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Results of a Two-Year Chronic Toxicity and Oncogenicity Study of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin in Rats

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Results of a Two-Year Chronic Toxicity and Oncogenicity Study of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin in Rats. KOCIBA, R. J., KEYES, D. G., BEYER, J. E., CARREON, R. M., WADE, C. E., DITTENBER, D. A., KALNINS, R. P., FRAUSON, L. E., PARK, C. N., BARNARD, S. D., HUMMEL, R. A., AND HUMISTON, C. G. (1978). *Toxicol. Appl. Pharmacol.* 46, 279–303. Rats were maintained for 2 years on diets supplying 0.1, 0.01, and 0.001 μg of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)/kg/day. Analysis of these diets indicated 2200, 210, and 22 parts per trillion (ppt) of TCDD. Ingestion of 0.1 μg/kg/day caused an increased incidence of hepatocellular carcinomas and squamous cell carcinomas of the lung, hard palate/nasal turbinates, or tongue, whereas a reduced incidence of tumors of the pituitary, uterus, mammary glands, pancreas, and adrenal gland was noted. Other indications of toxicity at this dose level included increased mortality, decreased weight gain, slight depression of erythroid parameters, increased urinary excretion of porphyrins and δ-aminolevulinic acid, along with increased serum activities of alkaline phosphatase, γ-glutamyl transferase and glutamic–pyruvic transaminase. Gross and histopathologic changes were noted in the hepatic, lymphoid, respiratory, and vascular tissues. The primary hepatic ultrastructural change at this high dose level was proliferation of the rough endoplasmic reticulum. Terminal liver and fat samples from rats at this high dose level contained 24,000 and 8100 ppt of TCDD, respectively. Rats given 0.01 μg/kg/day for 2 years had a lesser degree of toxicity than that seen at the highest dose level. This included increased urinary excretion of porphyrins in females, liver lesions (including hepatocellular nodules), and lung lesions (including focal alveolar hyperplasia). Terminal liver and fat samples from rats of this dose level contained 5100 and 1700 ppt of TCDD, respectively. Ingestion of 0.001 μg of TCDD/kg/day (~22 ppt in the diet) caused no effects considered to be of any toxicologic significance. At this lower dose level, terminal liver and fat samples each contained 540 ppt of TCDD. These data indicate that continuous doses of TCDD sufficient to induce severe toxicity increased the incidence of some types of tumors, while reducing other types. During the 2-year study in rats, no increase in tumors occurred in those rats receiving sufficient TCDD to induce slight or no manifestations of toxicity.

The compound 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a highly toxic impurity that may be formed under certain conditions during the production of 2,4,5-trichlorophenol. TCDD has been considered one of the causes of chloracne, which has been

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associated with the industrial production of 2,4,5-trichlorophenol and other products made from 2,4,5-trichlorophenol.

Most of the earlier toxicologic studies with TCDD were concerned with the assessment of its short-term toxicity and teratogenic potential. Results of these earlier studies have been summarized in a previous publication by Kociba *et al.* (1976). This same publication also reported the results of a subchronic study in which rats were given 1.0, 0.1, 0.01, 0.001, or 0 μg of TCDD/kg 5 days/week for 13 weeks. Doses of 1.0 μg/kg/day caused multiple toxicologic effects, including mortality and morphologic changes in liver, thymus, and reproductive organs. A dose level of 0.1 μg/kg/day caused lesser degrees of toxicity, and rats given 0.01 or 0.001 μg/kg/day had no alterations considered of any toxicologic significance.

More recently, toxicologic studies of TCDD have also been conducted in the monkey. McConnell *et al.* (1978) reported a single oral LD50 in monkeys of <70 μg TCDD/kg. These monkeys died with loss of hair or nails, keratinization of Meibomian glands and hair follicles, and hyperplasia of the epithelium of the renal pelvis, stomach, and bile duct.

Allen *et al.* (1977) reported on a subchronic study in which monkeys consumed a diet containing 500 ppt of TCDD for 9 months. It was calculated that these monkeys ingested 2 to 3 μg TCDD/kg over the course of the 9-month study. Clinically, these monkeys showed changes similar to those described by McConnell *et al.* (1978) as well as hematologic depression and hemorrhages in various tissues. Hypertrophy, hyperplasia, and/or metaplasia were noted in the epithelium of the bile ducts, salivary glands, bronchi, pancreatic ducts, sebaceous glands, skin, gastric lining, and urinary tract.

In regard to long-term or carcinogenic studies conducted in rodents with TCDD, Innes *et al.* (1969) reported no increase in tumors in mice given 2,4,5-Trichlorophenoxyacetic acid contaminated with a level of TCDD sufficient to supply 0.27 μg of TCDD/kg/day. DiGiovanni *et al.* (1977) conducted a study in which TCDD was reported to be only a weak tumor initiator in the two-stage system of mouse skin carcinogenesis with 7,12-dimethylbenz[a]anthracene (DMBA).

Van Miller and Allen (1977) issued a preliminary report on a study of small groups of male rats fed diets containing TCDD for 65 weeks. All 10 rats of each group receiving 1.0, 0.5, or 0.05 ppm of TCDD in the diet died within 4 weeks, with acute toxic effects, including severe liver necrosis, bile duct hyperplasia and edema, atrophy of spleen and thymus, gastrointestinal hemorrhages, and decreased spermatogenesis. Groups of male rats on diets containing 5000 or 1000 ppt experienced increased mortality, decreased weight gain, and liver toxicity. Dietary levels of 500, 50, 5, and 1 ppt of TCDD were also studied. Various neoplasms were found in some rats at all dose levels of 5 ppt and higher, with only the lowest dose level of 1 ppt reportedly free of any neoplasms. These same results on tumorigenesis were included in an updated report by Van Miller *et al.* (1977), which included the data generated through the end of their 95-week study. No tumors were reported in the group given 1 ppt of TCDD or in a total of 50 control rats.

In view of the need for an evaluation of the chronic toxicity and potential for carcinogenicity of TCDD, the study reported herein was conducted. In this study, groups of male and female rats were maintained for 2 years on diets supplying

various dose levels of TCDD, and numerous parameters were evaluated in order to assess the potential chronic toxicity associated with long-term ingestion of the material.

METHODS

Experimental design. Male and female Sprague–Dawley rats, Spartan substrain,[1] 6 to 7 weeks old, were randomly placed (two/cage) into suspended wire-bottomed cages for this study. Food[2] and water were available *ad libitum*. Groups of 100 rats (50 males, 50 females) were maintained for up to 2 years on diets supplying 0.1, 0.01, or 0.001 μg of TCDD/kg/day. The diet of the control group of 172 rats (86 males, 86 females) contained the vehicle.

Test material. The TCDD sample used for this 2-year study was prepared by the Dow Chemical Company. Purification of the crude TCDD was followed by gas chromatography and mass spectrometry. The final product had a purity exceeding 99%, as determined by electron-capture gas chromatography. This sample was used to prepare the premixes and test diets according to the following general procedure: Approximately 1 mg of TCDD was weighed on a microbalance and dissolved in 40 ml of reagent-grade acetone. This solution was then added to 1000 g of control feed and mixed for 30 min to prepare a stock premix. A sufficient sample of the stock premix was then mixed with control feed to produce a working premix to be used in preparing the test diets. The stock premix was prepared six times during the course of the study. The test diets were prepared by diluting the working premix with sufficient control feed to provide dose levels of TCDD as required by body weight and food consumption determinations in order to maintain the designated dosages on a microgram per kilogram per day basis. Samples of the working premix and the test diets were analyzed periodically to ascertain that the dietary levels were being maintained as scheduled. The concentration of TCDD in the premix samples was determined using electron-capture gas chromatography and gas chromatography–mass spectrometry. The concentration of TCDD in the test diets was determined by gas chromatography-mass spectrometry after extraction and suitable cleanup.

