLER, Department of Pharmacology, University of Rhode Island, Kingston, Rhode Island.

Recent experiments [Lal and Shah, *Pharmacologist* **10**, 153 (1968)] have established that MC inhalation reduces hexobarbital sleeping time and increases hepatic oxidation of this drug in mice. These studies were extended to young male rats. Twenty-four-hour inhalation of MC (≈ 3000 ppm; concentration-dependent effect) significantly reduced hexobarbital sleeping time and enhanced *in vitro* hepatic oxidation of hexobarbital as well as demethylation of aminopyrine. All parameters were measured 24 hours after termination of exposure. MC inhalation also shortened the duration of loss of righting reflex induced by meprobamate or zoxazolamine. MC inhalation did not alter total body weight, but significantly increased liver weight of animals. The effects of MC inhalation on hexobarbital sleeping time were not altered by pretreatment with morphine sulfate (20 mg/kg, ip, daily, for 3 days prior to MC inhalation) or adrenalectomy.

The effect of MC inhalation on pharmacologic sensitivity is of interest in view of the fact that MC is an industrial and household solvent and is a chemical analog of several chlorinated hydrocarbon-type insecticides and of anesthetic drugs.

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33. *Some Aspects of the Interaction of Mitochondrial and Microsomal Fractions of Liver from Several Species.* E. BACHMANN, L. GOLBERG, and P. DIGIACOMO, Institute of Experimental Pathology and Toxicology, Albany Medical College, Albany, New York.

The interaction of liver mitochondria and a crude microsomal fraction from liver was investigated, using the hydroxylation of biphenyl as a measure of processing-enzyme activity. Mitochondria isolated in iso-osmolar mannitol-sucrose do not affect processing enzyme activity in the 10,000 *g* supernatant. Mitochondria damaged by freezing, prolonged storage at 4°, preincubation at 38°, or isolation in salt medium produce a reduction of microsomal enzyme activity. This adverse effect can be alleviated by adding an excess (up to 100 times the regular amount, 0.8 mM) of NADP⁺ or NADPH to the incubation medium. The inhibitory effect of mitochondria can therefore be attributed to competition for the energy source in the incubation medium. Liver fractions from rat, dog, or monkey (*Macaca mulatta*) exhibit

similar properties in this respect. At 24 hours after a single oral dose of 2,6-dichloro -4-nitro-aniline (Botran®) to rats, there is an increase in processing-enzyme activity in the 10,000 g liver supernatant which is not affected by addition, to the assay system, of intact or damaged mitochondria from the treated animal but is diminished by addition of similarly treated mitochondria from control animals. However, prolonged preincubation at 38° of mitochondria from Botran-treated rats elicits an inhibitory effect on the microsomal fraction from the same animals. Processing enzymes from control animals are affected in the same way by mitochondria from either Botran-treated or control rats.

34. *Too Much Protein, Too Slow Shaking, or Using Air instead of Oxygen Can Minimize Amount of Hepatic Microsomal Drug-Metabolizing Enzyme Induction Measured in Vitro.* JAMES R. FOUTS and LYSLE R. WATERS, Oakdale Toxicology Center, Department of Pharmacology, University of Iowa College of Medicine, Iowa City, Iowa.

Hepatic microsomal drug-metabolizing enzyme activity is assayed by various "standard" techniques in different laboratories. Incubation volumes of 3-5 ml often contain 9000 g supernatant fractions or microsomes from 0.33 to 1 g of liver. Microsomal protein concentration in such incubation mixtures will often exceed 2 mg/ml. Most workers do not specify rate of shaking or gas flow rates used in incubations. Air rather than oxygen is often the gas phase. We found that hepatic fractions from phenobarbital-treated rats did not have higher drug-metabolizing enzyme activity than control samples when incubated in one particular machine. Yet before sacrifice, these phenobarbital-treated animals had hexobarbital sleeping times that were less than those of control rats. The "defective" incubator differed from one in which the same enzyme solutions from phenobarbital-treated rats gave the "correct" results (evidence for enzyme induction) in shaking rate, shaking vigor, and gas flow. With relatively high microsomal protein concentrations (seen in preparations from phenobarbital-treated animals), optimal incubation conditions included rapid shaking (> 100 oscillations per minute), vigorous stirring (a glass marble added to the incubation beaker), and oxygen rather than air. These factors are still important, though not as critical, when microsomal protein concentration is kept below 2 mg/ml in the incubation mixture.

(Supported by NIH grant GM 12675).

35. *The Effect of Simultaneous Application of Chlorinated Hydrocarbon and Antioxidant on Enzyme Induction. A Biochemical and Electron Microscopic Investigation.* K. L. STEMMER and R. D. GREENLAND, Kettering Laboratory, University of Cincinnati College of Medicine, Cincinnati, Ohio. (Sponsor: M. Eisler)

Rats were exposed to heptachlorepoide, BHA, a combination of both, corn oil without antioxidant, and a control group. The administration was by ip injections. The concentration of heptachlorepoide was adjusted to that dose which gave induction of enzymatic activity after 4 injections. The antioxidant was the allowable concentration of the daily intake of humans. The liver was homogenized. The microsomal fraction was obtained by centrifugation. This fraction was then incubated with labeled testosterone. The metabolites were extracted and chromatographed. The strips were scanned for radioactivity. The relative amounts of metabolites were related to the protein content of the fraction. Pieces of tissue were prepared from each animal for electron microscopy. The morphologic alterations were compared with the biochemical results.

In each of the animals the induction of enzymatic activity after heptachlorepoide showed also an increase in the endoplasmic reticulum. The findings were not as well distinguished after the exposure to BHA. The morphologic alterations were not indicative of increased enzyme activity in this case. The combined application of both compounds did not yield a relationship between morphologic changes and biochemical data. Neither an antagonistic nor synergistic effect was clearly present. However, the dose of heptachlorepoide in this

acute study was very high as compared with the dose for the antioxidant. Long-term experiments would probably give a more definite answer.

36. *Some Biochemical Effects in Squirrel Monkeys on Repeated Administration of p,p'-DDT.*

M. F. CRANMER and A. PEOPLES, Pharmacology Section, Pesticides Research Laboratory, Pesticides Program, Food and Drug Administration, Perrine, Florida. (Sponsor: C. Cueto, Jr.)

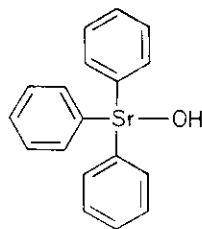
The present investigation was undertaken to evaluate the squirrel monkey as an experimental animal for the study of possible biochemical effects produced by oral administration of *p,p'*-DDT. Thirty squirrel monkeys were separated into 5 groups of 6 animals. Baseline values of biochemical parameters were obtained weekly for each animal for 3 months prior to the initiation of treatment with the insecticide. The parameters measured included hematologic values, and the plasma enzymes, SGOT, ICD, amylase, and aldolase.

The compound *p,p'*-DDT was administered orally in peanut oil to each of 4 groups of monkeys at the respective dosage of 50, 5.0, 0.5, 0.05 mg/kg/day. The fifth group of animals served as controls and received peanut oil without the insecticide. One-third of each group was sacrificed at the end of 2.5, 5, or 7.5 months of treatment with *p,p'*-DDT. The 50 mg/kg level of administration was the only level which resulted in a fatality. Few or no biochemical effects were observed in the group receiving 0.05 mg/kg/day. No marked hematologic change nor changes in plasma ICD or aldolase were observed in any of the groups. However, SGOT and amylase levels were significantly elevated in the higher dosage groups. At the time of sacrifice, the *in vitro* liver microsomal enzyme activity was determined using *p*-nitroanisole and ethyl *p*-nitrophenyl phenylphosphonothioate as substrates. The degree of enzyme stimulation was shown to be dose related.

37. *Toxicity of Triphenyltin Hydroxide (Vancide KS).* M. J. MARKS, C. L. WINEK, and S. P.

SHANOR, Department of Pharmacology and Toxicology, Duquesne University School of Pharmacy, Pittsburgh, Pennsylvania.

The compound triphenyltin hydroxide (Vancide KS) is an experimental industrial bacteriostatic and fungistatic agent with the following structure:



Vancide KS is a white powder extremely insoluble in all solvents. The acute oral LD₅₀ in rats was 171 mg/kg (100–295) in males and 268 mg/kg (205–344) in females. Abnormality was observed in the spleen in the form of extramedullary hematopoiesis. A 90-day chronic feeding study in male and female Sprague-Dawley rats at levels of 0, 1, 10, and 100 ppm was conducted. Reports on the parameters included in the study will be given. Acute 24-hour and subacute 14-day repeated application of the compound to rabbit skin was without gross effect. The compound was nonirritating to rabbit skin and did not sensitize male guinea pigs. Eye irritation studies showed that the compound was an extreme irritant even when the eyes were washed 2 sec after application. The compound produced corneal opacities in all animals. A teratogenic study in Sprague-Dawley rats at a dosage of 15 mg/kg showed that the compound prevented implantation when given from day 1 through 7. When given from day 8 or beyond, the compound was fetidical. The compound caused a lowering of body temperature in rats and gave a positive Robishaud test. Pathologic findings, including extramedullary hematopoiesis, are reported.

38. *Changes in Tissue Distribution of Beryllium Induced by Metopirone.* J. J. CLARY, C. R. HOOPER, and H. E. STOKINGER (Sponsor), Consumer Protection and Environmental Health Service Bureau of Occupational Safety and Health, USPHS, Cincinnati, Ohio.

The toxicity of beryllium is well established, but more information on the causes of the latency of the chronic disease is needed. In many human cases, years elapse from the time of exposure to the onset of the chronic disease. This onset appears to be triggered often by stress such as pregnancy, respiratory infection, surgery. Metopirone (2-methyl-1,2-di-3 pyridyl-1-propanone) acts by selectively inhibiting 11- β -hydroxylation in corticosteroid biosynthesis. Hence, metopirone can create a temporary adrenal insufficiency, mimicking a stress condition similar to that found in man. Mice and guinea pigs received beryllium in the form of BeSO₄ and BeO via intrathoracic or intratracheal injection. One month later, half of the animals received metopirone subcutaneously, the other half saline. Animals were sacrificed 1 month after metopirone treatment, and beryllium was determined in the lung, liver, spleen, heart, kidney, adrenal, and femur. Most of the beryllium was found in the lung, liver, and bone. Changes in distribution were greater in males than in females treated with metopirone, suggesting that there may be a sex difference in beryllium mobilization. In general, metopirone treatment caused a transfer of beryllium from the lung and bone into the liver. It is our hypothesis that dissemination of beryllium from the lung and bone to other body sites is the first step in the onset of the latent, chronic, systemic disease in man. The simulation in animals of systemically distributed beryllium, which apparently occurs in man in adrenal stress, has not heretofore been demonstrated in animals.

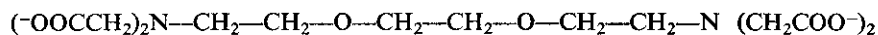
39. *Effects of Cadmium on the Minute Blood Vessels.* E. J. YOUKILIS, R. C. MARTZ, P. D. HARRIS, P. A. NICOLL, and R. B. FORNEY, Department of Toxicology and Pharmacology and Department of Physiology, Indiana University School of Medicine, Indianapolis, Indiana.

The effects of cadmium on the minute blood vessels were studied in the subcutaneous vasculature of the wing of unanesthetized female bats (*Myotis lucifugus*). A compound microscope equipped with a beam splitter permitted an image of the vessels to impinge upon the photocathode of a closed circuit television system. The video information was analyzed on a 23-inch monitor (total magnification of 1000 \times). Venule and arteriole diameters were measured at 30-sec intervals, and venule vasomotion (cycles of alternating constriction and relaxation) was counted over 1-min intervals. Rectal temperatures were measured with a thermistor. Electrocardiograms were observed on some of the animals. The experimental procedure consisted of a 10-min control period followed by an intraperitoneal injection of the test solution. Vascular responses were observed for an additional 50 min. Two groups, one having innervated arterioles and venules and the other having denervated vessels, were dosed with cadmium, 3 mg/kg body weight, as the acetate. The innervated venules and arterioles significantly dilated and the venule vasomotion markedly decreased. Denervated vessels showed no change from control values. The pretreatment vascular diameters for the denervated animals were larger than control values for the innervated group; however, they were significantly less than the diameters of the innervated vessels dilated following cadmium treatment.

In both groups there were significant decreases in rectal temperature and heart rate after cadmium treatment. Since wing denervation does not affect the basic mechanism controlling these variables, this similar result was not unexpected in both groups. Thus our data suggest that cadmium inhibits central activity.

40. *Effect of EDTA and EGTA on Bladder Stone Formation in Rats.* BART VAN'T RIET, CHARLES E. O'REAR, JAMES E. WYNN, and JOSEPH F. BORZELLECA, Departments of Pharmaceutical Chemistry and Pharmacology, Medical College of Virginia, Richmond, Virginia.

A slight decrease of the free calcium level in rat urine is observed after oral administration of either disodium-EDTA or -EGTA. The tetravalent ion of the latter compound



complexes calcium more than 10^6 times stronger than magnesium, and it is less toxic than EDTA in acute and chronic toxicity studies. Male Sprague-Dawley rats usually produce deposits of magnesium ammonium phosphate hexahydrate on zinc pellets in the bladder. The deposits change to smooth calcium oxalate monohydrate when the drinking water contains 0.5–1.5% ethylene glycol. A combination of 1% ethylene glycol in the water and 1% disodium EDTA or EGTA in Purina Rat Chow was chosen to study the effect of these complexing agents on the growth of calcium oxalate deposits on zinc pellets. Test feeding was done for 3 weeks after a period of 5 weeks in which the rats were kept on a regular diet and 1% ethylene glycol solution ad libitum. The weight and kind of deposit were determined both after the initial 5 weeks and the 3-week test period. There was no significant effect of either EDTA or EGTA on the growth rate of deposit; however, the composition of the deposit changed from the mono- to the dihydrate of calcium oxalate in 50% of the rats in each group, causing sharp-edged crystals to be formed. These results suggest a potential hazard connected with the use of complexing agents in the management of urolithiasis.

41. *Treatment of Drug Poisonings with the Capillary Artificial Kidney.* R. D. STEWART, E. D. BARETTA, E. A. BACHAND, and R. T. BACHAND, JR., Department of Environmental Medicine, Marquette School of Medicine, Milwaukee, Wisconsin.

The capillary artificial kidney which is currently being evaluated for the treatment of chronic renal disease has several features which make it attractive for use in the treatment of acute drug intoxications: (1) The dialysis unit is presterilized and can be quickly placed into operation. (2) The pressure required to operate the unit is sufficiently low so as not to require an external blood pump. (3) The internal priming volume is 130 ml, making the unit suitable for infant as well as adult use. (4) A single unit provides, a dialyzing area of 9000 cm² with a membrane thickness of 25–30 μ . This affords drug clearance rates comparable to those of the Travenol Ultracoil®. (5) When it is clinically desirable to remove a drug more rapidly than is possible with the Ultracoil, the low priming volume allows the use of multiple capillary kidney units.

Mongrel dogs were poisoned with sodium salicylate, pentobarbital, phenobarbital, or glutethimide. One group of animals served as untreated controls while the others were hemodialyzed with the capillary kidney. The optimum blood and dialyzate flow rates for single and multiple capillary kidney units to achieve the maximum drug dialysis were determined for each compound studied.

42. *Effects of Cobalt Salts in Cyanide and Sulfide Poisoning and on Methemoglobinemia.* R. P. SMITH, Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, New Hampshire.

Pretreatment of mice with cobaltous chloride (I) produces significant protection against the subsequent injection of potentially lethal doses of sodium cyanide or sulfide. This dose of compound I does not generate methemoglobin in mice or in red cell suspensions. It is presumed that I protects against cyanide or sulfide by directly reacting with them. Pretreatment of mice with sodium cobaltinitrite (II, Na₃Co(NO₂)₆) also protects them against death by cyanide or sulfide. In contrast to I, however, II generates significant levels of methemoglobin in mice and in red cell suspensions. The shape of the methemoglobinemic response to II (duration and peak) exactly parallels that produced by sodium nitrite, but the peak level generated indicates that only 3 of the 6 equivalents of nitrite are released per mole of II injected. Thus, II may protect against cyanide or sulfide by a "direct" reaction like I and/or by the formation of methemoglobin. Methemoglobin formation alone can account for the entire protective effect against sulfide if II, like sodium nitrite, generates sulfide binding sites in addition to the ferric iron of methemoglobin. In the case of cyanide, however, more than

half of the cyanide inactivated by pretreatment with II cannot be ascribed to methemoglobine-mia. Neither I nor II significantly inhibits spontaneous methemoglobin reductase activity in mouse red cells, but II appears to block methylene blue-stimulated methemoglobin reductase activity. This latter effect may be shared by I, but when tested in concentrations of 2 to 8 mM, intense hemolysis results. Indeed, hemolysis appears to account for the inhibition of methemoglobin reductase activity previously ascribed to I by others.

(Supported by Grant AP 00260, National Center for Air Pollution Control and USPHS Research Career Program Award 1-K3-GM-31, 784, NIH).

43. *Oral Toxicity of 3-Tritylthio-L-Alanine in Mice and Dogs.* PAUL E. PALM and MARGARET S. NICK, Life Sciences Division, Arthur D. Little, Inc., Cambridge, Massachusetts.

3-Tritylthio-L-Alanine has strong antitumor activity against lymphocytic leukemia L1210 in mice following ip, sc, or oral administration and is of interest as a possible cancer chemotherapeutic agent. The single-dose oral LD50 value in the mouse and dog was 1406.4 mg/kg and ~2000-4000 mg/kg, respectively. Delayed deaths were observed in both species. In dogs, doses of 125, 500, 2000, and 4000 mg/kg \times 1 produced elevated serum glutamic-pyruvic transaminase and alkaline phosphatase values. Severe leukopenia was observed with doses \geq 500 mg/kg. The maximum tolerated repeated-dose in dogs was approximately 31.25 mg/kg following either a 5-day or a 5-day on, 9-day off repeated course regimen. Body weight loss, marked reversible leukopenia, and pronounced elevations in serum glutamic pyruvic transaminase and alkaline phosphatase levels were observed in dogs receiving doses \geq 125 mg/kg \times 5. Serum enzyme levels in survivors returned to normal limits during week 5-7. Micropathology observed in dogs administered repeated doses \geq 125 mg/kg included: liver degeneration characterized by diffuse reduction in PAS-positive glycogen, fatty metamorphosis, and parenchymal cloudy swelling; lymphoid hypoplasia in the spleen and lymph nodes, and hypoplastic bone marrow, primarily involving the leukopoietic elements. Sections of intestinal tract showed focal congestion and hemorrhage in animals which received dosages of 1 g/kg \times 3 or 4.

(Investigation supported by Contract No. PH 43-65-61 with the Chemotherapy Program, National Cancer Institute, USPHS.)

44. *Species Differences in the Disposition of Cyclo-leucine.* GARETH OWEN, H. W. RUELIIUS, F. JANSSEN, and J. J. POLLOCK, Wyeth Laboratories, Inc., Radnor, Pennsylvania. (Sponsor: H. P. K. Agersborg, Jr.)

