General Biological Effects of TCDD in Laboratory Animals

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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is reported to be one of the most toxic chemicals known. There has been variation in such reported toxic parameters as single oral LD_{50} dose, range of time interval from dosing until death, and toxic manifestations which an animal exhibits (*1–4*). It seemed most appropriate, therefore, that the mean values and ranges of these and other general biological parameters be determined under the experimental condition to which animals are exposed at this Institute.

Materials and Methods

The TCDD utilized in the studies was graciously supplied by the Dow Chemical Company and was found by analyses to contain more than 99% TCDD. The majority of the studies reported by NIEHS scientists at this conference utilized a single stock solution prepared by dissolving 5 mg of chemical in 67.5 ml of reagent grade acetone. Once dissolved, this TCDD acetone solution was added to 432.5 ml of corn oil purchased at a local retail store. This solution was calculated to contain 10 μg TCDD/ml. Chemical analysis of an aliquot of this solution performed by the Dow Chemical Company yielded a value of 11 μg/ml ± 2 μg/ml. This compares favorably with the calculated value. By using appropriate dilutions of the stock solution with corn oil, the actual dosing solution was prepared in a volume appropriate for the requirements of the experiment.

All animals used, rats, guinea pigs, and mice, were housed in animal quarters maintained under a rigid sanitary regimen. Temperature was maintained at 70 ± 2°F and 50 ± 5% RH. Food and water were available at all times. All animals were administered the TCDD–acetone-corn oil solution via gastric intubation. The volume administered ranged from 0.2 ml to 1.6 ml in rats, from 0.17 to 0.22 ml in guinea pigs, and from 0.1 to 0.2 ml in mice. The volume administered in any one experiment was the same. Control animals received an equal amount of acetone–corn oil, the actual proportion of each used being equal that contained in the TCDD solution. All animals were weighed at least once weekly. Rat food consumption as measured by disappearance of the blocks from a suspended stainless steel feeder was determined at least twice each week. Rats and mice were dosed according to a mean average weight of the dose group. Guinea pigs were dosed according to the weight of each animal. In multiple-dose experiments, dose was recalculated each week based on the weights of the animals on the date of recalculation. All animals were assigned to a given dose group according to a table of random numbers.

Rats used were of both sexes, 6 to 8 weeks of age, specific pathogen-free, and had been

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acclimated to NIEHS conditions at least 2 weeks prior to use. All were random bred albino rats, CD stock, purchased from Charles River Breeding Laboratories, Wilmington, Massachusetts. Female albino guinea pigs, Hartley strain, were purchased from Carworth, Inc., New City, New York. Mice used were of two types, random bred albino CD-1 stock from Charles River or inbred C57B1/6Sch strain reared at the Institute.

The data obtained in the four experiments are presented as mean values and standard errors or deviations. Dunnett's multiple comparisons test (*8*) was used to make treatment control comparisons, usually two-sided, except for organ weights. In addition, a nonparametric test (Jonckheere's test) (*9*) was used to test for monotonic dose–response relationships.

Results

Live Animal Effects

In the course of various rat experiments the frequency of dose administration varied from a single administration to daily dosing for 30 consecutive days or weekly administration for a period of 6 weeks. The effects of a single dose of TCDD at 1, 5, 25, 50, or 100 μg/kg on body weight and survival in rats are illustrated in Figures 1–3; the actual body weights and statistical values are given in Tables 1–4. Male and female rats at the 1 or 5 μg/kg dose gained weight at the same rate as the controls. At the 25 μg/kg dose an actual weight loss was observed (for 1 week) in females (Fig. 1, Table 1). Subsequent to week 1 until the end of the experiment (week 9) animals at this dose gained weight at the same rate as controls. Male rats at the same dose had a significantly decreased weight gain 1 and 2 weeks subsequent to the TCDD dose (Fig. 2, Table 3). Their weight gain after 2 weeks was equivalent to control rats. In a later experiment (Fig. 3, Table 4), male rats showed a dose-related decrease in body weight gain in the first two weeks subsequent to a 25 or 50 μg/kg dose with the reduced weight gain observed at the higher