Clinical observations. All rats were palpated on a monthly basis, with a recording of the number of rats bearing palpable masses. Body weights and food consumption of 20 rats/sex/treatment level and controls were routinely recorded for each week of the first 3 months of the study and at approximately monthly intervals thereafter. All remaining rats were weighed monthly throughout the study.

Blood samples for hematological determinations were collected from eight rats/sex/group at 3, 12, and 23 months of treatment. The total erythrocyte count (RBC), total and differential leukocyte counts (WBC), thrombocyte and reticulocyte counts, packed cell volume (PCV), and hemoglobin (Hgb) concentration were determined using automated[3] or manual procedures. Urine samples were collected from seven to eight rats/sex/group at these same time intervals. Urine specific gravity, pH, and the presence

[1] Spartan Research Animals, Haslett, Michigan.
[2] Purina Laboratory Chow, Ralston-Purina Co., St. Louis, Missouri.
[3] Coulter Counter Model ZB-1, Coulter Electronics, Hialeah, Florida.

or absence of sugar, protein, ketones, bilirubin, and occult blood were determined[4] at each of these times, and urinary urobilinogen was also evaluated at Month 23.

Urinary excretion of creatinine, coproporphyrin, uroporphyrin, and δ-amino levulinic acid (δ-ALA) was determined by a consulting laboratory[5] on samples collected from four to five rats/sex/group at Months 3–4, 12, and 23.

Serum samples were collected from seven rats/sex/group by orbital puncture at Month 22 for determination of urea nitrogen (BUN), glutamic pyruvic transaminase (SGPT), bilirubin (total, direct and indirect), cholesterol, and triglycerides. Automated procedures were used for these determinations.[6] Serum samples were similarly collected from seven rats/sex/group at Month 23 for determination of alkaline phosphatase (AP) activity, total protein, albumin and globulin.[6]

At terminal necropsy, serum samples were collected from all survivors or a maximum of 10 rats/sex/group for determination of BUN, SGPT, AP, and total bilirubin.[6] A consulting laboratory[5] also made determinations of serum γ-glutamyl transferase activity (GGT).

All rats dying or culled during the course of the study were subjected to a gross pathologic examination. Representative portions of the major organs and any gross lesion suggestive of a significant pathologic process or tumor formation were collected from each rat and preserved in buffered 10% formalin.

Terminal necropsy examination was conducted at the end of 2 years of treatment (105th week). All rats were deprived of food overnight prior to killing by decapitation. The eyes of all rats were examined by gently pressing a glass slide against the cornea under bright fluorescent illumination. Any observations on the eyes were recorded as part of the gross necropsy observation records. The eyes for a maximum of five rats/sex/group were preserved in Zenker's fixative. Eyes from remaining rats were fixed in formalin fixative.

The weights of the liver, kidney, brain, heart, thymus, spleen, testes, and ovaries/uterus were recorded for a maximum of 10 rats/sex/group. A bone marrow smear was prepared from most rats, and filed for future reference, if indicated. Portions of fat, liver, and kidney from a maximum of five rats/sex/group were frozen for possible TCDD analysis, with subsequent analysis of liver and fat samples from three females/dose level, using gas chromatography–low-resolution mass spectrometry. Portions of esophagus, salivary glands, stomach, small intestine, large intestine, pancreas, liver, kidneys, urinary bladder, prostate, accessory sex glands, epididymis, testes, ovaries, uterus, brain (cerebrum, cerebellum, brain stem), pituitary gland, spinal cord, peripheral (sciatic) nerve, trachea, lungs, spleen, thymus, lymph nodes, heart, aorta, skeletal muscle, mammary tissue (females), adrenal glands, thyroid, parathyroid, tongue, lower jaw, and skull (including nasal turbinates, ear canal), together with any additional gross lesions were preserved in formalin fixative.

Histologic examination of tissues was conducted on paraffin-embedded sections of tissues which were stained with hematoxylin and eosin. All rats from the control and top dose level, regardless of whether they died during the study or were killed at the

[4] Ames Bililabstix or Multistix, Ames Co., Elkhart, Indiana and TS Meter, AO Optical, Buffalo, New York.

[5] Bioscience Laboratories, Van Nuys, California.

[6] Technicon AutoAnalyzer, Technicon Corp., Rye, New York.

termination, were subjected to histologic examination of an extensive list of tissues, intended to include a majority of those tissues listed above as those collected at the time of terminal necropsy. All rats from the two lower dose levels were subjected to histologic examination of those selected tissues identified as possible target organs and all gross lesions suggestive of tumor formation. The actual number of tissues specimens from each group examined histologically is on file and available from the authors. Additional sections of liver from selected females from the terminal necropsy were stained for lipid content using Oil Red O or Sudan IV stains.

Liver tissue collected at the terminal necropsy was examined using an electron microscope[*] to characterize qualitatively the ultrastructure of hepatocytes from three females/group. The liver tissue was fixed in 2.5% phosphate-buffered glutaraldehyde and then postfixed in 1% phosphate-buffered osmium tetroxide, dehydrated through graded ethanol solutions, washed in propylene oxide, infiltrated with Epon 812, and embedded in polyethene capsules. Sections of 1 μm thickness were stained with toluidine blue and examined using light microscopy. Thin sections were stained with uranyl acetate and lead citrate prior to examination by electron microscopy.

Statistical evaluation of data. The significance of differences between control and test values for hematology, urinary and clinical chemistry parameters, body weights, organ weights, and organ/body weight ratios was statistically determined by one-way analyses of variance followed by the Dunnett test (Steel and Torrie, 1960). A significance level of $p < 0.05$ was used. Data on mortality, palpable masses, gross pathology, histopathology, and tumor incidences were analyzed using the Fischer exact probability test, $p < 0.05$, one-sided test (Siegel, 1956). Mortality data were also analyzed using the Mantel–Haenszel Test. Repeated measures analyses across time were not appropriate because of mortality and because the assumptions of the statistical tests were not valid.

Statistical evaluation of gross pathology data collated for the entire study compared the data of each of the treatment groups with the data of the control group of that sex. Statistical evaluation of histopathologic observations and tumor incidences compared the data of the high-dose group with the data of the control group of that sex. The same evaluation was conducted on the lower dose levels in instances in which comparable numbers of tissues were subjected to microscopic examination (apparent target organs).

RESULTS

Dietary Content of TCDD

Analyses of feed samples indicated the dosage levels of 0.1, 0.01, and 0.001 μg of TCDD/kg/day equated with approximately 2193, 208, and 22 ppt of TCDD in the diet. Six repeated analyses of the feed samples indicated good agreement between the intended content of TCDD and results of analysis for TCDD content.

Clinical observations

Females given 0.1 μg/kg/day had statistically increased cumulative mortality during the latter half of the study, whereas those given 0.01 or 0.001 μg/kg/day had mortality

[*] Carl Zeiss, Inc., New York.

rates comparable to that of the controls. In males, there were some isolated instances of statistical differences between the treated and control groups. However, as the mortality of only the group of males given 0.01 μg/kg/day was significantly different from control using the Mantel–Haenszel test, these deviations in the male rats were considered of questionable toxicologic significance. Mean body weights of males and females given 0.1 μg/kg/day were statistically decreased from control values throughout the major portion of the study, from Month 6 to the end of the 2-year test period. Mean body weights of females given 0.01 μg/kg/day were decreased to a lesser degree during this same time interval. The mean body weights of males given 0.01 or 0.001 μg/kg/day and females given 0.001 μg/kg/day were sometimes lower than controls during the middle of the study, but only occasionally were the differences statistically significant. During the last quarter of the study body weights of these groups were comparable to those of controls.