Cyclo-leucine (1-aminocyclopentanecarboxylic acid) has antitumor activity, which is good in rodents but poor in man, and immunosuppressive properties which have been demonstrated in this laboratory. Its excretion is delayed, depending upon the species. Half-life values of 22 days for rats and 3-4 days for man have been demonstrated, with ^{14}C -labeled compound, by Christensen *et al.* [*Biochim. Biophys. Acta.* **62**, 160-162 (1962)], and figures of 23-27 days in dogs, 8-9 days in squirrel monkeys, 3-4 days in African Green monkeys and 15-20 days in miniature swine have been obtained in this laboratory. Toxic effects which might be expected from repeated dosing have occurred. In groups of 20 rats given cyclo-leucine once weekly for 12 weeks, all those receiving 200, ten receiving 100, but none receiving 50 mg/kg, died. In dogs given 3 doses 1 week apart followed by 4 doses 2 weeks apart, all four at 100 and 200 mg/kg levels but none at 50 mg/kg died. In contrast, all dogs treated with the same doses, in a regimen adjusted to maintain half-life blood levels, survived 20 weeks. Six African Green and 8 squirrel monkeys tolerated daily doses of 800 mg/kg for at least 3 weeks, but all were dead by the week 10. Some deaths occurred in 400 and 200 mg/kg groups, but at 100 mg/kg, mortality was limited to 3 African Greens. The correlation of these results with serum, urinary and fecal levels will be discussed.

45. *Investigation of the Acute Toxicity and Mechanism of Action of 1,2-Bis(difluoramino)isobutane and Related NF Compounds.* F. W. WEIR and F. H. MEYERS, Department of Pharmacology and Experimental Therapeutics (Toxicology Activity), School of Medicine, University of California, San Francisco Medical Center, San Francisco, California. (Sponsor: C. H. Hine.)

An investigation was made of the pharmacologic effects of 1,2-bis(difluoramino)isobutane (I), 1,2-difluoramino propane (II), and 2,3-dimethyl-2,3-difluoramino butane (III) in an effort to elucidate the mechanisms of acute toxic actions of these reactive chemicals. The ip LD50 in mice were 99, 45, and 134 mg/kg for I, II, and III, respectively. These doses represent 0.72, 0.37, and 0.71 millimoles/kg for I, II, and III. The acute effects of iv doses of all compounds in dogs were similar to the effects of equipotent doses of nicotine and included central nervous system stimulation, greatly increased rate and depth of respiration, bradycardia, peripheral vasoconstriction, and death from respiratory paralysis. Similarity of the effects of these compounds to the ganglionic action of nicotine was also suggested by the fact that the contraction of guinea pig ileum could be blocked by tetramethylammonium iodide. Rat phrenic nerve-diaphragm response to any of the compounds showed a facilitation of the nerve-mediated contraction followed by neuromuscular blockade.

(Supported in part by USPHS Training Grant 01304.)

46. *β -Glucuronidase Activity in Serum and Liver of Rats Treated with Parathion.* CLARA H. WILLIAMS, Division of Pharmacology and Toxicology, Food and Drug Administration, Consumer Protection and Environmental Health Service, Washington, D.C.

Earlier studies [Williams, C. H., *Toxicol. Appl. Pharmacol.* **11**, 7 (1968)] showed that serum β -glucuronidase (β -Glu) activity of rats was markedly increased after administration of single doses of parathion, paraoxon, or Banol (6-chloro-3,4-xylylmethyl carbamate). The increased activity was not present 18 hours after dosing. In the present study, rats of either sex were administered 0.25, 0.50, or 1.0 mg/kg parathion daily for 4, 10, or 15 days. Twenty-four hours after the last dose, serum β -Glu activity in the females was increased above control activity at each time period and at each parathion dose, with the greatest increase after 15-day administration. In the males, activity was increased only after 15-day treatment. β -Glu activity in liver was inhibited at all time periods, and the amount of inhibition was approximately the same for both sexes. Serum cholinesterase in the females was inhibited after 4, 10, and 15 days of parathion dosing; in the males the enzyme was inhibited only after 10 and 15 days. In both sexes, the amount of inhibition was dose related. Liver sections from these rats were examined histochemically for β -Glu activity with 6-bromo-2-naphthyl- β -D-glucopyruroniside as substrate. The β -Glu staining was less in all of sections from parathion-treated rats at each time period than in the section from control livers. The effect of paraoxon and Banol and Mobam (4-benzothienylmethyl carbamate) in concentrations of 4×10^{-4} to 4×10^{-6} M on the *in vitro* β -Glu activity of rat serum and of liver homogenates was measured. No changes in activity were noted.

47. *Behavioral Indices of Dieldrin Toxicity in Sheep.* B. E. SANDLER, G. A. VAN GELDER, W. B. BUCK, J. MALAND, and G. G. KARAS, Psychotoxicology Laboratory, Iowa State University, Ames, Iowa.

In a Y maze visual discrimination apparatus sheep given a daily oral dose of 10 mg technical dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo,exo-5,8-dimethanonaphthalene) per kg body weight required significantly more trials, and had significantly greater latencies, in the relearning of a visual discrimination problem than did untreated controls. A daily oral dose of 20 mg technical dieldrin per kg body weight caused a large decrement in the performance of a vigilance task by sheep within two days. Three of four sheep recovered their pretreatment response levels within 10 days after the termination of

treatment. Retreatment of these animals to 5 mg technical dieldrin per kg body weight also resulted in a response decrement.

48. *The Oral Toxicity of Mirex in Adult and Suckling Rats.* T. B. GAINES and R. D. KIMBROUGH, Toxicology Laboratory, Pesticides Program, Food and Drug Administration, Consumer Protection and Environmental Health Service, Public Health Service, U.S. Department of Health, Education, and Welfare, Atlanta, Georgia. (Sponsor: William F. Durham.)

The oral toxicity of the pesticide mirex (dodecachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta[*c,d*]pentalene), which is used in the control of fire ants, was studied in Sherman strain rats. The single-dose LD50 was 365 mg/kg and the 90-dose LD50, 6.0 mg/kg in adult female rats. The chronicity factor, which is the ratio of single-dose LD50 to 90-dose LD50 [Hayes, *Toxicol. Appl. Pharmacol.* **11**, 327-355 (1967)] was 60.8. The highest dietary level of mirex tolerated without symptoms of poisoning was 50 ppm (3.1 mg/kg/day). The livers of adult male and female rats fed 25 ppm mirex in the diet for 166 days showed microscopic changes, and were significantly larger than the livers of the control rats ($p < 0.001$ and < 0.025 , respectively). Male and female rats fed 25 ppm mirex in the diet were bred to nontreated rats on days 45 and 102 of treatment. Nontreated females bred to the treated males produced normal, healthy litters. In comparison, there was a significant decrease ($p < 0.05$) in the number of offspring born alive to treated females (102 days of dosing) bred to nontreated males, and in the percent of offspring that survived to weaning ($p < 0.025$, 45 days dosing; $p < 0.001$, 102 days). Thirty-three percent of the offspring from the first breeding (45 days) and 46.2% of those from the second breeding (102 days) developed cataracts before weaning. The percent survival of offspring born to treated mothers (fed 25 ppm mirex for 6 weeks) and transferred to nontreated foster mothers at birth was 70.8%, and 1 of 64 survivors developed cataracts. Only 61.0% of the offspring born to nontreated mothers and transferred at birth to treated foster mothers (fed 25 ppm mirex for 6 weeks prior to breeding and during gestation and lactation) survived to weaning, and 39.1% of these developed cataracts. No cataracts developed in offspring born to control rats, and 95.8% survived to weaning when they were transferred to foster mothers at birth.

49. *Comparison of Biochemical Effects of Various Halogenated Hydrocarbons.* C. D. KLAASSEN and G. L. PLAA, Oakdale Toxicology Center, Department of Pharmacology, College of Medicine, University of Iowa, Iowa City, Iowa.

Chlorinated hydrocarbons differ in their capacity to produce liver damage. The comparative effects of carbon tetrachloride (CCl₄), chloroform, 1,1,1-trichloroethane, and 1,1,2-trichloroethane were studied in rats. CCl₄ produced the highest increase in hepatic triglycerides (34 mg/g liver) and also produced elevated levels at the lowest dose (0.03 ml/kg). Chloroform was intermediate in producing elevated liver triglyceride levels; with 1,1,2-trichloroethane, enhanced triglyceride levels were demonstrated only at near-lethal doses. No enhanced hepatic triglycerides levels was demonstrated with 1,1,1-trichloroethane. Decrease in hepatic glucose-6-phosphatase activity was demonstrated in rats treated 3-36 hours previously with CCl₄. Significant dose-related decreases in glucose-6-phosphatase were demonstrated with doses of 0.3 ml/kg or greater of CCl₄. With the other chlorinated hydrocarbons, no decrease in hepatic glucose-6-phosphatase activity was detected. When the hydrocarbons were added directly to liver homogenates, only those incubations containing CCl₄ exhibited increased lipid peroxidation (enhanced TBA reactants). *In vivo*, peak levels of lipid peroxides (diene conjugates) in the liver were detected 30 minutes after CCl₄ treatment. Enhanced diene conjugates were detected with doses of 0.3 ml/kg of CCl₄ or greater. However, with the other three hydrocarbons, no increase in diene conjugates was detected. Thus, this study shows that while the temporal relationships of CCl₄-induced hepatotoxicity are compatible with the lipid peroxidation hypothesis, the dose relationship is weak. Also the lack of qualitative similarity in the results obtained with chloroform is disturbing.

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50. *Studies on the Toxicity and Mode of Action of Morestan.* G. P. CARLSON and K. P. DuBOIS, Toxicity Laboratory, University of Chicago, Chicago, Illinois.

The present investigation consisted of a study of the acute and subacute toxicity of morestan (6-methyl-2,3-quinoxalinedithiol cyclic carbonate) and a metabolite, 6-methyl-2,3-quinoxalinedithiol, and their effects on various enzyme systems. The acute ip LD50 values for morestan were 192 mg/kg and 95 mg/kg for female and male rats, respectively, and the corresponding values for the metabolite were 85.6 mg/kg and 38.1 mg/kg for female and male rats. The approximate ip LD50 of morestan for weanling rats was 320 mg/kg with no apparent sex difference in susceptibility. The sex difference in adult rats could not be eliminated by castration or by administration of testosterone to females or estradiol to males. Mice were less susceptible to both agents and showed no sex difference. Morestan has a rather high cumulative toxicity since the highest daily dose that could be given intraperitoneally for 60 days without mortality was 25 mg/kg. Liver damage was detected histologically after repeated dermal application, and it was also indicated by elevated SGOT values after acute poisoning. Both compounds inhibited sulfhydryl enzymes. Thus succinic dehydrogenase, malic dehydrogenase, and α -ketoglutarate oxidase were significantly inhibited. Pyruvate utilization was inhibited by both compounds with the citric acid cycle operative and when it was blocked by malonate (acetoacetate formation). Reduced glutathione levels in the liver were lower. Glycolysis was slightly inhibited. Nitroreductase activity was reduced. Feeding of morestan in the diet for 90 days at 500 ppm resulted in decreased body weight, enlarged livers, and inhibition of acetoacetate synthesis, EPN detoxification, and *O*-demethylase activity.

51. *Oral Toxicity of Ethylene Chlorohydrin, A Potential Reaction Product of Ethylene Oxide Fumigation.* STEVEN CARSON, and BERNARD L. OSER, Food and Drug Research Laboratories, Inc., Maspeth, New York.

Ethylene oxide fumigation of food materials may result in the formation of traces of ethylene chlorohydrin by reaction with sodium chloride. The response to graded oral dosage of ethylene chlorohydrin over a 90-day period was investigated in rats, dogs (beagles), and monkeys (rhesus). Special precautions were taken to avoid loss of ethylene chlorohydrin by evaporation. Observations included body weight, food consumption, physical condition, hematologic, biochemical, and gross and microscopic pathological examinations. Growth depression occurred in all three species at high dosage levels. Subacute myocarditis was observed in rats that died at the highest dosage fed (67.5 mg/kg). Neither myocardial nor any other significant histopathologic alterations were found after sacrifice of the rats at the lower dosage levels or in any of the dogs or monkeys. The dogs showed a marked emetic response to ethylene chlorohydrin, regardless of how it was administered. Many variations of dosing procedure, including the parenteral route, were tried before an acceptable concentration range in food (600-900 ppm) could be retained. The "no adverse effect" level of ethylene chlorohydrin was estimated to be approximately 45 mg per kg body weight.

52. *Relationship between Sulfhydryl Reactivity and Inhibition of in Vitro Oxygen Uptake of Molluscicidal Nitrostyrene Derivatives.* G. C. FULLER and R. T. LOUIS-FERDINAND, Department of Pharmacology, University of Rhode Island, Kingston, Rhode Island. (Sponsor: H. Lal.)

Attempts to control schistosomiasis on a worldwide basis are usually directed at chemical control of the intermediate host. The present study was undertaken to elucidate the mode of action of a series of molluscicidal B-nitrostyrene derivatives in order to provide a sound basis for predicting the toxicity of these compounds on marine organisms. B-Nitrostyrene (BNS) (0.15-1.20 mM) and derivatives of BNS were found to inhibit oxygen uptake by fortified homogenates of *Australorbis glabratus*, the intermediate host for *S. mansoni*. Incubation of homogenates with cysteine (1.2 mM) prior to the addition of BNS decreased the effect of BNS on oxygen consumption. Derivatives of BNS which inhibit oxygen uptake were found to

interfere in the reaction of cysteine with 5,5'-dithiobis(2-nitrobenzoic) acid (DTNB), indicating sulfhydryl reactivity. Total tissue sulfhydryl content of the homogenate, as determined by the DTNB reaction, was significantly reduced after exposure to BNS. A positive correlation between the level of inhibition of oxygen uptake by BNS, *in vitro* sulfhydryl reactivity, and decreased tissue sulfhydryl content was observed. These data suggest that the mode of action of the BNS series of molluscicidal compounds is via interaction with tissue sulfhydryl groups. (Supported by U.S. Department of the Interior under P.L. 88-379.)

53. *The Effects of Ethylene Glycol on the Formation of Urine by the Cat.* J. H. WILLS, J. M. CHOLAKIS, and F. COULSTON, Institute of Experimental Pathology and Toxicology of Albany Medical College and New York State Department of Health, Albany, New York.

The effects of ethylene glycol on renal processes have been studied in the cat in experiments in which the glycol was administered either during ("acute") or on the second day before ("2-day") the experiment. The doses of ethylene glycol were 1.5 ml/kg iv and 0.5 ml/kg ip in the acute and the 2-day experiments, respectively. Estimated were the clearances of creatinine and *p*-aminohippuric acid (PAH) and the transport by tubular epithelium of glucose, sodium, and PAH in addition to measurement of the gross production of urine. The immediate effects of ethylene glycol were an increase in the production of urine, inconstant changes in the clearance of creatinine, and transtubular transport of glucose and decreases in the clearance of PAH and the transtubular transports of sodium and PAH. The principal findings from the 2-day experiments were that the rate of urine production remained within normal limits for the experimental conditions despite marked reductions in the clearances of both creatinine and PAH. The transtubular transports of both glucose and sodium, but not that of PAH, were reduced. These various findings suggest that a cardinal means of attack of ethylene glycol on the urinofac systems of the kidney is interference with blood flow through the nephron, possibly by partial blockage of the peritubular network of capillaries. Experiments to test this possibility are in progress.

54. *Changes in the Lens of Aging Rats.* T. BALAZS, S. OHTAKE, and J. F. NOBLE, Toxicology Research Section, Lederle Laboratories, American Cyanamid Co., Pearl River, New York.

Changes in the lens of Sherman rats of the Lederle Colony were observed by biomicroscopic and histologic examinations. Approximately 100 rats of either sex were examined on several occasions from 4-5 months of age to 2 years. Heightening of the anterior Y suture, striations in the anterior cortex, accentuation of posterior cortical suture lines surrounded by vesicles, and increased nuclear density were the most common findings. The changes were similar to those found during the early period of administration of cataractogenic compounds [Gordon and Balazs, *Toxicol. Appl. Pharmacol.* **10**, 393 (1967)]. The incidence of the changes due to aging increased linearly from 5% in 4-5-month-old rats to 90% in the 2-year-old rats. Although the changes were marked in the older animals, frank opacity did not occur as it did after the administration of cataractogens. Biomicroscopic and histologic findings are correlated and discussed in detail.

55. *The Cataractogenic Activity of 2,4-Dinitrophenol in Ducks and Rabbits.* P. J. GEHRING, The Dow Chemical Company, Midland, Michigan. (Sponsor: V. K. Rowe.)

The concentration of 2,4-dinitrophenol (DNP) in serum, aqueous humor, vitreous humor, and lens following a single intraperitoneal administration has been determined for ducklings and mature and immature rabbits. A higher concentration of DNP for a longer duration was present in the aqueous humor, vitreous humor, and lenses of animals susceptible to the cataractogenic activity of DNP, ducklings and immature rabbits, than in unsusceptible animals, mature rabbits. DNP persists in these compartments for a longer duration in immature rabbits than in ducklings. This observation is congruent with the finding that cataracts produced in immature rabbits are more persistent than those produced in ducklings. Evidence is

presented showing that the concentration of DNP attained in the compartments of the eye is related to the concentration of nonprotein-bound DNP in serum. To establish this relationship, the rate of clearance of DNP from serum and the rate of association and dissociation of DNP and serum protein were used. The apparent first order disappearance of DNP from serum was fastest in mature rabbits and slowest in immature rabbits. In ducklings, the biphasic disappearance of DNP from serum was best described by two apparent first-order rate constants; the rates of clearance of DNP from the serum of this species were intermediate to those of mature and immature rabbits. A blood aqueous barrier maintaining a lower concentration of DNP in the aqueous humor compartment is suggested. This barrier is most effective in the mature rabbit and least effective in the duckling.

56. *Cataracts and Other Lesions in Rats Receiving a Synthetic Progestin-Estrogen*. JAMES A. RAY and A. L. SCHUT, Pathology and Toxicology Research Unit, The Upjohn Co., Kalamazoo, Michigan; 401 Bronson Medical Center, Kalamazoo, Michigan. (Sponsor: J. E. Gray.)

U-13,851 (7 α -methyl-17 α -ethynyl-19-nortestosterone), a synthetic progestin-estrogen, was administered orally to Simonson, Sprague-Dawley rats 7 days/week for up to 18 months. Slit lamp examinations, conducted once every 3 months (A. L. Schut), disclosed cataracts at 12, 15, and 18 months of dosing. At dose levels of 1, 3, and 10 mg/kg/day for 12 months, the incidence among the females was 0/9¹ (0%), 0/11 (0%), and 6/14 (43%), respectively. The incidence rose by 18 months to 3/8 (38%), 4/8 (50%), and 3/3 (100%). Among male rats cataracts were found only at the 10 mg/kg/day dose level; the incidence increased with time. No cataracts were found in control rats dosed with vehicle only. In a second study, with 5 male and 5 female Upjohn Sprague-Dawley rats, the dose rate was doubled each month for 4 months, ranging from 120 mg/kg/day to 960 mg/kg/day. One female died 5 days before the end of the study. No cataracts were found in the remaining rats. Other apparently drug-related findings include: (1) hepatomegaly and variable degenerative liver changes; (2) mammary glands developed and secreting; (3) spleen and bone marrow active with increased megakaryocytes; (4) anterior pituitary adenomas increased. In conclusion, low dose levels (1 mg/kg/day) of U-13,851 appeared to cause cataracts in female rats in 15-18 months, whereas no lenticular abnormalities were found when high dose levels (120-960 mg/kg/day) were administered for 4 months.

57. *Toxicologic Evaluation of Oral and Dermal Treatment of Primates with DMSO*. E. E. VOGIN, S. CARSON, and G. CANNON; C. R. LINEGAR; and L. F. RUBIN, Food and Drug Research Laboratories, Inc. Maspeth, New York, Squibb Institute for Medical Research New Brunswick, New Jersey; and University of Pennsylvania School of Veterinary Medicine Philadelphia, Pennsylvania.