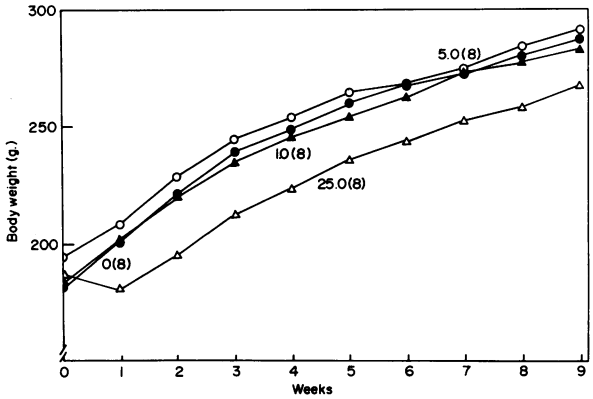

FIGURE 1. Body weights of female rats receiving a single dose of (●) 0; (△) 1.0 μg/kg; (○) 5.0 μg/kg; (△) 25.0 μg/kg. TCDD: There were 8 animals per dose group. All animals survived.

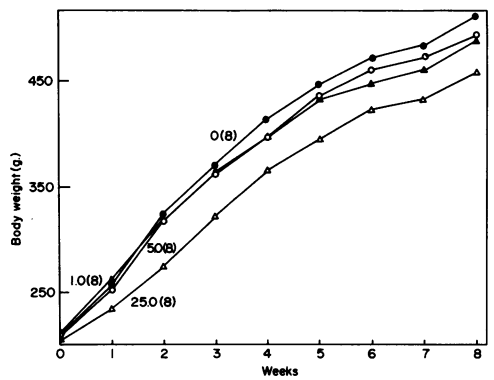

FIGURE 2. Body weights of male rats receiving a single dose of TCDD: (●) 0; (△) 1.0 μg/kg; (○) 5.0 μg/kg; (△) 25.0 μg/kg. There were 8 animals per dose group. All animals survived.

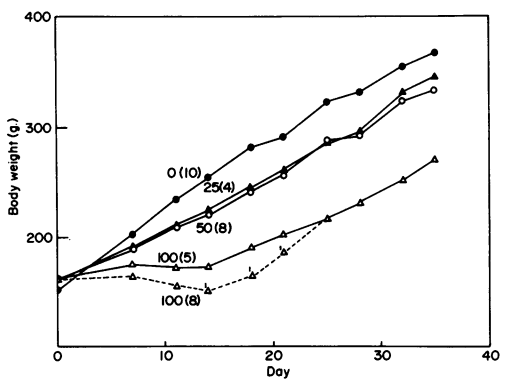

FIGURE 3. Body weights of male rats receiving a single dose of TCDD: (●) 0; (△) 25 μg/kg; (○) 50 μg/kg; (△) 100 μg/kg. The number of animals is shown in parentheses. In the 100 μg/kg group, the mean weight of the animal surviving the experimental period is also plotted (. .). The superscript 1 denotes days on which one animal died.

Table 1. Weight gain of female rats receiving a single dose of TCDD.[a]

Single TCDD dose, μg/kg	Number of animals	Initial body weight, g	Weight gain, g	
			Week 0–1	Week 1–9
0	8	181.5 ± 7.2	20.3 ± 5.5	85.3 ± 16.8
1.0	8	183.8 ± 7.1	18.3 ± 2.3	81.3 ± 12.2
5.0	8	194.3 ± 6.9[b]	14.3 ± 5.2	82.8 ± 13.6
25	8	187.3 ± 5.7	−6.4 ± 11.4[b]	86.5 ± 20.6

[a] Mean values ± SD.
[b] $P < 0.01$.

