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Three-Generation Reproduction Study of Rats Given 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD) in the Diet

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Three-Generation Reproduction Study of Rats Given 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin in the Diet. MURRAY, F. J., SMITH, F. A., NITSCHKE, K. D., HUMISTON, C. G., KOCIBA, R. J., AND SCHWETZ, B. A. (1979). *Toxicol. Appl. Pharmacol.* 50, 241–252. A three-generation reproduction study was conducted to evaluate the effects of chronic, low-level ingestion of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Sprague–Dawley rats were maintained continuously on diets providing dose levels of 0, 0.001, 0.01, or 0.1 μg TCDD/kg/day. No significant toxicity was noted in the f_0 rats of either sex during the 90 days of TCDD ingestion prior to mating. Significant decreases in fertility and neonatal survival were observed in the f_0 generation rats receiving 0.1 μg TCDD/kg/day; these effects precluded continuation of this high dose level in subsequent generations. At 0.01 μg TCDD/kg/day, fertility was significantly decreased in the f_1 and f_2 but not f_0 generations. Other indications of toxicity seen at 0.01 μg TCDD/kg/day included decreases in litter size at birth, gestation survival (proportion of pups born alive), and neonatal survival and growth. Among the rats receiving 0.001 μg TCDD/kg/day, no effect on fertility, litter size at birth, or postnatal body weight was observed in any generation. No consistent effect on neonatal survival was observed at 0.001 μg TCDD/kg/day. In summary, the reproductive capacity of rats ingesting TCDD was clearly affected at dose levels of 0.01 and 0.1 μg TCDD/kg/day, but not at 0.001 μg TCDD/kg/day, through three successive generations.

e compound 2,3,7,8-tetrachlorodibenzo-ioxin (TCDD) may be found as a low-l contaminant in products made from 5-trichlorophenol, such as the herbicide 5-trichlorophenoxyacetic acid. Toxico-cal studies in laboratory animals indicate TCDD is extremely toxic when given as ngle oral dose (Schwetz *et al.*, 1973; Vos ., 1974; McConnell *et al.*, 1978) or when n repeatedly (Kociba *et al.*, 1976, 1978; n *et al.*, 1977; Van Miller and Allen,).

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TCDD has been shown to be teratogenic in several strains of mice. Courtney and Moore (1971) noted increased incidences of cleft palate and kidney abnormalities among the litters of mice given 3 μg TCDD/kg/day subcutaneously. Similar results were obtained in mice given oral doses of 1 to 3 μg TCDD/kg/day (Neubert and Dillman, 1972; Moore *et al.*, 1973; Smith *et al.*, 1976). No evidence of teratogenicity was observed in mice given 0.1 μg TCDD/kg/day (Smith *et al.*, 1976). In rats, TCDD was more fetotoxic than teratogenic, producing intestinal hemorrhage and edema (Sparschu *et al.*, 1971), kidney

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abnormalities (Courtney and Moore, 1971), and internal hemorrhages (Khera and Ruddick, 1972).

The postnatal effects of exposure to TCDD also have been investigated. Moore *et al.* (1973) reported a high incidence of hydronephrosis in the pups of female mice given a single dose of TCDD on the day of parturition; presumably, the pups were exposed to TCDD by it's presence in the dam's milk.

To assess the postnatal effects of prenatal exposure to TCDD, Khera and Ruddick (1972) gave 0, 0.125, 0.25, 0.5, or 1.0 μg TCDD/kg/day to pregnant Wistar rats from Days 6 to 15 of gestation. Dose-related decreases in the average litter size and pup weight at birth were noted at all but the lowest dose level. The survival of the pups to 21 days of age was significantly decreased at 0.5 μg TCDD/kg/day, and there were no surviving pups at 1.0 μg TCDD/kg/day. Upon mating the offspring of the dams given TCDD, decreases in the incidence of pregnancy and in the average litter size were observed at 0.5 μg TCDD/kg/day.

The purpose of the study presented herein is to evaluate the effects of TCDD on the reproductive capacity of rats given the compound continuously throughout three generations. For this study, groups of male and female rats were maintained on diets supplying levels of 0, 0.001, 0.01, or 0.1 μg TCDD/kg/day.

