Toxicology of Chlorinated Dibenzo-*p*-dioxins

by B.A. Schwetz,* J.M. Norris,* G.L. Sparschu,* V.K. Rowe,* P.J. Gehring,* J.L. Emerson,† and C.G. Gerbig †

Severe toxicological responses have been associated with certain chlorodibenzodioxins. One of these responses is chloracne, a folliculosis first associated with skin contamination by chlorohydrocarbons in 1899 (*1*). Serious outbreaks of chloracne-like lesions associated with runaway reactions in the production of 2,4,5-trichlorophenol occurred in Germany in the early 1950's (*2*). 2,4,5-Trichlorophenol itself does not cause acne (*3*), but the contaminants which may be formed in the uncontrolled production of 2,4,5-trichlorophenol are extremely potent acnegens (*2*). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and tri- and tetrachlorodibenzofuran were isolated from the contaminants formed in 2,4,5-trichlorophenol production and were demonstrated to be strongly positive acnegens when applied to rabbit ears (*3*). By using the rabbit ear test, the acnegenic potency of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) was confirmed in 1962 (*4*). In addition, 2,3,7,8-TCDD is extremely toxic in the chick embryo assay (*5*) and is highly embryotoxic in rats (*6*). Another chlorodibenzodioxin, hexachlorodibenzo-*p*-dioxin (HCDD), is known to be positive for the chick edema factor, a condition characterized by hydropericardium, ascites, and anasarca (*5*, *7*).

*Chemical Biology Research, The Dow Chemical Co., Midland, Michigan 48640.

† Human Health Research and Development Center, The Dow Chemical Co., Zionsville, Indiana 46077.

Experimental

Materials

The chlorodibenzodioxin samples used in these studies are identified and described in Table 1. Studies were limited in some cases by availability of pure samples.

Acute Lethality

Samples of 2,7-dichlorodibenzo-*p*-dioxin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, hexachlorodibenzo-*p*-dioxin, and octachlorodibenzo-*p*-dioxin were evaluated for acute oral lethality in several animals as summarized in Table 2.

Test materials were administered as suspensions in corn oil or as corn oil: acetone (9:1) solutions in single doses by gavage. The animals were deprived of feed for 16 hr before dosing. After dosing, they were observed for signs of toxicity including body weight changes for two to eight weeks.

Lethality of 2,3,7,8-TCDD via skin absorption was tested on rabbits of mixed sexes with doses of 31.6, 63, 126, 252, and 500 μg/kg body weight. The compound was applied as a 0.01% solution in acetone to the abdominal skin which had been shorn. After the acetone evaporated, the trunk of each rabbit was wrapped in cotton to prevent ingestion. The rabbits were housed in individual holding cages and were observed for signs of toxicity including body weight changes for three weeks.

Parenteral lethality was determined by injecting rabbits of mixed sexes intraperi-

Table 1. Purity of samples used in the toxicology studies.

Sample no.	Sample identification	Source	Purity [a]	Tests [b]
	2,7-Dichlorodibenzo-*p*-dioxin (2,7-DCDD)			
1a	#104, shelf 142	Dow Chem. Co.	99.8%	1, 2, 3
1b	AR-570 [c]	Dow Chem. Co.		3
1c	340-2-13A	Dow Chem. Co.	99.6%	1, 2, 3
1d	340-2-69A	Dow Chem. Co.	>99%	4
	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD)			
2a	Caustic insoluble isolate 1965	Dow Chem. Co.	96.4%	1
2b	851-142-24	Dow Chem. Co.	98%	1
2c	Skelly 11/11/64	Dow Chem. Co.	91%	1, 3, 5
2d	340-2-54B	Dow Chem. Co.	>99%	1, 2, 3, 5
	1,2,3,4-Tetrachlorodibenzo-*p*-dioxin (1,2,3,4-TCDD)			
3a	FDA-F990	FDA	98.5%	3
	Hexachlorodibenzo-*p*-dioxin (HCDD)			
4a	252-44-12B-AL22	Dow Chem. Co.	65:35, 2 isomers	1, 3
4b	252-44-12B-AL11	Dow Chem. Co.	99%, 65:35, 2 isomers	3
4c	340-2-82A	Dow Chem. Co.	>99%, 89:11, 2 isomers	1, 2, 3, 4, 5
4d	FDA-F911	FDA	95.1%, 3 isomers	3
	Octachlorodibenzo-*p*-dioxin (OCDD)			
5a	251-1-142A	Dow Chem. Co.	98%	1, 2, 3
5b	340-2-29A	Dow Chem. Co.	94%	1, 3
5c	AR-570 [d]	Dow Chem. Co.		3
5d	340-2-57A	Dow Chem. Co.	98.86%	1, 3, 4, 5