Table 2. Weight gain of female rats receiving a single dose of TCDD.[a]

Single TCDD dose, μg/kg	Number of animals	Initial body weight, g	Weight gain, g	
			Week 0–1	Week 1–6
0	6	166.0 ± 1.8	22.5 ± 7.8	57.2 ± 12.3
50	6[b]	162.2 ± 8.8	−11.5 ± 14.8[d]	66.2 ± 11.4
100	6[c]	168.8 ± 4.9	−20.5 ± 12.8[d]	32.7 ± 31.8

[a] Mean values ± SD.
[b] One animal died on day 14.
[c] Three animals died between 18 and 21 days.
[d] $P < 0.01$.

Table 3. Weight gain of male rats receiving a single dose of TCDD.[a]

Single TCDD dose, μg/kg	Number of animals	Initial body weight, g	Weight gain, g	
			Week 0–2	Week 2–8
0	8	209.1 ± 7.7	115.3 ± 13.0	184.4 ± 30.8
1.0	8	211.0 ± 12.1	107.0 ± 13.6	169.5 ± 23.1
5.0	8	212.8 ± 9.7	107.6 ± 18.5	172.9 ± 29.3
25	8	204.0 ± 5.6	72.3 ± 23.6[b]	182.1 ± 36.0

[a] Mean values ± SD.
[b] $P < 0.01$.

Table 4. Weight gain of male rats receiving a single dose of TCDD.[a]

Single TCDD dose, μg/kg	Number of animals	Initial body weight, g	Weight gain, g	
			Week 0–2	Week 2–5
0	10	153.6 ± 9.8	100.7 ± 14.1	113.9 ± 22.6
25	4	158.8 ± 2.5	66.5 ± 39.5	122.5 ± 27.7
50	8	163.6 ± 9.2	58.0 ± 30.5[c]	112.8 ± 22.1
100	8[b]	161.0 ± 9.9	−9.9 ± 30.4[c]	97.6 ± 51.6

[a] Mean values ± SD.
[b] Three animals died between 14 and 21 days.
[c] $P < 0.01$.

dose significant at the 1% level. At the 100 μg/kg dosage, 3 of 8 rats died between 14 and 21 days. The mean weight gain of the survivors at the high dose between weeks 2 and 5 was not significantly different from other TCDD dose levels or controls. Female rats which received 50 or 100 μg/kg exhibited a dose-related absolute decrease in body weight during the first week (Table 2). One of six and three of six rats died between 14 and 21 days at the 50 and 100 μg/kg dose, respectively. Weight gain between weeks 1 and 6 was not significantly different from that of controls; however, as the large standard deviation at the high dose suggests, appreciable variation among the survivors did exist.

The mean time interval until death was 18.3 days in the 6 of 14 male and female rats which died at the 100 μg/kg dose. Five of six deaths occurred between the 18th and 21st day; the female rat at the 50 μg/kg dose which died, survived 14 days.

Clinical symptoms of toxicity, which were seen at the 100 μg/kg dose, were ruffled hair coat, hunched posture, and inactivity (depression). Jaundice was observed for several days in those rats that died. Food consumption in male rats which received 25, 50, and 100 μg/kg was recorded and analyzed for the initial 2-week period subsequent to TCDD intubation (Table 5). Although the average amount of food consumed decreased in all treatment groups when compared to controls, the difference is significant only at the 100 μg/kg dose ($P<0.05$). If the food consumption of the three rats which subsequently died in the 100 μg/kg group is excluded and the mean recomputed, the value is no longer significantly different from controls. The standard deviation for food consumption in all TCDD treatment groups is relatively large which suggests marked variation within a dose group.

In the daily dose experiments, female rats received either 0, 0.1, 1, or 10 μg/kg TCDD for 31 consecutive days. The body weight changes which occurred in one of these experiments are given in Table 6. Although not included in the table because it was not part of this particular experiment, the effect on body weight in rats receiving daily TCDD at 0.1 μg/kg was negligible. Rats at the 10 μg/kg dose lost 21.8 g weight during the first 7 days of the experiment. Fifteen of 16 rats died or became moribund a mean of 21.8 days after the study commenced. Weight gain at the 1 μg/kg dose group was significantly less than that of controls for the 1–35 day time period. Weight gain during the 35–63 day time period exceeded that of controls by 14 g.