METHODS

Test material. The test sample of TCDD used for this study was prepared by The Dow Chemical Company, Midland, Michigan. The purity of the test material was greater than 99% as determined by electron capture gas–liquid chromatography.

Animals. Male and female Sprague–Dawley rats (Spartan substrain),[2] 6–7 weeks of age were randomly placed in suspended wire-bottom cages (two per cage). The rats were allowed laboratory chow[3] and tap water *ad libitum.*

[2] Spartan Research Animals, Haslett, Mich.

[3] Purina Laboratory Chow, Ralston–Purina Company, St. Louis, Mo.

Experimental design. Throughout three generatio the test animals were continuously maintained diets containing sufficient TCDD to provide dc levels of 0, 0.001, 0.01, or 0.1 μg TCDD/kg/day. T f_0 rats were randomized into test groups consisting 16 males and 32 females each (control and high do groups) or 10 males and 20 females each (low a intermediate dose groups). The f_0 rats were placed the test diets at about 7 weeks of age. After 90 days the test diets, the f_0 rats were mated to produce t f_{1a} litters. Thirty-three days after the last f_{1a} litte were weaned, the f_0 rats were remated to produce t f_{1b} litters. Some of the f_0 rats were mated for a thir time in a cross-mating study, as described in a lat section. The f_{1a} and f_{1b} rats were mated at an averag age of 130 days to produce the f_2 and f_1 litters, respec tively. The rats which served as parents for thes generations were selected by a table of rando numbers.

Preparation of test diets. The test diets were pre pared by the procedure described by Kociba *et al.* (1978). The concentration of TCDD in the diet wa adjusted as required by body weight and food con sumption determinations in order to maintain th designated dosages on a microgram per kilogram pe day basis. The concentration of TCDD in the tes diets was confirmed by gas chromatography–mas spectrometry after extraction and suitable clean-up

Mating procedure. For the initial mating of the f_0 rats, each male was placed with two females from the same dose group for a period of 15 days (three estrous cycles). Because the incidence of pregnancy following the initial mating of the f_0 rats was unusually low in all groups, including the controls, the f_0 rats were mated again approximately 1 month after the last f_{1a} litters were weaned. The mating procedure was altered in an effort to improve the overall pregnancy rate. Each female was placed with a male for two 6-day mating periods, each with a different male, separated by a 6-day resting period. The pan beneath the cage was examined each morning for the presence of a vaginal plug. This mating procedure was used for all subsequent matings with the exception of the cross-mating study.

During the mating period, animals of both sexes were maintained on diets containing TCDD at concentrations calculated for the females. After the mating period, males were returned to their original cages and given the TCDD-containing diets as prepared for the males. The females were placed in individual cages containing ground corn cob litter for nesting. A screen was placed in the bottom of each cage at the end of the first week of lactation. The females continued to receive the test diets throughout gestation and lactation.

Observations. For each generation, body weights of the male and female rats were recorded weekly until

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the time of mating. The amount of food consumed was recorded at 3- to 4-day intervals until the time of mating. After mating, the females were observed daily for signs of normal and abnormal parturition. The date of parturition was recorded, and the number of days from the first cohabitation to parturition was calculated. The numbers of live and dead newborn pups were recorded on the day of parturition (Day 0). The number of live pups was noted on Days 1, 7, 14, and 21 after delivery. Each litter was weighed on Days 1, 7, and 14. On Day 21, individual body weights of the weanlings were recorded.

Cross-Mating study. After receiving the test diets for 12 months, the f_0 rats were cross-mated with younger, untreated rats as follows.

Males		Females
Control	×	Untreated
0.1 μg TCDD/kg/day	×	Untreated
Untreated	×	Control
Untreated	×	0.1 μg TCDD/kg/day
Untreated	×	Untreated
Control	×	Control

Each male was placed with a single female for 10 days. Vaginal smears were examined daily for the presence of sperm during the period of mating. Females were sacrificed by carbon dioxide inhalation 20 or 21 days after sperm were found in the vaginal smear. Those females which did not exhibit sperm in the vaginal smear were sacrificed 21 days after the first day of cohabitation. The uterine horns were exteriorized through a midline incision in the abdominal wall, and the number and position of live, dead, and resorbed fetuses were noted. The uterus of each apparently nonpregnant female was stained with a 10% solution of sodium sulfide and examined for evidence of implantation sites (Kopf, 1964); this procedure was conducted solely for the purpose of determining the incidence of pregnancy (not the incidence of resorptions).

