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2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD): RESULTS OF A 13-WEEK ORAL TOXICITY STUDY IN RATS[1]

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Running Title: TOXICITY OF TCDD IN RATS

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ABSTRACT

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD): Results of a 13-Week Oral Toxicity Study in Rats. KOCIBA, R. J., KEELER, P. A., PARK, C. N. and GEHRING, P. J. (1975). Toxicol. Appl. Pharmacol. , - . Rats were given 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) at dose levels of 0, 0.001, 0.01, 0.1 or 1.0 µg/kg 5 days/week for 13 weeks. Doses of 1.0 µg TCDD/kg/day caused some mortality, inactivity, decreased body weights and food consumption, icterus, increased serum bilirubin and alkaline phosphatase, pathomorphologic changes in the liver, lymphoid depletion of the thymus and other lymphoid organs, increased urinary excretion of porphyrins and delta-aminolevulinic acid, and minimal alterations of some hematopoietic components. Morphological evidence of a functional suppression of the reproductive organs was consistent with either a direct toxicological effect of this dose of TCDD, or an indirect toxicological effect associated with the poor physical condition of these rats. Doses of 0.1 µg TCDD/kg/day caused decreased body weights and food consumption, and slight degrees of liver degeneration and lymphoid depletion. Other effects seen only in females given this dose level included increases in urinary excretion of coproporphyrin and delta-aminolevulinic acid and increases in serum alkaline phosphatase and bilirubin. Effects seen only in males

given this dose level included a depression of some hematologic parameters (packed cell volume, red blood cells and hemoglobin). In rats given 0.01 or 0.001 µg TCDD/kg/day, all parameters were essentially unaffected, except for a slight increase in the mean liver-to-body-weight ratio in rats given 0.01 µg TCDD/kg/day. This slight increase in relative liver weight was not considered of any toxicological significance. These data indicate that no discernible ill effects occurred in rats given 0.01 or 0.001 µg TCDD/kg 5 days/week for 13 weeks.

INTRODUCTION

The compound 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a highly toxic impurity that may be formed during the production of 2,4,5-trichlorophenol prior to subsequent manufacture into the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). TCDD may be one of the causes of chloracne which has been associated with the industrial production of 2,4,5-trichlorophenol.

Most of the recent interest in TCDD began when Courtney et al. (1970a) described teratogenic effects in mice treated with 2,4,5-T containing about 30 ppm of TCDD. This and subsequent teratological studies in mice (Courtney et al., 1970b; Courtney and Moore, 1971; Hart and Valerio, 1972; Roll, 1971; Neubert and Dillmann, 1972; Bage et al., 1973) have shown that higher doses of 2,4,5-T containing from 0.02 to 30 ppm TCDD could affect prenatal development in mice, specifically causing cleft palate and in some cases an effect on the kidney structure. The incidence of cleft palate in mice was significantly increased only at high dose levels of 2,4,5-T. In some experiments these high dose levels caused the death of many fetuses and dams (Roll, 1971). As determined by various investigators, the experimental no-adverse-effect dose level in several strains of mice ranged from 20 to 50 mg 2,4,5-T/kg/day.

Other species in which 2,4,5-T has been tested for teratogenic potential include the rat (Courtney et al., 1970a; Courtney et al., 1970; Courtney and Moore, 1971; Khera and McKinley, 1972; Emerson et al., 1971; Sparschu et al., 1971a; Stotzer and Niggeschulze, 1970 and 1971; Hall, 1972; Sjoden and Soderberg, 1972), the hamster (Collins et al., 1971), the rabbit (Emerson et al., 1971), the monkey (Wilson, 1971; Dougherty et al., 1973), sheep (Binns and Balls, 1971) and reindeer in late gestation only (Erne et al., 1972). In most of these studies no skeletal deformities were noted. In two instances - one a rat study (Khera and McKinley, 1972) and the other a hamster study (Collins et al., 1971) the investigators observed delayed ossification of bones among litters delivered by Caesarean section. However, when the litters (rat) were delivered normally, the offspring were not impaired in their development.

TCDD has been studied for its effects on embryo and fetal development in the mouse (Courtney et al., 1970b; Courtney and Moore, 1971; Neubert and Dillmann, 1972) and in the rat (Courtney et al., 1970b; Courtney and Moore, 1971; Sparschu et al., 1971b; Khera and Ruddick, 1973). In mice, cleft palate occurred, with some strains more susceptible than others. In rats, cleft palate was not observed even though maternal and fetal toxicity was observed. The experimental no-adverse-effect dose level was 0.03 (3 ppb) µg TCDD/kg/day in rats.

3 ppb

Combinations of 2,4,5-T and TCDD have been given to pregnant rats (Sparschu et al., 1971c) and mice (Neubert and Dillmann, 1971). Based on the results of these studies, it appears that the effects of the TCDD contaminant of 2,4,5-T will be discernible only when the concentration of TCDD exceeds 10 ppm. As now manufactured, 2,4,5-T contains less than 0.1 ppm TCDD.

In addition to teratological studies, a few toxicological studies have been conducted with TCDD in which a single dose or a few repeated doses have been administered to experimental animals. In a paper on the toxicological properties of various chlorinated dioxins, Schwetz et al. (1973) reported the oral LD50 of TCDD to be 22 µg/kg and 45 µg/kg for male and female rats, respectively. In male guinea pigs the oral LD50 was reported to be 0.6 - 2.1 µg/kg and in rabbits of mixed sex the oral LD50 was reported to be 115 µg/kg.

Vos et al. (1974) recently reported an oral LD50 of 114 µg TCDD/kg in C57/Bl/6 male mice. Six weekly doses of 0.2, 1.0, 5.0 or 25 µg/kg produced liver changes discernible by microscopic examination, suggesting that in mice the liver is most sensitive to TCDD. Harris et al. (1973) studied some biological effects of TCDD in rats, guinea pigs and mice given single or a few repeated oral doses. The thymus appeared to be the most sensitive organ as indicated by its reduced weight.

In rats, Buu-Hoi et al. (1972a, 1972b) reported the main target organs of a material referred to as "dioxin" were the liver, thymus and heart. Gupta et al. (1973) reported the main target organs to be the liver and thymus of rats and the thymus of guinea pigs and mice. TCDD caused atrophy of the thymus in all 3 species. Degenerative, necrotic and regenerative changes, including multinucleated giant hepatocytes were observed in the livers of rats given up to 31 daily doses of 10 µg TCDD/kg. Multinucleated hepatocytes were also observed in rats examined 60 days after a single oral administration of 100 µg TCDD/kg (Greig et al., 1973).

Zinkl et al. (1973) reported alterations in some clinical chemistry values and hematological measurements in rats, mice and guinea pigs given up to 31 daily doses of TCDD. They concluded that the liver and hematopoietic system were major sites of TCDD toxicity. Weisberg and Zinkl (1973) reported female rats given 10 or 14 daily doses of 10 µg TCDD/kg had elevated packed cell volumes and erythrocyte counts, probably due to dehydration and consequent hemoconcentration. They also reported a depression of blood platelets, but the megakaryocytes in the bone marrow were not diminished. Clot retraction was reduced but prothrombin consumption times were increased.

Ultrastructure

Liver cells of rats given a single oral dose of 5 or 25 µg TCDD/ kg have been examined sequentially for ultrastructural changes by Fowler et al. (1973). By day 3 after treatment with either dose, increased amounts of smooth endoplasmic reticulum were noted. Later this was accompanied by massive increases in the amount of rough endoplasmic reticulum. By day 28, most liver cells of treated rats were indistinguishable from controls.

