Hematologic and Clinical Chemistry Effects of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin in Laboratory Animals

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Introduction

Chlorodibenzo-*p*-dioxins, especially 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), are among the most toxic compounds known. These compounds are found as contaminants of technical chlorophenols and their derivatives. A variety of pathologic condition have been associated with the injestion of products containing chlorodibenzo-*p*-dioxins.

Toxic fat, the cause of chick edema disease (*1, 2*) and a variety of pathologic manifestations in monkeys (*3*), has been found to contain chlorodibenzo-*p*-dioxins, among which is TCDD (*4, 5*). TCDD has been implicated in a variety of other toxicoses, including outbreaks of chloracne in chemical workers (*6*), hepatonecrosis in rabbits (*6*), and hepatonecrosis and thymic atrophy in rats (*7*). However, only in the studies in which monkeys were fed toxic fat (*3*) and in which rats were given TCDD orally (*7, 8*) were substantial clinical pathologic analyses performed.

Toxic fat caused in the monkeys, among other lesions, anemia, leukopenia and hypoproteinemia. The hypoproteinemia was primarily due to decreased serum albumin concentration. The blood urea nitrogen (BUN), serum bilirubin, cholesterol, sodium and potassium concentrations, and the prothrombin times were not altered (*3*).

Rats given 10 mg TCDD/kg body weight had, among the other lesions, increased activities of serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvate transaminase (SGPT), lactic dehydrogenase (LDH), and hydroxybuturate dehydrogenase as well as decreased arylesterase and cholinesterase activities. In addition, the rats had decreased serum glucose, sodium, and protein concentrations, and increased serum urea, lipid and bilirubin concentrations, hemoconcentration, and neutrophilia. Serum cholesterol, potassium and chloride concentrations and aldolase and alkaline phosphatase (AP) activities were not altered (*7, 8*).

Because the clinical pathologic and pathologic changes found in these and other studies suggest that the most profound effect of chlorodibenzo-*p*-dioxins are on the liver and various hematopoietic organs, we decided to determine the sequential clinical pathologic changes which might occur in rats given different doses of TCDD over a period of time. These methods might also determine which functions are the most sensitive to TCDD. In addition, hematologic studies were conducted as an adjuvant to studies of immunologic effects of TCDD in

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guinea pigs (*9*) and in mice in which the effects of TCDD on oögenesis are being determined (J. McLachlan, personal communication).

Materials and Methods

Animals

Female CD rats weighing 150–175 g were given, orally, 0.1, 1.0, or 10.0 μg TCDD/kg daily for 30 days. Blood for the clinical pathologic studies was obtained from the heart 3, 6, 10, 13, 17, 24, and 31 days after the TCDD dosing commenced.

Blood was obtained from the retroorbital sinus for hematologic studies 1, 3, and 5 weeks after 8-week-old female CD-1 mice were given oral doses of TCDD of 1.0, 10.0, or 50 μg/kg.

Female Hartley strain guinea pigs weighing about 250 g received oral weekly doses of 0.008, 0.04, 0.2, or 1.0 μg TCDD/kg for 8 weeks. The 1.0 μg TCDD/kg group became moribund between 3 and 5 weeks. Half the animals were injected with tetanus toxoid (group A) and half with killed *Mycobacterium tuberculosis* (group B) in order to determine the effects of TCDD on humoral and cell-mediated immune responses. The details of these experimental procedures can be found elsewhere in this issue (*9*). The highest dose guinea pigs when moribund and the other animals at 8 weeks were bled from the heart for hematologic analysis.