The oral administration or dermal application of dimethyl sulfoxide (DMSO) to several species of laboratory animals resulted in ocular abnormalities characterized by a change in lenticular relucency and progressive lenticular myopia. These changes were observed consistently in all subprimate species tested, but their occurrence in subhuman primates has been controversial. In order to shed further light on this question and to ascertain the toxicology and pathologic manifestations of chronic administration of pharmaceutical grade DMSO, rhesus monkeys (*Macaca mulatta*) were treated with various doses for 18 months. Daily doses of 1, 3, and 9 ml/kg body weight of a 90% (v/v) aqueous DMSO solution were given in equally divided portions each morning and afternoon to different groups of monkeys either topically or intragastrically. The topical applications were made to the entire abdominal area of the monkeys. Control animals received 9 ml/kg of water by the same routes of administration.

Observations were made of body weight, blood pressure, heart rate, respiratory rate, body temperature, water consumption, reflexes, electrocardiograms, hematology, chemical analyses

¹ Numerator = number of rats having cataracts; denominator = total number of rats.

of blood and urine, and gross and microscopic anatomic and ophthalmologic examinations. The animals could not tolerate the high dosage of DMSO given orally, and all animals died between the 15th and 53rd weeks of study. Histologically, atelectasis and emphysema were the only pathologic changes seen. The possibility exists that in an oral regimen with a highly volatile irritating substance some regurgitation and/or tracheal inspiration may have occurred. With 90% DMSO no toxicologic or pathologic changes attributable to oral administration of 1 or 3 ml/kg or topical application of 1, 3, or 9 ml/kg were found in the monkeys. None of the ophthalmologic alterations previously reported in subprimate species were seen in these monkeys during the entire period of observation.

59. *Mechanisms of Chloroquine and Phenothiazine Retinopathies.* H. REINERT and D. A. RUTTY, Pharmacology and Toxicology Group, Therapeutics Research Division, Pfizer Ltd., Sandwich, Kent, England.

Substituted phenothiazines, such as piperidylchlorophenothiazine (NP 207), have a high melanin affinity and accumulate in the pigment epithelium of the retina. The primary target for their toxic action is the ellipsoids of the rods, where they inhibit enzyme activity resulting in depression of oxidative phosphorylation. Phenothiazines are consequently retinotoxic only in those species whose retinas are rich in melanin and have a high metabolic rate, for example, the cat. In albino retinas the phenothiazines are nontoxic. The depression of retinal metabolism interferes with the functional unity between rods and pigment epithelium, and the inability of the latter to eliminate desquamated rod segments and metabolites leads gradually to a degeneration of the sensory receptors.

The retinotoxic action of chloroquine is claimed to be based on an affinity for the melanin of the pigment epithelium and an inhibitory action on the protein metabolism of the pigment epithelium. It is suggested that this results in permeability changes of the pigment epithelium, loss of melanin and degeneration of the sensory receptors (for references see Meier-Ruge and Werthemann, in *Medikamentöse Retinopathie*. Thieme, Stuttgart, 1967).

Our results with NP 207 are in agreement with other authors. In addition we have found that thioridazine produces similar lesions to those described for other substituted phenothiazines. We have found, however, that in contrast to the phenothiazine retinopathy, chloroquine produces retinal damage not only in the cat, but also in albino and hooded rats, albino mice, and dogs. Therefore affinity for melanin pigment and inhibition of the metabolism of the pigment epithelium cannot be the primary target of the toxic action of chloroquine in the retina. The primary lesion occurs in the soma of the optic ganglion cells. The lesion is characterised by intracytoplasmic membranous bodies which are present in large numbers and almost completely displace the soma of the ganglion cell. The morphologic appearance is

strikingly similar to the ballooning of the cerebral ganglion cells produced by lipid (ganglioside) accumulation in amaurotic family idiocy (Tay-Sachs disease). The primary ganglion cell lesion is then followed by morphologic changes of the visual cells and the pigment epithelium, similar in appearance to the phenothiazine retinopathy. The accumulation of the lipid crystalloids in the soma of the ganglion cells is most likely the reason for the irreversibility of the chloroquine retinopathy and the occurrence of amaurosis in contrast to the retinal damage produced by phenothiazines, which is reversible and does not involve the optic ganglion cells.

60. *Toxicosis in Miniature Swine Induced by Corn Cultures of Penicillium Viridicatum*. WILLIAM W. CARLTON and JOHN TUIITE, Department of Veterinary Physiology and Pharmacology and Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana.

Mycotoxigenesis induced in young miniature swine by feeding of corn cultures of *P. viridicatum* was studied. Cultures of the fungus were prepared by the inoculation of autoclaved corn with spores and incubation at 24°C for 2 weeks. Chloroform was added to flasks containing the cultures and was evaporated after 72 hours. The cultures were dried at 100°F for 5 days and ground. Ground corn cultures were mixed with a commercial pig starter at 1:1 concentration and fed ad libitum to miniature pigs weaned at 3 weeks of age. Toxicosis was observed after 10 days of feeding. Affected pigs were stunted and prior to death were dyspneic and cyanotic. At necropsy, gross lesions included subcutaneous edema, marked hydrothorax, ascites, hydropericardium and extensive edema of viscera, especially severe around pancreas and terminal colon. Lungs were collapsed, consolidated, and dark red. Striking lesions involved the perirenal tissues, which were hemorrhagic and edematous in some pigs, and semisolid gelatinous material was observed between kidney and its capsule. In more severely affected pigs, there was an extensive bulging of the perirenal area due to the accumulation of fluid between kidney and capsule. Kidney surface was pale and presented petechial and ecchymotic hemorrhages. Microscopic examination confirmed the extensive edema and established mild degenerative changes in the liver. Renal lesions varied in severity but included degeneration of tubular epithelium, interstitial edema and inflammatory cell infiltration and glomerulitis.

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61. *Rate of Pulmonary Excretion of Paraldehyde in Man*. H. H. BORGSTEDT and D. W. LANG, Department of Pharmacology, University of Rochester School of Medicine and Dentistry, Rochester, New York. (Sponsor: H. C. Hodge.)

Healthy volunteers were given 40, 60, or 80 mg/kg of paraldehyde orally. Minute ventilation, end-tidal and total CO₂ were measured periodically over the subsequent 4 hours. Paraldehyde concentrations in end-tidal and mixed expiratory air were assayed frequently by means of gas chromatography. Minute alveolar ventilation was estimated by a CO₂ dilution technique. Paraldehyde was the only volatile material that appeared in the expired air after drug administration. The mean percentage of the administered doses excreted over 4 hours was 7.0%. There was no significant dependence on dose. Hyperventilation produced no changes in the concentrations of paraldehyde in the expired air; the amounts, therefore, excreted during any period, depend on minute ventilation. A comparison of dead spaces determined by CO₂ and paraldehyde dilution techniques showed no significant differences. Therefore, pulmonary excretion of paraldehyde appears to take place in the alveoli only.

62. *Factors Influencing the Removal of Inhaled ²³⁹PuO₂ Particles by Pulmonary Washing*. CHARLES L. SANDERS, Biology Department, Battelle Memorial Institute, Pacific Northwest Laboratory, Richland, Washington. (Sponsor: H. A. Kornberg.)

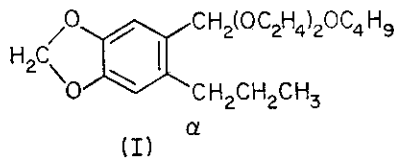
From 50 to 60% of inhaled ²³⁹PuO₂ particles were removed from rat lungs by pulmonary washing with 19 consecutive 12-ml saline washes. The most effective time for removal of particles follows the rapid tracheobronchial clearance phase. Nearly all particles found in

lung washes after the first day had been phagocytized by pulmonary macrophages. Phagocytosis of particles occurred within a few hours following deposition. About 50% of lung Pu was removed by washing at 4-7 hours, 20-30% at 1-11 days, and only about 10% at 15-23 days after lung deposition of 1-2 μCi . After lung deposition of 0.05-0.15 μCi , 50-60% of lung Pu was removed by washing at 1-8 days, and 30-40% at 9-15 days. The amount removed then increased to about 50% again by day 20. Values thereafter ranged from 30 to 60% of lung Pu in the washes. Considerable pulmonary damage was observed a few weeks after deposition of 1-2 μCi , only limited damage being seen in the low-exposure group during the first few months. High radiation doses probably resulted in pathologic relocation of particles found initially in pulmonary macrophages into the lung parenchyma, thus preventing their removal by washing. The ability to remove inhaled Pu particles by lung washing was therefore dependent on the amount of Pu deposited and the time after exposure. Plutonium particles were more rapidly cleared from the lung in the low exposure group than in the high exposure group. Thus the distribution and clearance of inhaled Pu particles were greatly influenced by the quantity of Pu initially deposited in the lung.

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63. *Detection of Unchanged Piperonyl Butoxide in the Lung of Rats after iv and Oral Administration.* L. FISHBEIN, H. L. FALK, J. FAWKES, S. JORDAN, and B. CORBETT, National Environmental Health Center, Research Triangle Park, North Carolina.

Piperonyl butoxide (3,4-methylenedioxy-6-propylbenzyl-*n*-butyl diethylene glycol ether) (I)



is widely employed as a potent synergist for pyrethrum, carbamate and chlorinated hydrocarbon, insecticides. The synergistic toxicity and carcinogenicity of Freons and piperonyl butoxide as well as its interference with benzo[*a*]pyrene metabolism have also been reported. The metabolism of both methylenedioxy- ^{14}C - and α -methylene- ^{14}C -piperonyl butoxide in the rat after iv oral administration was elaborated using both thin-layer and radioautographic techniques. In addition to a large number of metabolites detected in bile and urine (after iv administration), significant radioactivity (16-25%) from each isotope was detected in the lung and identified as free piperonyl butoxide in both cases. Similarly, free piperonyl butoxide has been detected after oral administration. The significance of this, as well as other tissue residues and rates of elimination of the respective isotopes, is discussed.

64. *The Use of an Automated Image Analyzer in the Quantitative Histologic Evaluation of Toxicologic Material, with Particular Reference to the Respiratory Tract.* L. E. MAWDESLEY-THOMAS and P. HEALEY, Department of Experimental Pathology, Huntingdon Research Centre, Huntingdon, England. (Sponsor: Professor A. N. Worden.)

An automated image analyzer, previously confined to the analysis of microscope images in metallurgy, is discussed in relation to quantitative histologic evaluation of mucus-secreting cells of the respiratory tract following irritation by sulfur dioxide. The accurate quantitative assessment of microscope images has long been a problem, particularly in toxicologic pathology. The image analyzer utilizes the principle of line scanning for counting and area-sizing any particle of sufficient contrast in a microscope image. The equipment consists of a television camera, monitor, detector circuit, and data-decode units. A 6-channel output enables information to be recorded on accumulating displays, tape, punch cards, or print out. The image analyzer operator is able to scan and record information from some 1000 microscope fields

an hour. The actual time for any parameter to be assessed is 0.6 sec, and up to 30 measurements may be simultaneously programmed.

A method of measuring a dose-related histologic response to irritants has long been sought, but the problem of time-consuming manual methods in relation to counting and area-sizing mucus-secreting cells has never been satisfactorily solved. The image analyzer answers this need and experiments in rats exposed to 50, 100, 200, and 300 ppm of sulfur dioxide (15×6 hour exposures) have shown a response related to dose. Rats were found to tolerate higher concentrations of sulfur dioxide than other species including cats, dogs, lambs, pigs, and baboons. Possible applications of the image analyzer are legion in the biological sciences and further research is being undertaken into increases in alveolar macrophages as an additional index of lung irritation, suppression of sebaceous glands in mouse skin as a short-term screening procedure for carcinogenic agents, alteration of number of cells in the central nervous system, and changes in the volume of thyroid colloid and pancreatic islet tissue under various physiologic and toxicologic conditions.

65. *Toxic Effects of High Concentrations of Bromobenzyl nitrile Vapor in Various Animal Species.* F. W. OBERST, J. W. CROOK, S. F. SWAIN, F. P. WARD, W. S. KOON, and N. P. MUSSELMAN, Toxicology Department, Medical Research Laboratory, Edgewood Arsenal, Maryland.

Eight animal species were exposed to bromobenzyl nitrile (BBN) vapor in concentrations ranging from 105 to 168 mg/m³, and the toxic effects and the LC₅₀ value for each species were determined. The exposure times were between 12 and 168 min, depending on the animal species and the effect desired. The primary local effect of BBN was irritation of the epithelial structures, particularly those in the respiratory tract, eyes, skin, and, possibly, the gastrointestinal tract. The predominant toxic signs were corneal ulceration, conjunctivitis, blepharitis, blepharospasm, photophobia, rhinitis with nasal discharge, laryngitis, tracheitis, bronchitis, pneumonia, chronic coughs or sneezes, parenchymal lung rales and wheezes, and hyperemia and desquamation of exposed skin. The severity of respiratory involvement was directly proportional to the Ct dose. The pig was the most sensitive species, the LC₅₀ value being 4900 mg/min/m³, while the rat was the most resistant, the LC₅₀ value being 18,900 mg/min/m³. The species order for increasing sensitivity to BBN vapor is as follows: rat, monkey, dog, guinea pig, goat, rabbit, mouse, and pig. It was concluded that BBN is a potent irritant and is lethal to animals when the concentration is high and the exposure time is sufficiently long.

66. *A Spontaneous Pulmonary Disease in Purebred Beagle Dogs Used in Toxicity Studies.* R. S. HIRTH and G. H. HOTTENDORF, Toxicology Department, Bristol Laboratories, Syracuse, New York. (Sponsor: M. H. Pindell.)

Up to the present time, 4 papers have been published on a total of only 4 cases of *Filaroides milksi* in the dog. The overall infection rate of purebred beagle dogs used by this laboratory for toxicity studies has been above 5% and was as high as 25% in some groups of dogs. Gross lesions consist of small, discrete gray or tan nodules on the surface of the lungs, along with coalescence of nodules, subpleural fibrosis, and emphysema. Histologically, any or all of the following changes may be present: peribronchiolitis, endobronchiolitis, perivasculitis, subpleural fibrosis and emphysema, and focal granulomatous interstitial pneumonia. The subpleural fibrosis and endobronchiolitis may be subacute or chronic and show various stages of resolution and repair. Since *F. milksi* is not usually seen in sections, it is fortuitous when they are found without making multiple slides from a lesion. This emphasizes the importance of making every attempt to determine whether this nematode is involved when suggestive lesions are encountered in dogs. It is important that the lesions not be interpreted as drug-induced changes, especially in chronic toxicity studies. Where doubt still exists, multiple sections are indicated in order to attempt to reveal the nematode in the lung parenchyma. These findings suggest that this lung worm infection can be enzootic in dogs raised on wire for pharmaceutical use. Thus this condition may be considered in any differential diagnosis when similar pulmonary lesions occur in dogs on toxicologic studies.

67. *Residual Lesions of Spontaneous Disease Observed in Dogs Utilized in Drug Safety Evaluations.* G. H. HOTTENDORF and R. S. HIRTH, Toxicology Department, Bristol Laboratories, Syracuse, New York. (Sponsor: M. H. Pindell.)

Recognition of the residual lesions of spontaneous disease in dogs utilized in drug safety evaluations and the differentiation of these lesions from those that may be drug-induced is of obvious importance. A definitive pathologic profile consisting of the residual lesions observed in control dogs is essential to this recognition and differentiation. The profile should be augmented by those sporadic lesions observed in treated dogs which have well established etiologies or display the etiologic agent in the tissue section. Such a profile, developed as the result of over 600 canine necropsies, is presented. Lesions are illustrated and the incidence, etiology, pathogenesis, significance, and interpretation are discussed briefly.

68. *Teratogenicity in Mice of Some Degradation Products of Cyclophosphamide.* J. E. GIBSON and B. A. BECKER, Oakdale Toxicology Center, Department of Pharmacology, University of Iowa College of Medicine, Iowa City, Iowa.

Proposed *in vivo* degradation products of cyclophosphamide were administered to pregnant Swiss-Webster mice (day 10 or 14), and minimal teratogenic dosages were determined: Cytoxyl alcohol [*N,N*-bis(2-chloroethyl)-*N'*-(3-hydroxypropyl) phosphorodiamidate], 290; cytoxyl amine [*N,N*-bis(2-chloroethyl)-*O*-(3-aminopropyl) phosphoramidate], > 270; phosphoramidate mustard [*N,N*-bis(2-chloroethyl)phosphorodiamidic acid], 154; nornitrogen mustard, 40; and nitrogen mustard, 1.6. Maternal toxicity was evident in each case. Malformations varied from agent to agent, and no agent paralleled cyclophosphamide, 20 mg/kg, teratogenicity which effected qualitatively and quantitatively greater soft tissue and skeletal malformations without maternal toxicity. Cyclophosphamide produced cleft palate, open eyes, aphakia, hydronephrosis, hydrocephalus, exencephaly, digital and rib anomalies, bone fusion, and bone curvature. Only phosphoramidate mustard and nitrogen mustard affected skeletal development; cytoxyl alcohol produced cleft palate, hydronephrosis and digital defects; phosphoramidate mustard produced digital defects; cytoxylamine and nitrogen mustard (day 10) were only slightly teratogenic. Only nitrogen mustard was teratogenic on day 14. Alkylating activities of these agents were determined by nitrobenzylpyridine reaction in plasma of mice at 2, 4, 8, 16, 32, and 64 min after ip dosages equimolar to cyclophosphamide. Nornitrogen mustard and nitrogen mustard exhibited trace activities possibly due to rapid excretion; over 50% of administered material was detected in urine in 16 min. Cytoxylamine, cytoxyl alcohol, and phosphoramidate mustard exhibited peak activities in 4 min equal to or greater than that of cyclophosphamide (peak: 16 min). No correlation between teratogenicity and alkylating properties of cyclophosphamide degradation products could be demonstrated. The teratogenicity of cyclophosphamide could not be attributed to any proposed metabolite studied. Cyclophosphamide teratogenicity appears to be independent of its alkylating property. (Supported by NIH Grant GM-12675 and NIH Training Grant GM-1308.)

69. *Phenobarbital and SKF 525-A Alteration of Diphenylhydantoin Teratogenicity in Mice.* R. D. HARBISON and B. A. BECKER, Department of Pharmacology, College of Medicine, University of Iowa, Iowa City, Iowa.