Consistent with the ultrastructural changes, Lucier et al. (1973), Poland and Glover (1973a and 1974) and Greig and DeMatteis (1973) reported stimulation of some hepatic microsomal enzymes by TCDD. TCDD had been reported to be an inducer of delta-aminolevulinic acid synthetase in the chick embryo liver (Poland and Glover, 1973a, 1973b and 1973c) but not in the rat liver (Woods, 1973).

The experiment reported here was designed to assess the biologic response associated with repeated doses of low levels of TCDD. TCDD was routinely administered by gavage daily Monday through Friday of each week for 13 weeks. In rats, it had been demonstrated that this regimen is sufficient to allow attainment of steady state amounts of TCDD in the tissues and body (Rose et al., 1974).

METHODS

Groups of 12 young adult Sprague-Dawley (Spartan substrain) rats/sex were given 0.0, 0.001, 0.01, 0.1 or 1.0 µg TCDD/kg of body weight via gavage 5 days/week for 13 weeks. The TCDD[2] was dissolved in acetone-corn oil, and the concentrations of TCDD in each dosing solution was confirmed by gas chromatography-mass spectrometry (Crummett and Stehl, 1973). Similar solutions prepared with ^{14}C-TCDD were stable with no evidence of either degradation or settling. The rats were housed in suspended wire-bottomed cages with food[3] and water available ad libitum. Five rats/sex/group were killed after 13 weeks treatment and the remainder are being held for post-treatment observation. This report includes data from the 13-week treatment period and initial 13-week post-treatment period.

All rats were observed daily for evidence of changes in demeanor and appearance. Body weights were recorded semi-weekly during treatment and weekly thereafter. Food consumption was recorded semi-weekly the first month and weekly thereafter. Blood samples for hematological determinations were collected from 4 or 5 rats per group after 36 to 37 and 85 to 86 days of treatment and 59 to 60 days after cessation of treatment. When fewer than 4 rats were available, blood samples were collected

from the survivors. Routine methods were used to determine total erythrocyte counts (RBC), total and differential leukocyte counts (WBC), thrombocyte and reticulocyte counts, packed cell volume (PCV) and hemoglobin (Hgb) concentration. After 26 and 85 to 86 days of the treatment period and 59 to 60 days after cessation of the treatment, urinalyses were conducted on 4 to 5 rats/group (or survivors, if fewer). Urinary specific gravity, pH, and the presence of sugar, protein, bilirubin, ketones and occult blood were evaluated. Total 48-hour urine samples were collected from randomly selected rats of each group on days 85 to 87 and 87 to 89 of the treatment period for males and females, respectively. This was repeated after cessation of treatment on days 52 to 54 or 54 to 56. Determinations of creatinine, uroporphyrin, coproporphyrin and delta-aminolevulinic acid (delta-ALA) in the samples were made by a consulting laboratory.[4]

All rats which died during the study were subjected to a pathological examination. At 92 days after initiating treatment, five rats per sex per group were killed by decapitation, collecting the blood for clinical chemistry determinations. A complete gross pathologic examination was conducted. After examining the eyes by gently pressing a glass microscope slide against the cornea, the eyes were

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removed and fixed in Zenker's fixative. The thymus, spleen, liver, kidneys, brain, heart and testes (male) were removed and weighed. For electron microscopic examination, selected portions of liver were cut into approximately 1 mm cubes and placed in 2.5% glutaraldehyde fixative. Electron microscopic examinations are being conducted by J. Fulfs and R. Abraham, Albany Medical College, Albany, New York.

For histopathological examination, portions of the following were collected routinely and fixed in buffered 10% formalin: integument, salivary glands, brain, spinal cord, aorta, lungs, thoracic lymph nodes, thymus, heart, heart valves, liver, kidney, adrenal, thyroid, parathyroid, trachea, esophagus, stomach, small intestine, large intestine, Peyer's patches, mesenteric lymph nodes, pancreas, testes, epididymis, accessory sex glands, uterus and ovaries, sciatic nerve, skeletal muscle, urinary bladder, spleen, pituitary gland, and any tissue having a discernible gross lesion.

All of the tissues listed above and the eyes were embedded in paraffin, sectioned and stained with hematoxylin and eosin. Sections of heart valves were prepared from rats of the control and top dose groups only. Additional sections of livers of all rats were cut with a freezing microtome and

were stained with Oil Red O to reveal the lipid content. Additional sections of the pituitary gland of female rats randomly selected from the control and the top dose groups were stained using the Glenner-Lillie procedure (1957).

For all rats, the blood obtained upon decapitation was allowed to clot, centrifuged and the serum collected and analyzed for glutamic-pyruvic transaminase (SGPT) and alkaline phosphatase (AP) activity, urea nitrogen (BUN), and total, direct and indirect bilirubin content. In addition, samples of heparinized blood were collected via orbital bleeding from 2-3 male rats per group 5 days after the last day of treatment. The ammonia content of the whole blood was determined with Conway diffusion plates and an Ammonia Specific Ion Electrode.

Selected samples of liver collected at time of necropsy examination following 13 weeks of treatment were subsequently analyzed for TCDD content. The gas chromatography-mass spectrometry method of Crummett and Stehl (1973) was used for these determinations.

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Data were statistically analyzed using analyses of variance (time, sex and dose) followed by Dunnett's test for specific dose-control comparisons (Steel and Torrie, 1960). For

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parameters which had multiple observations across time, a repeated measures analysis of variance followed by Dunnett's test was used. Increased variance was also used as an indication of statistical significance. A value of $P \leq 0.05$ was chosen for all analyses. The data have been tabulated separately by sex and time although statistical significance was generally determined on the basis of combined sexes and time periods. This was done to increase the sensitivity of the statistical tests.

RESULTS

Clinical Observations

Over the duration of treatment, male and female rats given the highest dose of 1.0 µg TCDD/kg/day, appeared to be less active but this became less apparent during the post-treatment phase. Icterus, indicated by a yellow discoloration of the external pinnae, tail and conjunctivae was noticed in some of the rats in this group both during and following treatment. Body weights (Figures 1 and 2) and food consumption (Table 1) of male and female rats given 1.0 or 0.1 µg TCDD/kg/day were significantly depressed relative to controls. There was no evidence of a dose-response effect at lower doses.

Spontaneous Deaths

Of the rats given 1.0 µg TCDD/kg/day, 4 females died during treatment and 2 females and 2 males died from 14 to 49 days after cessation of treatment (Table 2). Lesions found upon examination of these rats but not in rats which were killed after 13 weeks of treatment included aortic thrombosis and adrenal hemorrhage in one rat and anemia in another rat, PCV of 17% and Hgb of 6.3 gm/100 ml. Only one death occurred in rats given lower doses of TCDD and this death was attributed to accidental perforation of the esophagus.

Hematology (Table 3)

Significant differences between male and female rats existed in the hematologic parameters, so results will be presented separately for each sex. The PCV, RBC and Hgb concentrations were significantly decreased only in males given 1.0 or 0.1 µg TCDD/kg/day and elevated only for females given 1.0 µg TCDD/ kg/day. Reticulocyte counts were significantly increased only in males and females given 1.0 µg TCDD/kg/day.