Gross necropsy and histopathology of weanlings. A gross pathologic examination was conducted on the weanlings which were not selected as future parents. The liver, kidneys, and thymus of four to six 21-day-old pups per sex per dose level from the f_{1b}, f_2, and f_3 litters were weighed; histologic examination of these tissues was conducted on paraffin-embedded sections of tissues which were stained with hematoxylin and eosin.

Statistical evaluation. Statistical evaluation of the indexes of reproduction, such as fertility and survival indexes, was made by the Fisher exact probability test (Siegel, 1956). Analysis of the incidence of alterations among the weanlings was made by the Wilcoxon test as modified by Haseman and Hoel (1974). Analyses of the body weights and food consumptions were made by a one-way analysis of variance and Dunnett's test (Steel and Torrie, 1960). The level of significance chosen in all cases was $p < 0.05$.

RESULTS

Dietary Content of TCDD

During the 90-day period prior to mating of the f_0 rats, the concentrations of TCDD in the diet which were required to provide dose levels of 0.001, 0.01, and 0.1 μg TCDD/kg/day ranged between 12–20, 120–215, 1125–2075 ppt, respectively. After the initial 90 days on test, the concentration of TCDD in the diets ranged between 16–30, 150–290, and 2000–2500 ppt, respectively. Repeated analyses of the diets indicated good agreement between the intended concentration of TCDD and the analytical concentration.

Clinical Observations

Ingestion of the diets containing TCDD did not significantly alter the physical appearance, body weight, or food consumption of the f_0 rats during the 90 days prior to mating. During the mating period, one high-dose female, which exhibited pneumonic consolidation of the lungs, died, but no evidence of clinical toxicity was seen among any of the other f_0 rats.

In subsequent generations, the high-dose group (0.1 μg TCDD/kg/day) was discontinued since few females from this group delivered offspring. No effect was observed on the appearance, body weight, or food consumption of the three surviving pups (all males from the same litter) at the high dose. At the intermediate dose, 0.01 μg TCDD/kg/day, however, the body weights of the f_1 and f_2 rats (both sexes) were consistently lower than those of the control rats, and a trend toward a decrease in food consumption was noted. One f_2 male receiving 0.01 μg TCDD/kg/day died during the mating period. At the low dose, 0.001 μg TCDD/kg/day, no effect on appearance, body weight, or food consumption was discerned.

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TABLE 1

FERTILITY INDEX (FI) OF RATS GIVEN 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD)

	μg TCDD/kg/day							
	0		0.001		0.01		0.1	
Generation	FI[a]	%	FI	%	FI	%	FI	%
f_0								
First mating	14/32	44	10/20	50	12/20	60	3/31[b]	10
Second mating	21/32	66	14/19	74	15/20	75	1/30[b]	3
f_1	22/26	85	15/17	88	12/21[b]	57		
f_2	28/32	88	20/20	100	11/20[b]	55		

[a] Fertility Index (FI) = number of females delivering a litter/number of females placed with a male.

[b] Significantly different from the control value by Fisher's exact probability test, $p < 0.05$.

Fertility

Fertility was significantly reduced among the rats given 0.1 μg TCDD/kg/day (Table 1). After the initial mating of the f_0 rats, only 3 of 31 females receiving the high dose delivered a litter; one of these three dams died 11 days after parturition, with compression atelectasis of the lungs. Upon remating, the lowered fertility of the 0.1 μg TCDD/kg/day f_0 rats was even more pronounced, with only 1 of 30 females delivering offspring.

At 0.01 μg TCDD/kg/day, no effect on fertility was discerned among the f_0 rats, but a significant reduction in fertility was observed among the f_1 and f_2 rats (Table 1). Among the rats receiving 0.001 μg TCDD/kg/day, the fertility index was not significantly different from that of the control rats in any generation.