Table 5. Fourteen-day food consumption in male rats receiving a single dose of TCDD.

Single TCDD dose, μg/kg	Number of animals	Food consumption, g ± SD [a]
0	10	359.4 ± 19.3
25	4	313.2 ± 70.8
50	8	315.6 ± 56.4
100	8	257.5 [b] ± 87.2
100	5 [a]	275.0 ± 84.7

[a] Dose response test for food consumption significant at 0.01 level.
[b] $P<0.05$.
[c] Three rats in group which later died excluded.

Table 6. Weight gain of female rats receiving 31 daily doses of TCDD.

Daily TCDD dose, μg/kg	Initial no. of animals	Initial body weight, g ± SD	Weight gain, g ± SD		
			Days 1–7	Days [a] 1–35	Days [a] 35–63
0	16	183.3 ± 6.28	15.8 ± 6.6	65.8 ± 8.0	28.4 ± 9.7
1	12	185.6 ± 5.04	12.1 ± 6.4	34.8 ± 12.2 [b]	42.0 ± 11.9 [c]
10	16	184.0 ± 8.00	−21.4 ± 14.9 [b]	—	—

[a] Values based on 5 and 8 rats at 0 and 1 μg/kg respectively; 15 of 16 rats died or were killed when moribund at mean of 21.8 days.
[b] $P<0.01$.
[c] $P<0.06$.

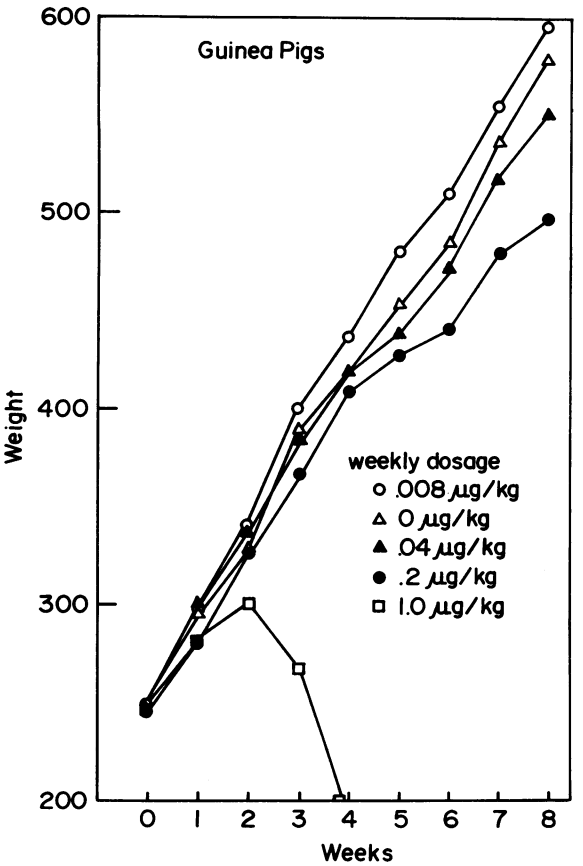

FIGURE 4. Body weight of guinea pigs receiving eight weekly doses of TCDD. There were 10 animals per dose group. All animals at the 1.0 μg/kg dose died.

Rats also received weekly doses of 0.02, 1.0, or 5.0 μg/kg for 6 weeks. During the dosing period, decreased body weight gain occurred in the 5.0 μg/kg dose group (*7*).

Guinea pigs were dosed weekly for 8 weeks with 0, 0.008, 0.04, 0.2, or 1.0 μg/kg. In other experiments, guinea pigs received a single 1.0 or 3.0 μg/kg dose of TCDD. Nine of ten animals died a mean 19.2 days after receiving the single 3 μg/kg dose. Severe weight loss preceded death. No guinea pigs died following the single 1.0 μg/kg dose, although a reversible decreased body weight gain was observed. All guinea pigs receiving the 1.0 μg/kg weekly dose 24–32 days after the study began died (mean survival time 28 days). Animals which received the weekly 0.2 μg/kg dose weighed significantly less at the end of the experiment (*7*). As Figure 4 illustrates, weight gain depression at this dose level primarily occurred during the fifth and sixth week of the study.