Thrombocyte counts were significantly decreased in male and female rats given 1.0 µg TCDD/kg/day. A trend toward lower thrombocyte counts, not statistically significant, was seen in female rats given 0.1 µg TCDD/kg/day. Thrombocyte counts in the lower dose groups were comparable to controls.

Female rats given 1.0 µg TCDD/kg/day showed significantly increased WBC counts while males given the same dose level showed a slight trend toward lower WBC counts. WBC counts in all remaining dose groups were comparable to controls. No deviations considered related to treatment were found in the differential WBC counts of male or female rats treated with all these dose levels of TCDD.

Urinary Analyses

At all sampling times, the specific gravity, pH, and the content of sugar, protein, ketones, occult blood and bilirubin

from treated and control animals were considered within normal limits.

Urinary Excretion of Creatinine, Coproporphyrin, Uroporphyrin and delta-ALA (Table 4).

Where male-female differences existed, statistical evaluation was done separately for each sex.

No significant differences were noted in total urine volume. Excretion of creatinine was significantly decreased only for male and female rats given 1.0 µg TCDD/kg/day. Corproporphyrin excretion was significantly increased only in males given 1.0 µg TCDD/kg/day and in females given 1.0 or 0.1 µg TCDD/kg/day. Uroporphyrin excretion was significantly increased only for male and female rats given 1.0 µg TCDD/kg/day. Delta-ALA excretion was significantly increased only in males given 1.0 µg TCDD/kg/day and in females given 1.0 or 0.1 µg TCDD/kg/day.

Clinical Chemistry Values (Tables 5 and 6)

Total and direct bilirubin levels were significantly increased only in males given 1.0 µg TCDD/kg/day and in females given 1.0 or 0.1 µg TCDD/kg/day. The indirect bilirubin level was increased only in one male of the group given 1.0 µg TCDD/kg/day; this increase was statistically significant.

No statistically significant dose-related differences were found in SGPT, although some inconsistently lowered values were noted. There was no evidence of a dose-response relationship. BUN was statistically increased in males given 1.0 µg TCDD/kg/day; this increase was not considered to be toxicologically significant. Serum AP was significantly increased in males given 1.0 µg TCDD/kg/day and in females given 1.0 or 0.1 µg TCDD/kg/day. No evidence of dose-related effects was seen at lower dose levels.

Determination of ammonia in the blood of male rats 5 days after cessation of treatment with TCDD revealed normal levels.

Terminal Organs Weights (Table 7)

The absolute weights of the brain, heart and kidney were significantly decreased and the organ weight:body weight ratios of the brain, spleen and testes were significantly increased in the group of rats given 1.0 µg TCDD/kg/day. Relative kidney:body weight ratios were slightly, but not significantly increased in rats given 1.0 µg TCDD/kg/day. These organ weight changes may be attributed to the depression of body weight in rats treated with this toxic dosage level.

Both the absolute weight of the thymus and its weight relative to body weight were significantly decreased only in rats given 1.0 or 0.1 µg TCDD/kg/day.

Absolute liver weights were significantly increased in rats given 0.1 µg TCDD/kg/day and in females but not males given 1.0 µg TCDD/kg/day. On a relative basis, liver:body weight ratios were significantly increased for rats given 1.0, 0.1 or 0.01 µg TCDD/ kg/day.

Gross and Microscopic Examination of Tissues

Gross examination revealed thymuses of both sexes of rats given 1.0 µg TCDD/kg/day to be reduced in size. A slight reduction in the size of the thymus was also observed in 3 of 5 male rats given 0.1 µg TCDD/kg/day. Microscopic examination revealed almost complete involution of the cortical region of the thymus of rats given 1.0 µg TCDD/kg/day (Figure 3). This was due to a pronounced decrease in cortical thymocytes. The number of cortical thymocytes was slightly decreased in both sexes of rats given 0.1 µg TCDD/kg/day. Other lymphoid tissues of male and female rats given 1.0 µg TCDD/kg/day had a slight to moderate decrease in lymphoid cells. There was a slight but discernible decrease in the lymphoid cells in the lymphoid tissues of male and female rats given 0.1 µg TCDD/kg/ day. Occasional pyknotic

lymphoid cells were noted within the lymphoid tissues of some affected rats. Typically, a pooling of edematous fluid, RBC's, and pigment was present in the lymphoid-depleted areas. The thymus and other lymphoid tissues of both males and females given 0.01 or 0.001 µg TCDD/kg/day were considered within normal limits relative to control rats.

With the exceptions of an occasional pyknotic lymphoid cell, and a very slight increase in extramedullary hematopoiesis in the spleens of some male rats given 1.0 µg TCDD/kg/day, the histological appearance of the spleens was normal.

Upon gross examination of some male and female rats given 1.0 or 0.1 µg TCDD/kg/day, a slightly edematous appearance of the mesentery and mesenteric lymph nodes was noted; sometimes adjacent areas of the gastrointestinal tract also appeared edematous. Histologic examination revealed a pooling of edematous fluid within the affected lymphoid tissues, such as the mesenteric lymph nodes and Peyer's patches. Grossly, edema was also noted in the subcutaneous tissues of 1 of 5 male rats given 1.0 µg TCDD/kg/day. Some male rats given 1.0 µg TCDD/kg/day had a slight focal congestion of gastric blood vessels.

The testes of 1 of 5 male rats and the accessory sex glands of 2 of 5 male rats given 1.0 µg TCDD/kg/day were decreased

in size. Microscopically the 1 rat with testes of decreased size had decreased testicular spermatogenic activity, and 1 epididymis had occlusive stasis of spermatozoa with a decreased content of spermatozoa. Microscopic examination also revealed decreased amounts of secretory material within the accessory sex glands of the 5 males given 1.0 µg TCDD/kg/day and in 1 of 5 male rats given 0.1 µg TCDD/kg/day. The testes, epididymides and accessory sex glands of all other male rats were comparable to those of the controls.

The uteri of 4 of 5 rats given 1.0 µg TCDD/kg/day were lined predominantly by cuboidal epithelium. The diameter of these uteri was reduced and there was a decrease in the size of the uterine glands. In the same rats, the corpora lutea were decreased in number and size. The ovarian stroma of all 5 rats in this group contained interstitial gland cells which had cytoplasmic foaminess and nuclear hyperchromatism (Figure 4A, B). The uteri and ovaries of females given 0.1, 0.01 or 0.001 µg TCDD/kg/day were normal in appearance.

Grossly visible hepatic alterations were limited principally to rats given 1.0 µg TCDD/kg/day. These consisted of diffuse paleness, accentuated lobular patterns, and isolated white pinpoint foci. Icterus was noted in 3 of 5 male and 3 of 5 female rats given 1.0 µg TCDD/kg/day. A slight accentuation

of the hepatic lobular pattern was observed in 1 of 5 male rats given 0.1 µg TCDD/kg/day.

Microscopically, the livers of both male and female rats given 1.0 µg TCDD/kg/day had some hepatic lobules which appeared to be more prominently delineated and slightly to moderately distorted in size and shape. There were wide variations in size and shape of some hepatocytes, with some multinucleated hepatocytes present (Figure 5). Some hepatocytes were undergoing necrosis and were accompanied by focal reticuloendothelial aggregations. Some hepatocytes contained vacuolations or inclusion-like structures, and there were moderate variations in the staining density of hepatocytes. An Oil Red O stain revealed a slight increase in lipid content of the hepatocytes. The hepatic changes appeared to be more pronounced near the periphery of lobules. There was slight hyperplasia of bile ducts and ductular epithelial cells. Aggregations of inflammatory cells were present in portal areas (Figure 6) and near central veins. There was a slight hyperplasia of Kupffer cells and some increased amounts of golden-brown pigment.