Compared to the control group, the copulatory index (the percentage of females exhibiting a vaginal plug in the pan beneath the cage) was significantly decreased at the high dose. However, this decrease in copulatory activity was not of sufficient magnitude to fully explain the decreased fertility noted at that dose. No effect on the copulatory index was observed among the low or intermediate dose groups.

The average number of days from cohabitation to delivery was not significantly altered by ingestion of 0.1 μg TCDD/kg/day in the diet (Table 2). However, at 0.01 μg TCDD/kg/day, significant increases in the average number of days from cohabitation to parturition were noted among the f_1 and f_2 rats but not among the f_0 rats. Ingestion of 0.001 μg TCDD/kg/day did not alter the period from cohabitation to delivery in any generation.

TABLE 2

AVERAGE NUMBER OF DAYS BETWEEN FIRST COHABITATION AND PARTURITION AMONG RATS GIVEN 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD)

	μg TCDD/kg/day			
Generation	0	0.001	0.01	0.1
f_0				
First mating	26±5[a]	25±2	26±3	26±1
Second mating	26±3	27±3	26±4	31
f_1	25±1	25±2	27±4[b]	
f_2	25±2	25±3	28±4[b]	

[a] Mean ± SD.

[b] Significantly different from the control value by Dunnett's test, $p < 0.05$.

Litter Size at Birth

Compared to the control value, the average number of pups (live and dead) per litter at birth was significantly decreased among the few f_{1a} litters delivered at 0.1 μg TCDD/kg/day (Table 3). Similarly, the size of the single f_{1b} litter from the animal re-

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TABLE 3

AVERAGE NUMBER OF PUPS PER LITTER AT BIRTH AMONG RATS GIVEN 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD)

	μg TCDD/kg/day			
Generation	0	0.001	0.01	0.1
f_{1a}	8±4[a]	10±3	8±4	4±1[b]
f_{1b}	11±4	10±3	10±4	6[c]
f_2	11±3	11±4	8±3[b]	
f_3	11±3	11±4	9±4[b]	

[a] Mean number of pups (live and dead) ± SD.
[b] Significantly different from the control value by Dunnett's test, $p < 0.05$.
[c] One litter only.

ceiving the high dose also was smaller than that of the control group. At 0.01 μg TCDD/kg/day, statistically significant decreases in litter size were noted among the f_2 and f_3 litters but not among either set of f_1 litters. At 0.001 μg TCDD/kg/day, no effect on litter size at birth was observed in any generation.

Gestation Survival

Among the offspring of rats given 0.1 μg TCDD/kg/day, the gestation survival index (the percentage of pups alive at birth) was significantly lower than that of the control pups; in fact, all of the pups were stillborn in each of the three f_{1a} litters delivered (Table 4). In the single f_{1b} litter delivered at that dose, one of six pups was stillborn. At 0.01 μg TCDD/kg/day, statistically significant decreases in the gestation survival index were noted among the f_2 and f_3 pups but not among the f_{1a} or f_{1b} pups. At 0.001 μg TCDD/kg/day, the gestation survival index was significantly lower than the control value in the f_2 generation, but not in earlier or later generations.

Postnatal Survival

The postnatal survival index (the percentage of liveborn pups surviving to 21 days of age) for the single 0.1 μg TCDD/kg/day litter was comparable to that of the control group (Table 5). Ingestion of 0.01 μg TCDD/kg/day resulted in statistically significant decreases in the postnatal survival index among the f_{1a} and f_2 pups but not among the f_{1b} or f_3 pups. At 0.001 μg TCDD/kg/day, the postnatal survival index among the f_{1a} and f_{1b} litters was significantly decreased and increased, respectively; in subsequent generations, no effect on the postnatal survival index was seen at this dose.

