Abstracts of Papers for the Tenth Annual Meeting of the Society of Toxicology, Washington, D.C. March 7–11, 1971

1. *Sex-Related Disposition of Cyclacillin (Wy-4508) and Associated Renal Changes in Rats.* W. E. Tucker, H. W. Ruelius, F. W. Janssen, and H. P. K. Agersborg, Jr., Wyeth Laboratories, Inc., Radnor, Pennsylvania.

In chronic and subchronic dietary studies of cyclacillin [6-(1-aminocyclohexanecarboxamido)-penicillanic acid, Wy-4508] in rats, a nephrotoxic effect was observed in male, but not in female, rats. This was the only important toxicologic effect at the dietary levels used. Comparable time-dose treatment of dogs and rhesus monkeys did not result in nephrotoxicity in either sex. The principal morphologic changes in the male rat after chronic (6 mo) treatment were marked renal enlargement, extreme dilatation of convoluted tubules with cast formation and focal degeneration, and luxuriant regenerative hyperplasia of tubular epithelium. Chronic inflammation and fibrosis were also present in the more severely affected kidneys. Since the nephrotoxic effects are completely sex-related the metabolic disposition of cyclacillin was compared for both sexes. The unchanged drug and Wy-4708 (1-aminocyclohexanecarboxylic acid) were identified among the renal excretion products of cyclacillin-treated animals. A marked sex-related difference in the disposition of these two compounds was observed: the ability to excrete Wy-4708 and cyclacillin is much lower in the male rat than in the female rat. Consequently, these two compounds accumulate in the blood of the male. Wy-4708, an unnatural amino acid which is not further metabolized accumulates to a far greater extent than cyclacillin. Prolonged treatment with Wy-4708 produces renal changes less severe but qualitatively similar to those observed with the parent compound. It is considered that the renal changes probably result from the low excretion, and ensuing accumulation, of cyclacillin and Wy-4708. These two compounds are possibly reabsorbed by the renal tubules and, therefore, recirculated. No sex difference in the disposition of cyclacillin was observed in dog, monkey, and man. In view of these observations, the male rat appears to be an inappropriate model for the safety assessment of cyclacillin.

2. *Chromomycin A_3 (NSC-58 514): Pharmacologic Evaluation in Dogs and Monkeys.* U. Schaeppi, G. R. Thompson, R. W. Fleischman, V. Ilievski, and H. Rosenkrantz, Mason Research Institute, Worcester, Massachusetts; D. A. Cooney and R. D. Davis, National Cancer Institute, Bethesda, Maryland.

Chromomycin A_3 (Toyomycin), an antibiotic from *Streptomyces griseus* with a molecular weight of 1183 has potent antitumor activity against Ehrlich carcinoma, Yoshida sarcoma, and leukemias L-1210 and P388. A pharmacologic evaluation was carried out in 14 dogs, 3 monkeys, and 120 mice. The LD50, for 5 daily consecutive iv injections in mice, was 0.6 mg/kg. Seven dogs injected iv with 400 or 200 μg/kg (single dose) or with 100 μg/kg (5 consecutive daily treatments or 1 treatment on days 1 and 5) became acutely moribund or died. Histopathologic evaluation revealed focal necrotic areas in lymphoid tissues (6/7), bone marrow (5/7), liver (1/7), renal tubules (1/7), pancreas (1/7), salivary glands (2/7), epithelium of the urinary bladder (1/7), cervix of the uterus (1/7) and esophagus (1/7) and necrosis of the intestinal plasma cells in the lamina propria (3/7). The intestines exhibited submucosal hemorrhages without important epithelial changes (6/7). Other generalized scattered hemorrhages and pulmonary edema occurred in 2/7 dogs. Prior to death, the dogs exhibited vomiting, hemorrhagic diarrhea, fever, weakness, and shock. Laboratory findings included leukocytosis, with neutrophilia and shift to the left, and elevation of alkaline phosphatase, transaminases, and α_2-globulins, all changes indicating an acute inflammatory process. One dog reacted with severe hypocalcemia. Dogs treated with a single dose of 100 μg/kg or 50 μg/kg or with 5 daily doses of 75, 50, or 25 μg/kg