Procedures

Routine methods were used for hematologic determinations. Platelet counts were determined in counting chambers (Unopette, Becton, Dickinson and Company, Rutherford, N. J.), fibrinogen by a heat-denaturation method (*10*); urea nitrogen was determined by a urease method (Boehringer-Mannhein Co., New York, N. Y.), cholesterol by a ferric chloride method (Hyland, Costa Mesa, California), serum proteins by the biuret technique (*11*), glucose by an *o*-toluidine method (Hyland, Costa Mesa, California), creatinine by an alkaline picrate method (*12*), bilirubin by a sulfanilic acid–caffeine–sodium benzoate method (Boehringer-Mannheim Co., New York, N. Y.), sodium and potassium by flame photometry, and chloride by coulometric titration. The serum enzymes, alkaline phosphatase (AP), lactic dehydrogenase (LDH), glutamic-oxaloacetic transaminase (SGOT), and glutamic pyruvate transaminase (SGPT) were determined by kinetic methods by use of commercially available kits (Sigma Chemical Co., St. Louis, Missouri) in a recording spectrophotometer.

Analysis of Data

Statistical analysis for differences between groups was done by Dunnett's test (*13*). Determination of a dose response was done by Jonckheere's method (*14*). The 5% level was selected as the value where variables would be considered significantly different.

Results

Rats

Enzymic alterations in the TCDD-treated rats consisted of increased SGPT activity in the high-dose (10.0μg TCDD/kg-day) rats at days 17, 24, and 31 (Fig. 1), and increased SGOT activity at days 13, 17, 24, and 31 in the high-dose rats as well as days 13 and 17 in the middle-dose (1.0 μg TCDD/kg-day) rats (Fig. 2). LDH was

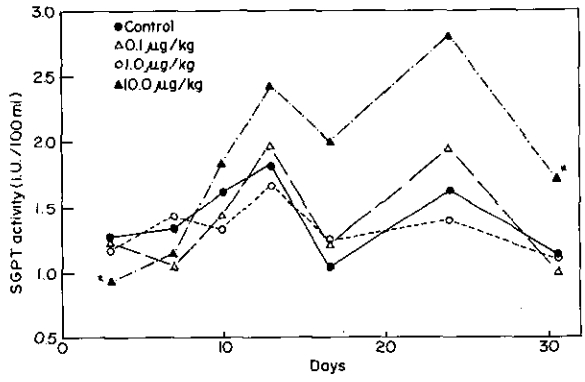

FIGURE 1. SGPT activity in rats treated with 0.1, 1.0, or 10.0 μg TCDD/kg-day. High-dose rats had increased SGPT activity at days 17 ($P < 0.01$) and 24 ($P < 0.05$). The asterisk (*) indicates a single observation. All other points are the means of three or four observations.

elevated only on day 24 in the high-dose rats, and AP was not altered at any time.

Serum cholesterol was increased on days 10, 17, 24, and 31 in the high-dose rats and day 24 in the middle-dose animals. Significant dose–response elevations for cholesterol concentrations were also found on days 17 and 24 (Fig. 3). Blood glucose concentration decreased with time in all the treated groups. At day 10, glucose concentrations in all treated animals were significantly decreased and on days 24 and 31 in the high and middle dose animals it was decreased. Significant dose–response blood glucose decreases were found at days 10, 17, and 24 (Fig. 4). Total serum protein fluctuated with the treatment. On days 24 and 31, the high-dose rats had decreased protein

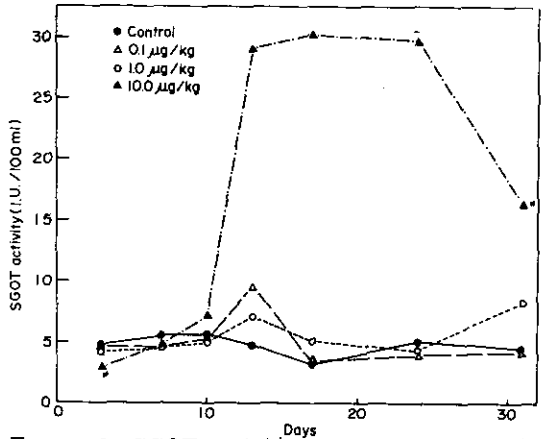

FIGURE 2. SGOT activity in rats treated with 0.1, 1.0, or 10.0 μg TCDD/kg-day. Significant increases were found in the high-dose rats on days 13, 17, and 24 ($P < 0.01$) and in the middle-dose rats on days 13 and 17 ($P < 0.05$). Significant dose responses were found on days 13 ($P < 0.01$), 17 ($P < 0.01$), and 31 ($P < 0.05$). The asterisk (*) indicates a single observation. All other values are means of three or four observations.