[a] Based on gas-liquid chromatographic (GLC) or GLC–mass spectrophotometric analysis.
[b] Test identifications: 1 = LD_{50}; 2 = eye irritation; 3 = chloracne; 4 = tetratogenicity; 5 = chick edema.
[c] Photolysis product of sample 1a.
[d] Photolysis product of sample 5a.

Table 2. Evaluation of acute oral lethality.

Test animal	Strain	Test material			
		2,7-DCDD	2,3,7,8-TCDD	HCDD	OCDD
Rat	Sprague-Dawley	X		X	X
Rat	Sherman (Spartan)		X		
Mouse	Swiss Webster	X	X		X
Rabbit	New Zealand albino		X		
Guinea pig	Hartley		X		
Dog	Beagle		X		

toneally with 31.6, 63, 126, 252 and 500 μg/kg of 2,3,7,8-TCDD as a 0.01% corn oil suspension; control rabbits were injected with corn oil. The rabbits were housed in individual holding cages and were observed for signs of toxicity for four weeks. The LD_{50}'s were calculated by the Weil modification of the Thompson method (*8, 9*) or by the Litchfield and Wilcoxon method (*10*). The acute lethality studies were terminated when it was evident that the survivors were not showing signs of toxicity.

Eye Irritation

Rabbit eyes were examined prior to experiments and found to be free from defects or irritation. Approximately 2 mg of 2,7-DCDD, 2,3,7,8-TCDD, HCDD, or OCDD were instilled in the conjunctival sac of one eye; the contralateral eye served as a control. The eyes were examined at various times after treatment for conjunctival redness and chemosis, iritis, and corneal injury. Responses were categorized according to intensity.

Rabbit Ear Bioassay For Acnegenic Activity

Acnegenic activity of 2,7-DCDD, 1,2,3,4-TCDD, 2,3,7,8-TCDD, HCDD, and OCDD was tested by applying 0.1 ml of either a solvent solution or the supernatant of a solvent suspension of each compound to the inner surface of the rabbit's ears five days a week for four weeks. The ears were examined weekly for signs of chloracne, inflammation and hyperkeratosis. The responses were divided into five categories: (1) none, (2) very slight, (3) slight, (4) moderate, and (5) severe.

Responses in the first three categories include no response to mild irritation, increased ear thickness, slight enlargement of the follicular aperture, slight exfoliation and slight crust formation. These responses alone are not considered indicative of chloracnegenic activity. Categories 4 and 5 are indicative of acnegenic response and are characterized by comedo formation, increased ear thickness and hyperkeratosis.

Teratology

Pregnant adult Sprague-Dawley (Spartan strain) female rats weighing approximately 250 g were used to study teratogenicity of the chlorinated dibenzo-*p*-dioxins. The day sperm were first present in a vaginal smear was considered day zero of pregnancy. The animals were housed individually in wire-bottom cages in a room controlled for temperature, humidity, light cycle and noise. Commercial laboratory rat chow and water were provided with choice.

Corn oil: acetone (9:1) solutions with varying amounts of test material were given in 2.5 ml/kg dosages by gavage. Dosages were calculated using daily body weights. Rats were treated with 100 mg of 2,7-DCDD/kg-day, 0.1, 1.0, 10, or 100 μg HCDD/kg-day and 100 or 500 mg OCDD/kg-day on days 6 through 15 of gestation. Control rats received 2.5 ml/kg of corn oil: acetone (9:1) orally. All rats were observed daily throughout pregnancy and were weighed on days 6, 13, and 21 of gestation. Pregnant females were sacrificed by carbon dioxide anesthesia on day 21 of gestation; 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. After being weighed and sexed, the fetuses were examined for external anomalies; the crown-rump length was measured with a vernier caliper. Half of each litter was preserved in Bouin's solution and later examined for soft tissue anomalies (*11*); the other half was preserved in alcohol, cleared and stained with Alizarin Red-S, and examined for skeletal abnormalities (*12*).