TABLE 4

GENERATION SURVIVAL INDEX (GSI) AMONG LITTERS OF RATS GIVEN 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD)

	μg TCDD/kg/day							
	0		0.001		0.01		0.1	
Generation	GSI[a]	%	GSI	%	GSI	%	GSI	%
f_{1a}	106/111	95	87/97	90	93/103	90	0/7[b,c]	0
f_{1b}	215/225	96	120/135	89	138/144	96	5/6	83
f_2	235/244	96	148/166[c]	89	87/101[c]	86		
f_3	296/318	93	208/224	93	83/100[c]	83		

[a] Gestation Survival Index (GSI) = the proportion of newborn pups which were alive at birth.
[b] Seven dead pups were observed among two of the three litters delivered by animals receiving the high dose; in the case of the third litter, it was recorded that "all" of the pups were dead at birth, but the exact number of dead pups was not recorded.
[c] Significantly different from the control value by Fisher's exact probability test, $p < 0.05$.

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TABLE 5

POSTNATAL SURVIVAL INDEX (PSI) AMONG LITTERS OF RATS GIVEN 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD)

	μg TCDD/kg/day							
	0		0.001		0.01		0.01	
Generation	PSI[a]	%	PSI	%	PSI	%	PSI	%
f_{1a}	99/106	93	73/87[b]	84	63/93[b]	68	—[c]	—[c]
f_{1b}	160/215	74	110/120[b]	92	93/138	71	4/5	80
f_2	205/235	87	126/148	85	51/87[b]	59		
f_3	235/296	79	163/208	78	64/83	77		

[a] Postnatal survival Index (PSI) = number of liveborn pups surviving to 21 days of age/number of liveborn pups.
[b] Significantly different from the control value by Fisher's exact probability test, $p < 0.05$.
[c] No liveborn pups.

TABLE 6

POSTNATAL BODY WEIGHT AMONG THE OFFSPRING OF RATS GIVEN 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD)

		μg TCDD/kg/day			
Generation	Day	0	0.001	0.01	0.1
f_{1a}	1	8±1[a]	7±1	7±1	—[b]
	7	17±3	14±2	15±2	—
	14	32±5	27±5	30±5	—
	21-M	49±12	45±6	46±8	—
	21-F	47±8	44±6	43±6	—
f_{1b}	1	7±1	7±1	7±1	6
	7	15±2	16±2	14±3	15
	14	29±3	28±4	28±4	33
	21-M	46±9	43±6	46±9	50
	21-F	43±7	43±7	44±8	51
f_2	1	7±1	7±1	6±1[c]	
	7	15±2	14±3	14±2	
	14	26±4	25±5	24±5	
	21-M	40±8	40±9	37±8	
	21-F	36±6	39±8	36±8	
f_3	1	7±1	8+2	6±1	
	7	14±2	16±5	12±2	
	14	28±6	29±8	22±5[c]	
	21-M	43±8	45±14	37±7	
	21-F	42±8	41±7	34±6[c]	

[a] Mean of litter means ± SD (g).
[b] No liveborn offspring.
[c] Significantly different from the control value by Dunnett's test, $p < 0.05$.

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Postnatal Body Weight

The average body weight of the pups of rats given 0.1 μg TCDD/kg/day was not significantly different from that of the control pups (Table 6). At 0.01 μg TCDD/kg/day, significant decreases in postnatal body weight were observed among the f_2 and f_3 litters but not among the f_1 litters. At 0.001 μg TCDD/kg/day, no effect on postantal body weight was detected in any generation.

The body weight of the dams during the period of lactation was not significantly affected by the ingestion of any of the TCDD-containing diets.

Necropsy

A gross necropsy was performed on the 21-day-old pups that were not saved as parents for the next generation. The few pups from animals receiving the high dose level which survived to 21 days of age were not sacrificed and were allowed to survive to adulthood. Upon necropsy as adulte, dilated renal pelvis was seen in all three rats which survived at that dose; in one case, a subcapsular cyst in the kidney also was observed. No other abnormalities were noted in these rats.

Necropsy of the 21-day-old pups revealed a statistically significant increase in the incidence of slightly dilated renal pelvis at 0.001 μg TCDD/kg/day in the f_1 generation but not in subsequent generations. At the intermediate dose, 0.01 μg TCDD/kg/day, the incidence of slightly dilated renal pelvis was not significantly different from the incidence in the control group in any generation. No other alteration occurred significantly more often among the pups of rats receiving TCDD than among the control pups.