Adult CD-1 mice received a single oral dose of TCDD at 0, 1.0, 10, or 50 μg/kg. No effect on body weight was observed during the subsequent 5-week observation period. Adult C57B1/6 mice received weekly doses of 25, 5, 1, or 0.2 μg/kg for four weeks. Significant weight loss occurred at the 25 μg/kg dose, with one of seven mice dying on the day 25 of the study.

Organ Weights

In some experiments, rats were killed and organ weights recorded at predetermined intervals after a single 0, 5, or 25 μg/kg TCDD dose. These organ weights from randomly selected rats were obtained 1, 3, 8, 9, 16, and 28 days after TCDD intubation.

Table 7. Liver and thymus weights of male rats receiving a single dose of TCDD.

Time since dose, days	No. of animals	0 μg/kg		5 μg/kg		25 μg/kg	
		Liver, g ± SE	Thymus, mg ± SE [a]	Liver, g ± SE	Thymus, mg ± SE [a]	Liver, g ± SE	Thymus, mg ± SE [a]
1	5	9.02 ± 1.15	934 ± 53	10.34 ± 0.76	866 ± 89	8.65 ± 0.67	768 ± 29
3	5	11.06 ± 0.44	864 ± 62	11.94 ± 0.84	724 ± 108	11.14 ± 0.53	568 ± 36 [a]
8	5	13.65 ± 0.88	826 ± 99	14.08 ± 0.62	644 ± 65	13.92 ± 0.59	334 ± 25 [b]
9	5	14.96 ± 0.47	836 ± 107	14.28 ± 0.84	646 ± 62	13.38 ± 0.81	280 ± 30 [b]
16	5	16.22 ± 0.70	790 ± 57	16.40 ± 1.27	570 ± 55 [a]	14.28 ± 1.99	264 ± 45 [b]
28	5	17.99 ± 0.95	764 ± 60	15.61 ± 0.93	664 ± 45	15.72 ± 1.60	494 ± 54 [b]

[a] Dose-response tests for reduced thymus significant on each day at $P < 0.05$.
[b] $P < 0.05$.
[c] $P < 0.01$.

Table 8. Liver and thymus weights of females rats receiving daily doses of TCDD.

No. of doses	No. of animals	0 μg/kg		0.1 μg/kg		1.0 μg/kg		10 μg/kg	
		Liver, g ± SE	Thymus, mg ± SE [a]	Liver, g ± SE	Thymus, mg ± SE [a]	Liver, g ± SE	Thymus, mg ± SE [a]	Liver, g ± SE	Thymus, mg ± SE [a]
3	4	7.80 ± 0.48	—	8.62 ± 0.41	—	9.32 ± 0.45	—	9.58 ± 0.43	—
6	4	8.41 ± 0.36	—	10.35 ± 0.45 [b]	—	9.00 ± 0.14	—	10.84 ± 0.97	—
10	4	9.14 ± 0.21	—	10.78 ± 0.41 [b]	—	11.53 ± 0.40 [c]	—	10.53 ± 0.36 [b]	—
13	4	7.90 ± 0.31	530 ± 48	10.17 ± 0.94 [b]	492 ± 81	9.76 ± 0.62	390 ± 58	9.80 ± 1.08	142 ± 12 [c]
17	4	9.26 ± 0.42	615 ± 59	10.91 ± 0.37 [b]	468 ± 22	11.33 ± 0.29 [c]	270 ± 11 [c]	7.52 ± 1.37	110 ± 48 [c]
24	4	9.45 ± 0.31	525 ± 43	11.40 ± 1.05	412 ± 28 [b]	11.39 ± 0.98	372 ± 28 [b]	4.67 ± 0.21 [c]	32 ± 10 [c]
31	4	9.70 ± 0.49	558 ± 87	11.78 ± 0.81	475 ± 49	12.84 ± 0.84 [b]	260 ± 41 [c]	—	—

[a] All dose response tests for thymus significant at $P < 0.01$.
[b] $P < 0.05$.
[c] $P < 0.01$.