Phenobarbital and SKF 525-A (2'-diethylaminoethyl 2,2-diphenylpentanoate HCl), which enhance and inhibit, respectively, microsomal drug-metabolizing systems, have been found to antagonize and enhance, respectively, the teratogenic effect of diphenylhydantoin in mice. Diphenylhydantoin, 50, 75, and 87.5 mg/kg, ip, was administered to groups of 8-10 primigravid Swiss-Webster mice during late embryogenesis (days 11, 12, and 13). Phenobarbital, 60 mg/kg, ip, and SKF 525-A 40 mg/kg, ip, were administered on gestational days 9, 10, 11, 12, and 13. Fetuses were delivered by cesarean section on gestational day 18, weighed, examined, and measured. Soft tissue malformations were found following Bouin's fixation and freehand

cross section. Diphenylhydantoin effected a dose related reduction in litter yield, fetal body weight, and size. Fetal long-bone lengths, measured in alizarin red-stained preparations, were shortened in diphenylhydantoin-treated mice. Diphenylhydantoin-induced fetal resorption rate at a dosage of 87.5 mg/kg was significantly ($P > 0.05$) reduced from 69% (62/89) to 10% (6/59) after phenobarbital pretreatment. SKF 525-A pretreatment significantly increased diphenylhydantoin-induced fetal resorptions at a dosage of 50 mg/kg from 8% (8/96) to 48% (31/64). The incidence of cleft palate at diphenylhydantoin dosage schedules of 50, 75, and 87.5 mg/kg was 27% (7/26), 57% (30/53), 85% (22/26), respectively, and 0% (0/56) for the respective controls. Phenobarbital pretreatment also reduced the incidence of diphenylhydantoin-induced cleft palate. The incidence of diphenylhydantoin-induced cleft palate at a dosage of 87.5 mg/kg was significantly reduced from 85% (22/26) to 47% (25/53) in the phenobarbital pretreated group. Similarly at a lower dosage of diphenylhydantoin (50 mg/kg), phenobarbital pretreatment reduced the incidence of diphenylhydantoin-induced cleft palate from 27% (7/26) to 15% (6/39). SKF 525-A pretreatment increased the incidence of diphenylhydantoin-induced cleft palate, at a dosage of 50 mg/kg, from 27% (7/26) to 52% (17/33) following pretreatment. In summary, diphenylhydantoin-induced cleft palate and fetal resorptions were decreased by phenobarbital pretreatment and increased by SKF 525-A pretreatment.

(Supported by NIH Grant GM-12675 and NIH Training Grant GM-1308).

70. *Teratogenic Effects in Hypervitaminosis A in the Hamster and the Guinea Pig.* JANE F. ROBENS, Division of Pharmacology and Toxicology, Food and Drug Administration, Washington, D.C. (Sponsor: Keith H. Jacobson.)

Previous studies [Cohlan, *Science* **117**, 535 (1953); Cohlan, *Pediatrics* **13**, 556 (1954); Deuschle *et al.*, *J. Dental Res.* **38**, 149 (1959); Kalter and Warkany, *Am. J. Pathol.* **38**, 1 (1961); Giroud and Martinet, *Compt. Rend. Soc. Biol.* **153**, 201 (1959); Mizutani *et al.*, *Japan. J. Genet.* **41**, 141 (1966)] have shown that high doses of vitamin A cause terata in animals. This investigation confirms and extends these previous studies in hamsters and guinea pigs. Hamsters were treated with 75,000–400,000 USP units of vitamin A palmitate per kilogram as single or multiple oral doses during organogenesis. Guinea pigs were treated with 200,000 USP units/kg as single oral doses during organogenesis. Similar but not identical soft tissue and skeletal anomalies were produced in each species. The percentage of fetal resorptions and the nature of the anomalies produced depended on the time of administration and the dose administered.

71. *Fetal Rabbit Ductus Arteriosus Assessed in a Teratologic Study on Isoproterenol and Metaproterenol.* R. L. HOLLINGSWORTH, W. J. SCOTT, M. W. WOODARD, and G. WOODARD, Woodard Research Corporation, Herndon, Virginia.

Five groups, including an untreated control group, of mated New Zealand White female rabbits were used for a teratologic study in which isoproterenol sulfate (Aludrin®) and metaproterenol sulfate (Alupent®) were fed separately in the diet each at 50 and 16 mg/kg/day, expressed in terms of free base, on days 7–16 after mating. Twenty-eight-day-old fetuses were delivered by cesarean section, and their survival during 6 hours in an incubator prior to sacrifice was determined. Of the 237 viable rabbit fetuses delivered, 201 survived the 6-hour incubation. Of these survivors, 93 had red-colored latex injected properly into the pulmonary arch and had heart and lungs preserved satisfactorily for examination to determine that the ductus arteriosus was open in 70 and barely open in 23. Injection of dyed latex into the aortic arch of 8 similar fetuses, however, did not determine the status of the ductus arteriosus. Suitable magnification near the junction of the fetal rabbit's ductus arteriosus with the aorta showed a pair of flaps which are valvelike and permit only unidirectional blood flow. Latex injected into the aortic arch thus could not enter the ductus arteriosus. Findings of Hamilton *et al.* [*Am. J. Physiol.* **119**, 206–212 (1937)] were confirmed. No evidence of any teratogenic effect was found with either isoproterenol or metaproterenol.

72. *Teratology Studies in Rabbits with Captan, Folpet, and Thalidomide.* J. McLAUGHLIN, JR., E. F. REYNALDO, J. K. LAMAR, and J.-P. MARLIAC, Division of Pharmacology and Toxicology and Bureau of Medicine Food and Drug Administration, Washington, D.C.

Captan, Folpet, and thalidomide were given in gelatin capsules to pregnant New Zealand White rabbits from day 6 through day 16 of gestation with day of mating counted as day zero. On day 29 the does were cesarean sectioned. Corpora lutea, implantation sites, fetuses (nonviable), pups (live), and other pertinent data were recorded. Dosages of 150, 75, and 37.5 mg/kg body weight for Captan and 150 and 75 mg/kg body weight for Folpet and thalidomide were administered. Controls were given empty gelatin capsules from day 6 through 16 of gestation. Thalidomide, but not Folpet, showed teratologic responses. Captan at 75 mg/kg body weight of the mother caused 9 malformed individuals from 75 implantations of 9 pregnant does. At 37.5 mg/kg dosage of Captan, one malformed individual from 49 implantation sites of 6 does was observed. Among the malformations were deformed limbs, cleft lip, fused upper lip at the 75 mg/kg dosage, and at the 37.5 mg/kg dosage the one malformed individual was acephalic. A discussion of the results obtained is given.

73. *Reproduction Studies in Guinea Pigs and Rabbits Following Clothiapine Administration.* F. E. KOHN, D. L. KAY, H. CERVENKA, J. H. KAY, and F. H. SCHULTZ, JR., Lifestream Laboratories, Libertyville, Illinois and Dorsey Laboratories, Lincoln, Nebraska.

Two dose levels of clothiapine (2-chloro-11-(4'-methyl)piperazinodibenzo[*b,f*] [1,4]-thiazepine), i.e., 6 and 15 mg/kg body weight per day were investigated for potential harmful effects on the reproductive processes in guinea pigs and rabbits. A total of four experiments were conducted to determine the hazard associated with ingestion of clothiapine at selected intervals both before and during mating, during gestation, and during the lactation period. Parameters evaluated included mating performance and fertility (in both males and females), fetal growth, examination of fetuses for external, visceral and skeletal teratogenic effects, number of resorptions, number of stillbirths, litter size, and growth and survival of progeny during the lactation period. All these parameters were investigated in guinea pigs, and rabbits were utilized principally for the evaluation of potential teratogenic effects. No harmful effects were noted.

74. *Reproduction Toxicity Studies of SK&F 14336-D in Rats and Mice.* K. T. SZABO, H. A. BIRKHEAD, A. M. DEGIORGIS, and M. C. FLOREK, Pathology and Toxicology, Smith Kline & French Laboratories, Philadelphia, Pennsylvania. (Sponsor: R. L. Steelman.)

Reproduction toxicity studies (fertility, general reproductive performance, teratology, perinatal and postnatal) of the tranquilizing compound, 2-chloro-9-[3-(dimethylamino)-propyl] acridane phosphate, SK&F 14336-D, were conducted in rats and mice in accordance with current FDA guidelines. In addition, specially designed experiments were performed to obtain detailed information on estrous cycles, sexual maturation, onset of puberty, labor and delivery.

The results and conclusions are based on over 4000 test and 2000 control progeny. In neither rats nor mice was there any evidence of drug-induced teratogenicity. Onset and maintenance of lactation in the rats were not adversely affected by the compound. The compound, when given above a determined dose to female rats, affected sexual rhythmicity, onset of puberty, and delivery.

75. *Effect of Nutrient Intake on CCl₄ Toxicity.* E. M. BLENDERMANN and LEONARD FRIEDMAN, Division of Nutrition, Food and Drug Administration, Washington, D.C. (Sponsor: Lawrence R. Weiss.)

A study has been made to determine the influence of fasting upon the hepatotoxic response to carbon tetrachloride. Rats were fed ad libitum a semipurified diet with 10% casein for

5 weeks from weaning. They were dosed orally with CCl_4 at 2 ml/kg body weight (as an olive oil solution, 1 : 1) and killed 24 hours later. The animals were either fed ad libitum or fasted 24 hours before CCl_4 administration, and then either force-fed a liquid diet or fasted until death 24 hours later.

The following changes due to CCl_4 were intensified by fasting instead of feeding *before* CCl_4 administration, regardless of postdosage food intake: increased total liver lipids, reduced oxygen uptake by liver slices, reduced $^{14}\text{CO}_2$ production from ^{14}C -1-lactate, necrosis, and reticuloendothelial cell invasion. A net production of pyruvic and lactic acids by liver slices from CCl_4 rats in the presence of exogenous glucose was noted when rats were fasted, but not when fed, *before* dosage. *Post*-dosage fasting caused an increase in degree of liver enlargement induced by CCl_4 but no other changes in signs of toxicity. In general, the prefasted rats showed greater CCl_4 changes than rats allowed food before CCl_4 ; these differences were not due to differences in absorption of the toxicant, since time studies showed no significant differences in blood $^{14}\text{CCl}_4$ levels between rats fasted and rats allowed food before administration.

76. *Ethanol Potentiation of Carbon Tetrachloride Hepatotoxicity*. E. T. WEI and L. C. K. WONG, Department of Pharmacology and Experimental Therapeutics (Toxicology Activity), School of Medicine, University of California, San Francisco Medical Center, San Francisco, California. (Sponsor: C. H. Hine.)

Ethanol is known to potentiate carbon tetrachloride hepatotoxicity in man and in experimental animals. Experiments were initiated to elucidate the possible mechanisms involved in the potentiation phenomenon. Female Sprague-Dawley rats fasted for 8 hours were gavaged with either 4 g/kg of ethanol or isocaloric amounts of glucose, but were otherwise fasted throughout the experimental period. Liver triglyceride, plasma triglyceride, plasma glucose, and liver glycogen contents were measured at 0, 6, 18, and 24 hours after administration of the solutions. Paired controls were used for each experiment, and the time of drug administration was standardized to minimize diurnal variation. Subsequent experiments in animals administered carbon tetrachloride were run under identical conditions. Blood triglyceride increased to a maximum at 6 hours. Liver triglyceride was found to be maximally elevated at 18 hours after ethanol. Blood glucose and liver glycogen levels were not different between the ethanol-treated and control groups.

A dose response effect was obtained with different doses of CCl_4 as measured by prolongation of hexobarbital induced sleeping time 2 hours after CCl_4 treatment. At a dose of 0.01 ml/kg given subcutaneously, CCl_4 did not prolong sleep time whereas 0.05 ml/kg tripled the duration of the loss of the righting reflex. From the dose-response curve obtained, it was estimated that 0.02 ml CCl_4 /kg might be the threshold dose for potentiation. Four groups of animals were given one of the following treatments: isocaloric glucose CCl_4 (0.02 ml/kg subcutaneously), ethanol or ethanol plus CCl_4 (0.02 ml/kg subcutaneously). CCl_4 was given 18 hours after either ethanol (4 g/kg, intragastric) or glucose. No potentiation was observed in the duration of hexobarbital sleep time. The results suggest that ethanol does not potentiate the early stages of CCl_4 -induced hepatic changes.

(Supported in part by USPHS Training Grant 01304.)

77. *The Hepatic Disposition of Sulfobromophthalein (BSP) and Other Compounds as Influenced by Probenecid*. R. M. McCLAIN and G. L. PLAA, Oakdale Toxicology Center, Department of Pharmacology, College of Medicine, University of Iowa, Iowa City, Iowa.

Probenecid-induced alterations in hepatic function were studied in rats using BSP, BSP-glutathione, phenol-3,6-dibromophthalein disulfonate (DBSP), indocyanine green (ICG), and bilirubin. Dose-related responses were observed when BSP 60 mg/kg, iv, was given immediately after probenecid, 62.5, 125, and 250 mg/kg, iv; 30 min after 250 mg/kg, these included increasing plasma BSP retention (5.4 vs 1.8 mg%), decreasing rate of excretion (12.5 vs 27.2 mg/kg/30 min), and decreasing percentage of conjugated BSP in the bile (77 vs 88%). During infusions of BSP at rates above the maximal biliary excretory rate, probenecid

had no effect on BSP relative hepatic storage capacity but significantly reduced maximal biliary excretory rate (1.31 vs 0.82 mg/kg/min after probenecid 250 mg/kg). *In vitro* conjugation of BSP with glutathione was also inhibited by addition of probenecid to liver homogenates. However, probenecid, 125 mg/kg, produced significant plasma retention and decreased biliary excretion of BSP-glutathione given in a dosage equimolar to BSP. The effect of probenecid, 125 mg/kg, on exogenous bilirubin disposition was similar; plasma retention, decreased biliary excretion, and decreased percentages of conjugated bilirubin in the bile were observed. ICG and DBSP, which are secreted without alteration, also exhibited plasma retention and decreased biliary excretion when given in dosages equimolar to BSP following probenecid, 250 mg/kg. These studies indicate that probenecid exerts an effect on a biliary excretory process common to all these compounds.

(Supported by USPHS Research Grant GM-12675).

78. *Influence of Dioxin on Liver Function in the Rabbit*. M. L. KEPLINGER, G. P. SCHOENIG, O. E. FANCHER, E. E. CHRISTOFANO, and J. P. FRAWLEY, Industrial Bio-Test Laboratories, Inc., Northbrook, Illinois; and Hercules Incorporated, Wilmington, Delaware.

It was suggested that the presence of dioxin (2,3,7,8-tetrachlorodichlorobenzo-*p*-dioxin) as a contaminant at very low concentrations in industrial chemicals can be detected by changes in liver function following oral administration to rabbits. It was thought that this might be a sensitive bioassay for the presence of dioxin. Therefore dioxin was added to industrial chemicals at concentrations of 0, 0.3, 1.0, 3.0, and 10.0 ppm and the contaminated materials were administered orally, in doses of 80 or 100 mg/kg, to rabbits. Determinations of SGOT, SGPT, and BSP retention were made before administration and at 5 and 15 days after administration. No effects indicating clinical evidence of liver damage were found. In a second experiment dioxin itself was administered at doses of 0.01 or 0.1 mg/kg (10 and 100 times the highest dose above). Carbon tetrachloride was administered at doses of 100, 250, and 500 mg/kg as a positive control. The SGOT, SGPT, and BSP retention were measured every 2 or 3 days for 18 days. All three doses of carbon tetrachloride caused significant increases in SGOT, SGPT, and BSP retention. Increases in these three were found in some, but not all animals receiving dioxin. The changes were neither well correlated with dose nor consistent in onset and duration, even in animals showing apparently significant effects. Although some positive results were obtained with dioxin itself, the usefulness of this technique as a bioassay for the presence of dioxin is questionable.

79. *Malathion Potentiation and Inhibition of Liver Esterases by Triorthotolylphosphate (TOTP) in Mice*. S. D. COHEN and S. D. MURPHY, Department of Physiology, Harvard School of Public Health, Boston, Massachusetts.

It has been suggested that aliesterase inhibition might serve as an indicator of possible potentiation among various organic phosphates. In this investigation we compared the time- and dose-response relationships for TOTP inhibition of liver hydrolysis of various esters with its potentiation of malathion toxicity in mice. Inhibition of brain cholinesterase (CHE) was used as the index of malathion toxicity. Groups of 8 male mice were injected intraperitoneally with various doses of TOTP. Four mice in each group were sacrificed 16-18 hours after TOTP, and liver aliesterase activity was assayed manometrically with diethyl succinate (DES), triacetin (TA), and malathion as substrates. The remaining 4 mice were challenged with malathion (50 mg/kg, ip) and were sacrificed 2 hours later for brain CHE assays. Potentiation of the anticholinesterase activity of malathion was dose dependent between 10 and 25 mg/kg of TOTP. Inhibition of hydrolysis of TA and malathion showed a similar dose-response relationship, but DES hydrolysis was maximally inhibited throughout this dose range. Similar experiments were performed in which the dose of TOTP was held constant (125 mg/kg) but the pretreatment interval was varied from 18 hours to 10 days. The potentiating effect of

TOTP on malathion anticholinesterase action decreased between 24 and 48 hours and could not be detected at 5 days after TOTP. Reversal of inhibition of TA hydrolysis occurred at the same time; however, inhibition of DES hydrolysis remained maximal for longer than 5 days. These experiments indicate that the substrate selected for measuring liver aliesterase inhibition can influence the accuracy of this test for predicting the potentiating capacities of organo-phosphates.

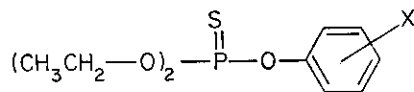
(Supported by Training and Research Grants TO1-ES-00045 and RO1-ES-00084 from the Division of Environmental Health Sciences, NIH.)

80. *Studies of the Hepatic Effects of Clofibrate (Ethyl Chlorphenoxisobutyrate) in Rats.*
H. A. HARTMAN, R. E. BAGDON, R. J. VAN RYZIN, and A. J. TOUSIMIS, Sandoz Pharmaceuticals, Hanover, New Jersey, and Biodynamics Research Corp., Rockville, Maryland.

Rats were administered 0.300% clofibrate for 6 and 12 weeks; additional groups were continued on a nonmedicated diet for 3 and 6 weeks after cessation of clofibrate treatment for 6 weeks. Food intake, weight gain, and serum cholesterol levels were comparable to controls. Serum triglycerides were decreased but returned to control levels 3 and 6 weeks after treatment. Relative and absolute liver weights were increased at 6 or 12 weeks but also reverted to normal 3 or 6 weeks after clofibrate was withdrawn. Serum bilirubin, SGPT, and serum ornithine carbamyl transferase were normal. Hexobarbital metabolizing activity (μg metabolized per gram liver per hour) and total liver hexobarbital metabolizing activity ($\text{g liver} \times \text{enzymatic activity in } \mu\text{g/g/hr}$) were $1.7 \times$ and $2.2 \times$ controls after 6 weeks but were similar to controls at 12 weeks. Light microscopic findings showed increased cytoplasmic granularity and cell diameter and a decrease of perinuclear clear-spaces of hepatocytes that were only partially reversible. Electron microscopy showed an increase in microbody: mitochondria ratio (Mb : M) and decreased glycogen pools; lysosomes were increased, but mitochondria, smooth and rough endoplasmic reticulum (SER and RER) were unchanged. After 12 weeks, the Mb : M ratio was also increased, the RER was disorganized and evenly distributed throughout the cytoplasm; other changes were minor. Ultrastructural changes also largely reverted to normal after cessation of clofibrate treatment. Thus, hepatotoxic effects were not observed based upon measurements of liver function, drug-metabolizing activity, light and electron microscopy. The hepatomegaly was readily reversible and may represent an adaptive response to administration of clofibrate.

81. *Effect of Structure on the Metabolism of Dialkylarylphosphorothionates by Rabbit Liver Microsomes.* MICHAEL WOLCOTT and R. A. NEAL, Department of Biochemistry, Division of Toxicology, Vanderbilt University, Nashville, Tennessee.

In the metabolism of dialkylarylphosphorothionates by rabbit liver microsomes, there are two major metabolites formed: dialkylphosphorothioic acid and dialkylaryl phosphate. These metabolites are formed oxidatively by what appear to be two separate enzymes. The purpose of the present study was to examine the effect of altered electrophilicity of the phosphorus atom on the rate of formation of these two metabolites. Compounds of the general



O,O-diethyl-*O*-aryl phosphorothionate

structure were used in the study. The substituents on the aromatic ring (X) ranged from strong electron withdrawers to strong electron donators. Relationships between both the Michaelis-Menten constant, K_m , and V_{max} vs the Hammett substituent constant, σ , were examined.