The livers of male and female rats given 0.1 µg TCDD/kg/day had a microscopically visible slight distortion of some hepatic lobules due to slight variations in size and shape

of hepatocytes. Some hepatocytes had slight cytoplasmic vacuolation and slight to moderate variations in staining density. The livers of male and female rats given 0.01 or 0.001 µg TCDD/kg/day had the same morphology as those of the control group. There were no gross or microscopic changes in the livers from these 2 lower dose groups (0.01 or 0.001 µg TCDD/kg/day) that could be attributed to the toxic effects of TCDD.

Focal aggregations of golden-brown pigment were observed microscopically in the livers of all rats given 1.0 µg TCDD/ kg/day. The pigment was also observed in the kidneys and lungs of some of these rats. The pigment was seen in the lungs of 1 of 5 male rats given 0.1 µg TCDD/kg/day. The pigment was not found in the tissues of other rats used in the study.

No discernible changes were noted upon examination of pituitary glands; however, no attempt was made to quantitate the relative ratios of the different cellular components of the pituitary glands.

Additional morphological observations of doubtful toxicological significance included 1 focus of periarteriolar mineralization

in the lung of 1 of 5 male rats given 0.1 µg TCDD/kg/day and a possible thickening of blood vessel walls in the lung of 1 of 5 female rats given 1.0 µg TCDD/kg/day.

Analytical Concentrations of TCDD in Livers (Table 8)

Combination gas chromatographic-mass spectrometric analyses of livers collected from male and female rats given 1.0 µg TCDD/kg/day Monday through Friday for 13 weeks revealed mean concentrations of 0.3240±0.0541 and 0.2840±0.0207 µg TCDD/g liver (wet weight), respectively. Mean concentrations of TCDD in livers from male and female rats given 0.1 µg TCDD/kg/day were 0.0360±0.0044 and 0.0346±0.0038 µg TCDD/g liver (wet weight). Mean concentrations of TCDD in livers of male and female rats given 0.01 µg TCDD/kg/day were 0.0026±0.0006 and 0.0037±0.0004 µg TCDD/g liver (wet weight).

DISCUSSION

Generally, the findings in this study of rats given daily oral doses of TCDD, Monday through Friday for 13 weeks are consistent with those of previously reported studies of shorter duration. Higher doses than used in this study produced inactivity and depression of body weights (Greig et al., 1973; Harris et al., 1973; Vos et al., 1974). In our study, depression of body weights was noted in rats given 1.0 or 0.1 µg TCDD/kg/day. Associated with the depression of body weight was decreased consumption of food. A persistent decrease in food consumption has been reported by Greig et al. (1973) in rats given a single oral dose of 200 µg TCDD/kg.

An unexpected finding was greater lethality in female than male rats given repeated daily oral doses of 1.0 µg TCDD/kg/day. The single oral dose LD50 of TCDD in male and female rats is 22 µg/kg and 45 µg/kg, respectively (Schwetz et al., 1973). Two male and 2 female rats died 14 to 49 days after daily administration of 1.0 µg TCDD/kg/day had been discontinued. This is consistent with the persistence of the compound in the body as reported previously (Piper et al., 1973; Rose et al., 1974).

Pathological changes in the rats that died spontaneously were observed consistently in liver, thymus, and other lymphoid tissues. In addition, the isolated observations of aortic thrombosis, adrenal hemorrhage and anemia suggest that 1.0 µg TCDD/kg/day had an untoward effect on the hematopoietic system in this study. Evidence for an effect of TCDD on the hematopoietic system was also noted upon hematologic examination of rats given 1.0 or 0.1 µg TCDD/kg/day. Thrombocyte counts were decreased in male and female rats given 1.0 µg TCDD/kg/day. In male rats given 1.0 or 0.1 µg TCDD/kg/ day, the PCV, RBC and Hgb concentrations were decreased. In females given 1.0 µg TCDD/kg/day, PCV, RBC, Hgb and WBC values were increased overall; however, after cessation of treatment, most of these parameters tended to be decreased. Although these trends in the female rats are difficult to interpret, they may be due to dehydration and hemoconcentration during treatment superimposed on a depression of hematopoiesis. After cessation of treatment, the depression of hematopoiesis becomes dominant because normal hydration is once again attained. Increased reticulocyte counts found in male and female rats given 1.0 µg TCDD/kg/day indicate that TCDD does not markedly inhibit the production of red blood cells in bone marrow. No significant hematological alterations were observed in rats given 0.01 or 0.001 µg TCDD/kg/ day for 13 weeks.

The hepatic alterations observed in rats given 1.0 µg TCDD/kg/day are in accord with those reported by others (Gupta et al., 1973; Buu-Hoi et al., 1972b; Greig et al., 1973; and Vos et al., 1974). Alterations associated with the effects of this dose level of TCDD on the liver were icterus, increased serum bilirubin, increased serum AP and multiple hepatic pathomorphological alterations, including multinucleated hepatocytes and biliary hyperplasia. Multinucleated hepatocytes have been described as a spontaneous finding in aged mice by Tucker and Baker (1967). Foy et al., (1966) have described multinucleated hepatocytes in pyridine-deficient baboons, and Svoboda et al. (1971) have reported similar findings in marmosets given aflatoxin, especially if in conjunction with infectious viral hepatitis. Thus, multinucleated hepatocytes are not indicative of a specific pathologic response to a chemical stimulus.

The increases in serum AP and direct bilirubin are probably indicative of an impairment, physical or otherwise, of biliary excretion and is consistent with the hepatocellular changes and biliary hyperplasia observed microscopically. Hwang (1973) has reported that TCDD inhibits the hepatobiliary excretion of indocyanine green. He suggests that the excretion of other anionic compounds, bilirubin and porphyrins may be inhibited and subsequently lead to jaundice

and porphyria. The lack of increase in SGPT activity was consistent with the minimal degree of frank hepatic necrosis observed in this study. Also associated with the effect of TCDD on the liver was an increased urinary excretion of porphyrins and delta-ALA. Vos et al. (1974) and Goldstein et al. (1973) have reported TCDD to be porphyrogenic in the mouse, but Gupta et al. (1973) and Woods (1973) reported no indication of porphyria in rats and guinea pigs. TCDD has been shown to be a potent inducer of ALA synthetase in chick embryo liver (Poland and Glover, 1973a, 1973b and 1973c).

The morphological changes in livers of rats given 0.1 µg TCDD/ kg/day were of a much lesser degree, and were accompanied by increases in serum AP and bilirubin, and urinary coproporphyrin and delta-ALA in females only.

Liver morphology of rats given 0.01 or 0.001 µg TCDD/kg/day was not discernible from controls, as were all serum and urinary components monitored in this study. In rats given 0.01 µg TCDD/kg/day, 5 days per week for 13 weeks, there was an increase in the liver-to-body weight ratio when compared to the controls. The mean liver weight for this group was not significantly increased when expressed on an absolute basis. In the absence of any discernible histological changes in these livers, this slight increase in the mean

relative liver weight of rats given 0.01 µg TCDD/kg/day for 13 weeks is considered to be a physiological adaptation rather than a toxicological effect. Barka and Popper (1967) list a variety of substances that may cause some increase in size of the liver without pathological alterations. Included are substances foreign to the body, such as drugs, as well as normal constituents of the body such as thyroxine, cortisone, estradiol and testosterone. An increase in size of the liver occurs in rats on diets high in protein (Hurvitz and Freedland, 1968) or carbohydrates (Allen and Leahy, 1966) as well as during pregnancy and lactation, when relative liver weights are increased as much as 50% (Wilson et al., 1970).