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controls. The animals, numbering 40 per group, were approximately 8 wk of age at initiation, and observations were recorded for periods ranging up to 91 to 104 wk. After sacrifice, target organs and all tissue masses were subjected to histopathologic examination. Data on all histologically identified tumors were then analyzed by the life-table technique to determine the probability of development of any type tumor. These data were further classified into 4 categories: any mammary tumor, only mammary carcinomas, only benign tumors, and any malignant tumor. Data indicated that probability of tumor development ranged from 25 to 45% for control male rats and from 48 to 69% for control female rats. Generally, the probabilities determined for the steroid-treated animals were not significantly increased in female rats and were increased in male rats in only certain categories at higher dosage levels.

105. *Toxicity of Metolazone in Meat-Fed Beagle Dogs.* D. J. Sullivan and R. E. Kane, Pennwalt Corporation, Rochester, New York. (Z. Hadidian.)

Beagle dogs fed an all meat diet were medicated with metolazone for 14 wk (beginning after 9 wk on the diet). Clinical chemistry studies were done once each week throughout the experiment. Meat-fed dogs were the first animals to give any evidence of the effect of medication. Diuresis with associated hemoconcentration was seen in meat-fed dogs medicated with metolazone after the first week of medication. Diminished serum electrolyte values (Na, K, Cl) were most prominent in meat-fed dogs, although serum K and Cl values of medicated dogs fed Purina Canine Checkers also declined. Serum glucose levels of meat-fed male dogs rose during the first week of medication. The results of this experiment suggest that the effects of medication with metolazone are more severe in dogs receiving suboptimal diets.

106. *Toxicology and Fate of 2,2,4-Trimethyl-1,3-pentanediol Diisobutyrate in the Rat.* Bernard D. Astill, C. J. Terhaar, and D. W. Fassett, Eastman Kodak Company, Rochester, New York.

Since Kodaflex® (TXIB) may be an indirect food additive from use in food packaging, a study of the toxicity and fate was done. Acute oral, ip, skin irritation, sensitization, and inhalation studies showed it to be practically nontoxic. Rats were fed for 103 days at concentrations of 0.1 and 1.0% in the diet. The only consistent finding was a slight but significant increase in the weight of the livers of rats fed 1.0% TXIB. Single oral 250 mg/kg doses of 3-^{14}C-labeled-TXIB were eliminated in the urine (64–88%) and feces (13–31%) almost completely within 8–10 days of dosing, 80% being eliminated within 3 days. $^{14}CO_2$ excretion was insignificant. At sacrifice after 14 days these doses, and one of 900 mg/kg, gave negligible increments of radioactivity in liver, brain, kidneys, lungs, and fat. After TXIB dosing, free 2,2,4-trimethylpentane-1,3-diol, 2,2,4-trimethyl-3-hydroxyvaleric acid, the glucuronides, and a monoisobutyrate of 2,2,4-trimethylpentane-1,3-diol, were found in the urine. Feces contained the same monoisobutyrate and unchanged TXIB. Large doses of TXIB are thus completely eliminated, with negligible retention by the organism. They appear to be handled by partial hydrolysis in the gut, and after incomplete absorption, by normal pathways such as hydrolysis, oxidation, and esterification.

107. *Toxicologic Studies with Polychlorinated Biphenyls.* M. L. Keplinger, Otis E. Fancher, and J. C. Calandra, Industrial BIO-TEST Laboratories, Inc., Northbrook, Illinois.

Polychlorinated biphenyl compounds (PCB) have been reported to be in the environment, and the chronic toxicologic properties have not been reported. PCB compounds with 42, 54, or 60% chlorine (1242, 1254, and 1260) are being fed to rats and dogs, and rat and chicken reproduction studies are being conducted at 1, 10, and 100 ppm. After 18 mo in rats (interim sacrifice and histology at 3, 6, and 12 mo), compound 1242 produced no effects. Rats fed compounds 1254 or 1260 had increased liver weights at 100 ppm, but not at 1 or 10 ppm. No other effects have been observed. Dogs fed 1242 have shown no adverse effects, while those fed 100 ppm 1254 or 1260 have not gained weight as well as controls. In the rat reproduction study there was decreased survival of pups at 100 ppm of 1242 or 1254, and decreased mating indices with

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1242. No adverse effects at any of the 3 levels of 1260 or at 1 or 10 ppm of the other two compounds have been observed. There was anorexia, loss of body weight, decreased thickness of egg shells, and poor hatchability of eggs from chickens fed 10 or 100 ppm of 1242, or 100 ppm of 1254. At 1, 10, and 100 ppm of 1260 and at other levels of 1242 and 1254 there were no effects.