A 2 × 2 contingency table was used to evaluate the frequency of anomalies and resorptions within the fetal population and between litters. Body weight and body measurements were statistically analyzed by an analysis of variance and Tukey's test (*13*). In all cases, the level of significance was $P<0.05$.

Chick Bioassay for Chick Edema Factor

The bioassay for chick edema factor was

conducted according to the Association of Official Agricultural Chemists method (*14*). Three-day-old white leghorn, single-comb cockerels were used. 2,3,7,8-TCDD, HCDD, and OCDD were the compounds studied. The diet used in the study was formulated specifically for conducting the chick edema bioassay (Nutritional Biochemicals, International Chemical and Nuclear Corp., Cleveland, Ohio). Body weights were recorded twice weekly for the oral intubation studies and at the start and termination of the dietary study. The chicks were observed daily for signs of toxicity, and food consumption was recorded weekly. After 20 or 21 days of treatment, all chickens were sacrificed by cervical dislocation and examined for gross lesions. The amount of pericardial and peritoneal fluid was measured, and all gross lesions were recorded. If the calculated t was greater than +1.3, the mean logarithm (100 × ml pericardial fluid) was greater than 1.1461 for the chicks receiving the test compound, and the mean logarithm of the negative control was less than 1.1460, the compound was considered positive for chick edema.*

Pathology

Toxicology studies were not designed to study the pathological changes associated with chlorodibenzodioxin administration, but in some cases, gross pathological and histopathological examinations were performed. For microscopic examination, tissues were fixed in 10% buffered formalin and were stained with hematoxylin and eosin. Sections of fetuses of control dams and dams treated with 100 mg 2,7-DCDD/kg-day were stained with hematoxylin and eosin, hematoxylin–phloxine–saffron, Masson's trichrome stain, and Mallory's phosphotungstic acid-hematoxylin stain.

*The calculated mean of logarithms of pericardial fluid voluemes of the test group and of concurrent negative control group x_t and x_c, respectively, is given by

$$t = (x_t - x_c)/[(s_t^2/n_t) + (s_c^2/n_c)]^{1/2}$$

where n_t and n_c are the number of chicks in the test and control groups, respectively, and s_t^2 and s_c^2 are variances of test and control groups, respectively (*14*).

Results

Acute Lethality

The lethality of 2,3,7,8-TCDD is presented in Table 3. The data reveal that the single oral LD50 ranges from 0.0006 mg/kg in male guinea pigs to 0.115 mg/kg in rabbits of mixed sex. Data on rats indicate that males are more sensitive than females; lethality is essentially the same following intraperitoneal, oral or skin administration for rabbits. Limited data show that dogs are less sensitive to 2,3,7,8-TCDD than rabbits. For female and male mice, single oral doses ranging from 0.001 to 0.130 mg/kg produced a few sporadic deaths without any definitive dose-response relationship; therefore the data are not presented in the table.

Limited lethality data are available for 2,7-DCDD, HCDD, and OCDD. HCDD (sample c) killed 1 of 2 and 0 of 2 male rats given oral doses of 100 and 10 mg/kg, respectively. No deaths occurred in four male mice given 2.0 g/kg of 2,7-DCDD (sample a or b) orally or in two female rats given 1 g/kg (sample a). For OCDD, oral doses of 1 g/kg (sample d) to five female rats did not cause death; in four male mice, doses of 4 g/kg also did not cause death. No signs of toxicity were observed in animals treated with either 2,7-DCDD or OCDD. The only sign of toxicity among animals treated with HCDD was loss of body weight.

While all species lost body weight following treatment with 2,3,7,8-TCDD, other signs of toxicity were species dependent. Ascites was seen in mice. Anorexia, dehydration, depression, emaciation, intestinal hemorrhage and alopecia were seen in dogs. Certain rabbits treated intraperitoneally with 2,3,7,8-TCDD developed skin lesions typical of those associated with acnegens.