In addition to the liver, the thymus and other lymphoid organs (other than the spleen) appeared to be the organs most sensitive to long-term treatment of rats with TCDD. Other workers have also reported lymphoid depletion to be a sensitive indicator of TCDD toxicity (Harris et al., 1973). In view of the report by Vos et al. (1973) showing a correlation between TCDD-induced lymphoid depletion and suppression of cell-mediated immunity, it is important to point out that all lymphoid organs of male and female rats given 0.01 or 0.001 µg TCDD/kg/day were considered normal in this study.

In female rats given 1.0 µg TCDD/kg/day for 13 weeks the ovaries and uteri had morphologic changes which were interpreted as indicative of a suppression or inhibition of the estrus cycle. Decreased spermatogenic activity was noted in the testes of 1 of 5 male rats given this dose level and a decreased amount of secretory material was noted in the accessory sex glands of all male rats given this dose level. These changes observed in the reproductive organs of male and female rats could have been due to the direct toxic effect of TCDD or to the poor physical condition of the rats given this high dose level.

The overall results of this study are interpreted to indicate no discernible ill effects in male and female rats given 0.01 (100 ppb) or 0.001 (1 ppb) µg TCDD/kg 5 days/week for 13 weeks. It has been demonstrated in other studies conducted in our laboratory that steady-state amounts of TCDD are attained in tissues of the body after 13 weeks of treatment with TCDD. Therefore, the untoward effects reported herein are not likely to be intensified by longer durations of administration and further accumulation of TCDD in the tissues (Rose et al., 1974).

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[2] Synthesized by O. Aniline, The Dow Chemical Company, Midland, Michigan

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FIGURE LEGENDS

Figure 1 — Mean Body Weights of Male Rats Given TCDD Daily via Oral Gavage, Monday Through Friday for 13 Weeks

Figure 2 — Mean Body Weights of Female Rats Given TCDD Daily via Oral Gavage, Monday Through Friday for 13 Weeks

Figure 3 — A photomicrograph to show pronounced decrease in number of thymocytes present within cortical region of the thymus of a rat given 1.0 µg TCDD/kg/day 5 days/week for 13 weeks. Hematoxylin and Eosin stain x 125.

Figure 4A, B — A photomicrograph of an ovary of a rat (4A) given 1.0 µg TCDD/kg/day 5 days/week for 13 weeks. The interstitial gland cells of the ovarian stroma show cytoplasmic foaminess and nuclear hyperchromatism. Ovary from control rat (4B) included for comparison. Hematoxylin and Eosin stain x 400.

Figure 5 — A photomicrograph of the liver of a rat given 1.0 µg TCDD/kg/day 5 days/week for 13 weeks. Note the multinucleated hepatocyte and variations in size, shape and tinctorial properties of hepatocytes. Hematoxylin and Eosin stain x 400.

Figure 6 — A photomicrograph of the liver of a rat given 1.0 µg TCDD/kg/day 5 days/week for 13 weeks. Note chronic inflammatory cells in portal region, cholangiolar proliferation and the slight distortion of hepatic lobular pattern. Hematoxylin and Eosin stain x 125.

TABLE 1

MEAN DAILY FOOD CONSUMPTION OF RATS GIVEN TCDD VIA ORAL GAVAGE, MONDAY THROUGH FRIDAY FOR 13 WEEKS[a]

Sex and TCDD Dose µg/kg/day	Respective Days of Treatment							Respective Days After Cessation of Treatment						
	0-4	11-14	28-35	42-49	63-70	78-84	84-91	7-14	21-28	28-35	35-42	42-49	70-77	84-91
M 0.0	25 ±1	30 ±4	25 ±2	26 ±1	25 ±1	25 ±2	25 ±2	28 ±3	28 ±3	32 ±8	31 ±2	29 ±3	30 ±5	29 ±4
M 0.001	26 ±2	28 ±2	27 ±2	26 ±1	26 ±1	27 ±1	25 ±1	27 ±1	27 ±2	28 ±2	28 ±3	27 ±4	27 ±3	28 ±3
M 0.01	26 ±1	30 ±2	28 ±1	26 ±1	26 ±1	26 ±1	26 ±1	27 ±1	27 ±2	29 ±2	26 ±4	30 ±2	27 ±2	26 ±5
M 0.1	24 ±1	27 ±1	25 ±1	24 ±1	24 ±1	24 ±2	24 ±2	25 ±1	25 ±2	26 ±3	25 ±2	26 ±3	26 ±4	26[b] ±2
M 1.0	24 ±1	26 ±2	22 ±2	21 ±3	19 ±4	18 ±5	19 ±5	21 ±5	23 ±2	23 ±4	20 ±7	20 ±12	25 ±2	26[b] ±2
F 0.0	17 ±2	20 ±3	19 ±1	19 ±1	19 ±1	20 ±1	19 ±1	21 ±1	20 ±3	22 ±1	19 ±1	20 ±1	20 ±1	21 ±1
F 0.001	20 ±3	24 ±10	20 ±2	21 ±4	19 ±1	22 ±6	19 ±1	21 ±1	22 ±3	23 ±5	21 ±2	24 ±6	21 ±3	21 ±3
F 0.01	18 ±2	20 ±2	22 ±1	20 ±2	19 ±2	22 ±4	19 ±11	22 ±2	21 ±1	23 ±2	21 ±2	22 ±1	22 ±2	21 ±3
F 0.1	17 ±3	20 ±2	19 ±1	18 ±1	17 ±1	19 ±1	19 ±2	20 ±1	18 ±0.3	19 ±1	18 ±0.1	19 ±1	19 ±2	19[b] ±1
F 1.0[c]	16 ±2	18 ±2	14 ±2	14 ±1	13 ±1	13 ±2	14 ±4	12 ±4	10 ±6	21	21	20	18	17[b]

a. Expressed as grams/rat/day for these time periods, mean ±S.D.

b. Significantly different from control mean, $P<0.05$. To increase the sensitivity, the statistical evaluations have been based on analyses of data combined across sexes and time although the data for each sex and time period are listed separately in this table.

c. Only one rat alive after day 28 of post-treatment period.

TABLE 2

DEATHS IN RATS GIVEN TCDD DAILY, MONDAY THROUGH FRIDAY FOR 13 WEEKS [a]

TCDD Dose Level µg/kg/day	Number of Rats Dying, and Time of Death	
	Males	Females
0	None dead	None dead
0.001	None dead	1 moribund, day 9 of treatment period (accidental perforation of esophagus during oral gavage)
0.01	None dead	None dead
0.1	None dead	None dead
1.0	1 dead, day 20 of post-treatment period 1 dead, day 49 of post-treatment period	1 moribund, day 44 of treatment period 1 dead, day 44 of treatment period 2 dead, day 73 of treatment period 1 dead, day 14 of post-treatment period 1 dead, day 29 of post-treatment period

a. Rats found in a moribund condition were killed.