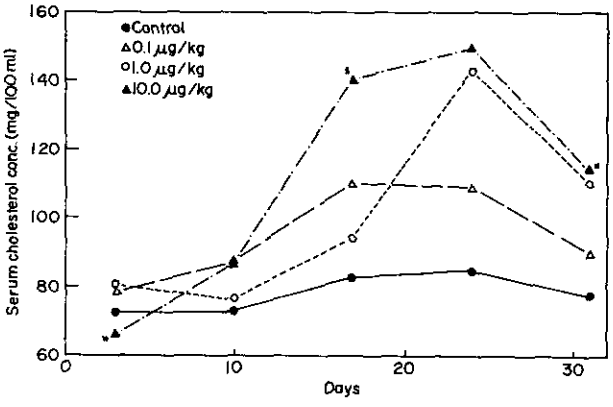

FIGURE 3. Serum cholesterol concentration in rats given 0.1, 1.0 or 10.0 μg TCDD/kg-day. Significant elevations occurred on days 10 ($P < 0.05$) and 24 ($P < 0.01$) in the high-dose rats and on day 24 ($P < 0.05$) in the middle-dose rats. Significant ($P < 0.05$) dose responses were found on days 17 and 24. The asterisk (*) indicates a single observations. All other values are the means of three or four observations.

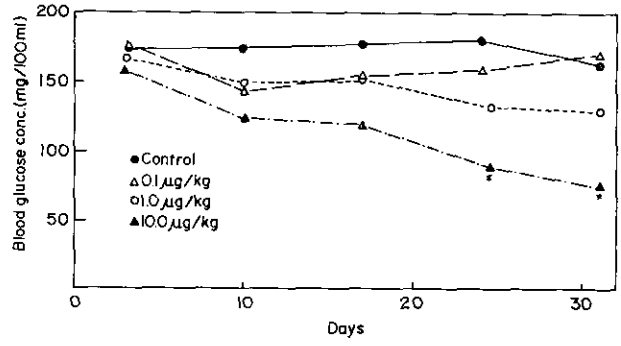

FIGURE 4. Blood glucose concentration in rats treated with 0.1, 1.0, or 10.0 μg TCDD/kg-day. Significant ($P < 0.05$) decreases occurred on day 10 in the low-dose rats and the middle-dose rats. Highly significant ($P < 0.01$) decreases occurred in the middle-dose rats on day 24 and in the high-dose rats on days 10 and 17. Significant dose responses occurred on days 10 ($P < 0.01$), 17 ($P < 0.05$ and 24 ($P < 0.01$). The asterisk (*) indicates a single observation. All other values are the means of three or four observations.

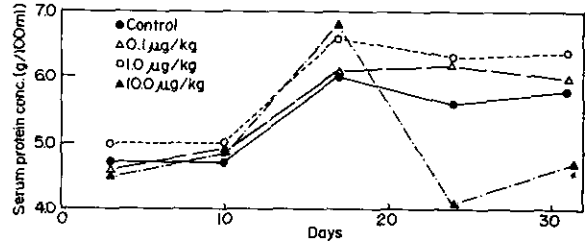

FIGURE 5. Serum protein concentration in rats treated with 0.1, 1.0, or 10.0 μg TCDD/kg-day. Significant ($P < 0.05$) increased concentrations occurred in the middle-dose rats on days 31 and decreased concentration in the high-dose rats on day 24. The asterisk (*) indicates a single observation. All other values are the means of three or four observations.

concentration while at day 31 it was increased in the middle-dose rats (Fig. 5). In addition, serum bilirubin concentrations were increased in the high-dose animals on days 17, 24, and 31 (Table 1). No significant changes were found for serum creatinine, sodium, potassium, chloride, fibrinogen, and BUN concentrations.