Rabbit Eye Irritation

Instillation of the chlorodibenzodioxins into the conjunctival sac caused slight, trans-

Table 3. Lethality of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [a]

Species and sex	Sample [b]	Route of administration	Time of death, days postadministration	LD_{50}, mg/kg	Dose, mg/kg	Number deaths/ number treated
Rat, male	c	Oral	9–27	0.022	0.008	0/5
					0.016	0/5
					0.032	10/10
					0.063	5/5
Rat, female	c	Oral	13–43	0.045 (0.030–0.066)		
Guinea pig, male	c	Oral	5–34	0.0006 (0.0004–0.0009)		
Guinea pig, male	d	Oral	9–42	0.0021 (0.0015–0.0030)		
Rabbit, mixed	c	Oral	6–39	0.115 (0.038–0.345)		
	c	Skin	12–22	0.275 (0.142–0.531)		
	c	Intraperitoneal	6–23	—	0.032	0/5
					0.063	2/5
					0.126	2/5
					0.252	2/5
					0.500	3/5
Dogs, male	c	Oral	9–15		0.30	0/2
					3.00	2/2
Dogs, female	c	Oral	—		0.03	0/2
					0.10	0/2

[a] Responses to individual doses are given in those cases in which an LD_{50} could not be calculated. The LD_{50} for oral administration to rabbits was calculated by using the method of Litchfield and Wilcoxon (*9*); the remaining values were calculated by using the Weil modification of the method of Thompson (*15, 16*).

[b] Letters refer to sample identification in Table 1.

ient pain and conjunctival inflammation, initially. Treatment with 2,3,7,8-TCDD was associated with delayed conjunctival chemosis 13-22 days later. By day 27, the chemosis had subsided, but the rim of the eyelid was thickened and encrusted. In rabbits treated with HCDD, the rim of the eyelid was encrusted 27 days after treatment. Neither corneal injury nor iritis was observed in any of the animals following instillation of the chlorodibenzodioxins in the conjunctival sac.

Acnegenic Response

Both 2,3,7,8-TCDD and HCDD produced acne in the rabbit ear bioassay as indicated by the formation of comedones. Solutions of 2,3,7,8-TCDD (sample c) in benzene ranging in concentration from 0.04 to 400 μg/ml produced a positive response with severity increasing with concentration. A negative response was obtained with a solution of 0.004 μg/ml. In contrast, a chloroform solution of 1,2,3,4-TCDD, 50 μg/ml, did not produce a positive response. With HCDD (samples a, b, c, and d), a response was produced by solutions of 10 to 50 μg/ml in chloroform and dimethoxyethane. Chloroform extracts from 10% suspensions of 2,7-DCDD or OCDD were negative, indicating that these have a low order or possibly no acnegenic activity.

Teratogenicity

The effects of chlorodibenzodioxins on maternal and fetal body measurements, incidence of fetal resorptions and anomalies are given in Tables 4 and 5.

2,7-DCDD. Rats treated with 100 mg/kg-day on days 6 through 15 of gestation gained slightly more weight during pregnancy than controls but showed no toxicity. There was no effect on fetal body measurements, or incidence of resorptions, or gross, soft tissue or skeletal anomalies.

HCDD. Administration of 0.1–100 μg HCDD/kg-day was associated with a dose-related decrease in maternal weight-gain

during gestation. Gross necropsy examination at the time of cesarean section revealed evidence of maternal toxicity only among dams receiving 100 μg/kg-day (pale, friable liver 3/20 dams; serous atrophy of fat, 1/20 dams).

Treatment with 10 or 100 μg HCDD/kg-day was highly lethal to fetuses during late gestation. While the incidence of early resorptions was not increased at any dose level of HCDD (5–7% in the treated versus 7% in the controls), there was a significant increase in late resorptions (0% at 0.1 μg/kg-day to 79% at 100 μg/kg-day). The weight and length of surviving fetuses were significantly decreased.

A significant increase in the incidence of fetal soft-tissue and skeletal anomalies was seen following treatment of pregnant rats with HCDD at the 100 μg/kg-day dose level. The incidence of cleft palate, subcutaneous edema, vertebrae with split or unfused centra, and split sternebrae was significantly greater than among control litters or the control fetal population. Among dams treated with 1 or 10 μg/kg-day, only subcutaneous edema occurred at a significantly greater incidence than in the control litters or fetal population. Treatment with 0.1 μg/kg-day of HCDD did not increase fetal anomalies among the litters or the fetal population. The incidence of delayed ossification of sternebrae was significantly increased among the fetal population but not among litters.

OCDD. Signs of maternal toxicity were not observed in rats given 100 or 500 mg/kg-day OCDD. Examination of the fetuses did not reveal changes in fetal body measurements, incidence of fetal resorptions, or incidence of any fetal anomaly among litters or the fetal population. At 500 mg/kg-day, the incidence of subcutaneous edema was significantly increased among the fetal population (23/100 compared with 8/156 in controls) but not among litters (9/18 compared with 6/28 in controls).