TABLE 3

MEAN HEMATOLOGICAL VALUES OF RATS GIVEN DAILY ORAL DOSES OF TCDD, MONDAY THROUGH FRIDAY FOR 13 WEEKS [a]

Sex & µg/kg/day	Packed Cell Volume%			Red Blood Cells $10^6/mm^3$			Hemoglobin g/100 ml			Reticulocytes %			Thrombocytes $10^3/mm^3$		
	Days														
	36-37T	85-86T	59-60PT	36-37T	85-86T	59-60PT	36-37T	85-86T	59-60PT	36-37T	85-86T	59-60PT	36-37T	85-86T	59-60PT
M 0.0	53.6 ±2.6	55.2 ±2.2	54.8 ±0.8	9.06 ±0.51	9.20 ±0.44	9.04 ±0.68	17.1 ±0.5	18.1 ±0.5	16.7 ±0.4	1.4 ±0.4	1.7 ±0.5	1.8 ±0.5	956 ±422	1074 ±104	1231 ±218
0.001	53.8 ±1.8	53.5 ±1.3	53.5 ±1.9	8.64 ±0.32	9.24 ±0.13	9.04 ±0.44	17.2 ±0.5	17.7 ±0.5	16.9 ±0.4	1.9 ±0.6	1.8 ±0.3	2.2 ±0.6	1107 ±190	976 ±194	1120 ±195
0.01	53.4 ±1.7	52.6 ±1.5	52.8 ±2.3	8.79 ±0.66	9.12 ±0.13	9.00 ±0.33	17.2 ±0.7	17.2 ±0.2	16.6 ±0.6	2.1 ±0.5	1.7 ±0.9	2.0 ±0.3	1160 ±167	1102 ±98	1233 ±143
0.1	(51.2 ±1.3	51.0 ±2.7	49.8 ±2.3)[b]	(8.86 ±0.48	8.49 ±0.58	8.10 ±1.50)[b]	(16.7 ±1.2	15.7 ±0.7	15.8 ±1.0)[b]	1.6 ±0.5	1.9 ±0.4	1.6 ±0.3	1163 ±237	1001 ±183	1105 ±257
1.0	(47.2 ±2.2	45.8 ±6.6	46.4 ±2.1)[b]	(8.39 ±0.61	8.50 ±1.06	7.77 ±0.92)[b]	(15.3 ±0.7	14.7 ±1.6	14.4 ±0.7)[b]	(2.2 ±0.2	2.5 ±0.7	2.5 ±0.6)[b]	(765 ±218	489 ±167	832 ±171)[b]
F 0.0	49.2 ±0.8	49.0 ±0.7	50.2 ±0.8	8.30 ±0.30	7.89 ±0.35	7.42 ±0.23	15.7 ±0.3	15.8 ±0.7	15.1 ±0.6	1.9 ±0.5	1.7 ±0.6	1.8 ±0.6	1006 ±109	1004 ±106	1089 ±142
0.001	49.8 ±1.5	49.8 ±2.5	51.0 ±2.6	8.30 ±0.27	8.03 ±0.49	7.25 ±0.44	15.5 ±0.7	16.0 ±0.4	15.9 ±0.5	2.1 ±0.3	1.9 ±0.3	1.6 ±0.6	1130 ±137	1046 ±199	1171 ±353
0.01	49.8 ±1.5	50.4 ±3.0	53.2 ±4.7	8.45 ±0.19	8.29 ±0.49	7.58 ±1.05	16.1 ±0.4	16.5 ±0.8	16.6 ±1.2	1.9 ±0.3	2.5 ±0.7	2.6 ±0.6	860 ±148	916 ±214	1212 ±283
0.1	47.8 ±1.9	47.4 ±3.0	49.6 ±3.3	8.11 ±0.62	8.07 ±0.12	7.20 ±0.27	14.9 ±0.5	15.4 ±0.5	15.4 ±0.8	1.9 ±0.4	3.1 ±0.8	1.8 ±0.2	825 ±478	831 ±241	923 ±179
1.0 [c]	(56.0 ±5.8	57.8 ±6.6	44.0)[b]	(9.66 ±1.19	9.69 ±0.81	6.96)[b]	(17.5 ±1.7	17.6 ±2.0	13.2)[b]	(1.8 ±0.8	3.1 ±0.4	2.2)[b]	(535 ±144	608 ±99	715)[b]

a. Blood samples for hematological evaluation were collected after 36 to 37 and 85 to 86 days of treatment, and 59 to 60 days after cessation of treatment. The treatment and post-treatment periods are indicated by T and PT in the table.

b. Significantly different from control mean by Dunnett's test, $P<0.05$. To increase the sensitivity, statistical evaluations have been based on analyses of data combined across time.

c. Only one rat alive at post-treatment bleeding.

TABLE 3 (continued)

MEAN HEMATOLOGICAL VALUES OF RATS GIVEN DAILY ORAL DOSES OF TCDD, MONDAY THROUGH FRIDAY FOR 13 WEEKS [a]

Sex and µg/kg/day	Leukocytes $10^3/mm^3$ Days 36-37T	85-86T	59-60PT	Average Leukocyte Differential Counts % Day 36-37T N	L	M	E	B	Day 85-86T N	L	M	E	B	Day 59-60PT N	L	M	E	B
M 0.0	18.1 ±1.6	20.0 ±2.1	13.6 ±1.6	6.0	92.4	1.0	0.6	0	7.0	89.0	2.6	1.4	0.0	6.6	92.6	0.8	0.0	0
0.001	17.0 ±2.6	21.3 ±5.9	11.9 ±1.8	9.4	89.4	0.2	1.0	0	18.5	78.7	1.8	1.0	0.0	9.7	87.0	1.5	1.8	0
0.01	18.7 ±2.9	17.2 ±1.9	13.9 ±2.2	9.6	89.2	0.2	1.0	0	8.2	89.2	1.8	0.8	0.0	7.2	91.2	1.0	0.6	0
0.1	21.0 ±2.0	22.4 ±5.6	16.4 ±4.7	10.2	88.0	1.6	0.2	0	11.6	86.8	1.6	0.0	0.0	21.4	76.4	1.8	0.4	0
1.0	18.9 ±2.1	16.3 ±1.5	10.4 ±0.6	7.2	92.2	0.6	0.0	0	11.6	87.0	1.2	0.0	0.2	10.2	88.2	0.8	0.8	0
F 0.0	15.9 ±2.9	18.3 ±9.4	10.9 ±1.8	11.8	86.6	0.4	1.2	0	10.4	86.8	1.4	1.4	0.0	10.8	87.2	0.4	1.6	0
0.001	13.9 ±2.6	14.8 ±5.4	10.2 ±1.0	13.7	84.8	0.7	0.8	0	8.8	87.2	2.0	1.7	0.3	8.5	89.2	1.0	1.3	0
0.01	16.8 ±3.5	15.0 ±1.5	12.4 ±1.2	14.8	83.0	0.6	1.6	0	7.0	90.6	1.4	1.0	0.0	9.8	88.6	0.8	0.8	0
0.1	15.3 ±3.5	13.4 ±2.7	9.6 ±1.4	18.6	79.4	1.2	0.8	0	10.8	87.2	1.6	0.4	0.0	10.6	88.6	0.0	0.8	0
1.0 [c]	(24.3 ±3.7	25.2 ±4.3	17.4)[b]	13.8	84.6	1.0	0.2	0	15.2	83.2	1.6	0.0	0.0	2.0	95.0	2.0	1.0	0

N = Neutrophiles, L = Lymphocytes, M = Monocytes, E = Eosinophiles, B = Basophiles.

a Blood samples for hematological evaluation were collected after 36 to 37 and 85 to 86 days of treatment, and 59 to 60 days after cessation of treatment. The treatment and post-treatment periods are indicated by T and PT in the table.

b Significantly different from control mean by Dunnett's test, $P<0.05$. To increase sensitivity, statistical evaluations have been based on analyses of data combined across time.

c Only one rat alive at post-treatment.