82. *The Reduction of Nitro and Azo Compounds by House Fly Microsomes.* LEON SHARGEL, SHOSHANA AKOV, and PAUL MAZEL, Department of Pharmacology, George Washington University Medical School, Washington, D.C., and the Entomology Research Division, United States Department of Agriculture, Beltsville, Maryland.

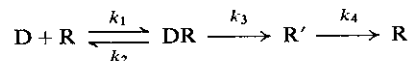
Microsomes obtained from abdomens of 6-day-old female house flies, *Musca domestica*, were compared with liver microsomes from male, Sprague-Dawley rats for their ability to reduce an azo compound (1,2 dimethyl-4-(*p*-carboxyphenylazo)-5-hydroxybenzene, CPA), an aromatic nitro compound (*p*-nitrobenzoic acid, PNB) and cytochrome *c*, as well as for their respective cytochrome P-450 content. The spectra of activity for house fly and rat hepatic microsomes were qualitatively similar. Specific enzyme activities when based on a $\mu\text{mole/mg}$ protein basis were found to be less in microsomes from house flies than from rat liver. House fly microsomes required reduced NADPH for both azo and nitroreductase activities. Both microsomal preparations had NADPH cytochrome *c* reductase activity. FMN, 2×10^{-5} M stimulated microsomal azoreductase and nitroreductase activities of both house fly and rat. Carbon monoxide (CO) inhibited the reduction of CPA and PNB to a smaller degree in microsomes from house flies than from rat liver. Moreover, house fly microsomes had far less cytochrome P-450 than did rat hepatic microsomes. The data indicate that house fly microsomal azo and nitroreductase activities are less sensitive to CO inhibition than are rat hepatic microsomal enzymes due to a smaller level of cytochrome P-450 in the former. These studies indicate that the microsomal enzymes involved in the reduction of xenobiotic compounds show a great universality among animal species.

(Supported by USPHS Grants GM 13749 and GM 26.)

83. *Receptor Kinetics: and Examination of Several Theoretical Models.* R. E. GOSSELIN, Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, New Hampshire.

Toxicologists are usually concerned solely with quantal (all-or-none) responses, such as death, the occurrence or nonoccurrence of tissue lesions, etc. Graded responses, however, cannot be ignored. To account for the shape of dose-response curves and of time-responsive curves, various receptor models have been proposed and analyzed. Most models are recognizable as variants of the classical occupation theory (OT) or of Paton's rate theory (RT).

The following new hypothesis is of particular interest:



In this scheme DR represents the combination of a receptor (R) with a drug or poison (D). The complex (DR) is converted irreversibly to inactive receptor (R'). R' cannot react with D but can be reactivated to generate R. Stimulus for the effector and the response intensity at any moment are assumed to be proportional to the rate of formation of R' ($= k_3[DR]$). Thus the model is a modified occupation theory (OT) and becomes identical to the classical OT whenever $k_2 \gg k_3 \ll k_4$. In contrast, when k_3 is much larger than the other 3 rate constants, the model becomes formally equivalent to the rate theory (RT). Therefore the OT and RT represent special cases of the present proposal.

In general, this model possesses most of the dynamic (transient state) characteristics of the RT. For example, overshoot and fade are often encountered, but highly damped oscillations are predicted to occur under some circumstances. In the steady state the intrinsic activity (α) can be represented by $k_3 \cdot k_4 / (k_3 + k_4)$. General properties of the new model are examined for both agonists and antagonists.

(Supported by USPHS-NIH Research Grant GM 11598.)

84. *Dietary Protein and Pesticide Toxicity: A Review.* ELTON M. BOYD, Department of Pharmacology, Queen's University, Kingston, Ontario, Canada.

A deficiency of protein in the diet produces a variety of syndromes from minor stunting of growth to severe kwashiorkor in many countries of the world. The ultimate eradication of the

condition increased involves dietary protein intake through increased crop production, which today means the use of agricultural chemicals including pesticides. The widespread use of pesticides involves the risk of pesticide poisoning. Is pesticide poisoning more—or less—likely to occur among exposed workers whose diet is deficient in protein? In an initial attempt to get information on this subject, the clinicopathologic syndrome of intoxication from a series of pesticides given orally at the range of the LD50 was determined in albino rats fed from weaning on protein-deficient diets. The results were compared with those in control rats fed what are usually considered as normal diets. Results to date suggest that protein-deficient diets do not appreciably affect the clinicopathologic features of pesticide intoxication but may significantly lower the dose at which toxicity occurs for some pesticide. For example, the LD50 of Captan in protein-deficient rats is 4% of that in controls, of endosulfan 20–25%, of lindane, diazinon, and malathion some 50%, and of dicophane and carbaryl slightly less.

(Assisted by a grant from the World Health Organization.)

85. *Persistence of Enzyme Induction with Various Dietary Levels of Pesticides and Drugs.*
F. K. KINOSHITA and K. P. DuBOIS, Toxicity Laboratory, University of Chicago, Chicago, Illinois.

Previous rat feeding studies conducted in this laboratory on the hepatic microsomal enzyme inducing capacity of various low dose levels of the chlorinated hydrocarbon insecticides, DDT and toxaphene [Kinoshita *et al.*, *Toxicol. Appl. Pharmacol.* **9**, 505 (1966)], and the substituted urea herbicides, Herban and Diuron [Kinoshita and DuBois, *Toxicol. Appl. Pharmacol.* **10**, 410 (1967)], demonstrated dose-related increases in enzyme activity. In addition, similarities were observed in the rate and persistence of induction by the various compounds. At all feeding levels that caused induction, the greatest amount of stimulation occurred during the first week followed by a gradual decline of activity during 13 weeks of continued treatment. The present investigation was initiated to ascertain whether this rise and decrease in induced enzyme activity occurs when drugs and other pesticides are fed to rats. Various dietary levels of phenobarbital, a known broad-spectrum enzyme inducing agent, and 4-fluoro-4'-trifluoromethylbenzophenone guanylhydrazone (WR 9792) an antimalarial agent, previously shown to stimulate only *O*-demethylase of rats [Kinoshita *et al.*, *Toxicol. Appl. Pharmacol.* **12**, 285 (1968)], were fed to male and female weanling rats. Both compounds produced dose-related increases in enzyme activity that were maximal after 1 week of feeding. Phenobarbital stimulated all the enzyme systems studied, but had the greatest effect on the *N*-demethylase system of male rats. WR 9792 stimulated only the *O*-demethylase system in both male and female rats. Enzyme activity remained elevated over a period of 6 weeks of continued feeding, but there was a gradual decline in the amount of stimulation.

86. *Nitrite Toxicosis in the Ascorbic Acid-Deficient Guinea Pig.* R. J. KOCIBA and S. D. SLEIGHT, Department of Pathology, Michigan State University, East Lansing, Michigan. (Sponsor: T. M. Brody.)

Subcutaneous administration of 50 mg/kg NaNO₂ consistently produced higher levels of methemoglobin in ascorbic acid-deficient guinea pigs than in controls. This dosage was fatal to a high percentage of the deficient group, whereas all control guinea pigs survived. Pretreatment of deficient guinea pigs with 10 mg/kg methylene blue protected against high levels of methemoglobin formation following the administration of 50 mg/kg NaNO₂.

Abortions were caused in a high percentage of ascorbic acid-deficient female guinea pigs following the subcutaneous administration of 45 mg/kg NaNO₂. This same dosage caused no abortions in the control females. Ascorbic acid-deficient females not given NaNO₂ had normal litters.

Maternal blood levels of methemoglobin were consistently higher in ascorbic acid-deficient pregnant females following the subcutaneous administration of 40 mg/kg NaNO₂. The accompanying levels of methemoglobin in fetuses derived by laparohysterotomy were similar in both the deficient and control groups. This data indicated that the fetal deaths were related

to higher levels of methemoglobin in maternal blood of deficient females and not to increased placental passage of nitrite.

87. *Hepatic Damage by Diet and Lasiocarpine in Rats*. PAUL M. NEWBERNE, ADRIANNE E. ROGERS, and NORA KULA, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Lasiocarpine, a plant alkaloid that results in liver damage, was administered to rats with livers previously injured by a low lipotrope diet. Each animal was intubated with 35 mg/kg body weight on alternate days for a total of 105 mg/kg. Body weight was decreased by low lipotrope diet but was affected little by lasiocarpine in either group. After 20 weeks on experiment, rats fed the low lipotrope diet had fatty liver and early fibrosis. Lasiocarpine enhanced the fatty and fibrotic changes and resulted in large, bizarre nuclei and moderate to severe cirrhosis. The control animals with lasiocarpine superimposed had fatty infiltration, large numbers of megalocytes, minimal fibrosis but no cirrhosis. Lasiocarpine had a much more severe effect on livers previously damaged by dietary means, thereby pointing out the importance of nutrition to the response of animals to toxic materials.

88. *Impairment of Organochlorine Pesticide Induction of Hepatic Detoxifying Enzymes in Ascorbic Acid Deficiency*. D. J. WAGSTAFF and J. C. STREET, Department of Veterinary Science, Utah State University College of Agriculture, Logan, Utah. (Sponsor: J. L. Shupe.)

Activity of hepatic detoxifying enzymes is dependent on an adequate dietary supply of compounds required in enzyme synthesis and function. As one example, ascorbic acid was reported to be required for normal levels of activity of coumarin hydroxylase. The effect of ascorbic acid deficiency on the induction of certain hepatic microsomal enzymes by organochlorine pesticides has been investigated. *In vitro* aniline hydroxylation, EPN detoxication, and *p*-nitroanisole *O*-demethylation in liver homogenates were the enzymatic reactions studied. The activity of each was depressed in ascorbic acid deficiency. Marked induction of these enzymes was produced by feeding dieldrin (HEOD; 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-exo-1,4-endo-5,8-dimethanonaphthalene) at a level of 25 ppm in the diet for 4 days or longer. DDT (2,2-bis-(*p*-chlorophenyl)-1,1,1-trichloroethane) and lindane (γ -benzenehexachloride), despite their effectiveness as inducers in rats at such dosage, were relatively ineffective in quinea pigs. Enzyme induction by these compounds was absent or severely depressed when the animals were deficient in ascorbic acid. Impairment of enzyme induction by dieldrin was observed as early as day 4 on an ascorbic acid-deficient diet and was quite pronounced by day 15 when body weight loss and subcutaneous hemorrhage of the hind legs became obvious. Thus, it would appear that ascorbic acid deficiency may be associated in some manner with impairment of normal and induced activity of hepatic detoxifying enzymes.

89. *Experience with Infusions of Various Concentrations of Xylitol in Monkeys and Rabbits*. L. E. BLOCKUS, J. F. DONAHOE, T. A. CROWLEY; and J. W. KEATING and M. S. WEINBERG, Abbott Laboratories, North Chicago, Illinois; and Biological Science Laboratory, Foster D. Snell, Inc. 800 Dowd Avenue, Elizabeth, New Jersey.

Xylitol is a pentahydroxy alcohol, a normal intermediary of carbohydrate metabolism. It is metabolized via an insulin-independent pathway and yields 4 cal/g. Experience in several species of animals, a prerequisite of clinical evaluation of any new drug, indicated that the rhesus monkey and the rabbit were the best models for studying xylitol. In the dog, xylitol produces and apparent species-specific hypoglycemia and hyperinsulinemia. After the development of techniques for continuous infusions in unanesthetized, uncooperative animals, graded concentrations of xylitol in saline were administered to 18 monkeys and 30 rabbits, daily, for 30

days. A 40% glucose infusion served as positive control. Effects on biochemical and hematologic parameters, serum insulin levels, and histology were compared among groups.

	Mortality		
	Xylitol, 6 g/kg	Glucose, 6 g/kg	Xylitol, 3 g/kg
Monkeys	2/6	2/6	0/6
Rabbits	7/10	2/10	2/10

Blood glucose levels varied predictably after glucose administration. No significant changes in blood glucose occurred with xylitol. Serum insulin values suggested a hormonal response different from that reported in man; however, the sample timing was also different from that of human studies. The applicability of hormonal studies in infrahuman species to the prediction of effects in man is questionable. The test materials, even in high concentrations, caused no significant effects on the vascular intima. Histopathologic examination of internal organs and the eyes was negative. The results and their interpretation are discussed. The development of techniques for simulating human use in animals is reviewed. The need for such studies is evident, but the difficulty of design and implementation must be recognized.

90. *Strain-Dependent Renal Toxicity of a Nonsteroid Anti-inflammatory Agent*. D. L. BOKELMAN, W. J. BAGDON, P. A. MATTIS, and P. F. STONIER, Merck Institute for Therapeutic Research, West Point, Pennsylvania.

Manor Farm SPF and Charles River CD rats were given a nonsteroid anti-inflammatory agent (1-*p*-chlorobenzylidene-5-methoxy-2-methyl-3-indeneacetic acid) orally, at dosage levels of 3, 6, or 12 mg/kg/day, for periods up to 29 weeks. Hematuria occurred in all groups of male Manor Farm SPF rats, both control and drug-treated. The incidence of hematuria was dose dependent and occurred 6, 12, or 24 times more often in male rats receiving 3, 6, or 12 mg/kg/day than in the controls. In contrast, hematuria was not observed in any of the control females and was extremely infrequent in drug-treated females. The hematuria observed in the Manor Farm SPF rats was due to a series of lesions in the renal papilla. The initial lesion consisted of foci of hyperplasia in the epithelium covering the renal papilla, most often located near the fornix. These single, tiny foci progressed to form large, multiple, polypoid hyperplastic lesions which projected into the pelvis of the kidney. Areas of necrosis were frequently observed within the hyperplastic foci which led to hemorrhage from the vulnerable underlying vasa rectae. Scars were also frequently found in the affected areas and represented sites of previous necrosis. Basically, the lesions were identical in the control and treated rats, but were more severe in the latter.

In contrast, untreated Charles River CD rats did not show renal lesions. When this strain of rat was treated with the same compound at the same doses, hematuria was rarely seen and no lesions of the renal papillary epithelium were found. On the other hand, very slight interstitial edema and necrosis were observed in a few of the drug-treated rats. These observations suggest that problems in evaluation of the safety of a compound may arise due to strain-specific lesions.

91. *Subsensitivity to Cholinergic Drugs Following Treatment of Rats with a Cholinesterase Inhibitor*. JOSEPH J. McPHILLIPS, Department of Pharmacology, West Virginia University Medical Center, Morgantown, West Virginia.

It has been demonstrated previously [McPhillips and Dar, *J. Pharmacol. Exptl. Therap.* **156**, 507-513 (1967)] that repeated injections of *O,O*-diethyl *S*-2-(ethylthio) ethyl phosphorodithioate (Di-Syston) to rats produces subsensitivity of the ileum to carbachol. In the present

study, daily injections of 0.9 mg/kg of Di-Syston for 10 days produced subsensitivity of the ileum to furfurethonium. There was, however, no change in the sensitivity of the ileum to methacholine or to potassium chloride. The sensitivity of the ileum to butyrylcholine was increased. The subsensitivity which develops, therefore, is apparently specific for certain types of cholinergic drugs.

(Supported by USPHS grant No. 1R01 HD 1816.)

92. *Long-Term Toxicologic and Tumorigenesis Studies on an Oral Contraceptive Agent in Albino Rats.* J. L. SCHARDEIN, D. H. KAUMP, E. T. WOOSLEY, and M. M. JELLEMA, Pathology and Toxicology Department, Parke, Davis & Co., Ann Arbor, Michigan.

Albino rats were fed 98% norethindrone acetate plus 2% ethynylestradiol¹ in the diet at dose levels about 10- and 100-fold the recommended human dose for a period of 2 years. The treated rats had dose-related growth retardation, hair loss, mastopathy, liver hyperplasia, and gonadal atrophy, but fewer and less severe degenerative lesions of aging, and an increased survival rate compared to the control rats. Females given the higher dose had a significant delay in onset of tumor development, and males had a significant increase in the number of tumors developed. There was an increased incidence of hepatocellular adenomas, uterine polyps, pituitary adenomas, and mammary gland tumors in animals given the higher dose, but there was no significant difference between treated and control rats in the number which developed tumors, or in the cumulative probability of tumor development.

93. *The Feeding of Aflatoxin to Beagle Dogs.* C. G. DURBIN, J. N. GELETA, W. T. SHALKOP, and B. H. ARMBRECHT, Division of Veterinary Research, Food and Drug Administration, Beltsville, Maryland.

Young adult Beagle dogs were orally dosed with aflatoxin (B₁ 37.5, B₂ 5.4, G₁ 17.1, G₂ 1.0%) at a dose rate (DR) of 1, 5, or 20 µg/kg whole body weight. The animals received this dose 5 days per week for a period of 10 weeks. Each group contained 2 females and 1 male. During these tests, 2 other dogs were challenged with 500 µg/kg/day for 2 days. A DR of 500 was lethal. Clinical signs attributable to aflatoxicosis were generally absent among the dogs in the DR 5, 1, and controls. Icterus, inappetence, and yellow-orange colored urine were noted in dogs receiving a DR of 20. Histopathologic examinations revealed no hepatic morphologic changes in the 2 groups of dogs on the lower levels of aflatoxin (DR 5, 1), or in the controls. In livers of the DR 20 dogs, there was moderate bile duct proliferation, bile pigment accumulation in the portal areas, and proliferation of multiple vascular channels around the central and portal veins.

94. *The Effects Produced in Brood Sows by Feeding Aflatoxin.* B. H. ARMBRECHT, W. T. SHALKOP, H. G. WISEMAN, J. E. JACKSON, and H. E. ZIMMERMAN, JR., Food and Drug Administration, Agricultural Research Center, Beltsville, Maryland.

Brood sows and breeding boars were fed sublethal amounts of aflatoxin (B₁, 37.5; B₂, 5.4; G₁, 17.1; G₂, 1.0%) to assess the chronic toxicity on adult animals and the effects on their offspring. Mixed-bred Poland-China, Duroc, and Berkshire sows over a period of 2.4 years were fed daily 10 µg of toxin per kg of body weight during gestation and 40 µg during lactation. Normal appearing piglets were born in 4 successive farrowings. These piglets ingested about 0.4 µg of mixed aflatoxins per kg of body weight from the sow milk. A transitory loss of growth was observed in test piglets at various weights prior to weaning. After weaning, these test animals were reared to market weight on control diet. Suckling piglets were withdrawn from other litters at various ages for a pathologic examination. In both instances, no histologic liver injury was detected. The clinical appearance of the sows was normal but histopathologic changes were characterized by many regenerative hyperplastic nodules and a few benign

¹ Norlestrin®, Parke, Davis & Company

adenomatous nodules with no indication of carcinoma. Yorkshire or Duroc gilts were fed 40 μg of toxin per kg body weight daily for 65 days during their growing period, and 17 or 32 μg of toxin per kg weight for 142-159 days which included pregnancy. Still other Yorkshire sows were fed 7-40 μg of toxin per kg body weight through the first farrowing, followed by 4-8 $\mu\text{g}/\text{kg}$ for the second farrowing, and finally basal diet for 3 more farrowings. Signs attributable to aflatoxicosis were not observed.

95. *Toxicologic Significance of UDP-Glucuronyltransferase Deficiency in the Newborn Gunn Rat.* ROGER A. YEARY and RONALD H. GROTHAUS, Department of Veterinary Physiology and Pharmacology, The Ohio State University, Columbus, Ohio.