Chick Edema Bioassay

Chick edema was produced in groups of birds treated with 1 and 10 μg/kg-day of 2,3,7,8-TCDD and 10 and 100 μ/kg-day of HCDD (Table 6). The mean logarithm for pericardial fluid volume of the negative control groups was greater than 1.1460 and could negate the results if the guidelines for interpreting chick edema bioassay studies were rigidly followed. However, since the volume of pericardial fluid was markedly increased by the treatments indicated above, the treatments were considered to be positive for the production of chick edema. A positive response was not observed in chicks maintained on a diet containing 0.5% OCDD.

Severe dyspnea, subcutaneous edema, and distended abdomens were observed in some birds receiving 1 or 10 μg 2,3,7,8-TCDD/kg-day. Dyspnea and mucus accumulation in the mouth prior to death were observed in birds receiving 100 μg 2,3,7,8-TCDD/kg-day. No overt clinical signs were observed in birds receiving OCDD.

The gross lesions seen in chicks treated with chlorodibenzodioxins are summarized in Table 7. The most consistent gross lesions were increased pericardial and peritoneal fluid, subcutaneous and pulmonary edema, hepatomegaly and a mottled appearance of the liver.

Histopathologic examination of tissues of selected birds from the 2,3,7,8-TCDD (1 and 10 μg) and HCDD (10 and 100 μg) groups revealed similar lesions consisting of: atrophy of germinal centers of the spleen, a paucity of lymphocytes in the bursa of Fabricius, pulmonary edema, intersititial edema of the myocardium, fatty degeneration and coagulation necrosis of the liver. Many birds died as a result of pulmonary edema.

Pathology

Gross necropsy and histological examinations were conducted on relatively few mammals treated with the chlorinated dibenzo-*p*-dioxins. Therefore, the results reported here are incomplete and preliminary. The liver of animals treated with 2,3,7,8-TCDD and HCDD was most consistently affected.

Table 4. Effect of treatment with chlorinated dibenzo-*p*-dioxin on maternal and fetal body measurements and the incidence of fetal resorption.

Test compound (sample) [a]	No. of litters	Maternal weight gain, g [b]			Fetal body weight, g [c]	Fetal crown–rump [c] length, mm	Fetal resorptions, %	
		Days 6–13	Days 13–21	Days 6–21			Population [d]	Litter [e]
Control	30	36 ± 2	101 ± 6	137 ± 8	5.68 ± 0.05	44.5 ± 0.1	7 (22/337)	47 (14/30)
2,7-Dichlorodibenzo-*p*-dioxin (d)								
100.0 mg/kg-day	7	31 ± 1	122 ± 4	152 ± 5	5.80 ± 0.09	44.2 ± 0.2	6 (5/ 86)	57 (4/ 7)
Hexachlorodibenzo-*p*-dioxin (c)								
0.1 μg/kg-day	19	28 ± 2	102 ± 5	130 ± 5	5.73 ± 0.04	43.8 ± 0.1	5 (10/217)	47 (9/19)
1.0 μg/kg-day	19	27 ± 3	99 ± 5	126 ± 6	5.93 ± 0.16	45.7 ± 0.5	9 (20/218)	74 (14/19)
10.0 μg/kg-day	18	22 ± 3 [f]	97 ± 5	119 ± 6	5.12 ± 0.05 [f]	42.6 ± 0.2 [f]	25 [f] (57/229)	94 [f] (17/18)
100.0 μg/kg-day	19	6 ± 2 [f]	13 ± 7 [f]	19 ± 9 [f]	3.65 ± 0.28 [f]	35.2 ± 0.7 [f]	85 [f] (194/227)	100 [f] (19/19)
Octachlorodibenzo-*p*-dioxin (d)								
100.0 mg/kg-day	12	32 ± 2	100 ± 8	131 ± 7	5.73 ± 0.09	43.6 ± 0.4	8 (11/131)	42 (5/12)
500.0 mg/kg-day	17	35 ± 3	115 ± 4	150 ± 5	5.69 ± 0.05	44.5 ± 0.2	5 (9/199)	41 (7/17)

[a] Sample identified in Table 1; administered on days 6–15 of gestation as a corn oil: acetone (9:1) solution.
[b] Mean ± S.E. for various gestation times.
[c] Mean of litter means ± S.E.
[d] % (number resorptions/number implantations).
[e] % (number litters with at least one resorption/number litters).
[f] Significantly different from control by an analysis of variance and Tukey's test (measurements) or the 2 × 2 contingency table (resorptions), $P < 0.05$.