TABLE 4

MEAN 48-HOUR URINE VOLUME AND URINARY EXCRETION OF CREATININE, COPROPORPHYRIN, UROPORPHYRIN, AND delta-AMINO-LEVULINIC ACID OF RATS GIVEN TCDD VIA ORAL GAVAGE, MONDAY THROUGH FRIDAY FOR 13 WEEKS [a]

Sex and TCDD Dose µg/kg/day	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		delta-Amino-levulinic Acid mg/48 hr.		mg delta-ALA / mg Creatinine	
	T	PT	T	PT	T	PT	T	PT	T	PT	T	PT	T	PT	T	PT
M 0.0	34.6 ±3.8	34.2 ±7.7	30.8 ±2.6	36.4 ±4.6	20.0 ±6.4	14.1 ±7.2	0.655 ±0.234	0.380 ±0.160	1.9 ±0.8	1.9 ±0.4	0.062 ±0.031	0.053 ±0.014	0.14 ±0.02	0.14 ±0.03	0.0044 ±0.0009	0.0040 ±0.0007
M 0.001	48.8 ±9.6	39.3 ±7.6	32.3 ±1.3	34.5 ±3.3	21.0 ±2.8	8.3 ±3.8	0.654 ±0.110	0.249 ±0.129	2.7 ±1.1	1.4 ±0.6	0.085 ±0.036	0.042 ±0.021	0.14 ±0.03	0.15 ±0.04	0.0045 ±0.0010	0.0045 ±0.0007
M 0.01	44.8 ±3.6	46.4 ±4.4	32.8 ±3.1	40.4 ±2.3	20.8 ±10.3	12.6 ±8.2	0.653 ±0.340	0.314 ±0.199	2.7 ±1.1	2.7 ±0.9	0.085 ±0.035	0.068 ±0.025	0.14 ±0.02	0.12 ±0.02	0.0044 ±0.0005	0.0032 ±0.0003
M 0.1	35.2 ±5.4	41.4 ±3.9	30.0 ±2.8	37.0 ±2.4	23.5 ±12.3	14.6 ±7.8	0.781 ±0.414	0.402 ±0.217	2.6 ±1.2	2.2 ±1.1	0.087 ±0.037	0.061 ±0.032	0.15 ±0.03	0.13 ±0.04	0.0050 ±0.0007	0.0030 ±0.0010
M 1.0	42.4 ±9.7	31.8 ±13.3	(22.2 ±6.7	29.0 ±5.5)[b]	(39.0 ±19.3	27.3 ±10.7)[b]	(1.987 ±1.279	0.910 ±0.429)[b]	(2.2 ±0.1	4.1 ±3.5)[b]	(0.110 ±0.041	0.135 ±0.111)[b]	(0.22 ±0.06	0.15 ±0.04)[b]	(0.0100 ±0.0012	0.0058 ±0.0017)[b]
F 0.0	47.4 ±26.6	45.8 ±12.2	17.0 ±0.0	17.6 ±4.2	9.5 ±1.3	9.1 ±5.3	0.561 ±0.075	0.619 ±0.580	1.6 ±0.2	2.9 ±2.9	0.096 ±0.014	0.217 ±0.291	0.097 ±0.012	0.086 ±0.025	0.0056 ±0.0005	0.0048 ±0.0011
F 0.001	42.0 ±17.5	44.8 ±15.0	15.0 ±7.1	19.0 ±4.7	8.8 ±2.8	9.9 ±5.5	0.665 ±0.240	0.515 ±0.231	1.3 ±0.4	2.5 ±1.8	0.095 ±0.032	0.125 ±0.079	0.100 ±0.030	0.100 ±0.020	0.0080 ±0.0030	0.0055 ±0.0006
F 0.01	40.8 ±4.5	45.6 ±14.5	18.2 ±2.3	13.6 ±6.0	15.7 ±3.6	9.3 ±3.5	0.872 ±0.217	0.987 ±0.900	1.9 ±0.4	2.8 ±1.7	0.104 ±0.017	0.257 ±0.175	0.112 ±0.020	0.094 ±0.017	0.0060 ±0.0010	0.0124 ±0.0154
F 0.1	36.4 ±12.0	32.4 ±6.4	16.2 ±2.4	12.6 ±6.1	(20.9 ±6.2	13.2 ±6.3)[b]	(1.268 ±0.248	1.380 ±0.913)[b]	1.9 ±0.7	1.8 ±1.6	0.113 ±0.033	0.153 ±0.164	(0.143 ±0.005	0.084 ±0.016)[b]	(0.0090 ±0.0010	0.0092 ±0.0078)[b]
F 1.0[c]	45.6 ±23.1	28.0	(12.6 ±1.9	12.0)[b]	(31.6 ±8.5	15.0)[b]	(2.563 ±0.806	1.250)[b]	(2.7 ±0.8	0.8)[b]	(0.221 ±0.072	0.067)[b]	(0.502 ±0.790	0.090)[b]	(0.0420 ±0.0660	0.0080)[b]

a "T" indicates samples were collected after 85 to 87 days (M) and 87 to 89 days (F) of treatment. "PT" indicates samples were collected 52 to 54 days (M) and 54 to 56 days (F) after cessation of treatment.

b Significantly different from control group by Dunnett's test, $P<0.05$. To increase sensitivity, statistical evaluations have been based on analyses of data combined across time (and sex, where applicable).

c Only one female rat alive on days of post-treatment collection.

TABLE 5

MEAN CLINICAL CHEMISTRY VALUES OF RATS GIVEN TCDD DAILY VIA ORAL GAVAGE, MONDAY THROUGH FRIDAY FOR 13 WEEKS

TCDD µg/kg/day	Total Bilirubin mg/100 ml		Direct Bilirubin mg/100 ml		Indirect Bilirubin mg/100 ml		SGPT K units/ml		BUN mg/100 ml		AP, KA Units/100 ml	
	M	F	M	F	M	F	M	F	M	F	M	F
0.0	0.35 ±0.05	0.33 ±0.03	0.26 ±0.04	0.26 ±0.04	0.09 ±0.02	0.07 ±0.03	37.2 ±7.7	35.5 ±7.1	17.2 ±2.5	26.0 ±4.8	18.1 ±4.6	14.7 ±4.4
0.001	0.26 ±0.04	0.38 ±0.13	0.20 ±0.00	0.26 ±0.09	0.06 ±0.04	0.12 ±0.05	41.0 ±8.1	27.7 ±5.6	17.9 ±0.7	23.7 ±3.0	14.9 ±2.2	13.5 ±3.4
0.01	0.31 ±0.09	0.33 ±0.13	0.24 ±0.05	0.28 ±0.13	0.07 ±0.04	0.05 ±0.00	31.6 ±3.0	25.2 ±2.2	16.9 ±1.3	22.8 ±3.5	16.4 ±3.0	15.7 ±4.1
0.1	0.30 ±0.05	0.49[a] ±0.29	0.23 ±0.04	0.42[a] ±0.27	0.07 ±0.03	0.07 ±0.03	24.7 ±3.2	37.2 ±6.2	16.2 ±1.0	23.6 ±3.0	14.7 ±2.6	31.2[a] ±12.7
1.0	2.55[a] ±4.17	1.11[a] ±0.60	2.32[a] ±3.91	1.00[a] ±0.57	0.23[a] ±0.27	0.11 ±0.05	35.6 ±12.9	27.3 ±2.9	23.4[a] ±1.9	22.8 ±2.8	35.4[a] ±17.0	26.8[a] ±8.9

[a]Significantly different from controls by Dunnett's test, $P<0.05$. To increase sensitivity, statistical evaluations have been based on combined Male-Female data except where pooling of the data was not appropriate.