Table 1. Bilirubin concentration in TCDD-treated rats.[a]

Time, days	Bilirubin concentration, mg/100 ml serum		
	Control	1.0 μg/kg-day	10.0 μg/kg-day
13	0.41 ± 0.10		0.83 ± 0.28 [b]
17	0.25 ± 0.08	0.22 ± 0.09	1.94 ± 0.42 [c]
24	0.64 ± 0.51	0.44 ± 0.15	5.62 ± 1.07 [c]
31	0.52 ± 0.15	0.40 ± 0.09	2.16 [d]

[a] Mean ± 1 S.D.
[b] $P < 0.05$.
[c] $P < 0.01$.
[d] Single value.

Hematologic changes were confined to hemoconcentration, as previously noted in rats (7), in the high-dose animals on days 17 and 24 (Figs. 6–8), and to striking thrombocytopenia in all the groups. After only 3 days treatment, the high and middle-dose animals had depressed platelet counts which remained depressed throughout the study. In the low-dose rats (0.1 μg TCDD/kg-day), platelets were decreased

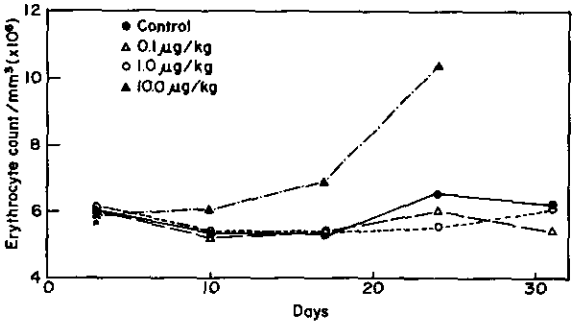

FIGURE 6. Erythrocyte count in rats given 0.1, 1.0, or 10.0 μg TCDD/kg-day. Significant increases occurred in the high-dose rats on days 17 and 24 ($P < 0.01$). All values are the means of three or four observations.

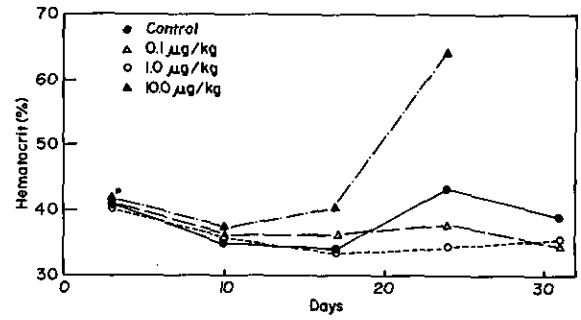

FIGURE 7. Hematocrit in rats given 0.1, 1.0, or 10.0 μg TCDD/kg-day. Highly significant ($P < 0.01$ increases occurred in the high-dose rats on days 17 and 24. All values are the means of three or four observations.

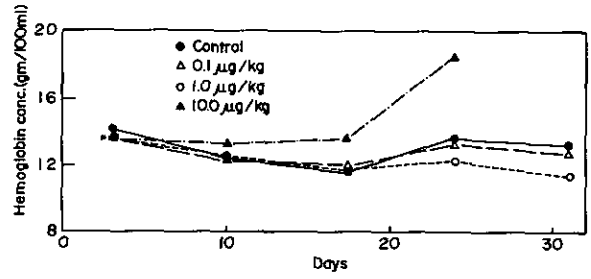

FIGURE 8. Hemoglobin concentration in rats given 0.1, 1.0, or 10.0 μg/kg-day. Highly significant ($P < 0.01$) increases occurred on days 17 and 24 in the high-dose rats. All values are the means of three or four observations.

significantly only on day 17. Significant dose response decreases in platelets occurred throughout the study (Fig. 9). No significant changes occurred in leukocyte counts or differentials (in particular, lymphocytes) in these rats.