Organ Weights and Histopathology

Organ weights of 21-day-old pups were not obtained at the high dose or at the intermediate dose in the f_2 generation due to a lack of available pups. A significant decrease in the average thymus weight (absolute and relative) was seen at 0.01 μg TCDD/kg/day in the f_3 generation but not the f_1 generation (Table 7). Also, the relative liver weight of the 0.01 μg TCDD/kg/day male weanlings was significantly greater than that of the controls in the f_3 but not the f_1 generation; a similar trend was noted among the females. No effect on the average weight of the kidneys was discerned at 0.01 μg TCDD/kg/day. At 0.001 μg TCDD/kg/day, organ weights were not altered significantly, but a trend toward an increase in relative liver weight was observed. Light microscopic examination of the thymus, liver, and kidneys of the weanlings revealed no histopathological alterations considered to be related to the ingestion of TCDD.

Cross-mating Study

To ascertain whether one sex was preferentially affected by TCDD, male and female f_0 rats (control and high dose groups) were cross-mated with younger untreated rats of the opposite sex. The incidence of pregnancy among the untreated females bred with the f_0 males which received the high dose was not significantly different from that of untreated females bred with f_0 control males (Table 8); in fact, 60% of the untreated females were pregnant when bred with the same males receiving the high dose which earlier had produced fertility indexes of only 10 and 3% when mated with the high-dose f_0 females. Among the f_0 females, the incidence of pregnancy at the high dose was slightly greater than that of the controls. However, the incidence of pregnancy among both the control and high-dose females was extremely low due probably to their advanced age. The number of implantations per dam among the cross-mated high-dose males or females was not significantly different from their respective controls. No effect on the incidence of resorptions was discerned among the cross-mated high-dose males. However, among the cross-mated females receiving the high dose, there was a statistically significant increase in the incidence of resorptions compared to the control

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TABLE 7

ORGAN WEIGHTS OF 21-DAY-OLD OFFSPRING OF RATS GIVEN 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD)

Generation	Sex[a]	μg/TCDD kg/day	*n*	Body weight (g)	Thymus		Liver		Kidney	
					g	g/100 g	g	g/100 g	g	g/100 g
f_1	M	0	5	58±10[b]	0.23±0.02	0.41±0.07	3.04±0.89	5.17±0.64	0.67±0.13	1.16±0.11
	M	0.001	5	51±5	0.20±0.04	0.38±0.06	2.92±0.35	5.72±0.46	0.61±0.07	1.20±0.10
	M	0.01	5	49±4	0.18±0.05	0.36±0.07	2.75±0.41	5.58±0.39	0.59±0.08	1.21±0.11
	F	0	5	49±9	0.22±0.07	0.45±0.14	2.65±0.63	5.36±0.42	0.60±0.16	1.22±0.10
	F	0.001	5	45±7	0.17±0.05	0.37±0.06	2.39±0.18	5.38±0.53	0.55±0.06	1.23±0.09
	F	0.01	5	47±6	0.18±0.04	0.37±0.06	2.67±0.25	5.67±0.47	0.57±0.08	1.20±0.05
f_2	M	0	5	36±8	0.12±0.04	0.32±0.08	1.82±0.38	5.11±0.47	0.41±0.08	1.17±0.11
	M	0.001	5	41±9	0.15±0.06	0.35±0.09	2.40±0.71	5.79±0.60	0.52±0.15	1.25±0.11
	M	0.01	0[c]	—	—	—	—	—	—	—
	F	0	5	36±6	0.14±0.04	0.37±0.06	1.99±0.41	5.46±0.53	0.47±0.06	1.31±0.09
	F	0.001	5	42±10	0.13±0.06	0.31±0.10	2.54±0.66	6.00±0.59	0.52±0.15	1.21±0.11
	F	0.01	0[c]	—	—	—	—	—	—	—
f_3	M	0	5	42±5	0.19±0.01	0.45±0.06	1.96±0.36	4.60±0.29	0.47±0.07	1.11±0.06
	M	0.001	5	42±11	0.19±0.06	0.43±0.04	2.26±0.47	5.39±0.47	0.50±0.10	1.19±0.12
	M	0.01	4[d]	30±9	0.08±0.02[e]	0.26±0.02[e]	1.72±0.53	5.96±1.04[e]	0.38±0.08	1.34±0.24
	F	0	5	41±8	0.18±0.04	0.45±0.07	1.96±0.63	4.73±0.60	0.47±0.12	1.14±0.08
	F	0.001	5	42±8	0.18±0.03	0.42±0.04	2.42±0.53	5.69±0.46	0.54±0.10	1.28±0.08
	F	0.01	5	30±9	0.07±0.03[e]	0.25±0.11[e]	1.65±0.54	5.72±1.38	0.37±0.09	1.31±0.29