There were no consistent deviations in food consumption of males or females at any dose. The few sporadic cases in which there was a statistical increase or decrease between the control and treatment groups followed no consistent trend, and were considered of no toxicologic significance. The first palpable mass was noted at Month 5 in a male of the control group. There were no statistically significant differences between the control and treated groups except during Months 15 and 16, when the males given 0.01 μg/kg/day had an increased incidence of palpable masses. This was considered of no toxicologic significance because of its isolated occurrence and lack of dose response. During the last 12 months of the study, females ingesting 0.1 μg/kg/day had a consistent trend toward a decrease in the number of rats with palpable masses. This observation was not noted at lower dosage levels in the females.

Hematology

The hematology data collected after 23 months of treatment are listed in Table 1 and are similar to the patterns observed during the study. In rats given 0.1 μg/kg/day, there were statistically significant decreases in the PCV and Hgb values for males after 3 months as well as decreases in the Hgb values for males and decreases in PCV, total RBC, and WBC counts and Hgb values for females after 1 year. At the preterminal examination, this high dose group again had statistically significant decreases in RBC and Hgb values (males) and PCV and Hgb values (females); reticulocyte counts also appeared to be slightly increased. Thrombocyte and WBC differential counts appeared to be unaffected at all dose levels of TCDD. Rats given 0.01 or 0.001 μg/kg/day had no hematologic changes considered related to treatment.

Urinalyses

Repeated examination of urinary parameters revealed no consistent alterations that could be attributed to any of the dose levels of TCDD.

Urinary porphyrins and δ-ALA

Porphyrin data collected after 23 months of treatment are listed in Table 2 and are representative of the patterns observed during the study. Urinary excretion of coproporphyrin was statistically increased in female rats at a dose level of 0.1 μg/kg/day after each evaluation at 3–4, 12, and 23 months. Coproporphyrin excretion was also

TABLE 1

MEAN HEMATOLOGIC VALUES OF MALE RATS (DAY 681) AND FEMALE RATS (DAY 682) ON DIETS CONTAINING TCDD

Dose of TCDD (μg/kg/day)	Sex	Number of rats/group	PCV (%)	RBC (×10⁶/mm³)	Hgb (g/100 ml)	Reticulocytes (%)	Thrombocytes (×10⁶/mm³)	WBC (×10³/mm³)	WBC differential count (%)				
									Neut	Lymph	Mono	Eosin	Baso
0	M	8	46.9 ± 4.7[a]	7.99 ± 0.64	15.6 ± 1.6	1.0	1.195 ± 0.350	14.9 ± 5.4	27	65	7	1	0
0.100	M	8	43.4 ± 4.3	7.19 ± 0.65[b]	13.9 ± 1.5[b]	2.2	1.214 ± 0.358	12.1 ± 5.2	33	62	4	1	0
0.010	M	8	47.6 ± 2.8	7.65 ± 0.46	16.0 ± 0.8	1.0	1.482 ± 0.342	15.8 ± 4.0	34	60	5	1	0
0.001	M	8	47.4 ± 1.8	7.73 ± 0.27	15.7 ± 0.6	0.5	1.042 ± 0.143	18.7 ± 8.0	30	65	4	1	0
0	F	8	43.6 ± 1.4	6.84 ± 0.66	14.1 ± 1.1	0.8	1.046 ± 0.210	9.1 ± 1.5	34	60	4	2	0
0.100	F	8	38.9 ± 3.8[b]	6.38 ± 0.90	12.5 ± 1.2[b]	1.2	1.176 ± 0.358	7.1 ± 2.1	30	67	2	1	0
0.010	F	8	45.1 ± 1.1	7.35 ± 0.32	15.1 ± 0.4	0.5	0.962 ± 0.161	9.4 ± 2.2	36	60	3	1	0
0.001	F	8	46.9 ± 4.7	7.56 ± 0.69	15.7 ± 1.5[b]	0.5	0.857 ± 0.239	9.5 ± 1.9	23	72	3	2	0

[a] Mean ± SD.

[b] Statistically significant from control mean using analysis of variance and Dunnett's test, $p < 0.05$.

TABLE 2

URINARY EXCRETION OF CREATININE, COPROPORPHYRIN, UROPORPHYRIN, AND δ-AMINO LEVULINIC ACID FOR MALE AND FEMALE RATS (DAYS 678–680) ON DIETS CONTAINING TCDD

Dose TCDD (μg/kg/day)	Sex	Number of rats/group	Total urine vol, 48 hr (ml)	Creatinine (mg/48 hr)	Coproporphyrin (μg/48 hr)	μg Coproporphyrin / mg Creatinine	Uroporphyrin (μg/48 hr)	μg Uroporphyrin / mg Creatinine	δ-amino-levulinic acid (mg/48 hr)	mg δ-ALA / mg Creatinine
0	M	4	51.0 ± 21.6[a]	27.6 ± 3.2	18.0 ± 3.7	0.69 ± 0.19	5.4 ± 2.2	0.200 ± 0.092	0.27 ± 0.38	0.010 ± 0.015
0.100	M	5	63.4 ± 40.5	19.6 ± 9.1	19.6 ± 11.7	1.22 ± 1.08	7.3 ± 4.0	0.418 ± 0.295	0.08 ± 0.02	0.005 ± 0.003
0.010	M	5	61.4 ± 26.1	24.4 ± 5.7	16.5 ± 8.0	0.64 ± 0.25	5.7 ± 2.1	0.228 ± 0.056	0.26 ± 0.42	0.009 ± 0.013
0.001	M	5	41.0 ± 6.4	30.8 ± 3.2	23.8 ± 4.8	0.78 ± 0.19	5.3 ± 1.6	0.174 ± 0.053	0.06 ± 0.01	0.002 ± 0.001
0	F	5	60.0 ± 34.9	23.3 ± 6.2	9.8 ± 1.3	0.43 ± 0.49	3.8 ± 1.7	0.157 ± 0.050	0.07 ± 0.03	0.003 ± 0.001
0.100	F	5	51.2 ± 22.8	18.6 ± 4.3	17.4 ± 4.0[b]	0.98 ± 0.41[b]	5.7 ± 2.3	0.296 ± 0.074[b]	0.12 ± 0.05	0.006 ± 0.002[b]
0.010	F	5	54.2 ± 20.5	19.4 ± 2.3	16.4 ± 4.7[b]	0.83 ± 0.18	3.5 ± 1.1	0.181 ± 0.053	0.08 ± 0.03	0.004 ± 0.002
0.001	F	5	57.2 ± 20.0	20.8 ± 4.7	8.6 ± 2.0	0.42 ± 0.06	3.0 ± 1.1	0.143 ± 0.037	0.08 ± 0.02	0.004 ± 0.001

[a] Mean ± SD.

[b] Statistically significant from control mean by analysis of variance and Dunnett's test, $p < 0.05$.

statistically increased in female rats at a dose level of 0.01 μg/kg/day after 3 and 23 months. Urinary excretion of uroporphyrin was statistically increased in females after 3 and 23 months of receiving 0.1 μg/kg/day and after 3 months of receiving 0.01 μg/kg/day. Urinary excretion of δ-ALA was statistically increased in females after 3 and 23 months of receiving 0.1 μg/kg/day. Total urine volume or creatinine excretion was not affected by any of these dose levels in the females. Males had no alterations considered treatment-related in any of these parameters at any of the dose levels of TCDD.