The Gunn rat is a mutant of the Wistar strain that has a homozygous recessive trait for deficiency of hepatic UDP-glucuronyltransferase (UDPG). The homozygous recessive rats deficient in UDPG are icteric at birth and often develop kernicterus. The heterozygotes and dominants for UDPG are not icteric and cannot be distinguished from each other by visual observation. Matings were made by pairing homozygous recessive males with heterozygous females. Shortly after birth, the icteric homozygotes were separated from the nonicteric heterozygotes by visual examination. Littermates from one litter were exchanged with littermates from another litter and foster nursed so that one female nursed an entire litter of icteric UDPG-deficient rats and the other nursed a litter of nonicteric heterozygotes. The acute toxicities of various compounds known to be metabolized, at least in part, by glucuronidation were then determined within the first 3 days after birth for comparison of the LD50 values between the UDPG-deficient and nondeficient newborn rats. Compounds that are metabolized primarily by glucuronidation were generally found to be significantly more toxic in the UDPG-deficient newborn rat as compared with nondeficient littermates. These investigations are being extended in an attempt to correlate UDP-glucuronyltransferase substrate specificity *in vitro* with acute toxicity.

96. *Hydrazine and Protein Metabolism.* W. L. BANKS, JR. and N. L. SMITH, Department of Biochemistry, Medical College of Virginia, Richmond, Virginia. (Sponsor: Joseph F. Borzelleca.)

A time-course study of the effect of a single subconvulsive dose of hydrazine on liver protein metabolism revealed that protein/DNA, RNA/DNA, and the uptake of leucine- ^{14}C into protein were greater in hydrazine-treated than in control rats from 24 to 48 hours after the treatment. The difference in these parameters between control and experimental groups was maximal at 24 hours. At this dose level (40 mg/kg body weight) the livers from the hydrazine-treated rats contained considerably greater quantities of triglyceride and cholesterol than the control animals at 24 hours. When rats were fasted for 48 hours prior to hydrazine treatment in order to reduce their hepatic protein reserves to a minimal value, hydrazine treatment produced a marked increase in protein/DNA and RNA/DNA over the control values. These results taken together might suggest that hydrazine treatment stimulated hepatic protein biosynthesis. These responses to hydrazine treatment appear to be unlike those recorded for other hepatotoxins that produced a fatty liver.

97. *The Hyperglycemic Effect Following Inhalation of Carbon Tetrachloride.* L. C. K. WONG and C. H. HINE, Department of Pharmacology and Experimental Therapeutics (Toxicology Activity), School of Medicine, University of California, San Francisco Medical Center, San Francisco, California.

Hyperglycemic effect of CCl_4 in the cat has been recently reported by Wong and DiStefano [*J. Pharmacol. Exptl. Therap.* **162**, 344-351 (1968)]. They observed an immediate fall in blood glucose levels when the cat inhaled CCl_4 at a concentration of 10,000 ppm, and found that neither plasma nor adrenal catecholamine levels were altered. A prompt drop in blood pressure in the cat within a few minutes after administration of CCl_4 (10,000 ppm by inhalation) has been observed in the present study which is coincident with the CCl_4 -induced hyperglycemia

reported by Wong and DiStefano (1968). Cat blood pressure returned to normal within an hour after the withdrawal of CCl_4 whereas the blood glucose levels remained elevated throughout the experimental period. When cats were exposed to a concentration of CCl_4 at 3000 ppm, although the blood pressure remained within normal limits, a delayed hyperglycemic response was observed. Prior administration to cats of the β -adrenergic blocking agent, MJ1999 [4-(2-isopropylamino-1-hydroxyethyl)methane-sulfonanilide hydrochloride] failed to block CCl_4 -induced hyperglycemia. The present experimental results indicate that the hyperglycemic effect of CCl_4 is not due to the release of catecholamine.

(Supported in part by USPHS Training Grant 01304.)

98. *Studies on the Mechanism of the Barbiturate-Ethanol Interaction.* G. S. WIBERG, B. B. COLDWELL, and H. L. TRENHOLM, Pathology and Toxicology Section, Research Laboratories, Department of National Health and Welfare, Ottawa, Canada.

In young adult male rats, the ip 24-hour LD50 for five representative barbiturates were, in mg/kg: thiopental 53, pentobarbital 69, amobarbital 155, phenobarbital 171, and barbital 312 [Trenholm, H. L., Wiberg, G. A. and Coldwell, B. B., *Proc. Can. Fed. Biol. Sci.* **11**, 41 (1968)]. Significant dose-dependent decreases in the LD50 of all five barbiturates were obtained by the simultaneous administration of ethanol by the same route at doses of 2, 3, or 4 g/kg. The increased toxicity was most marked with phenobarbital and barbital—the barbiturates least subject to biotransformation. A nonhypnotic dose of ethanol greatly prolonged the sleeping times of threshold hypnotic doses of the 5 barbiturates and reduced the induction times. Consideration of the data suggested that at least 3 factors could be operative: (1) a concerted CNS depressant action, (2) and inhibition of ethanol metabolism, and (3) alterations in the rate of uptake and storage of barbiturates by the tissues. Evidence favoring these postulates has been obtained by studying blood concentration decay curves in rats and pigs, tissue distribution levels, tissue slice metabolism studies, and kinetics of isolated enzyme systems.

99. *Effects of Ethionine, Dimethyl Sulfoxide, and Route of Administration on Methylchloroform-Induced Alteration of Barbiturate Action.* H. LAL and H. C. SHAH, Department of Pharmacology, University of Rhode Island, Kingston, Rhode Island.

Shortening of the sleeping-time and enhancement of hepatic oxidation of hexobarbital was previously reported to occur in mice exposed to methylchloroform (MC) (3000 ppm or more; concentration-dependent effect) containing air mixtures [Lal and Shah, *Pharmacologist* **10**, 153 (1968)]. In subsequent experiments DL-ethionine (100 mg/kg, ip) administered prior to exposure blocked the effect of 24-hour MC inhalation. Higher doses (160–410 mg/kg) of ethionine prolonged the sleeping time and reduced the rate of hepatic hexobarbital oxidation. In contrast to the effects of MC through the inhalation route, ip injection of MC (1 ml/kg) prolonged the sleeping time and decelerated the hepatic oxidation of hexobarbital, suggesting that the effects of MC are specific to the route of administration. Olive oil, either as solvent or injected prior to MC administration afforded partial protection against ip MC. Dimethyl sulfoxide, on the other hand, markedly enhanced the toxicity of ip MC when used as a solvent for MC or injected prior to ip administration. The observation of an interaction of MC with usual solvents and the specificity of the route of administration is of considerable interest in view of the fact that MC is an industrial and household solvent and is a chemical analog of several chlorinated hydrocarbon insecticides and anesthetic drugs.

(Supported at University of Kansas by ONR Contract N00014-66-C006.)

100. *Effects of Chronic Nicotine, Acute Hypoxia, and Their Interactions on Myocardial Enzymes.* D. G. WENZEL and M. H. RICHARDS, Department of Pharmacology and Toxicology, University of Kansas, Lawrence, Kansas.

As cigarette smoking has been demonstrated to produce hypoxemia, and because the metabolic and circulatory effects of nicotine on the heart may be aggravated by hypoxemia,

certain effects of chronically administered nicotine and of acute hypoxia on the rat heart were studied separately and together. Male Sprague-Dawley derived rats were treated for approximately 28 weeks with either 1.14 or 4.56 mg/kg/day of nicotine in the drinking water. Both untreated controls and the nicotine-treated animals were then exposed to 6% O₂ for 12 hours. Immediately after exposure to the hypoxic environment and at intervals thereafter, separate groups were studied for changes in cardiac lesion grades and for activities of glucose-6-phosphate dehydrogenase, LDH isoenzymes, isocitric dehydrogenase, acid phosphatase, and β -glucuronidase activities of the hearts. Hematocrits and mortalities were also determined. Hypoxia alone, when compared to control values by the *t* test, affected most of these measurements. Treatment with nicotine alone increased cardiac isocitric dehydrogenase activity and decreased the soluble β -glucuronidase activity. Nicotine appeared to interact with hypoxia in some effects of the latter on enzyme activities.

(This research was supported by a grant from the American Medical Association Education and Research Foundation.)

101. *Toxicologic Studies with a Halogenated Salicylamide*. S. M. KURTZ, J. L. SCHARDEIN, J. E. FITZGERALD, and D. H. KAUMP, Department of Pathology and Toxicology, Parke, Davis & Company Research Laboratories Ann Arbor, Michigan.

When rats were fed a diet containing 0.4% (about 350 mg/kg, or 10–14 times the single dose proposed for veterinary use) of the halogenated benzanilide, 2-acetoxy-4'-chloro-3,5-diodobenzanilide,¹ extreme vacuolation was noted by light microscopy in the white matter of the central nervous system and peripheral nerves after 3 weeks. Remarkably, this change was not accompanied by clinical signs of neurologic malfunction or microscopic damage to other structures in the nervous system. Examination, by electron microscopy, of specially prepared specimens disclosed that the vacuoles were formed by a separation of myelin layers near the surface of the myelin with subsequent ballooning of the outer involved layers. The vacuoles contained no electron dense material, suggesting that the contents were composed chiefly of water and possibly electrocytes. There was no evidence of damage to other structures in the region of the vacuoles. When the treated animals were given normal ration, the vacuolation subsided steadily over a 6–8-week period.

102. *Toxicity Testing of Chemical Mutagens on Mouse Spermatozoa in Vitro*. S. R. JOSHI, K. FUJII, and S. S. EPSTEIN, Laboratories of Environmental Pathology and Carcinogenesis Children's Cancer Research Foundation, Inc., and Department of Pathology, Harvard Medical School, Boston, Massachusetts.

The mutagenic effects of drugs, pesticides, food additives, carcinogens, and air and water pollutants have been recently studied by their administration to male mice which were then mated with untreated females; chromosomal damage induced in spermatozoa by chemical mutagens resulted in early death of developing embryos [Epstein and Shafner, *Nature* **219**, 385 (1968)]. In an attempt to further elucidate basic mechanisms involved in this dominant lethal test, particularly the role of transport, cell-uptake and metabolic factors, it was considered of interest to analyze the toxicity of selected mutagens on mouse spermatozoa *in vitro*.

Adult ICR male mice were killed, and spermatozoa were collected by flushing each vas deferens with 0.5 ml of Eagle's basal medium at pH 8.0 and 37°; spermatid flushings from 4 mice were pooled. Aqueous solutions of drugs in 0.05 ml volume were added to 0.45 ml aliquots of cell suspensions, yielding final concentrations of 1, 5, 10, 50, and 100 mg/ml. Suspensions were incubated in the dark at 37° for 2 hours. Immobilization of sperm, used as a criterion of drug toxicity, was measured at 0, 1, and 2 hours. Using this system, the toxicity of a series of aziridine alkylating agents was determined. Toxicity was shown to be both dose and time dependent. LD50 values for TEPA and METEPA were 17.5 and 25 mg/ml

¹ Tremerad®, Parke, Davis & Company.

after 1-hour, and 9 and 22.5 mg/ml after 2-hour incubation, respectively; corresponding slope values were 0.5 and 0.4 at 1-hour and 0.5 and 0.6 at 2-hours, respectively.

(These studies were supported by Grants C-6516 and FR-05526 from the N.I.H. and Contract PH-86-66-169 from the National Air Pollution Control Administration.)

103. *A Practical Test for Chemical Mutagens in Mammals.* S. S. EPSTEIN, Laboratories of Environmental Pathology and Carcinogenesis Children's Cancer Research Foundation, Inc., and Department of Pathology, Harvard Medical School, Boston, Massachusetts.

Chemical mutagens in the environment pose hazards which have not been systematically explored. Dominant lethal mutants are convenient markers of major genetic damage which have been previously reported for measuring the effects in mammals of certain mutagens, notably X-rays. Such data can, probably, be meaningfully extrapolated to man in whom the majority of gene mutations are due to dominant autosomal traits. Modification of previously described procedures, together with data processing on a high speed computer, has permitted development of a sensitive and practical bioassay for chemical agents inducing dominant lethal mutations in the mouse. Using this system, selected environmental contaminants, drugs, pesticides, food additives, carcinogens, air and water pollutants, have been assayed for mutagenic activity [Epstein and Shafner, *Nature* **219**, 385 (1968)].

Test materials were administered, generally singly and ip at LD5 levels to mature male mice, which were then sequentially mated with untreated females over 8 weeks, covering the duration of the spermatogenic cycle. Females were dissected on day 13 of pregnancy and scored for early fetal deaths, as a measure of mutagenicity, and for living implants. The alkylating agents MMS, TEPA, METEPA, THIOTEPA, and TEM and the carcinogens, benzo[*a*]pyrene and aflatoxin, were all mutagenic. These effects were largely restricted to post meiotic cells, spermatozoa and spermatids, consistent with action on nonreplicating DNA; aflatoxin, however, acted on late spermatocytes. Dose-response studies for TEPA and METEPA suggested threshold levels below 1 mg/kg. Caffeine produced no mutagenic effects by itself nor did it enhance the mutagenicity of low doses of MMS; possible interactions with X-rays are being currently studied.

(These studies were supported by Grants C-6516 and FR-05526 from the National Institute of Health and Contract PH-86-66-169 from the National Air Pollution Control Administration.)

104. *The Effect of Methyl Salicylate on Rat Reproduction.* T. F. X. COLLINS, W. H. HANSEN, and H. V. KEELER, Division of Pharmacology and Toxicology, Food and Drug Administration, Washington, D.C.

The flavoring agent methyl salicylate (methyl ester of *o*-hydroxybenzoic acid) was fed at 0, 500, 1500, 3000, and 5000 ppm to Osborne-Mendel rats for three generations. Significant decreases were observed in the fertility, viability, survival, and lactation indexes, but the decreases did not show a constant dose-related response. Nonsignificant but apparently dose-related decreases were observed in average number of progeny born per litter in the second and third generations. In a supplementary study of the third-generation rats, calcium carbonate at 1500 ppm was added to the diet with methyl salicylate at all dose levels. No significant decrease was observed in any fertility, survival, or lactation index. A significant decrease was found in one viability index of the first litter. Nonsignificant but apparently dose-related decreases were observed in average number of progeny per litter. External examination of the newborn and weanling rats from all the litters disclosed no abnormalities. In autopsies of the third-generation weanlings, findings were negative.

105. *Chromosomal Aberrations of Lymphocytes Caused by Diepoxy Butane.* J. NEMENZO and C. H. HINE, Department of Pharmacology and Experimental Therapeutics (Toxicology Activity), School of Medicine, University of California, San Francisco Medical Center, San Francisco, California.

Low molecular weight diepoxy compounds are known to be active cross-linking agents, and some are carcinogenic. Diepoxy butane, the simplest member of the class, was tested for

its potential chromosomal damaging effects. The test species was the young male Long-Evans rat; the test system was lymphocytic chromosomes cultured from peripheral blood according to the method of Nemenzo and Hine. Mitosis was affected in lymphocytes harvested from animals injected subcutaneously with doses as low as 1.0 mg/kg. There was an original fall in the mitotic index at 48 hours post injection, and a rebound at 30 and 60 days. Stickiness and clumping of the chromosomes was noted at both 30 and 60 days at the 1 mg/kg dose. At doses of 3 mg/kg, greater fragmentation occurred in chromosomes harvested as late as 60 days post injection, without accompanying increase in the mitotic index. This test is suggested as a useful screen in the evaluation of the potential chromosomal damaging effects of this class of compounds.

(Supported in part by a grant from Shell Development Company Emeryville, California.)

106. *Factors Affecting Cyclohexylamine Lethality in Mice.* INSU P. LEE and ROBERT L. DIXON, Department of Pharmacology, University of Washington School of Medicine, Seattle, Washington.

Cyclohexylamine (CHA) is a metabolite of the artificial sweetening agent cyclamate. Previous studies have demonstrated that CHA is an indirect sympathomimetic amine with actions much like those of tyramine and/or amphetamine [*J. Pharm. Sci.* **57**, 1132 (1968)]. Results reported here are an attempt to further investigate the mechanism of CHA toxicity and lethality. Acute toxicity studies using male albino Swiss Webster mice (20–25 g) indicated that CHA lethality is both temperature and aggregation dependent. Toxic doses of CHA produced behavior characterized by general hyperactivity resembling the signs seen after amphetamine treatment. The LD₅₀ of CHA was 770 (658–900) mg/kg for mice singly and 520 (413–655) mg/kg for mice in groups of 10 maintained at 20°. These data indicated a potency ratio of 1.48 due to an aggregation effect. An increase in environmental temperature to 28° increased the lethality of CHA for both single and aggregated mice. This increase in lethality can be expressed as a potency ration of 1.66 for single mice and 1.73 for mice in groups of ten. At 28° the difference in lethality between single and aggregated animals produced a potency ratio of 1.55. However, the lethality after CHA could not be correlated with an increase in body temperature. Drug interaction studies demonstrated that chlorpromazine, reserpine, phenoxybenzamine, tolazoline, and α -methylmetatyrosine were each capable of protecting mice against CHA-induced lethality. In contrast, pretreatment with SKF 525-A potentiated CHA lethality. This potentiation is suggestive that CHA is further metabolized by mice. Metabolic studies with carbon-labeled acetate indicated that CHA-treated mice expired more ¹⁴CO₂ than control animals, suggesting an enhanced metabolic activity. These data fail to demonstrate the actual mechanism of death after CHA, but it appears to be related to the sympathomimetic action of CHA.

107. *Cyclamate Degradation in Mammals and in Vitro.* L. GOLBERG, C. PAREKH, A. PATTI, and K. SOIKE, Institute of Experimental Pathology and Toxicology, Albany Medical College, Albany, New York.

Mongrel dogs given cyclamate orally (100 mg/kg daily) were found to excrete cyclohexylamine (CHA) with some regularity after the first day, albeit in small amounts (up to 0.1% of the dose). More persistent retention of label from a single dose of cyclamate-¹⁴C was observed after prolonged consumption of cyclamate than when cyclamate was not given beforehand. Human excretion of CHA after daily ingestion of soda containing 512 mg of cyclamate ranged from a trace to 28% of the dose on days 3–5 after the start. Other metabolites present in human urine comprise cyclohexanone, cyclohexanol, and *N*-hydroxycyclohexylamine. A small conversion (0.13–0.25%) of cyclamate-¹⁴C to CHA-¹⁴C was achieved by anaerobic incubation of contents of canine large intestine and, to a lesser extent, of small intestine. Only strains of spore-forming bacteria, identified as *Clostridium perfringens*, isolated from canine gut contents cleaved the N—S bond of cyclamate even to a slight degree. This capacity was lost on repeated subculture. Of 7 strains of spore-forming anaerobes isolated from feces of a human excretor of CHA, only one strain formed trace amounts of CHA from cyclamate.

108. *Effects of Cyclamates and Related Chemicals upon Incorporation of Inorganic Sulfate-³⁵S into Protein Polysaccharides.* B. WORTMAN, R. K. LOCKE, and J. J. SCHROGIE, Bureau of Science and Medicine, Food and Drug Administration, Washington, D.C. (Sponsor: P. L. Harris.)