[a] M, males; F, females.
[b] Mean±SD.
[c] Due to a shortage of weanlings at 0.01 μg TCDD/kg/day in the f_2 generation, organ weights were not obtained.
[d] At 0.01 μg TCDD/kg/day, only females were available for necropsy in the fifth litter selected at random.
[e] Significantly different from the control value by Dunnett's test, $p < 0.05$.

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TABLE 8

RESULTS OF THE CROSS-MATING OF THE f_0 RATS GIVEN 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD)[a]

Experimental group			Percentage pregnant	Implants/dam[b]	Percentage implants resorbed
Male		Female			
Control	×	Untreated	58 (11/19)	9±3	10 (10/100)
TCDD	×	Untreated	60 (12/20)	10±4	9 (11/123)
Untreated	×	Control	5 (1/20)	13	15 (2/13)
Untreated	×	TCDD	15 (3/20)	7±3	70 (14/20)[c]
Untreated	×	Untreated	90 (18/20)	12±2	13 (28/219)
Control	×	Control	30 (3/10)[c]	7±7[d]	23 (5/22)

[a] Male and female f_0 rats (control and 0.1 μg TCDD/kg/day groups) were cross-mated with young untreated rats of the opposite sex.

[b] Mean ± SD.

[c] Significantly different from the control value by Fisher's exact probability test, $p < 0.05$.

[d] Significantly different from the control value by a one-way analysis of variance, $p < 0.05$.

value; half of the resorptions observed among the high-dose females were late resorptions.

Upon staining of the uterus with sodium sulfide stain at necropsy, approximately one-half of the high-dose females demonstrated evidence of having been pregnant at an earlier date, probably from one of the two previous matings. Of the control f_0 females which did not appear to be pregnant, all but two showed indications of having been pregnant when the uterus was stained with sodium sulfide.

DISCUSSION

Effects of TCDD on reproduction seen in the current study were qualitatively similar to those observed by Khera and Ruddick (1972) in the offspring of pregnant rats given TCDD from Days 6 to 15 of gestation. In that study, decreased neonatal survival was observed at dose levels of 0.5 and 1.0 μg TCDD/kg/day; dose-related decrease in litter size and pup weight at birth were noted at doses of 0.25 μg TCDD/kg/day and above. In the present study, similar effects were clearly evident at 0.01 and 0.1 μg TCDD/kg/day, as summarized in Table 9.

Accumulation of TCDD may have been responsible for the difference between the two studies in the dose levels required to produce these adverse effects. In the study by Khera and Ruddick, rats were given the compound for only 10 days, whereas in the current study TCDD was given throughout gestation and for at least 90 days prior to gestation. Pharmacokinetic studies in rats given 0.01–1.0 μg TCDD/kg/day have indicated that TCDD approaches steady-state concentrations in the body in 90 days (Rose *et al.*, 1976).