Clinical Chemistry

For sake of brevity, only the results obtained at terminal necropsy after 2 years of treatment are presented in Table 3. Analyses of serum samples collected by orbital

TABLE 3

MEAN TERMINAL (2-YEAR) CLINICAL CHEMISTRY VALUES FOR MALE AND FEMALE RATS GIVEN DIETS CONTAINING TCDD

Dose TCDD (μg/kg/day)	Sex	Number of rats/group	BUN (mg/100 ml)	SGPT (mU/ml)	AP (mU/ml)	Total bilirubin (mg/100 ml)	γ-glutamyl transferase (mU/ml)
0	M	10	33 ± 34[a]	49 ± 17	87 ± 30	0.2 ± 0	0 ± 0
0.100	M	5	28 ± 11	42 ± 6	105 ± 17	0.2 ± 0	1 ± 0
0.010	M	4	36 ± 19	47 ± 10	88 ± 26	0.2 ± 0	1 ± 0
0.001	M	10	20 ± 9	43 ± 9	86 ± 25	0.2 ± 0	0 ± 0
0	F	10	21 ± 9	39 ± 11	60 ± 29	0.2 ± 0	0 ± 0
0.100	F	4	20 ± 3	54 ± 12[b]	205 ± 146[b]	0.2 ± 0	14 ± 10[b]
0.010	F	10	17 ± 3	49 ± 7	61 ± 20	0.3 ± 0.1	1 ± 0
0.001	F	10	18 ± 5	42 ± 5	54 ± 28	0.4 ± 0.2	1 ± 0

[a] Mean ± SD.

[b] Statistically significant from control mean using analysis of variance and the Dunnett's test, $p < 0.05$.

puncture at 22 to 23 months of treatment revealed no alterations considered related to treatment in regard to BUN, SGPT, total, direct, or indirect bilirubin, cholesterol, triglycerides, total protein, albumin, and globulin. Serum AP was statistically increased in females given 0.1 μg/kg/day. A statistically significant increase in serum triglycerides noted in males given 0.01 μg/kg/day was considered of no toxicologic significance based on the lack of a dose-response relationship. Analyses of serum samples collected at terminal necropsy after 2 years indicated a statistical increase in SGPT, AP, and GGT activities for females given 0.1 μg/kg/day. Females at the lower dose levels and males at all dose levels were unaffected in these parameters. The BUN and total bilirubin values of either sex were unaffected by any level of treatment with TCDD.

Gross and Microscopic Observations on Tissues

Detailed descriptions of all gross and microscopic observations made on all rats killed or dying during the course of the 2-year study are on file and available from the authors. On account of the voluminous nature of the data, the results are summarized below. Tumor and tumor-like lesions are listed in Tables 4 and 5.

TABLE 4

TUMOR INCIDENCE IN MALE RATS MAINTAINED ON DIETS CONTAINING TCDD[a]

Time intervals during study:	Months 13-24				Terminal kill				Total			
Dose level in μg/kg/day:	0	0.1	0.01	0.001	0	0.1	0.01	0.001	0	0.1	0.01	0.001
Number of rats examined:	65	41	46	38	15	5	4	11	85	50	50	50
Rats with tumors/tumor like lesions:												
Hepatocellular hyperplastic nodule(s)	2	1	2	0	4	1	1	0	6	2	3	0
Hepatocellular carcinoma(s)	1	0	0	0	1	1	0	0	2	1	0	0
Bile duct adenoma	0	1	0	0	0	0	0	0	0	1	0	0
Stratified squamous cell carcinoma of hard palate or nasal turbinates	0	4	0	0	0	0	0	0	0	4[b]	0	0
Paravertebral or sub cutaneous malignant schwannoma	0	1	1	0	0	0	0	0	0	1	1	0
Carcinoma of renal tubules pelvis, or bladder	1	0	0	0	1	0	0	1	2	0	0	1
Adenoma of renal tubules or pelvis	0	0	0	1	0	0	0	1	0	0	0	2
Keratinizing squamous cell carcinoma of lung	0	1	0	0	0	0	0	0	0	1	0	0
Pulmonary adenoma	1	1	0	0	0	0	0	0	1	1	0	0
Pulmonary adenocarcinoma	1	0	0	0	0	0	0	0	1	0	0	0
Oligodendroglioma/astrocytoma of brain, or glioma of spinal cord	2	0	0	1	0	0	0	0	2	0	0	1
Interstitial cell adenoma of testes	2	0	0	1	0	0	0	1	2	0	0	2
Adenoma of prostate	0	0	0	0	0	0	0	1	0	0	0	1
Subcutaneous fibroadenoma/fibroma/lipoma	8	6	4	1	1	0	1	0	10	6	5	1[b]

Subcutaneous fibrosarcoma	0	1	1	0	0	0	0	0	0	1	1	0
Benign mammary neoplasms	0	1	0	0	0	0	0	0	0	1	0	0
Mammary gland adeno carcinoma	2	0	0	0	0	0	0	0	2	0	0	0
Cutaneous papilloma/basal cell tumor	3	0	0	0	0	0	0	6	3	0	0	0
Squamous cell carcinoma of integument	0	0	0	1	0	0	0	0	0	0	0	1
Zymbal gland carcinoma	0	0	1	1	0	0	0	0	0	0	1	1
Pituitary adenoma	19	10	11	5	7	2	0	1	26	13	11	6
Pituitary adenocarcinoma	1	0	0	0	1	0	0	0	3	0	0	0
Stratified squamous cell carcinoma of tongue	0	3	1	1	0	0	0	0	0	3[a]	1	1
Fibrosarcoma of tongue	1	0	0	0	0	0	0	0	1	0	0	0
Squamous papilloma/polyp of gastric mucosa	0	1	1	1	0	0	0	0	0	1	1	1
Mucocystaderocarcinoma of small intestine	1	0	0	2	0	0	0	0	1	0	0	2
Leiomyosarcoma of cecum	0	0	0	0	0	1	0	0	0	1	0	0
Intraabdominal schwannoma/sarcoma	0	2	0	0	0	0	0	0	0	2	0	0
Acinar adenoma of pancreas	11	2	4	3	3	0	1	4	14	2[a]	5	7
Acinar adenocarcinoma of pancreas	1	0	0	0	0	0	0	0	1	0	0	0
Islet adenoma of pancreas	6	3	2	1	6	0	1	2	12	3	3	3
Islet adenocarcinoma of pancreas	1	0	0	0	1	0	0	0	2	0	0	0
Adenoma of adrenal cortex	0	5	2	0	0	0	0	0	0	5[a]	2	0
Pheochromocytoma of adrenal	19	3	10	3	9	1	0	3	28	4[a]	10	6
Interfollicular C-cell adenoma of thyroid	2	4	0	0	6	0	0	0	8	4	0	0
Interfollicular C-cell adenocarcinoma of thyroid	2	1	0	0	1	0	0	0	3	1	0	0
Follicular adenoma of thyroid	0	1	1	0	0	0	0	0	0	1	1	0
Follicular adenocarcinoma of thyroid	0	0	0	0	0	1	0	0	0	1	0	0

TABLE 4—*continued*

Time intervals during study:	Months 13–24				Terminal kill				Total			
Dose level in μg/kg/day:	0	0.1	0.01	0.001	0	0.1	0.01	0.001	0	0.1	0.01	0.001
Number of rats examined:	65	41	46	38	15	5	4	11	85	50	50	50
Malignant lymphoreticular neoplasm	5	0	3	2	0	0	0	1	5	0	3	3
Hemangioma of lymph node	1	0	0	0	0	0	0	0	1	0	0	0
Fibrosarcoma/osteosarcoma of musculoskeletal system	1	0	0	0	0	0	0	0	2	0	0	0
Intraorbital malignant schwannoma	0	0	1	0	0	0	0	0	0	0	1	0
Mediastinal fibrosarcoma	0	0	0	1	0	0	0	0	0	0	0	1
Not available for pathological examination	0	0	0	0	0	0	0	0	1	0	0	0

[a] No tumors occurred during Months 1 through 6. Tumors occurring during Months 7 to 12 included 1 subcutaneous fibroadenoma (control), 1 pituitary adenoma (0.1 μg/kg/day), 1 pituitary adenocarcinoma (control), 1 osteosarcoma (control). These four tumors, which were present in the 10 males dying prior to Month 13, are included in the above total tabulation.

[b] Statistically different from control data when analyzed using the Fischer exact probability test, $p < 0.05$. Appropriate tumor data have been combined for the sake of brevity.