The incorporation of inorganic sulfate-³⁵S into corneal protein polysaccharides was followed. More than 98% of the radiolabel is incorporated into the macromolecules in this structure as the ester sulfate of mucopolysaccharides; the remainder is in sulfur-containing amino acids. This biologic test system assesses the possible role of sodium-potassium activated ATPase and is useful to study the effects of nutrients, drugs, and chemicals at the membrane and enzyme level.¹ To assess the influence of sodium-potassium ratios, fresh guinea pig corneas were incubated in a salt solution (Earle's medium) which contained either 116.2 mM NaCl (normal-Earle's-medium) or 41.9 mM KCl (low-Earle's-medium) and inorganic sulfate-³⁵S added as carrier-free sulfuric acid-³⁵S. Sodium and calcium cyclohexylsulfamate, cyclohexylamine, *N*-(3-aminopropyl)cyclohexylamine, sulfanilic acid, and sulfanilamide were added to media in concentrations up to about 1.2 mM. In low-sodium medium, cyclohexylsulfamic acid and sulfanilamide did not affect sulfate-³⁵S incorporation; however, sulfanilic acid was stimulatory, with maximal effects at low concentrations. *N*-(3-Aminopropyl)cyclohexylamine had a more inhibitory effect at low concentrations than did cyclohexylamine. In normal-sodium medium, effects were similar, except that sulfanilic acid stimulated only at higher concentrations. Order of inhibition is tertiary amines (e.g., chlorpromazine)¹ > secondary amines (e.g., *N*-(3-aminopropyl)cyclohexylamine) > primary amines (e.g., cyclohexylamine) > ammonium ion. A sulfonic acid group had a stimulatory effect in both low- and normal-sodium medium.

109. *Distribution of Inspired Air in Cynomolgus Monkeys.* C. M. BANERJEE, Y. ALARIE, L. BARLETT, and A. KRUMM, Hazleton Laboratories Inc., A Subsidiary of TRW Inc., Falls Church, Virginia. (Sponsor: R. J. Weir.)

The distribution of inspired air by an open circuit N₂ washout technique was studied in 88 male and 80 female unanesthetized cynomolgus monkeys weighing 1.6–3.5 kg. The animals were placed upright in specially designed restraining chairs and allowed to breathe room air through a face mask. After a 15-min rest period and the appearance of a steady breathing pattern, the inspired air was switched to 99.6% O₂ at the end of an expiration. The tidal volume and breath by breath N₂ concentration were recorded until the end tidal N₂ concentration fell to 1% or less. The number of breaths, cumulative tidal volume and time between 80 and 1% N₂, were recorded and analyzed. Also the two compartmental sequential distribution was studied by graphic analysis and is presented. Functional residual capacity (FRC) was also measured simultaneously by the N₂ dilution principle. The results are tabulated below:

Parameters	Male $\bar{X} \pm S.D.$	Female $\bar{X} \pm S.D.$
Respiratory rate (bpm)	38.3 ± 9.0	35.0 ± 9.8
Tidal volume (ml)	37.8 ± 9.8	41.4 ± 8.8
Number of breaths to 1% N ₂	42.6 ± 3.2	43.1 ± 3.3
Time to 1% N ₂ (secs)	79.3 ± 7.7	69.6 ± 7.1
Cumulative tidal volume to 1% N ₂ (l)	1.3 ± 0.2	1.4 ± 0.3
FRC (ml)	101.1 ± 6.1	90.7 ± 5.5

¹ Wortman, B. and Locke, R. K., Sodium-potassium dependent inorganic ³⁵S-sulfate incorporation into cornea, *Invest. Ophthalmol.* in press (1968).

110. *Reproduction Studies in Rats and Rabbits with Cyclohexylamine Sulfate.* G. L. KENNEDY, P. G. SANDERS, M. S. WEINBERG, D. W. ARNOLD, and M. L. KEPLINGER, Industrial Bio-Test Laboratories, Inc., Northbrook, Illinois; Biological Science Laboratories, Foster D. Snell, Inc., Elizabeth, New Jersey; and Abbott Laboratories, North Chicago, Illinois.

Three different studies were conducted to determine the effects of cyclohexylamine sulfate at doses of 1.5 or 15 mg/kg upon fertility, reproduction, embryogenesis, and perinatal and postnatal development. Three phases were included in each study, with rats being used in all three phases and albino rabbits used in the teratologic phase. In phase 1, the compound was administered to both males and females before and during mating, gestation, and lactation. In phase 2, the compound was administered to female rats and rabbits during the critical period of organogenesis. In phase 3, female rats received the compound from day 15 of gestation through weaning of the litter. The results of the interim sacrifice at day 14 (phase 1) revealed no differences between test and control animals with regard to the number of corpora lutea, implantation sites, resorption sites and viable fetuses. The ability of the animals to copulate, conceive, and reproduce was unaffected. In one study the rate of survival of pups from females receiving 15 mg/kg was slightly lower than controls. Complete external, internal, and skeletal examination of the pups revealed no abnormalities. There appeared to be significantly more resorption sites in treated rabbits in one of the studies than in controls. The rate of survival of rat pups from days 4 through 21 was lower than controls in phase 3 of one of the studies, but not in the other two studies. When the data from all three studies were examined, there were no consistently significant effects on reproduction or survival of the pups from the administration of cyclohexylamine sulfate at doses of 1.5 or 15 mg/kg/day.

111. *Toxicity and Metabolism of 2-Hydroxy-4-methoxybenzophenone.* Y. M. PATEL, G. J. LEVINSKAS, and C. B. SHAFFER, Environmental Health Laboratory, Central Medical Department, American Cyanamid Company, Princeton, New Jersey.

Substituted benzophenones are effective photostabilizers for synthetic resins. A previous study with 2-hydroxy-4-*n*-octoxybenzophenone (Cyasorb® UV 531) had shown that only a small portion of the orally administered compound was absorbed, and this was excreted conjugated with glucuronide. Similar studies were undertaken with 2-hydroxy-4-methoxybenzophenone (Cyasorb® UV 9), which has a shorter alkoxy side chain. The acute oral LD50 of UV 9 for male albino rats is greater than 5 g/kg, and the LD50 by continuous 24-hour contact with the clipped skin of male albino rabbits is greater than 16 g/kg. The substance is essentially nonirritating to rabbit skin and rabbit eyes. Male albino rats tolerated a dietary level of 1.0% for 27 days with no adverse effects. Dietary levels of 1.25%, 2.5%, and 5.0% fed to albino rats of both sexes for 98 days, resulted in lowered body weight gains, increased liver weights, gross hematuria or, if absent, a positive test for occult blood, and kidney damage. The kidney pathology could be attributed to blockage of the renal structures by glucuronide precipitates which were observed at autopsy in the tubules and pelves of the kidneys from rats fed 2.5% and 5.0% of UV 9. After 1.0% of UV 9 was fed for 30 days, about 60% of the UV 9 was excreted in the urine as a glucuronide, and the remainder could be found in the feces. Thus, the methoxy derivative (UV 9) is absorbed to a greater extent than the octoxy compound (UV 531). The length of the alkoxy side chain appears to influence the degree of absorption of substituted benzophenones.

112. *Toxic Manifestations of Cinchophen in the Ferret Stomach.* N. UMEDA and C. J. PFEIFFER, Institute of Gastroenterology, Presbyterian-University of Pennsylvania Medical Center, Philadelphia, Pennsylvania.

Previous reports on the toxic effects of cinchophen on the stomach have indicated numerous species differences; accordingly, the susceptibility of the ferret gastric mucosa was evaluated since the ferret gastric physiology is similar to that of man. Cinchophen was administered (po, in corn oil) in single, daily doses of 220 mg/kg for periods of 3-35 days. Cinchophen

induced deep, linear, multiple, hemorrhagic erosions of the antral mucosa, polymorphonuclear infiltration, and marked edema at 3-4 days. These manifestations subsided at 7 days and began to reappear at 14-35 days. Similar, but less severe reactions appeared in the fundus and followed a similar time course. Secretory studies of chronic fistula ferrets injected with a single 220-mg/kg dose of cinchophen indicated that the pepsin activity and the concentration of free acid were not altered by cinchophen during a 3-hour study period. Preliminary evidence in antral pouch ferrets demonstrated slight, acute inflammatory, antral changes following cinchophen administration into the pouch, and no antral changes following per os administration.

The interaction of sc steroids (hydrocortisone:corticosterone, 1.5:1.0) on cinchophen ulceration was evaluated at 4 days (15 mg steroids/kg plus 220 mg cinchophen/kg) and 3 weeks (7.5 mg steroids/kg plus 220 mg cinchophen/kg). Steroids alone induced small, multiple fundic and antral erosions after 3 weeks, but essentially no changes after 4 days. However, animals treated with both cinchophen and steroids revealed antral ulcers which penetrated the muscularis mucosa at 4 days. Steroids did not alter the course of cinchophen ulceration in animals treated for 3 weeks.

(This study has been supported by the William H. Rorer Gastroenterology Research Fund.)

113. *The Toxicology of Terbacil, A New Substituted Uracil*. HENRY SHERMAN, Haskell Laboratory for Toxicology and Industrial Medicine, E. I. du Pont de Nemours and Company, Newark, Delaware.

Terbacil [3-*tert*-butyl-5-chloro-6-methyluracil] is an herbicide that has found use for the selective control of many annual and some perennial weeds in crops such as sugarcane, apples, pears, peaches, citrus fruits, and mint hay. Terbacil has low acute oral toxicity; its approximate lethal dose is > 7500 mg/kg for rats and > 5000 mg/kg for dogs. The oral LD50 for fasted male and female rats is > 5000 mg/kg but < 7500 mg/kg. When administered to rats at doses of 1000 mg/kg/day for 10 days, it produced pathologic changes in the liver (heavier liver, liver cell enlargement in centrilobular area, increase in mitotic activity, and margination of larger cytoplasmic contents to cell membrane). A three-generation, 6-litter reproduction study with rats fed dietary levels of 50 or 250 ppm terbacil was without adverse effects upon reproduction and lactation performance; no pathologic changes were observed in weanling pups of the F_{3B} generation. Male and female rats have been fed nutritionally complete diets containing 50, 250, and 2500-10,000 ppm terbacil for 2 years. Except for a lower rate of weight gain among the animals that received 2500-10,000 ppm terbacil, there was no nutritional, clinical, hematologic, urinary, or biochemical evidence of toxicity in the test groups; however, there was evidence of liver enlargement and occasional vacuolation of the centrilobular hepatocytes in the animals that received the highest dietary level. Male and female beagle dogs, 1-2 years of age, fed nutritionally complete diets containing 50, 250, and 2500-10,000 ppm terbacil for 2 years showed no evidence of toxicity, except for a slight increase in relative liver weight at the highest dietary level.

114. *The Toxicity in Guinea Pigs of a Potent Antipsychotic Compound (Clothiapine) from the Dibenzothiazepine Group*. F. H. SCHULTZ, JR., D. L. KAY, H. CERVENKA, F. E. KOHN, and J. H. KAY, Dorsey Laboratories, Lincoln, Nebraska, and Lifestream Laboratories, Libertyville, Illinois.

Rats were unable to tolerate doses of clothiapine (2-chlor-11-(4'-methyl)piperazinodibenzo-[b,f][1,4]thiazepine) which were meaningful multiples of the proposed daily human therapeutic dose (3 mg/kg). Pilot studies indicated that the guinea pig could tolerate amounts which would establish acceptable margins of safety. The oral LD50 in the guinea pig was 150 mg/kg. Seven- and 30-day feeding tests, employing dietary levels corresponding to 15, 25, and 50 mg/kg/day, resulted in poor food consumption, marked weight loss, and a number of deaths due to malnutrition during the first week. However, after the start of the second week there was a sudden adaptation to the test diet. In the surviving animals, there was a marked reduction in overall weight gain which could be related to the very poor food consumption during the

first week of testing. After this time, the food consumption increased and the guinea pigs at all drug levels gained weight at rates which were essentially the same as the controls. An 18-month feeding study was conducted in guinea pigs at dietary levels corresponding to doses of 0, 6, 12, and 18 mg/kg/day. The mortality rates were as follows: controls 40%, low dose 46%, intermediate dose 33%, high dose 32%. Mortality was largely associated with respiratory infections. Clothiapine produced no significant effects with the exception of a reduction in the female thyroid gland weights in the intermediate and high dosage groups. No histopathologic changes were observed.

115. *Fluorine Toxicosis in Livestock*. JAMES L. SHUPE and ARLAND OLSON, Utah State University Logan, Utah.

Fluorine is universally present in varying amounts in soils, water, vegetation, the atmosphere, and animal tissues. Because of its chemical reactivity, it is found in nature in a combined (fluoride) form. (The terms fluorine and fluoride are used interchangeably in this report.)

Fluorine has beneficial effects when ingested in small amounts. Toxic and adverse effects occur when fluorides are ingested in excessive amounts.

Livestock normally ingest variable low-level amounts of fluoride throughout their lives. Fluorine accumulates in the body as long as the animal continues to ingest a constant or increasing amount of fluoride. The major problem of fluorine poisoning in livestock has involved chronic toxicosis (fluorosis), which may be caused by a variety of sources. With the expansion of certain types of industries into agricultural areas, fluorosis has become an important toxicologic problem in some areas of the United States and many other countries. Various factors influence biologic responses of livestock to ingested fluorides. Characteristic symptoms and lesions are associated with fluorosis of livestock, thus enabling it to be correctly diagnosed and evaluated. It is important to realize that not all cases involve adverse effects and economic losses, some cases are merely cosmetic and noneconomic in nature. Studies and research prove that fluorotic problems can be prevented and controlled and that agriculture and industry can cooperate to realize mutual benefits. A comprehensive guide for use in diagnosing and evaluating fluorosis in livestock has been compiled.

116. *Tolerance to Etorphine (M99)*. M. W. WILLIAMS, C. S. WILLIAMS, L. A. MEEKS, and B. E. GUNNING, Veterans Administration Hospital, Tucson, Arizona.

The analgesic effects of M99, 7 α (1-(R)hydroxy-1-methylbutyl)-6, 14 endoetheno tetrahydro-*oripavine* hydrochloride, have been tested by the tail flick method in the mouse. A significant acute tolerance to the drug has been demonstrated after a single dose of 2 μ g/kg, which has been found to be of equal potency with 4 mg/kg of morphine (potency differential 2000 times). The developed acute tolerance was maintained for over 2 weeks without further dosage in one experiment and for 6 weeks in another. Significant acute tolerance has been seen to occur within 24 hours with M99 and within 48 hours with morphine, the same daily doses being repeated at 24-hour intervals. After a 2-day rest period followed by double the last preceding dose, tolerance to the new level was again established within 24 hours, but at a slightly higher level. Placebo injections of water in both experimental and control groups after 5- and 12-day rest periods showed the 2 groups to be essentially alike in their sensitivity to pain. When M99 was again given at the same dose level to this group, analgesic effectiveness with some loss of tolerance during the no dosage interim was seen. The time course of analgesic effectiveness of the drug under several experimental conditions is discussed.

117. *Environmental Distribution of Biologically Active Methylenedioxyphenyl Compounds*. R. CSILLAG, K. FUJII, H. JAFFE, and S. S. EPSTEIN, Laboratories of Environmental Pathology and Carcinogenesis Children's Cancer Research Foundation, Inc., and Department of Pathology, Harvard Medical School, Boston, Massachusetts.

Since the discovery that certain methylenedioxyphenyl (MDO) compounds synergize the toxicity of pyrethrins, a number of potent derivatives, notably piperonyl butoxide (PB), have

been synthesized and widely used in commercial insecticide formulations. The efficacy of these synergists appears to depend on blockage of insect detoxifying microsomal systems similar to those well characterized in mammalian liver. Additionally, MDO derivatives and related compounds are widely distributed natural products. Although there are no available data on PB dietary residues, tolerances have been established at 8–20 ppm levels for fruits, vegetables, processed foods, and grain crops. Recently, MDO compounds have been shown to decrease microsomal hydroxylation of benzo[*a*]pyrene in the rat (Falk and Kotin, *Ann. N.Y. Acad. Sci.* in press), and to pose potential hazards to man by producing synergistic toxicity and carcinogenicity with certain environmental pollutants and drugs [Epstein *et al.*, *Nature* **214**, 526 (1967); *Toxicol. Appl. Pharmacol.* **11**, 442 (1967)].

This study was undertaken to survey the environmental distribution of MDO compounds and to study their activity, using prolongation of hexobarbital sleeping (HST) and zoxazolamine paralysis times (ZPT) in the mouse, and inhibition of rat liver microsomal hydroxylation of hexobarbital, aminopyrine and biphenyl, as pharmacologic and biochemical measures, respectively, of hepatic microsomal enzyme function. Activity was found in naturally occurring synergists, such as apiol and methyleugenol, besides synthetic synergists, such as PB, Sesamex[®], and piperonyl cyclonene. Natural product flavoring agents, such as safrole and eugenol derivatives, exhibited moderate or strong activity. However, therapeutic drugs containing an MDO moiety, such as narcotine, podophyllotoxin and some synthetic piperazine antitussives (Douarec), showed weak or negligible activity.

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

118. *Distribution of Oral Zinc Bacitracin-¹⁴C in Rats and Swine.* J. DONOSO, G. O. CRAIG, and R. S. BALDWIN, Woodard Research Corporation, Herndon, Virginia, and Research Department, Commercial Solvents Corporation, Terra Haute, Indiana. (Sponsor: M. Woodard.)

The biosynthesis and preparation of ¹⁴C-labeled zinc Bacitracin are described. Zinc Bacitracin-¹⁴C was administered to rats and pigs for 4 consecutive days at the rates of approximately 75 mg/kg of body weight and 200 g per ton of feed, respectively. The animals were housed in closed metabolism cages and radiometric measurements were performed on samples of feces, urine, and exhaled carbon dioxide collected at 24, 48, 72, and 96 hours after the first dose. The rats and pigs were sacrificed 24 hours after final dosage, and the concentration of zinc Bacitracin-¹⁴C in several of the animal tissues was determined by liquid scintillation counting. In rats, at 96 hours approximately 92% of the administered dose was found in the feces whereas in swine, approximately 93% of the administered dose was found in the feces (53.3%), large intestine (38.3%), and urine (1.6%). No radioactivity was found in expired carbon dioxide or selected tissues of either animal species. The recoveries of the administered doses for rats and pigs were 98% and 95%, respectively.

119. *Tissue Reactions to 2-Chloroethanol.* WALLACE L. GUESS, Drug Plastic Research and Toxicology Laboratory, College of Pharmacy, University of Texas, Austin, Texas.

2-Chloroethanol is a potential reaction product between ethylene oxide and a free chloride ion. Certain plastic devices, such as polyvinyl chloride, contain such free chloride ions and are often sterilized by ethylene oxide. Depending on the chloride ion content and residual ethylene oxide, varying amounts of 2-chloroethanol (a chlorohydrin) may be formed as the reaction product. Since this agent is less volatile than the ethylene oxide, standard degassing procedures may leave residual 2-chloroethanol in the plastic device. If these devices come into prolonged contact with tissues, such as might occur with tracheotomy tubes, the possibility exists for tissue damage from leaching 2-chloroethanol. This paper reports on the effect of pure 2-chloroethanol, and various dilutions of this material, on several of the tissue types that a tracheotomy tube might come in contact with in a patient. It was found that pure 2-chloroethanol is quite destructive to most of the tissues, including ophthalmic, mucosal, muscular, and subcutaneous tissues. However, on dilution, the toxicity potential decreased

sharply. Dilutions of 1 : 10 were still toxic, but in most cases, dilutions of 1 : 100 were practically innocuous. A cell culture evaluation correlated almost perfectly with the *in vivo* techniques.

120. *Percutaneous Absorption of Sodium Pyridinethione-³⁵S in Animals.* C. PAREKH and L. GOLBERG, Institute of Experimental Pathology and Toxicology, Albany Medical College, Albany, New York.