At the intermediate dose of 0.01 μg TCDD/kg/day, many adverse effects seen in the f_1 and f_2 adults and their litters were not evident in the f_0 adults or their offspring. The most obvious difference is that the f_0 rats were given TCDD beginning at about 7 weeks of age whereas subsequent generations were exposed to TCDD, at least theoretically, from the time of conception. This suggests that some of the effects observed were initiated, perhaps, during the neonatal period. This theory is supported by

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TABLE 9

SUMMARY OF EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) ON REPRODUCTION

Parameter	Generation	μg TCDD/kg/day[a]		
		0.001	0.01	0.1
Fertility	f_0	—	—	dec
	f_0	—	—	dec
	f_1	—	dec	
	f_2	—	dec	
Litter size	f_{1a}	—	—	dec
	f_{1b}	—	—	dec
	f_2	—	dec	
	f_3	—	dec	
Gestation Survival	f_{1a}	—	—	dec[b]
	f_{1b}	—	—	—[c]
	f_2	dec	dec	
	f_3	—	dec	
Postnatal survival	f_{1a}	dec	dec	[b]
	f_{1b}	inc	—	—[c]
	f_2	—	dec	
	f_3	—	—	
Postnatal body weight	f_{1a}	—	—	[b]
	f_{1b}	—	—	—[c]
	f_2	—	dec	
	f_3	—	dec	

[a] (—), unaffected; dec, decreased; inc, increased.
[b] No liveborn offspring.
[c] One litter only.

the fact that reduced fertility and smaller litter size was seen by Khera and Ruddick (1972) in the adult offspring of pregnant rats treated prenatally.

Cross-mating studies indicated that ingestion of TCDD did not interfere with the fertility of the male rats. Among the cross-mated females, however, a high incidence of resorptions, particularly late resorptions, was observed among the dams receiving TCDD. Thus, the decreased fertility index seen with TCDD may have been due, at least in part, to a number of litters being entirely resorbed. The decrease in the average number of fetuses per litter at birth might also be explained in terms of an increase in the incidence of resorptions. Sparschu *et al.* (1971) reported a high incidence of late resorptions and a decrease in litter size among pregnant rats given TCDD on Days 6 through 15 of gestation.

Other factors also appeared to contribute to the reduction in the fertility index. For example, there was a significant decrease in the number of females exhibiting a vaginal plug at 0.1 μg TCDD/kg/day, suggesting a decrease in copulatory activity. At 0.01 μg TCDD/kg/day, the average number of days from the first day of cohabitation to the day of parturition was significantly increased in the later generations, indicative of some interference with the estrous cycle. Kociba *et al.* (1976) reported morphologic alterations in the ovaries and uterus suggestive of a suppression of the estrous cycle in female rats given 1 μg TDCD/kg/day for 90 days. Allen

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et al. (1977) reported an irregular estrous cycle and a low incidence of pregnancy among female rhesus monkeys given a diet containing 500 ppt TCDD for 6 months.

In general, the reproductive performance of the f_0 rats, including the control rats, of this and a concurrent reproduction study on another chemical was poorer than that seen in previous studies. Attempts to explain the cause of the poor performance were fruitless. A marked improvement in reproductive performance was observed in subsequent generations (control and low-dose groups).

Slightly dilated renal pelvis was seen significantly more often than in controls among the weanlings at the low dose in the f_1 generations but not in the f_2 or f_3 generations. Because no increase in this alteration was observed in subsequent generations or at higher dose levels, the increase at the low dose was not considered to be compound related. TCDD-Induced hemorrhages in the gastro-intestinal tract of fetal rats as reported by Sparschu *et al.* (1971) and Khera and Ruddick (1972) were not seen in the current study.

The no-adverse-effect level in the present study, 0.001 μg TCDD/kg/day, was the same as that found in a chronic toxicity study of TCDD conducted in this laboratory by Kociba *et al.* (1978). In that study, the incidence of certain types of neoplasms was altered among rats given 0.01 or 0.1 μg TCDD/kg/day, dose levels which adversely affected reproduction in the current study. Of interest, a decrease in the incidence of tumors of the uterus and the mammary gland was noted among the female rats receiving TCDD; that effect on the tumor incidence, as well as some of the effects observed on reproduction in the present study, may have been mediated through a hormonal imbalance.

In conclusion, impairment of reproduction was clearly evident among rats ingesting 0.01 or 0.1 μg TCDD/kg/day; significant decreases were observed in fertility, litter size, gestation survival, postnatal survival, and postnatal body weight. No adverse effect on reproduction was discerned among rats given the low dose of 0.001 μg TCDD/kg/day.

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