TABLE 5

TUMOR INCIDENCE IN FEMALE RATS MAINTAINED ON DIETS CONTAINING TCDD[a]

Time intervals during study:	Months 13–24				Terminal kill				Total			
Dose level in μg/kg/day:	0	0.1	0.01	0.001	0	0.1	0.01	0.001	0	0.1	0.01	0.001
Number of rats examined:	60	36	34	32	25	4	14	16	86	49	50	50
Rats with tumors/tumor-like lesions:												
Hepatocellular hyperplastic nodules	2	20	8	1	6	3	10	2	8	23[b]	18[b]	3
Hepatocellular carcinoma(s)	0	10	1	0	1	1	1	0	1	11[b]	2	0
Bile duct adenoma	0	2	0	0	0	0	0	1	0	2	0	1
Stratified squamous cell carcinoma of hard palate or nasal turbinates	0	4	1	0	0	0	0	0	0	4[b]	1	0
Keratinizing squamous cell carcinoma of lung	0	7	0	0	0	0	0	0	0	7[b]	0	0
Pulmonary adenocarcinoma	0	0	1	0	0	0	0	0	0	0	1	0
Astrocytoma of cerebrum	0	0	0	0	1	0	0	0	1	0	0	0
Malignant schwannoma of pelvic canal	1	0	0	0	0	0	0	0	1	0	0	0
Nephroblastoma of kidney	0	1	1	0	0	0	0	0	0	1	1	0
Adenoma of renal tubules	0	0	0	1	0	0	0	0	0	0	0	1
Carcinoma of renal pelvis	0	0	0	0	0	0	1	0	0	0	1	0
Granulosal cell neoplasm of ovary	0	0	1	1	3	0	0	0	3	0	1	1
Benign tumor of uterus	16	3	7	5	12	2	4	7	28	7[b]	11	12
Malignant schwannoma of uterus	2	0	3	1	0	0	0	0	2	0	3	1
Adenocarcinoma of uterus	6	4	0	1	0	0	0	0	6	4	0	1
Fibroma of cervix/vagina	1	0	0	0	1	0	1	0	2	0	1	0
Subcutaneous fibroma/ fibrolipoma	1	0	0	1	0	0	0	0	1	0	0	1

TABLE 5—*continued*

Time intervals during study:	Months 13–24				Terminal kill				Total			
Dose level in μg/kg/day:	0	0.1	0.01	0.001	0	0.1	0.01	0.001	0	0.1	0.01	0.001
Number of rats examined:	60	36	34	32	25	4	14	16	86	49	50	50
Subcutaneous fibrosarcoma	0	0	0	1	0	0	0	0	0	0	0	1
Benign neoplasm of mammary gland	50	22	23	24	23	2	12	11	73	24[b]	36	35
Carcinoma of mammary gland	5	0	1	3	6	0	5	1	8	0[b]	4	4
Stratified squamous cell carcinoma of digit	0	1	0	0	0	0	0	0	0	1	0	0
Cystadenoma of Zymbal gland	0	0	0	0	0	0	0	1	0	0	0	1
Pituitary adenoma	26	12	8	12	17	0	5	6	43	12[b]	13	18
Pituitary adenocarcinoma	4	2	0	0	2	0	1	0	6	2	1	0
Stratified squamous cell carcinoma of tongue	1	2	0	0	0	0	0	0	1	2	0	0
Papilloma of esophagus	0	0	0	0	1	0	0	0	1	0	0	0
Squamous papilloma/polyp of gastric mucosa	0	2	0	0	1	0	0	1	1	2	0	1
Leiomyosarcoma/sarcoma of small intestine	1	0	0	0	0	0	1	0	1	0	1	0

Polypoid adenoma of large intestine	1	0	0	0	0	0	0	0	1	0	0	0
Acinar adenoma of pancreas	0	1	0	0	0	0	0	1	0	1	0	1
Islet cell adenoma of pancreas	1	0	0	1	2	0	1	2	3	0	1	3
Islet cell adenocarcinoma of pancreas	1	0	0	0	1	0	0	0	2	0	0	0
Adenoma of adrenal cortex	7	5	1	2	2	0	1	4	9	5	2	6
Pheochromocytoma of adrenal	4	3	0	1	3	0	1	1	7	3	1	2
Interfollicular adenoma of thyroid	9	4	1	1	5	0	1	0	14	4	2	1
Interfollicular adenocarcinoma of thyroid	3	2	0	1	1	0	0	1	4	2	0	2
Follicular adenoma of thyroid	0	1	0	0	0	0	0	0	0	1	0	0
Malignant lymphoreticular neoplasm	1	1	1	0	1	0	0	0	2	1	1	0
Hemangioma of abdominal muscle	1	0	0	0	0	0	0	0	1	0	0	0
Not available for pathologic examination	0	0	0	0	0	0	0	0	0	1	0	0

[a] No tumors occurred during Months 1 through 6. Tumors occurring during Months 7 to 12 included two benign tumors of the uterus (0.1 μg/kg/day) and one benign neoplasm of the mammary gland (0.01 μg/kg/day). These three tumors, which were present in the 14 females dying prior to Month 13, are included in the above total tabulation.

[b] Statistically different from control data when analyzed using the Fischer exact probability test, $p < 0.05$. Appropriate tumor data have been combined for the sake of brevity

Gross necropsy examination of the rats of the top dose level indicated the grossly visible target organs to include the liver, vascular system, respiratory system, and lymphoid organs; the general body condition was also consistently affected.

Microscopic examination of tissues from rats dying during the study or killed after 2 years revealed the following treatment-related affects:

Liver. The liver was the organ most consistently affected, and rats given 0.1 or 0.01 μg/kg/day had multiple hepatocellular degenerative, inflammatory, and necrotic changes noted upon light microscopy. These hepatic changes, which were more extensive in females than in males, were characterized by cytomegaly, distortion of

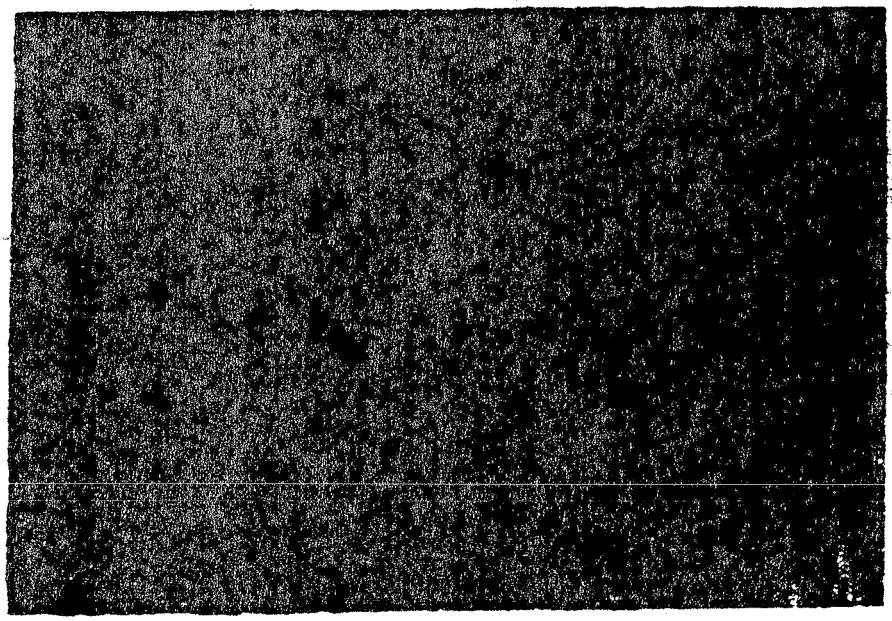

FIG. 1. Lesion classified morphologically as hepatocellular carcinoma in liver of rat given 0.1 μg of TCDD/kg/day. Note adjacent fibrosis, inflammation, and fatty infiltration on left. H & E stain. ×200.

lobular pattern, and resultant atrophy of hepatic cords, cytoplasmic vacuolization, fatty metamorphosis, altered tinctorial properties with increased basophilia, hepatic necrosis and inflammation, multinucleated hepatocytes, and foci or areas of hepatocellular alterations. They were accompanied by increased aggregates of pigment, bile duct hyperplasia, and some increase in fibrosis and periportal inflammation. During the latter phase of the study and at the terminal necropsy the females given 0.1 μg/kg/day also had hepatocellular proliferative lesions classified morphologically as hepatocellular carcinomas (Fig. 1) and hyperplastic (neoplastic) nodules. There was no evidence of metastasis of any liver neoplasms. Female rats given 0.01 μg/kg/day also had an increased incidence of these hepatocellular hyperplastic nodules. Upon examination using light microscopy, livers of female rats given 0.001 μg/kg/day had a statistical increase above the background incidence of foci or larger area of slight hepatocellular alteration (swollen hepatocytes). However, in male rats given 0.001 μg/kg/day, there was a statistically significant decrease in the number of livers with an area of hepatocellular alteration of this type.