Mice

Mice given a single oral dose of 1.0, 10.0, or 50.0 μg TCDD/kg had significantly decreased leukocyte (Table 2) and lymphocyte counts (Table 3) after 1 week. After 3 weeks, none of the treatment groups had significant differences from control mice; however, a significant dose-response lymphocyte depression remained. The leukocyte depression was on the borderline of being a

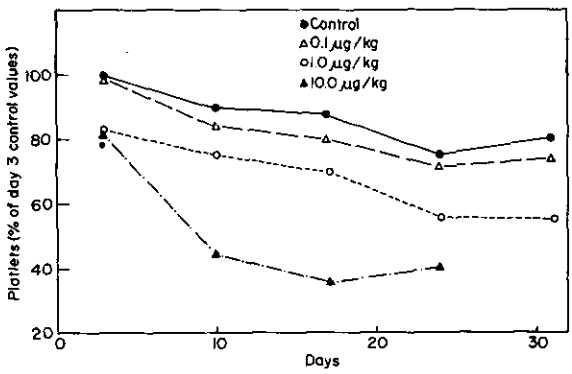

FIGURE 9. Relative platelet counts in rats treated with 0.1, 1.0, or 10.0 μg TCDD/kg-day. Significant ($P < 0.05$) decreases occurred in the low-dose rats on day 17, the middle-dose rats on days 3, 10, and 31 and in the high-dose rats on day 24. Highly significant ($P < 0.01$) decreases occurred in the middle-dose rats on day 24 and the high-dose rats on days 10 and 17. Significant dose-responses occurred on days 3 ($P < 0.05$), 10 ($P < 0.01$), 17 ($P < 0.01$), 24 ($P < 0.01$, and 31 ($P < 0.05$). The mean numbers of platelets at day 3 in the control rats were 1109 × 10^3/mm³ blood. All values are the means of three or four observations.

significant dose-response. After 5 weeks, no TCDD effects were detected except that the mice treated with 10 μg TCDD/kg had significantly elevated erythrocyte counts (Table 4). This difference is probably due to a lack of variability in the parameter rather than a real elevation and likely does not reflect an effect of TCDD.

Guinea Pigs

The TCDD-treated guinea pigs in the tetanus toxoid-injected group (group A) (*9*) had consistently lower leukocyte counts than the controls, but only for the middle-dose animals (0.04 μg TCDD/kg) was this decrease significant (Table 5). The lymphocyte counts were significantly decreased in all the TCDD-treated animals in this group. A significant dose response occurred for the leukocyte and lymphocyte decreases also, but these effects simply reflect the depression observed at all three TCDD treatment levels.

Table 2. Leukocyte counts in mice given a single oral dose of TCDD.

	Leukocyte count × 10^{-3} per mm³								
	Controls		1 μg/kg		10 μg/kg		50 μg/kg		
Week	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	Dose response [a]
1	12.05	2.58	8.35 [b]	1.72	8.78 [b]	0.74	6.08 [c]	0.90	$P_1 < 0.01$
3	8.07	2.50	6.53	0.65	5.43	0.91	6.10	2.29	$P_1 = 0.052$
5	8.70	3.67	8.23	3.82	7.30	1.49	7.27	3.95	$P_1 = 0.222$

[a] P_1 = one-side P value.
[b] P (one-sided) < 0.05.
[c] P (one-sided) < 0.01.

Table 3. Lymphocyte counts in mice given a single oral dose of TCDD.