SGPT = Serum Glutamic Pyruvic Transaminase; BUN = Blood Urea Nitrogen, AP = Alkaline Phosphatase
K Units = Karmen units/ml; KA Units = King-Armstrong units/100 ml.

TABLE 6

BLOOD AMMONIA LEVELS OF MALE RATS 5 DAYS AFTER CESSATION OF DAILY ORAL ADMINISTRATION OF TCDD, MONDAY THROUGH FRIDAY FOR 13 WEEKS

Dose, TCDD µg/kg/day	µg NH_3/ml Whole Blood	
0		3.9
0		2.4
	Mean ± S.D.	3.2 ±1.1
0.001		3.1
0.001		3.7
	Mean ± S.D.	3.4 ±0.4
0.01		3.2
0.01		4.8
0.01		7.7
	Mean ± S.D.	5.2 ±2.3
0.1		4.1
0.1		3.7
	Mean ± S.D.	3.9 ±2.3
1.0		3.2
1.0		3.8
	Mean ± S.D.	3.5 ±0.4

No statistically significant differences were found between treated and control groups by Dunnett's test, $P<0.05$.

TABLE 7

FINAL MEAN BODY WEIGHTS AND ORGAN WEIGHTS OF RATS GIVEN TCDD DAILY VIA ORAL GAVAGE, MONDAY THROUGH FRIDAY FOR 13 WEEKS

Dose TCDD µg/kg/day	Sex	Body wt.(g)	Brain		Heart		Liver		Kidney		Spleen		Thymus		Testes	
			g	g/100 g	g	g/100 g	g	g/100 g	g	g/100 g	g	g/100 g	g	g/100 g	g	g/100 g
0	M	438 ±25	1.84 ±0.05	0.42 ±0.03	1.36 ±0.11	0.31 ±0.01	12.47 ±1.20	2.84 ±0.14	3.02 ±0.22	0.69 ±0.03	0.85 ±0.05	0.20 ±0.01	0.52 ±0.16	0.12 ±0.03	3.97 ±0.30	0.91 ±0.08
0.001	M	463 ±15	1.78 ±0.06	0.39 ±0.02	1.39 ±0.11	0.30 ±0.02	13.59 ±1.13	2.93 ±0.17	3.25 ±0.36	0.70 ±0.05	0.86 ±0.09	0.19 ±0.02	0.51 ±0.09	0.11 ±0.02	3.95 ±0.45	0.85 ±0.10
0.01	M	476 ±29	1.85 ±0.05	0.39 ±0.03	1.47 ±0.06	0.31 ±0.01	14.14 ±1.49	2.97[a] ±0.17	3.26 ±0.24	0.68 ±0.02	0.98 ±0.12	0.19 ±0.02	0.50 ±0.08	0.10 ±0.01	3.78 ±0.24	0.79 ±0.05
0.1	M	450 ±26	1.78 ±0.04	0.40 ±0.03	1.36 ±0.16	0.30 ±0.03	15.65[a] ±1.69	3.47[a] ±0.20	2.94 ±0.31	0.65 ±0.06	0.83 ±0.14	0.18 ±0.03	0.41[a] ±0.10	0.09[a] ±0.02	3.90 ±0.30	0.87 ±0.08
1.0	M	297[a] ±48	1.65[a] ±0.10	0.56[a] ±0.07	0.97[a] ±0.14	0.33 ±0.01	11.57 ±3.22	3.83[a] ±0.56	2.29[a] ±0.31	0.77 ±0.04	0.73 ±0.08	0.25[a] ±0.04	0.13[a] ±0.04	0.05[a] ±0.01	3.37 ±0.89	1.12[a] ±0.18
0	F	262 ±23	1.71 ±0.08	0.66 ±0.07	0.88 ±0.05	0.34 ±0.04	6.43 ±0.62	2.45 ±0.07	1.78 ±0.13	0.68 ±0.06	0.65 ±0.12	0.24 ±0.03	0.40 ±0.01	0.15 ±0.02		
0.001	F	266 ±14	1.73 ±0.08	0.65 ±0.02	0.85 ±0.05	0.32 ±0.02	6.63 ±0.31	2.50 ±0.08	1.85 ±0.18	0.69 ±0.04	0.60 ±0.13	0.22 ±0.05	0.37 ±0.07	0.14 ±0.02		
0.01	F	259 ±15	1.69 ±0.06	0.65 ±0.04	0.89 ±0.08	0.34 ±0.01	6.88 ±0.38	2.66[a] ±0.07	1.87 ±0.16	0.72 ±0.05	0.64 ±0.10	0.25 ±0.04	0.34 ±0.05	0.13 ±0.02		
0.1	F	250 ±18	1.68 ±0.04	0.67 ±0.06	0.83 ±0.02	0.33 ±0.02	8.15[a] ±0.59	3.26[a] ±0.13	1.89 ±0.19	0.75 ±0.07	0.56 ±0.04	0.23 ±0.02	0.23[a] ±0.05	0.09[a] ±0.02		
1.0	F	211[a] ±17	1.57[a] ±0.09	0.74[a] ±0.07	0.71[a] ±0.05	0.34 ±0.04	7.46[a] ±0.44	3.53[a] ±0.11	1.51[a] ±0.11	0.72 ±0.06	0.58 ±0.08	0.27[a] ±0.05	0.09[a] ±0.01	0.04[a] ±0.01		

All values listed as mean ± S.D.

a Significantly different from control mean by Dunnett's test, $P<0.05$. To increase sensitivity, the statistical evaluations have been based on analyses of combined male and female data (except absolute liver weights at the top dose); all data are presented separately for each sex in this table.

TABLE 8

Concentrations of TCDD in Livers of Rats Given TCDD daily Via Oral Gavage, Monday Through Friday for 13 Weeks

Dose Level of TCDD	Sex	µg TCDD/g Liver		(Mean + S.D.
1.0 µg/kg/day	M	0.38	380ppb	0.3240
	M	0.29		±0.0541
1ppm	M	0.31		
	M	0.38		
	M	0.26		
	F	0.27		0.2840
	F	0.30		±0.0207
	F	0.26		
	F	0.28		
	F	0.31		
0.1 µg/kg/day	M	0.041	41ppb	0.0360
	M	0.038		±0.0044
	M	0.036		
	M	0.029		
	M	0.036		
	F	0.036		0.0346
	F	0.040		±0.0038
	F	0.035		
	F	0.031		
	F	0.031		
0.01 µg/kg/day	M	0.0028	3.8ppb	0.0026
	M	0.0026		±0.0006
	M	0.0030		
	M	0.0031		
	M	0.0017		
	F	0.0034		0.0037
	F	0.0033		±0.0004
	F	0.0043		
	F	0.0039		
	F	0.0038		