Sodium 1-hydroxypyridine-2-thione-³⁵S (SPT-³⁵S), in aqueous of 1% soap solution, is partially adsorbed on glass or metal surfaces, necessitating the use of polyethylene ware. When 1% soap solution containing SPT-³⁵S was applied topically for 15 min to shaved abdominal skin of rats, followed by thorough rinsing with water, 0.7–1.4% of the applied dose was retained by the skin and 0.09–0.31% was excreted in urine; the remainder of the applied radioactivity was recovered, essentially completely, in the rinses. The interpretation of effects of repeated daily application for 4 consecutive days was made difficult by growth of hair, to which SPT is substantive. When 4 applications were made at 1-hour intervals, skin retention was no greater than after a single application, but ³⁵S activity in urine rose to 4.8–18.8%. Skin retention and urinary excretion of the label were enhanced by pretreatment of the skin, for instance with 2% sodium lauryl sulfate. In monkeys, a single 1-hour topical application of SPT-³⁵S in 1% soap solution involved 0.009–0.358 mg SPT/cm², with areas of application ranging from 12.6 to 152 cm². Skin retention varied from 1.5 to 13.5% of the dose applied, and excretion from 0.0 to 1.6% in urine over 24 hours. Abraded skin of monkeys retained 9.0–33.9%, while 24-hour urinary excretion rose to 6.6–14.2% of the applied dose. Repeated 1-hour application to intact skin, daily for 4 consecutive days, produced 0.2–0.4% retention of label by skin 7 days after the last application, and a total of 2.1–3.8% of the label was excreted in urine over 11 days. Negligible ³⁵S activity was present in any major tissue of both rats and monkeys except in muscle subjacent to the skin application. Recoveries were in the range 93–100% of activity applied, SPT being excreted predominantly unchanged in urine as it is in bile. No manifestation of toxicity was seen in the course of these experiments.

121. *Convulsions Induced by 2-Di-n-butylaminoethanol.* R. HARTUNG, L. B. PITTLE, and H. H. CORNISH, The University of Michigan, Department of Industrial Health, School of Public Health, Ann Arbor, Michigan.

As part of a continuing study into the toxicity of the 2-*N*-alkyl-substituted aminoethanols, 2-di-*n*-butylaminoethanol (DBAE) was investigated. DBAE had been shown to be particularly toxic (ip LD₅₀ 0.14 g/kg) when compared with other aminoethanols. During acute and subacute studies it had been noted that the primary symptoms produced by DBAE, regardless of the route of administration, were tremors followed by clonic-tonic convulsions.

Death is due to respiratory arrest. DBAE has been shown to inhibit brain cholinesterase *in vivo* and *in vitro*. However, atropine is not effective in counteracting DBAE toxicity. Nevertheless, physostigmine, cholinesterase inhibitor, potentiates DBAE toxicity. *In situ* phrenic nerve-diaphragm preparations indicate that the rate of discharges of the phrenic nerve increases after DBAE, and that the respiratory arrest appears to be due to a subsequent neuromuscular blockade, possibly due to accumulation of acetylcholine at the neuromuscular junction.

The convulsions arise centrally. They disappear distally after spinal section. Barbiturates are not very effective in controlling these convulsions. However, mephensin, dilantin, and magnesium sulfate can be effective. Within limits, agents from the latter group can protect against convulsions and mortality produced by DBAE.

122. *Effects of Hydroxyzine on the Digitalis-Intoxicated Heart.* G. A. BOBB, A. L. WIT, and A. N. DAMATO, Cardiopulmonary Laboratory, U.S. Public Health Service Hospital, Staten Island, New York. (Sponsor: H. E. Stokinger.)

The effects of hydroxyzine on the digitalis-intoxicated heart was investigated both *in vivo* and *in vitro*. In the intact and bilaterally vagotomized dog, a pair of wire electrodes were

inserted into the His bundle to record a His bundle electrogram. Heart rate was controlled by atrial pacing. Atrioventricular conduction time (AVC) was measured from the pacing spike to His bundle electrogram and intraventricular conduction time (IVC) from the His bundle to S wave of lead II electrocardiogram. A single iv injection of 7.5 $\mu\text{g}/\text{kg}$ of ouabain was given, followed by a continuous infusion at a rate of 2.5 $\mu\text{g}/\text{kg}/\text{min}$ until a stable ventricular tachycardia was established. Hydroxyzine, 10–20 mg/kg iv, consistently converted this arrhythmia within 2 min to normal sinus rhythm which lasted 1–2 hours. This effect appeared at lower doses (5–10 mg/kg) when the vagi were cut. In the intact dog hydroxyzine markedly prolonged AVC (+61%) while IVC was slightly prolonged (+9%). The prolongation of AVC (+11%) and IVC (+3%) in vagotomized dogs indicates that these effects of hydroxyzine were mediated in part via the vagus. Blood pressure was transiently lowered (–18%) and returned to control values in 5–15 min. *In vitro*, glass microelectrodes were inserted into isolated perfused canine Purkinje fibers, and the cardiac action potential was recorded. Hydroxyzine (10^{-5} M) consistently decreased phase 4 depolarization induced by ouabain or anoxia while prolonging the effective refractory period, demonstrating that the antiarrhythmic effect of hydroxyzine is probably mediated through direct action on the heart, not through its known neural effects.

123. *Carcinogenic Activity of 4-Amino-4'-nitrobiphenyl, a Metabolite of 4,4'-Dinitrobiphenyl.*

SOUHEIL LAHAM and JOHN W. SINCLAIR, Environmental Toxicology Program, Division of Occupational Health, Environmental Health Center, Department of National Health and Welfare, Ottawa 3, Canada.

4,4'-Dinitrobiphenyl was reported a few years ago [S. Laham *et al.*, *Toxicol. Appl. Pharmacol.* 6, 352 (1964)] as a potent bladder carcinogen in the Sprague-Dawley rat. Metabolic studies on this compound showed also that it is reduced *in vivo* to produce 4-amino-4'-nitrobiphenyl which was isolated from the urine of rats force-fed or injected subcutaneously with 4,4'-dinitrobiphenyl [S. Laham *et al.*, *Symp. Chemi. Carcinogenesis, Unio Intern. Contra. Cancer*, Vienna, (1967)]. In order to find out whether this metabolite was responsible for induction of bladder cancer, it was necessary to test its biological activity. The present report describes the results obtained with 4-amino-4'-nitrobiphenyl over a period of 73 weeks. Corn oil suspensions of 4-amino-4'-nitrobiphenyl (4.38 mg/0.5 ml/rat) were force-fed once a week to 20 male and 20 female rats. A high incidence of bladder carcinoma developed in the males, whereas adenocarcinoma of mammary gland were observed in the female. No carcinoma was observed in the control animals.

Author Index

Abstract numbers are in *italics*; page numbers are in roman type.

- A**
Akoy, Shoshana, *82*, 67
Alarie, Y., *109*, 87
Anderson, R. C., *31*, 25
Armbrecht, B. H., *93*, 75;
94, 75
Arnold, D. W., *110*, 89
- B**
Bachand, E. A., *41*, 33
Bachand, R. T., Jr., *41*, 33
Bachmann, E., *33*, 25
Back, K. C., *13*, 11
Bagdon, R. E., *80*, 65
Bagdon, W. J., *90*, 73
Balazs, T., *54*, 43
Baldwin, R. S., *118*, 95
Banerjee, B. N., *1*, 1
Banerjee, C. M., *109*, 87
Banks, W. L., Jr., *96*, 77
Baretta, E. D., *41*, 33
Barlett, L., *109*, 87
Barth, M. L., *16*, 13
Bates, R., *3*, 1
Battista, S. P., *11*, 9
Becker, B. A., *68*, 55; *69*, 55
Birkhead, H. A., *74*, 59
Blendermann, E. M., *75*, 59
Blockus, L. E., *89*, 71
Bobb, G. A., *122*, 97
Bogden, A., *5*, 3
Bokelman, D. L., *90*, 73
Borgstedt, H. H., *61*, 49
Borzelleco, Joseph F., *40*,
31
Boyd, Eldon M., *84*, 67
Braunberg, R., *20*, 15
Braude, M. C., *20*, 15
Brodie, R., *23*, 17
Brouwer, J., *26*, 21
Buck, W. B., *47*, 37
- C**
Cannon, G., *57*, 45
Carlson, G. P., *50*, 41
Carlton, William W., *60*, 49
Carson, S., *10*, 9; *57*, 45
- Carson, Steven, *51*, 41
Cervenka, H., *73*, 59; *114*,
91
Cholakakis, J. M., *53*, 43
Christofano, E. E., *9*, 7; *78*,
63
Clary, J. J., *38*, 31
Cohen, S. D., *79*, 63
Coldwell, B. B., *98*, 79
Collins, T. F. X., *104*, 83
Coon, J. M., *25*, 19
Cooney, D. A., *6*, 5
Cotton, F. A., *7*, 5
Corbett, B., *63*, 51
Cornish, H. H., *16*, 13;
121, 97
Cottrell, J. C., *14*, 11
Coulston, F., *27*, 21; *53*, 43
Craig, G. O., *118*, 95
Cranmer, M. F., *36*, 29
Crook, J. W., *65*, 53
Crowley, T. A., *89*, 71
Cruz, E. A., *14*, 11
Csillag, R., *117*, 93
Cueto, C., Jr., *21*, 17, 22, 17
- D**
Dajani, Esam Z., *15*, 11
Damato, A. N., *122*, 97
Davis, R. D., *5*, 3; *6*, 5
DeGiorgis, A. M., *74*, 59
Denine, E. P., *11*, 9
Digiacomio, P., *33*, 25
Dixon, Robert L., *106*, 85
Dodson, V. N., *16*, 13
Dominguez, A. M., *13*, 11
Donahoe, J. F., *89*, 71
Donoso, J., *118*, 95
Drobeck, H. P., *58*, 47
DuBois, K. P., *19*, 15; *50*,
41; *85*, 69
Durbin, C. G., *93*, 75
- E**
Earl, F. L., *26*, 21; *28*, 21
Emmerson, J. L., *31*, 25
- Epstein, S., *4*, 3
Epstein, S. S., *30*, 23; *102*,
81; *103*, 83; *117*, 93
Ewinike, Helenah, *24*, 19
- F**
Falk, H., *3*, 1
Falk, H. L., *63*, 51
Fancher, O. E., *9*, 7; *78*, 63
Fawkes, J., *63*, 51
Fishbein, L., *3*, 1; *63*, 51
Fitzgerald, J. E., *101*, 81
Florek, M. C., *74*, 59
Flynn, M., *19*, 15
Frankel, H. H., *2*, 1
Frawley, J. P., *9*, 7; *78*, 63
Friedman, Leonard, *75*, 59
Forney, R. B., *39*, 31
Fouts, James R., *34*, 27
Fuller, G. C., *32*, 25; *52*, 41
Fujii, K., *4*, 3; *30*, 23; *102*,
81; *117*, 93
Fujimoto, J. M., *18*, 13
- G**
Gaines, T. B., *48*, 39
Gehring, P. J., *55*, 43
Geleta, J. N., *93*, 75
Gibson, J. E., *68*, 55
Golberg, L., *33*, 25; *107*, 85;
120, 97
Goldhamer, R. E., *10*, 9
Gordon, B. M., *6*, 5
Gosselin, R. E., *83*, 67
Greenblatt, M., *12*, 9
Greenland, R. D., *35*, 27
Grothaus, Ronald H., *95*,
77
Gunning, B. E., *116*, 93
Guess, Wallace L., *119*, 95
Gumaer, K. I., *58*, 47
- H**
Hansen, W. H., *104*, 83
Harbison, R. D., *69*, 55

Harris, P. D., 39, 31
 Hart, E. R., 3, 1
 Hartman, H. A., 80, 65
 Hartung, R., 121, 97
 Healey, P., 64, 51
 Hine, C. H., 97, 77; 105, 83
 Hassan, A., 21, 17
 Hirth, R. S., 66, 53; 67, 55
 Hoffman, D. G., 31, 25
 Hollingsworth, R. L., 71, 57
 Hopper, C. R., 38, 31
 Hottendorf, G. H., 66, 53; 67, 55
 Howard, D. J., 1, 1

I

Innes, J. R. M., 3, 1

J

Jackson, J. E., 94, 75
 Jaffe, H., 30, 23; 117, 93
 Janssen, F., 44, 35
 Jellema, M. M., 92, 75
 Jordan, S., 63, 51
 Joshi, S. R., 102, 81

K

Karas, G. G., 47, 37
 Kaump, D. H., 92, 75; 101, 81
 Kay, D. L., 73, 59; 114, 91
 Kay, J. H., 73, 59, 114, 91
 Keating, J. W., 89, 71
 Keeler, H. V., 104, 83
 Kennedy, G. L., 110, 89
 Kensler, C. J., 7, 5; 11, 9
 Keplinger, M. L., 9, 7; 78, 63; 110, 89
 Kimbrough, R. D., 48, 39
 Kinoshita, F. K., 19, 15; 85, 69
 Klaassen, C. D., 49, 39
 Klein, M., 3, 1
 Kociba, R. J., 86, 69
 Kohn, F. E., 73, 59; 114, 91
 Koon, W. S., 65, 53
 Krop, S., 20, 15
 Krumm, A., 109, 87
 Kula, Nora, 87, 71
 Kurtz, S. M., 101, 81

L

Laham, Souheil, 123, 99
 Lal, H., 32, 25; 99, 79
 Lamar, J. K., 72, 59
 Lang, D. W., 61, 49
 Lee, Insu P., 106, 85
 Levinskas, G. J., 111, 89
 Linegar, C. R., 57, 45
 Liss, R. H., 7, 5
 Locke, R. K., 108, 87
 Louis-Ferdinand, R. T., 52, 41

M

Magus, Raymond D., 29, 23
 Maland, J., 47, 37
 Marks, M. J., 37, 29
 Marliac, J. P., 72, 59
 Martz, R. C., 39, 31
 Mason, M. M., 5, 3
 Mattis, P. A., 90, 73
 Mauro, Jacqueline, 17, 13
 Mawdesley-Thomas, L. E., 64, 51
 Mazel, Paul, 82, 67
 McClain, R. M., 77, 61
 McLaughlin, J., Jr., 72, 59
 McPhillips, Joseph J., 91, 73
 Mennear, John H., 15, 11
 Meeks, L. A., 116, 93
 Meyers, F. H., 45, 37
 Miller, E., 26, 21
 Murphy, S. D., 8, 7; 79, 63
 Musselman, N. P., 65, 53

N

Neal, R. A., 81, 65
 Nemenzo, J., 105, 83
 Newberne, Paul M., 87, 71
 Nick, Margaret S., 43, 35
 Nicoll, P. A., 39, 31
 Noble, J. F., 54, 43

O

Oberst, F. W., 65, 53
 Ohtake, S., 54, 43
 Olshan, A., 32, 25
 Olson, Arland, 115, 93
 O'Rear, Charles E., 40, 31

Oser, Bernard L., 51, 41
 Owens, J., 22, 17
 Owen, Gareth, 44, 35

P

Pallotta, A. J., 3, 1
 Palm, Paul E., 43, 35
 Parekh, C., 107, 85; 120, 97
 Patel, Y. M., 111, 89
 Patti, A., 107, 85
 Peoples, A., 36, 29
 Petrucci, L., 3, 1
 Pfeiffer, C. J., 112, 89
 Pittle, L. B., 121, 97
 Plaa, G. L., 49, 34; 77, 61
 Pollock, J. J., 44, 35
 Puri, S., 32, 25

R

Rakieten, N., 6, 5
 Rall, D. P., 6, 5
 Ray, James A., 56, 45
 Reinwall, J., 26, 21
 Reinert, H., 59, 47
 Reynaldo, E. F., 72, 59
 Richards, M. H., 100, 79
 Riet, Bart van't, 40, 31
 Robens, Jane F., 70, 57
 Rogers, Adrienne E., 87, 71
 Root, M., 19, 15
 Rubin, L. F., 57, 45
 Ruelius, H. W., 44, 35
 Ruttly, D. A., 59, 47

S

Sanders, Charles L., 62, 49
 Sanders, P. G., 110, 89
 Sandler, B. E., 47, 37
 Santolucito, J., 22, 17
 Schaeppi, U., 5, 3
 Schardein, J. L., 92, 75; 101, 81
 Schein, P. S., 5, 3; 6, 5
 Schrogie, J. J., 108, 87
 Scott, W. J., 71, 57
 Serrone, D. M., 27, 21
 Shaffer, C. B., 111, 89
 Shah, H. C., 32, 25; 99, 79
 Shalkop, W. T., 93, 75; 94, 75
 Shanor, S. P., 37, 29
 Shargel, Leon, 82, 67

- Sherman, Henry, *113*, 91
 Schoenig, G. P., *78*, 63
 Schultz, F. H., Jr., *73*, 59;
114, 91
 Shupe, James L., *115*, 93
 Schut, A. L., *56*, 45
 Sinclair, John W., *123*, 99
 Sleight, S. D., *86*, 69
 Smith, E. J., *28*, 21
 Smith, N. L., *96*, 77
 Smith, R. P., *42*, 33
 Soike, K., *107*, 85
 Somsin, P., *17*, 13
 Sperling, Frederick, *24*, 19
 Stein, A., *27*, 21
 Stein, Arthur, *17*, 13
 Stemmer, K. L., *35*, 27
 Stewart, R. D., *41*, 33
 Stokinger, H. E., *38*, 31
 Stonier, P. F., *90*, 73
 Street, J. C., *88*, 71
 Swain, S. F., *65*, 53
 Switzer, J., *3*, 1
 Szabo, K. T., *74*, 59
 Szot, R. J., *8*, 7
- T
- Tegeris, A. S., *14*, 11
 Tousinis, A. J., *80*, 65
 Trenholm, H. L., *98*, 79
- Triolo, A. J., *25*, 19
 Tuite, John, *60*, 49
- U
- Ulland, B., *3*, 1
 Umeda, N., *112*, 29
- V
- Valerio, M., *3*, 1
 Van Gelder, G. A., *47*, 37
 Van Loon, E. J., *26*, 21;
28, 21
 Van Ryzin, R. J., *80*, 65
 Vogin, E. E., *10*, 9; *57*, 45
- W
- Wagstaff, D. J., *88*, 71
 Ward, F. P., *65*, 53
 Waters, Lysle R., *34*, 27
 Watrous, W. M., *18*, 13
 Wei, E. T., *76*, 61
 Weinberg, M. S., *89*, 71;
110, 89
 Weir, F. W., *45*, 37
 Weiss, L. R., *23*, 17
 Weisburger, J. H., *2*, 1
 Wenzel, D. G., *100*, 79
- Whitcomb, E., *22*, 17
 Wiberg, G. S., *98*, 79
 Wiland, J., *58*, 47
 Wills, J. H., *53*, 43
 Williams, Clara H., *46*, 37
 Williams, C. S., *116*, 93
 Williams, M. W., *116*, 93
 Winek, C. L., *37*, 29
 Wiseman, H. G., *94*, 75
 Wit, A. L., *122*, 97
 Wolcott, Michael, *81*, 65
 Wong, L. C. K., *76*, 61; *97*,
77
 Woodard, G., *71*, 57
 Woodard, M. W., *71*, 57
 Woosley, E. T., *92*, 75
 Worth, H. M., *31*, 25
 Wortman, B., *108*, 87
 Wynn, James E., *40*, 31
- Y
- Yamamoto, R. S., *2*, 1
 Yeary, Roger A., *95*, 77
 Youkilis, E. J., *39*, 31
- Z
- Zimmerman, H. E., Jr., *94*,
75