Table 9. Liver and thymus weights of CD-1 mice treated with a single dose of TCDD.[a]

Time since dose, weeks	No. of animals	0 μg/kg		1.0 μg/kg		10 μg/kg		50 μg/kg	
		Liver, g ± SD	Thymus, mg ± SD	Liver, g ± SD	Thymus, mg ± SD	Liver, g ± SD	Thymus, mg ± SD	Liver, g ± SD	Thymus, mg ± SD
1	4	1.68 0.16	82.5 19.8	1.65 0.15	75.5 16.3	1.90 0.26	74.2 19.2	2.27 [b] 0.16	60.8 17.7
3	3	1.89 0.38	81.7 8.1	2.05 0.26	83.3 3.2	1.94 0.41	60.3 [c] 5.9	2.43 0.19	60.7 [c] 14.2
5	3	1.77 0.17	51.0 18.0	1.61 0.13	47.0 8.5	1.63 0.10	54.7 6.7	1.83 0.04	38.7 22.0

[a] Dose-response test: $P < 0.01$ (liver) at week 1; $P < 0.05$ (thymus) at week 3.
[b] $P < 0.01$.
[c] $P < 0.05$.

Kidney, heart, lung, spleen, adrenal, testes and thymus weights were recorded and evaluated. Significant differences in organ weights were seen with the thymus. Wet weights for this organ and liver are given in Table 7. There is a tendency for thymus weights to decrease with age. A significant reduction in thymus weight was first observed 3 days after rats received 25 μg/kg TCDD. Thymus weights at this dose continued to decrease with the lowest weights observed on day 16. The day 28 mean value was 220 mg higher than day 16 but still far below control values. The only significant ($P<0.05$) decrease in thymus weight in the 5 μg/kg group occurred at day 16. However, the average thymus weight reduction at this dose level for the entire 28-day period is significant ($P<0.01$). Liver weights were not significantly different from controls at either dose level. Reduced spleen weights were observed on days 8, 9, and 16 at the 25 μg/kg dose.

Organ weights were also collected at predetermined times in some rats being intubated daily with 0, 0.1, 1.0, or 10 μg/kg. Liver and thymus weights for these doses are illustrated in Table 8. Mean liver weights were always greater at the 0.1 and 1 μg/kg dose; however, the statistical significance of these increases was inconsistent. At the daily 10 μg/kg dose livers were increased in size on the first four days organ weights were collected (days 3, 6, 10, and 13). There was a marked decrease in liver weight, falling below control mean values, on days 17 and 24. It should be remembered that body weight at this dose level markedly decreased and death occurred in 15 of 16 rats. Thymus weights were 20–25% of control values in the daily 10 μg/kg dose group when recorded on days 13 and 17. Weights were further reduced at death. At the 1 μg/kg level, thymus was first found significantly smaller than controls at day 17; it continued to remain so through the 31-day dosing period. Thymus weights at the 0.1 μg/kg dose were significantly reduced when evaluated for an overall dose response effect.

Weight changes were not seen in heart, lung, or kidney. Weight decreases occurred in spleen at the high dose group as they approached death.

Pathologic, hematologic, and clinical chemistry changes observed in rats, mice and guinea pigs used in these experiments are reported elsewhere (*5*, *6*). Experiments which measured humoral or cell-mediated immune capabilities are the subject of another report (*7*).

Table 9 lists the liver and thymus weights of CD-1 mice 1, 3, or 5 weeks after a single dose of TCDD. Liver weight was significantly increased after 1 week in mice which received 50 μg/kg TCDD. Thymus weight was decreased in the 10 and 50 μg/kg groups at week 3. The measurement of an overall dose response was significant for the liver increase at week 1 and thymus decrease at week 3. No weight effects on spleen or adrenal were observed.