	Lymphocyte count per mm³								
	Controls		1 μg/kg		10 μg/kg		50 μg/kg		
Week	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	Dose response [a]
1	9233.0	1551.7	6276.5 [b]	1405.8	6443.5 [b]	1112.9	3752.2 [b]	378.6	$P_1 < 0.01$
3	6170.7	1872.1	5363.0	422.2	3788.3	839.8	4414.3	1764.6	$P_1 = 0.037$
5	6353.3	3261.2	6587.7	3482.6	5024.3	405.7	4864.0	2679.8	$P_1 = 0.182$

[a] P_1 = one-sided P value.
[b] P (one-sided) < 0.01.

Table 4. Erythrocyte counts × 10^6 in mice given a single oral dose of TCDD.

Week	Erythrocyte count × 10^{-6} per mm³								Dose response
	Controls		1 μg/kg		10 μg/kg		50 μg/kg		
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	
1	7.28	0.320	7.30	0.141	6.90	0.356	7.33	0.479	NS [a]
3	7.63	0.115	7.57	0.306	7.43	0.058	7.33	0.252	NS [a]
5	7.53	0.058	7.73	0.153	7.80 [b]	—	7.83	0.569	$P_2 = 0.045$

[a] NS = not significant.
[b] P_2 = two-sided P value.

Table 5. Leukocyte, lymphocyte, and neutrophil counts in group A guinea pigs treated with TCDD for 8 weeks.

Variable	Count per mm³								Dose response
	Control		0.008 μg/kg-wk		0.04 μg/kg-wk		0.2 μg/kg-wk		
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	
Leukocytes	6407.8	1878.0	5050.0	1161.7	4850.0 [a]	1237.6	4914.3	990.7	$P = 0.022$
Lymphocytes	4159.7	1465.3	2390.9 [b]	726.8	2872.6 [a]	1031.4	2591.7 [a]	459.3	$P = 0.021$
Neutrophils	1885.6	559.2	2286.5	1269.5	1668.7	466.6	2096.7	634.7	NS [c]

[a] $P < 0.05$.
[b] $P < 0.01$.

Table 6. Hematologic parameters for group B guinea pigs treated with TCDD for 8 weeks.

	Control		0.008 μg/kg-wk		0.04 μg/kg-wk		0.2 μg/kg-wk		Dose response
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	
Leukocytes per mm³	8840.0	4205.1	8230.0 [a]	2482.9	7260.0	1659.5	6140.0 [a]	2910.2	$P < 0.01$
Lymphocytes per mm³	4270.5	2293.3	4229.1	1312.0	3717.5	1169.1	3628.2	2371.7	$P = 0.024$
Neutropil per mm³	3517.6	2127.6	3405.9	1204.4	3104.3	654.8	2179.3 [a]	718.7	$P = 0.020$
Platelets × 10^{-3} per mm³	796.500	103.496	—	—	—	—	645.000 [b]	84.755	—
Erythrocytes × 10^{-6} per mm³	4.16	0.28	4.24	0.25	4.18	0.30	4.26	0.25	NS [c]
Hemoglobin, g/100 ml	10.74	0.57	11.00	0.40	11.29	0.57	10.59	0.66	NS
Hematocrit, %	41.70	1.84	42.25	1.77	41.90	2.34	40.45	2.22	NS

[a] $P < 0.05$.
[b] $P < 0.01$.
[c] NS = not significant.

For Group B (*Mycobacterium tuberculosis* tuberculin-treated) (*9*) only the highest dose (0.2 μg TCDD/kg) caused significantly decreased leukocyte counts (Table 6). Neutrophil counts were also reduced in these guinea pigs, but there was no treatment versus control differences in lymphocyte counts. Significant dose-related decreases were found for leukocytes, neutrophils, and lymphocytes. In addition, the guinea pigs treated with 0.2 μg TCDD/kg also had lower platelet counts than the controls ($P < 0.01$).