As part of the ultrastructural evaluation of hepatocytes, light microscopy of toluidine blue-stained sections of liver from females given 0.1 μg/kg/day revealed an increased number of individual hepatocytes containing large accumulations of lipid droplets. Ultrastructural evaluation by electron microscopy of liver sections from this high dose

FIG. 2. Hepatocyte from female rat given 0.1 μg of TCDD/kg/day for 2 years. Note disorientation of RER and focal cytoplasmic vacuolization. Uranyl acetate–lead citrate stain. ×3350.

level revealed the most consistent change to be in the rough endoplasmic reticulum (RER), which appeared to be undergoing proliferation with some distortion and fragmentation (Fig. 2). Smooth endoplasmic reticulum (SER) and mitochondrial structures were within the range of variation observed in the control sections. Other changes noted at this high dose level included focal areas of cytoplasmic vacuolization, increased lysosomal activity with residual body formation, and an occasional multinucleated hepatocyte (Fig. 3). Upon ultrastructural examination of hepatocytes from

rats of the 0.01-μg/kg/day dose level, the most notable change was limited to a lesser degree of proliferation and disorientation of the RER and some proliferation of SER (Fig. 4). There was some slight increase in the number of individual hepatocytes with lipid droplet accumulations.

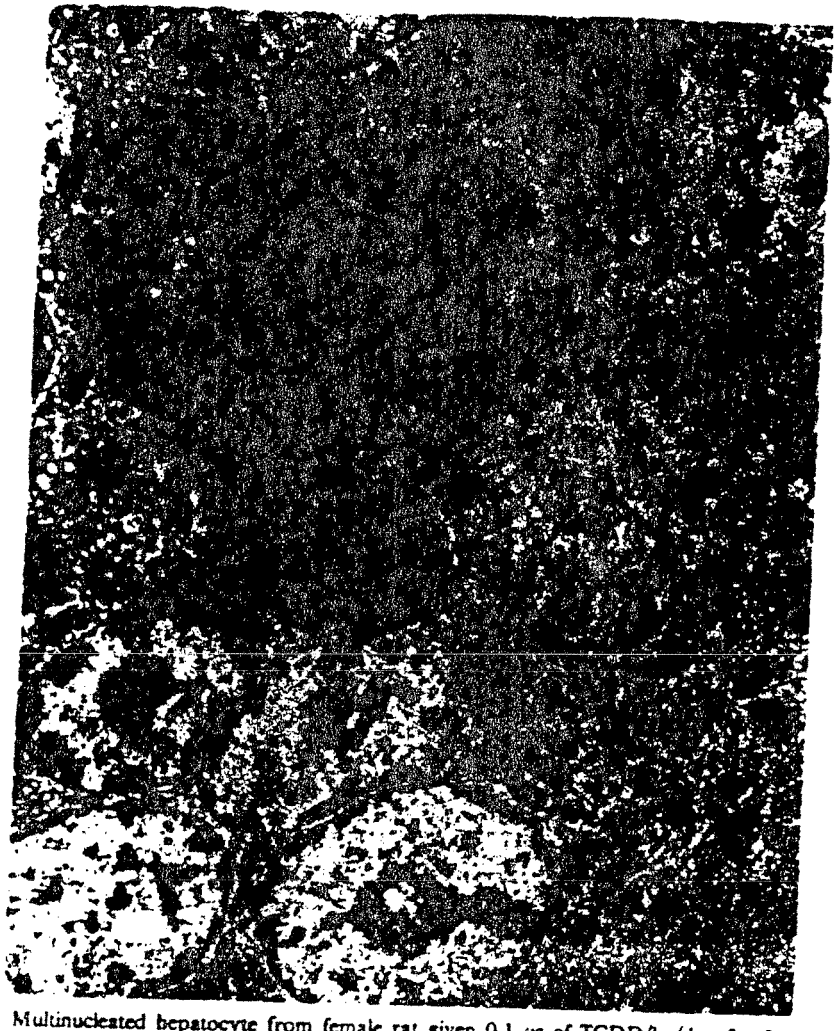

FIG. 3. Multinucleated hepatocyte from female rat given 0.1 μg of TCDD/kg/day for 2 years. Note focal area of cytoplasmic degeneration and distribution of RER. Uranyl acetate–lead citrate stain. x 1620.

The hepatocytes of female rats given 0.001 μg/kg/day were ultrastructurally within the limits of variation seen in the controls (Fig. 5). There was no general increase in the lipid droplet content, but an occasional cell contained increased numbers of lipid droplets.

Lymphoreticular tissues. Treatment-related effects, noted only in females of the 0.1-μg kg/day dose level, included isolated occurrences of thymic atrophy and/or splenic atrophy.

Respiratory system. Treatment-related effects were noted in both males and females at the 0.1-μg/kg/day dose level but were much more extensive in the female rats and included an increased incidence of focal alveolar hyperplasia (Fig. 6), aggregates of hematogenous pigment in lung and thoracic lymph nodes, focal

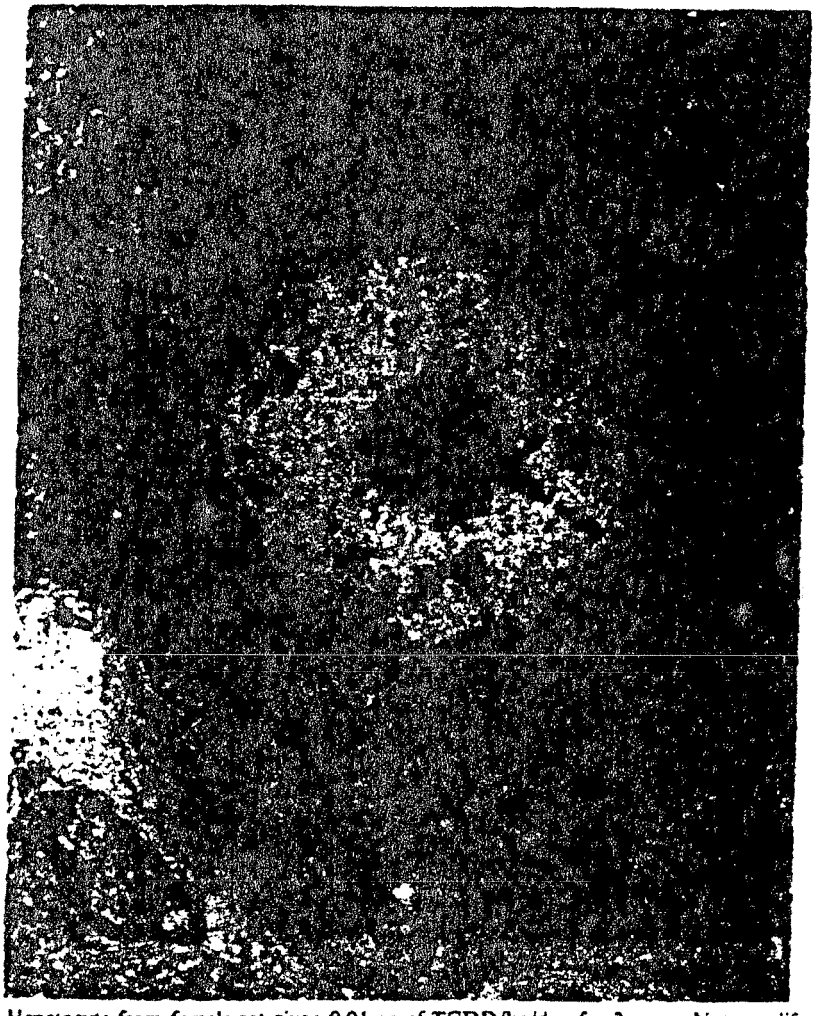

FIG. 4. Hepatocyte from female rat given 0.01 μg of TCDD/kg/day for 2 years. Note proliferation of SER and disorientation of RER. Uranyl acetate–lead citrate stain. ×2070.