Guinea pig liver, kidney, thyroid, and uterus weights collected after 8 weekly doses of 0.2, 0.04, or 0.008 μg/kg TCDD were not significantly lower than controls. Decreased thymus weight was observed at the 0.04 and 0.2 μg/kg dose level (Table 10). Marked thymic atrophy was also seen at time of necropsy in those guinea pigs which died in the weekly 1.0 μg/kg or single, 3.0 μg/kg dose groups.

Table 10. Liver and thymus weights of female guinea pigs receiving 8 weekly doses of TCDD and killed after 56 days.

Weekly dose of TCDD, μg/kg	No. of animals	Liver g ± SD	Thymus, mg ± SD [a]
0	10	29.4 ± 3.8	901 ± 246
0.008	10	29.5 ± 4.3	741 ± 163
0.04	10	26.9 ± 3.6	672 ± 161 [b]
0.2	7	26.9 ± 3.9	476 ± 70 [c]

[a] Thymus dose response $P<0.01$.
[b] $P<0.05$.
[c] $P<0.01$.

Discussion

Table 11 summarizes and contrasts some TCDD effects seen in the 3 species tested.

Table 11. Summary of biological effects of TCDD.

	Rats			Guinea pigs			Mice
	Dose, μg/kg	MTD, days [a]	Mortality [b]	Dose, μg/kg	MTD, days [a]	Mortality [b]	Dose, μg/kg
Lethal dose							
Single	100	18	6/14	3	18	9/10	>50
Weekly	4 × 25	28	2/10	5 × 1	28	10/10	>4 × 25
Daily	10	22	15/16	—			—
Body weight							
Lowest dose effect							
Single	25			1			—
Weekly	6 × 5			8 × 0.2			4 × 25
Daily	30 × 1			—			—
No effect							
Single	5			—			50
Weekly	6 × 1			8 × 0.04			4 × 5
Daily	30 × 0.1			—			—
Thymus weight							
Lowest dose effect							
Single	5			—			10
Weekly	6 × 5			8 × 0.04			4 × 5
Daily	30 × 0.1			—			—

[a] Mean time to death after first exposure.
[b] Number of animals dying/number of animals treated.

Guinea pigs are most sensitive to the lethal effects of TCDD with 90% dying from a single 3 μg/kg dose. In contrast, a 100 μg/kg dose was lethal to 6 of 14 rats. In both species the time interval until death is similar and a large weight loss over a period of days preceded death. We did not observe a great range in the time interval from exposure until death which was observed by others (*1*).

Female rats appear to be more sensitive to TCDD than males. Although death incidence at 100 μg/kg was similar, one female did die at the 50 μg/kg dose. Additionally, at the 25 and 50 μg/kg dose females underwent an actual weight loss, whereas males exhibited only a 30–40% reduction in weight gain. Body weight effects of a single dose are primarily observed during the first two weeks after exposure. Subsequently, weight gain equates with controls, although treated rats never narrow the actual weight divergence which appears during the first 2 weeks.

There is an overall tendency for feed consumption to be reduced following TCDD exposure, but this depression is not sufficient magnitude to account for the body weight changes which occurred. Food consumption at the 100 μg/kg dose was significantly decreased primarily due to the greater anorectic state observed in rats for the several days preceding death.

Administration of daily or weekly sublethal doses does not seem to raise the threshold level of TCDD toxicity. For example decreased weight gain at the 1 μg/kg daily dose occurred in rats once the total dose administered exceeded approximately 20 μg/kg; decreased weight gain first occurred at the single 25 μg/kg level. A comparison of the body weight gains of 5 μg/kg weekly dose and the 1 μg/kg daily dose also reveals a similar weight pattern change. Parallel growth rates after 1 or 2 weeks in the single dose rat experiments and weight gains exceeding that of controls subsequent to 30-day exposure at 1 μg/kg/rat suggest that toxicity

of TCDD, at least in some circumstances, are reversible. As Table 11 indicates, thymus appears to be a most sensitive indicator of TCDD exposure. Decreases in thymus weight consistently occurred in all species at a dose level below which body weight effects occurred.

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