The leukocyte and lymphocyte counts of the group A and group B control guinea pigs did not differ significantly (Table 7). However, the group B controls did have higher neutrophil counts than the group B controls ($P < 0.05$). When the group A control guinea pigs were compared with the moribund 1.0 μg TCDD/kg guinea pigs, decreased lymphocyte and increased neutro-

Table 7. Leukocyte counts of tetanus toxoid-treated (group A), *Mycobacterium tuberculosis* tuberculin-treated (group B), and TCDD-treated guinea pigs.

Variable	Group A controls		Group B controls		1.0 μg TCDD/kg-wk	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Leukocyte count per mm³	6407.8	1878.0	8840.0	4205.1	6800.0	4018.7
Lymphocyte count per mm³	4159.7	1465.3	4270.5	2293.3	1804.0 [a]	522.4
Neutrophil count per mm³	1885.6	559.2	2517.6 [b]	2127.6	4686.4 [a]	3534.7

[a] $P<0.01$.
[b] $P<0.05$.

phil counts were found in the treated animals. However, this comparison may not be valid because the TCDD-treated guinea pigs were 3 to 5 weeks younger than the group A controls and were in a moribund state in addition to not having been injected with tetanus toxoid. Hemoconcentration was also present in these dying animals, there being an average erythrocyte count of 5.1×10^6 per mm³ and a hematocrit of 48.9%. Platelet counts were decreased as well.

Discussion

The clinical pathologic findings of this and other studies (*3, 7, 8*) suggest that the major sites of the toxic action of TCDD and the chlorodibenzo-*p*-dioxins of toxic fat are the hematopoietic system and the liver. Mice and guinea pigs given TCDD and monkeys fed toxic fat (*3*) were leukopenic, which in mice and guinea pigs is characterized by lymphopenia. In mice which received a single dose of TCDD the lymphopenia was reversed 5 weeks after TCDD exposure. Perhaps the lymphopenia seen in mice and guinea pigs is related to the decreased cell-mediated immune response observed in these two species after TCDD exposure (*9*). It is interesting that lymphopenia and cell-mediated immunosuppression were not found in rats treated with TCDD.

Rats and guinea pigs were thrombocytopenic. The cause of this was not determined. Examination of rat bone marrow in one experiment did not reveal any differences in numbers or morphology of megakaryocytes following TCDD treatment (*17*), but in another experiment in rats, spleen and bone marrow megakaryocytes were degenerated and appeared to be reduced in numbers (*15*). Therefore, further work is indicated to determine, perhaps by radioisotope tagging, whether the lowered platelet counts are due to decreased production or increased distruction of platelets. Nevertheless, thrombocytopenia or defects in the clotting mechanism (*17*) might have played a role in the production of hemorrhages that were occasionally seen in rats that died (*15*).

Anemia was not found in these studies, but it was seen in a previous study in monkeys fed toxic fat (*3*). That anemia was accompanied by atrophic bone marrow and normal serum bilirubin suggesting that the anemia was aplastic or depression type rather than hemolytic anemia.

We observed only terminal hemoconcentration in rats and guinea pigs, similar to that previously reported in rats given TCDD (*7*). This is probably an effect of terminal shock and dehydration rather than increased erythrocyte production. Dehydration is further evidenced by the increased serum protein concentrations found in the middle-dose rats. However, hypoproteinemia was observed in the high-dose rats probably as a result of liver damage, and may have contributed further to the hemoconcentration by decreasing the colloidal osmotic pressure of the blood and allowing fluid to accumulate in the tissues.

Liver damage in rats was evidenced by increased SGOT and SGPT activity. The increased SGOT activity might also be due to, in part, myocardial necrosis (*15*). Hyperbilirubinemia, hypercholesterolemia, and hypoproteinemia (in the high-dose rats) are

probably other effects of the liver pathology. Since increased cholesterol concentrations have previously been seen in rats (*18*) and rabbits (*19*) which have sustained hepatocellular damage from polychlorinated biphenyls, perhaps metabolism of cholesterol is altered in liver damage caused by these chlorinated compounds. Hypoglycemia in the TCDD-treated rats might have been due to decreased food consumption (*16*), but there might also be an effect on the gluconeogenic ability of the damaged liver. Although clinical chemical parameters to assess liver damage were not determined in guinea pigs and mice, histopathologic evidence suggests that hepatocellular damage was minimal in these species (*15*).