Table 5. Effect of treatment with hexachlorodibenzo-*p*-dioxin on the incidence of fetal anomalies.

		Incidence with treatment on days 6–15 of gestation									
		0		0.1 μg/kg-day		1.0 μg/kg-day		10 μg/kg-day		100 μg/kg-day	
Soft tissue anomalies											
Cleft palate	P[a]	0	(0/156)	1	(1/104)	0	(0/99)	0	(0/86)	47	(8/17)[c]
	L[b]	0	(0/ 28)	5	(1/ 19)	0	(0/19)	0	(0/18)	73	(8/11)[c]
Dilated renal pelvis	P	0.6	(1/156)	0	(0/104)	2	(2/99)	6	(5/86)[c]	12	(2/17)[c]
	L	4	(1/ 28)	0	(0/ 19)	5	(1/19)	17	(3/18)	18	(2/11)
Subcutaneous edema	P	5	(8/156)	6	(6/104)	55	(54/99)[c]	100	(86/86)[c]	100	(17/17)[c]
	L	21	(6/ 28)	32	(6/ 19)	100	(19/19)[c]	100	(18/18)[c]	100	(11/11)[c]
Skeletal anomalies											
Split vertebral centra	P	6	(9/158)	2	(2/103)	1	(1/99)	7	(6/86)	31	(5/16)[c]
	L	19	(5/ 27)	5	(1/ 19)	6	(1/18)	29	(5/17)	56	(5/ 9)[c]
Split sternebrae	P	0.6	(1/158)	1	(1/103)	2	(2/99)	2	(2/86)	31	(5/16)[c]
	L	4	(1/ 27)	5	(1/ 19)	11	(2/18)	12	(2/17)	56	(5/ 9)[c]
Delayed ossification of sternebrae	P	11	(18/158)	28	(29/103)[c]	12	(12/99)	34	(29/86)[c]	56	(9/16)[c]
	L	44	(12/ 27)	74	(14/ 19)	50	(9/18)	71	(12/17)	56	(5/ 9)

[a] Incidence among fetal population; % (number of affected fetuses/number fetuses examined).
[b] Incidence among litters; % (number of affected litters/number litters examined).
[c] Significantly different from control by 2 × 2 contingency table, $P < 0.05$.

Table 6. Results of chick edema bioassay: body weight, food consumption, and pericardial fluid volume calculations of chicks treated with chlorodioxins.

Treatment (sample)[a]	n	Body weight, g[b] Day 0	Body weight, g[b] Day 21	Food consumption, g[c]	Pericardial fluid volume ml ± S.E.	Pericardial fluid volume Mean log (100 × ml)	Calculated t value	Positive for chick edema factor based on Calculations	Positive for chick edema factor based on Gross lesions
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (d)[d]									
0 μg/kg	10	46 ± 1	199 ± 5	17.4	0.16 ± 0.02	1.1717	—	—	—
0.01 μg/kg	10	44 ± 1	196 ± 7	16.7	0.14 ± 0.02	1.1181	−0.74	No	No
0.10 μg/kg	10	45 ± 1	203 ± 7	17.2	0.19 ± 0.01	1.2688	+1.69	No	No
1.0 μg/kg[e]	2	42 ± 1	196 ± 24	33.7	2.34 ± 0.08	2.3680	+21.7	No	Yes
10.0 μg/kg[f]	9	42 ± 1	No survivors	11.2	1.29 ± 0.62	1.5661	+1.47	No	Yes
Hexachlorodibenzo-*p*-dioxin (c)[d]									
0 μg/kg	9	38 ± 1	194 ± 5	17.4	0.15 ± 0.01	1.1771	—	—	—
0.1 μg/kg	10	42 ± 1	197 ± 4	17.8	0.11 ± 0.02	0.9978	−1.93	No	No
1.0 μg/kg	10	43 ± 1	196 ± 6	18.2	0.09 ± 0.01	0.9387	−4.57	No	No
10.0 μg/kg[g]	9	38 ± 1	187 ± 7	16.7	0.81 ± 0.01	1.7294	+3.82	No	Yes
100.0 μg/kg[h]	10	36 ± 1	No survivors	13.3	0.62 ± 0.24	1.5650	+2.72	No	Yes
Octachlorodibenzo-*p*-dioxin (d)[i]			(Day 20)						
0% of diet	12	45 ± 1	141 ± 8	13.3	0.08 ± 0.01	0.8053	—	—	—
0.1% of diet	11	45 ± 1	124 ± 5	9.5	0.06 ± 0.01	0.7889	−0.21	No	No
0.5% of diet	11	43 ± 1	196 ± 10	10.9	0.09 ± 0.01	0.9002	+0.91	No	No