accumulation of alveolar macrophages and cholesterol clefts, pulmonary edema, focal interstitial inflammation and fibrosis, keratinizing squamous metaplasia, or squamous cell carcinoma formation (Fig. 7) within the lung. Focal alveolar hyperplasia was also increased in females given 0.01 μg/kg/day. The lower dose level of 0.001 μg/kg/day had no discernible effect on the tissues of the respiratory system.

Cardiovascular system. Effects probably related to the ingestion of 0.1 μg/kg/day included an apparent increase in the incidence of hemorrhage in the brain and possibly

spinal cord of females, an increase above the background incidence rate of mesenteric/thoracic periarteritis with accompanying changes, such as thrombosis and hematoma formations in both males and females, and an increase above the background incidence of myocardial degenerative changes (females only). At the 0.01-

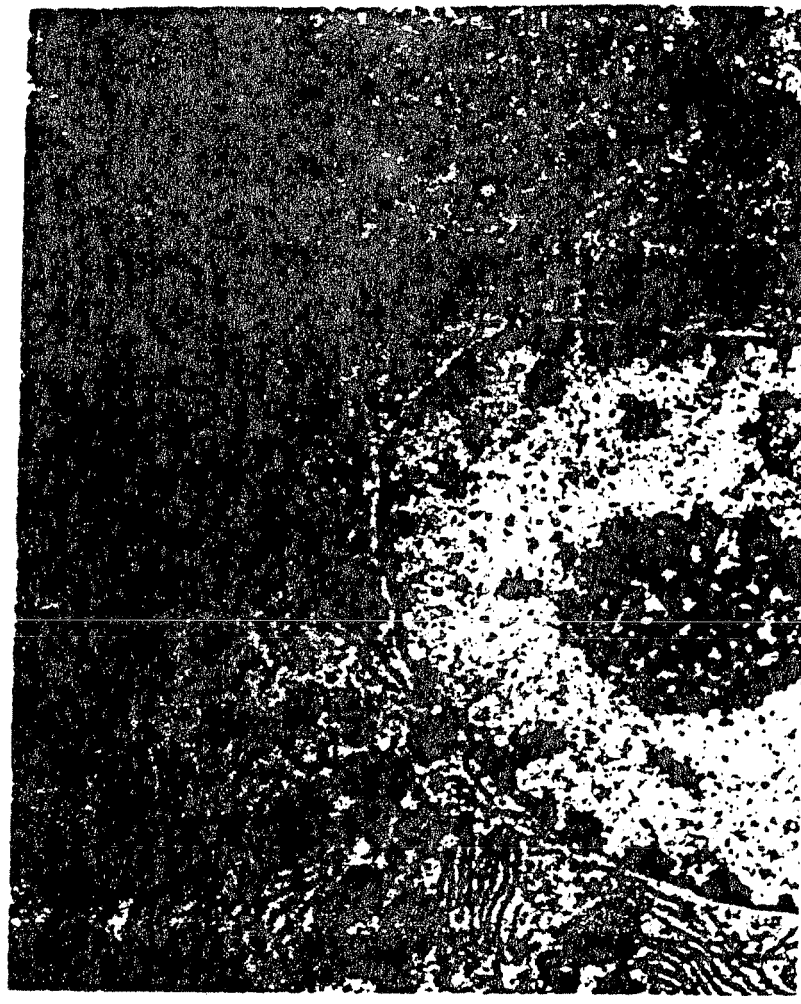

FIG. 5. Hepatocyte from female rat given 0.001 μg of TCDD/kg/day for 2 years. Morphology within normal limits of variation seen in controls. Uranyl acetate–lead citrate stain. ×4100.

μg/kg/day dose level, probable treatment-related lesions were limited to an increase above background incidence of periarteritis and thrombosis of testicular or thoracic/mediastinal vessels of male rats. There were no alterations considered related to treatment with 0.001 μg/kg/day.

Reproductive system and mammary gland. Female rats given 0.1 μg/kg/day had a statistically decreased incidence of uterine changes, including endometrial hyperplasia,

cyst formation, and adenomatous polyp formation. This same group of high dose level female rats also had a significantly decreased incidence of subcutaneous mammary tumors. These observations correlated well with a decreased incidence of pituitary

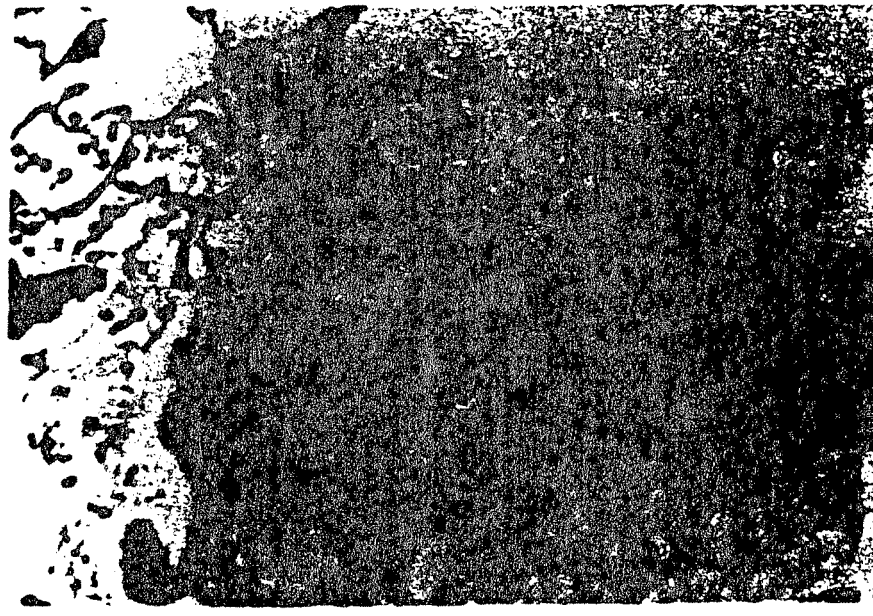

FIG. 6. Focal alveolar hyperplasia near terminal bronchiole within lung of rat given 0.1 μg of TCDD/kg/day. H & E stain. x 100.

FIG. 7. Lesion within lung of rat given 0.1 μg of TCDD/kg/day classified morphologically as squamous cell carcinoma. Note accumulation of keratinized material within lesion. H & E stain. x 100.

adenomas noted in this same high dose level of female rats. There were no discernible effects in female rats given 0.01 or 0.001 μg/kg/day.

The reproductive organs of male rats appeared to be unaffected by these dose levels, with similar degenerative, inflammatory, and proliferative lesions in all treated and control groups.