108. *Cataracts in Parachlorophenylalanine-Treated Rats: Development, Clinical Appearance and Antagonistic Effect of Phenylalanine.* E. J. GRALLA and L. D. RUBIN, Yale University School of Medicine and University of Pennsylvania School of Veterinary Medicine.

Parachlorophenylalanine, a serotonin biosynthesis inhibitor, causes distinctive cataracts and depressed body weight gain when fed to weanling rats. Our studies explored the effect of dietary phenylalanine on this toxicity, while cataract development was followed biomicroscopically. Phenylalanine feeding partially prevented the ocular lesions without significantly affecting the toxic effect on growth patterns. The cataracts were subcapsular, and matured in characteristic patterns; they were permanent and apparently age-related since mature rats showed a marked resistance to eye changes. The results suggest that depletion of serotonin, or serotonin metabolites, may be an important factor in the pathogenesis of these cataracts.

109. *Studies on the Mechanism of the Cataractogenic Activity of 2,4-Dinitrophenol.* J. E. LEBEAU and P. J. GEHRING, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan.

Using an in vitro lens culture system, 2,4-dinitrophenol (DNP) at a concentration of 2×10^{-5} M caused cataracts to develop in lenses obtained from rabbits 40 ± 5 days of age. Known metabolites of DNP (2 amino-4 nitrophenol, 2 nitro-4 aminophenol, and 2,4-diaminophenol) did not produce this effect even at a concentration of 1×10^{-3} M. The cataractogenic activity of DNP was found to have a positive temperature coefficient and was not influenced by the amount of oxygen in the system. The observation that control lenses maintain their clarity even in the absence of oxygen, eliminates the uncoupling of oxidative phosphorylation as the mechanism for the cataractogenic activity. Having eliminated this possibility, the effect of DNP on ATPase activity was determined. Although ATPase activity was stimulated and oligomycin blocked the production of cataracts, a cause-effect relationship was not established due to the observation that the ATP content of lenses treated with DNP and oligomycin was lower than that of lenses treated with only DNP. DNP did not alter either the production of lactic acid or sorbitol by the lens. Although DNP caused a significant depression of the hexose monophosphate shunt activity of lenses, this depression was not alleviated when cataract induction was blocked with oligomycin.

110. *Storage, Metabolism, and Urinary Excretion of DDT and DDT Metabolites in Human Subjects.* D. P. MORGAN and C. C. ROAN, University of Arizona, Tucson, Arizona. (George Ware.)

Eight human subjects ingested 5–20 mg per day of either *pp'*-DDA, *pp'*-DDD, *pp'*-DDE, or technical DDT for periods of 21 days to 6 mo for the purpose of studying efficiencies of storage and rates of metabolic conversion and excretion. Serum and fat concentrations of DDT and/or metabolites, plus urinary excretion of DDA, were measured before, during, and after dosing. *pp'*-DDA was efficiently absorbed and excreted in the urine. *pp'*-DDD underwent adipose storage, but was nearly all excreted (mainly as urinary DDA) within a year of the end of dosing. Adipose storage of *pp'*-DDE accounted for most of the *pp'*-DDE ingested during dosing, after which release from storage was extremely slow. None was excreted as urinary DDA. The *pp'*-DDT technical DDT was more efficiently stored than the *op'*-DDT. There was only very slow conversion of *pp'*-DDT to *pp'*-DDE during and after DDT dosing. In the course of 1 yr after termination of DDT dosing, urinary *pp'*-DDA excretion accounted for 30–50% of estimated *pp'*-DDT storage loss. Comparative urinary DDA excretions during and immediately after DDT dosing suggest an important role of the intestine in DDA synthesis.

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