It appears that hepatocellular necrosis is the main toxic action of TCDD in rats but with effects on platelets being important. The clinical pathologic changes are consistent with this hypothesis. Ultimately, hemoconcentration due to shock and dehydration occurs as a terminal event in rats as well as guinea pigs.

The lymphopenia observed in mice and guinea pigs are consistent with the immunosuppressive effects of TCDD in these species (*9*). Pathologic studies suggest that liver damage does not play a part in the death of guinea pigs given high doses of TCDD (*15*). However, in guinea pigs, atrophy of the adrenal zona glomerulosa (*9, 15*) suggests that electrolyte imbalance might occur. Perhaps a study of serum electrolyte and aldosterone concentrations in guinea pigs given lethal doses of TCDD is warranted.

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REFERENCES

1. Edgar, S. A., et al. The effect of a toxic substance in fat on poultry. Poultry Sci. 37: 1200 (1958).
2. Allen, J. R. The role of toxic fat in the production of hydropericardium and ascites in chickens. Am. J. Vet. Res. 25: 1210 (1964).
3. Allen, J. R., and Carstens, L. A. Light and electron microscope observations in *Macaca mulatta* monkeys fed toxic fat. Am. J. Vet. Res. 28: 1513 (1967).
4. Higgenbotham, G. R. et al. Chemical and toxicological evaluations of isolated and synthetic chloro derivatives of dibenzo-*p*-dioxins. Nature 220: 702 (1968).
5. Metcalfe, L. D. Proposed source of chick edema factor. J. Assoc. Offic. Anal. Chemists 55: 542 (1972).
6. Schultz, K. H. Clinical picture and etiology of chloracne. Arbeits-med.-Socialmed.-Arbeitshyg. 3: 25 (1968).
7. Buu-Hoi, N. P., et al. Organs as targets of "dioxin" (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) intoxication. Naturwiss. 4: 174 (1972).
8. Buu-Hoi, N. P., et al. Enzymatic functions as targets of the toxicity of "dioxin" (2,3,7,8-tetrachlorodibenzo-*p*-dioxin). Naturwiss. 4: 173 (1972).
9. Vos, J. G., Moore, J. A., and Zinkl, J. G. Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on the immune system of laboratory animals. Environ. Health Perspect. No. 5: 149 (1973).
10. Kaneko, J. J., and Smith, R. The estimation of plasma fibrinogen and its clinical significance in the dog. Cal. Vet. 21: 21 (1967).
11. Gornall, A. G., Bardawill, C. J. and David, M. M. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 177: 751 (1949).
12. Henry, R. J. *Clinical Chemistry Principles and Techniques.* Harper and Row, New York, 1962, p. 292.
13. Miller, R. G., Jr. *Simultaneous Statistical Inference.* McGraw-Hill, New York, 1966.
14. Jonckheere, A. R. A distribution free K-sample test against ordered alternatives. Biometrika 41: 133 (1954).
15. Gupta, B. N., et al. Pathological effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in laboratory animals. Environ. Health Perspect. No. 5: 125 (1973).
16. Harris, M., et al. General biological effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in laboratory animals. Environ. Health Perspect. No. 5: 101 (1973).
17. Weissberg, J. B., and Zinkl, J. G. Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on hematologic and blood coagulation function in the rat. Environ. Health Perspect. No. 5: 119 (1973).
18. Kuratsune, M. An abstract of results of laboratory examinations of patients with Yusho and of animal experiments. Environ. Health Perspect. No. 1: 129 (1972).
19. Koller, L. D., and Zinkl, J. G. Pathology of polychlorinated biphenyls in rabbits. Am. J. Path. 70: 363 (1973).