Endocrine organs. Female but not male rats given 0.1 μg/kg/day had a significantly decreased incidence of pituitary changes, including hemangiectasis and adenoma formation. Adrenal changes noted at this high dose level included a decreased incidence of medullary hyperplastic nodule formation (males and females), a decreased incidence of pheochromocytoma formation (males), an increased incidence of cortical necrosis and hemorrhage (females), and an increased incidence of adrenal hematocyst formation (males). A statistical increase in the incidence of adrenal cortical adenomas noted for males given 0.1 μg/kg/day may have been the result of normal biological variation of the incidence of this tumor, which does occur spontaneously in this strain of rat (approximate 10% incidence in the control group of female rats used in this study).

The pancreas of male rats given 0.1 μg/kg/day had a statistical decrease in the incidence of acinar adenoma formation. A statistically increased incidence of fibrosis of atrophic pancreatic tissue noted in the group of females given 0.1 μg/kg/day may or may not have been associated with the increased incidence of periarteritis noted in this group. The thyroid glands of the high dose group of male rats appeared to have a low incidence of various follicular changes that may or may not have been related to treatment; this included isolated cases of follicular cyst or microcyst formation, follicular adenoma, or follicular adenocarcinoma formation. The parathyroid gland was unaffected by treatment, except for a *decrease* in secondary parathyroid hyperplasia as a result of the decrease in severity of chronic renal disease of the males given 0.1 μg/kg/day.

Gastrointestinal system. A wide variety of degenerative or inflammatory lesions occurred in all control and treated groups, with no indications of a direct treatment-related effect in the salivary glands, esophagus, stomach, small intestine, or large intestine. However, the group of male rats given 0.1 μg/kg/day had a statistical increase above background incidence of stratified squamous cell carcinomas of the tongue, which were considered to be probably related to treatment. There was also a statistically significant increase in the incidence of squamous cell carcinomas of the hard palate/nasal turbinate region of male and female rats given 0.1 μg/kg/day. Historically, squamous cell carcinomas of the tongue and hard palate/turbinates have occurred at a spontaneous incidence rate of 1 to 3% in this strain of rat. It appears as if treatment with 0.1 μg/kg/day increased the incidence of this type of neoplasm. A secondary effect of treatment was noted in the stomach only of the high dose group of males, in which there was a decrease in the incidence rate of mineralization of the gastric muscularis and mucosa. This was secondary to the decreased incidence and severity of chronic renal disease and uremia in this high dose group of male rats.

Nervous system. The only observation considered as probably related to treatment was the increased incidence of focal hemorrhage in the brain (and possibly spinal cord) of female rats given 0.1 μg/kg/day. This was described previously in the description of the cardiovascular system. All groups of rats had the expected spectrum of degenerative, inflammatory, and proliferative lesions considered spontaneous in origin.

Urinary system. There appeared to be a *decrease* in the severity of the chronic nephropathy affecting the kidneys of the male rats given 0.1 μg/kg/day. Other degenerative, inflammatory, and proliferative changes occurred in the kidneys or urogenital tract of control or treated groups, with no observations considered related to treatment.

Musculoskeletal system, eye, and miscellaneous tissues. Various degenerative, inflammatory, or proliferative lesions occurred in a scattered pattern in all treated and control groups, with no indication of a treatment-related effect. No toxicologic significance was attached to the statistical decrease in the incidence rate of subcutaneous benign tumors noted in males given 0.001 μg/kg/day.

Organ Weights

Statistically significant differences in terminal organ weights considered related to treatment included (1) an increase in liver weight calculated on an absolute basis (males given 0.1 or 0.01 μg/kg/day, females given 0.1 μg/kg/day) and on a relative basis of liver/body ratio (females given 0.1 or 0.01 μg/kg/day) and (2) a decrease in the absolute weight of the thymus of females given 0.1 μg/kg/day. Additional changes in organ weights were considered to be secondary to decreased body weights due to treatment with 0.1 μg/kg/day.

TCDD Content of Tissues

Results of analysis of samples of fat and liver collected at terminal necropsy of female rats after 2 years of treatment indicated that rats given 0.1 μg/kg/day had an average TCDD content of 8100 ppt in the fat and 24,000 ppt in the liver. Rats given 0.01 μg/kg/day had an average TCDD content of 1700 ppt in the fat and 5100 ppt in the liver. Rats given 0.001 μg/kg/day had an average of 540 ppt of TCDD in the fat and also in the liver.

DISCUSSION

The findings of this chronic toxicity study on TCDD in rats are an extension of the studies of shorter duration reported previously from this laboratory (Kociba *et al.*, 1976). Continuous ingestion of diets containing approximately 2200 ppt of TCDD (0.1 μg of TCDD/kg/day) for 2 years caused multiple toxicologic effects, including increased mortality, decreased body weight gain, slight depression of certain hematologic parameters, increased urinary excretion of porphyrins and δ-ALA, increased serum activities of AP, GGT, and SGPT, and morphological changes primarily of the hepatic, lymphoid, respiratory, and vascular tissues of the body. This high dose level of 0.1 μg/kg/day also caused an increase in the incidence of hepatocellular carcinomas of the liver (females only) and squamous cell carcinomas of the lung, hard palate/nasal turbinates, or tongue. The occurrence of numerous age-related lesions usually encountered in this strain of rat, including tumors of the pituitary, uterus, mammary gland, pancreas, and adrenal gland was reduced at the high dose level. Also reduced was the incidence and severity of chronic renal disease in the aged male rats. Female rats given this high dose level for 2 years had 24,000 ppt present in the liver. This compares with 34,600 ppt of TCDD present in the liver of female rats given this

same dose level for 13 weeks (Kociba *et al.*, 1976) and indicates steady state concentrations were achieved during the early phase of this 2-year study.

Ingestion of the intermediate dose level of 0.01 μg/kg/day (~210 ppt in the diet) caused a lesser degree of toxicity. The primary effects noted at this dose level included (1) increased urinary excretion of porphyrins (females), (2) liver toxicity, including an increased incidence of hepatocellular nodules, and (3) increased incidence of focal alveolar hyperplasia in the lungs. Terminal liver and fat content of TCDD averaged 5100, and 1700 ppt, respectively. This compares with 3700 ppt present in the liver after 13 weeks of treatment with this dose level (Kociba *et al.*, 1976).

Lifetime ingestion of 0.001 μg/kg/day (~22 ppt in the diet) caused no effects considered to be of any toxicological significance. Light microscopy of livers from females of this group indicated a statistical increase above the background incidence of swollen hepatocytes; conversely, the livers of the males of the group had a decreased incidence of this observation. When liver tissue was examined using electron microscopy, the hepatocytes from the females were within the limits of variation seen in the controls with an occasional hepatocyte containing increased lipid droplets. The liver and fat each contained 540 ppt of TCDD at the end of the lifetime ingestion of 0.001 μg/kg/day.

If the results of this lifetime study in rats are compared to the preliminary results of the study in rats by Van Miller *et al.* (1977), it will be noted that both studies report neoplastic responses in the lung and liver of rats maintained for extended periods of time on high doses of TCDD. In the preliminary report by Van Miller *et al.* (1977), 5000 ppt produced both liver and lung neoplasms, while in this study, 2200 ppt produced both liver and lung neoplasms.

Thus, there is agreement between the results obtained in both studies at higher dose levels of 2200 to 5000 ppt of TCDD in the diet. However, at lower dose levels, there are differences in the two studies, with Van Miller *et al.* (1977) reporting a diverse spectrum of neoplasms in rats given as low as 5 ppt of TCDD, based on a zero incidence of neoplasms in a total of 50 control rats examined in their study. Conversely, in this study, there was no carcinogenic response in rats given 210 or 22 ppt of TCDD for 2 years. DiGiovanni *et al.* (1977) reported TCDD to be only a weak tumor initiator in studies of mouse skin carcinogenesis with DMBA.

In summary, data collected in the study reported herein indicate that doses sufficient to induce severe toxicity increased the incidence of some types of neoplasms in rats, while reducing the incidence of other types. No increase in neoplasms occurred in rats receiving sufficient TCDD during the 2-year study to induce slight or no manifestations of toxicity.

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