[a] Sample identified in Table 1.
[b] Mean ± S.E.
[c] Grams/chick/day.
[d] Administered orally as a corn oil: acetone solution.
[e] Animals died on days 9, 11, 11, 14, 15, 17, 18, and 19 of treatment.
[f] Animals died on days 3, 4, 4, 4, 5, 8, 8, 9, 12 and 15 of treatment.
[g] One animal died on day 19 of treatment.
[h] Animals died on days 5, 5, 6, 7, 8, 10, 11, 11, 15 and 17 of treatment.
[i] Fed in the diet (0.1% ≃ 100 mg/kg, 0.5% ≃ 500 mg/kg).

Table 7. Results of chick edema bioassay: summary of gross lesions observed in chicks treated with chlorodioxins.

Treatment (sample)[a]	Mortality	Pericardial fluid >0.2 ml	Peritoneal fluid	Subcutaneous edema	Pulmonary edema	Atrophy of spleen and/or bursa	Liver swollen and/or mottled	Gizzard erosions
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (d)[c]								
0 μg/kg	0/10[b]	2/10	0/10	0/10	0/10	0/10	0/10	0/10
0.01 μg/kg	0/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10
0.10 μg/kg	0/10	2/10	0/10	0/10	0/10	0/10	0/10	0/10
1.0 μg/kg	8/10	10/10	9/10	9/10	5/10	2/10	7/10	1/10
10.0 μg/kg	10/10	5/10	9/10	9/10	5/10	0/10	6/10	0/10
Hexachlorodibenzo-*p*-dioxin (c)[c]								
0 μg/kg	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
0.1 μg/kg	0/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10
1.0 μg/kg	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
10.0 μg/kg	1/10	9/10	3/10	1/10	1/10	1/10	3/10	0/10
100.0 μg/kg	10/10	6/10	3/10	8/10	8/10	0/10	4/10	0/10
Octachlorodibenzo-*p*-dioxin (d)[d]								
0% of diet	0/12	0/12	0/12	0/12	0/12	0/12	0/12	2/12
0.1% of diet	0/12	0/12	0/12	0/12	0/12	0/12	0/12	7/12
0.5% of diet	0/12	0/12	0/12	0/12	0/12	0/12	0/12	7/12

[a] Sample identified in Table 1.
[b] Number affected/total number in group.
[c] Administered orally as a corn oil: acetone solution.
[d] Fed in the diet (0.1% $\cong$ 100 mg/kg, 0.5% $\cong$ 500 mg/kg).

Microscopic examination of this organ revealed a highly variable pattern and degree of hepatic necrosis with various degrees of degeneration and regeneration of the hepatocytes, depending upon the post-treatment interval. Necrosis was observed both in the centrilobular and periportal areas. The degree of necrosis of the liver was not sufficient to conclude that it was responsible for death. Hepatic lesions were observed in rats, mice, rabbits, and dogs. In addition to hepatic involvement, other changes observed sporadically include fat necrosis, periarteritis, serous atrophy of fat, and ascites.

Discussion and Summary

The studies reported here confirmed the high toxicity of 2,3,7,8-TCDD. In addition, some perspective of the relative toxicities of 2,7-DCDD, HCDD, and OCDD has been obtained. 2,7-DCDD and OCDD failed to cause death in female rats given oral doses of 1 g/kg; even larger doses were given to mice without causing death. Limited data suggest that oral doses of approximately 100 mg/kg of HCDD are needed to cause death in male rats. In the teratology study, no deaths occurred following administration of 100 μg/kg of HCDD to female rats for 10 consecutive days.

2,3,7,8-TCDD is much more toxic than the other chlorodibenzodioxins studied; the LD_{50} ranged from 0.6 μg/kg in male guinea pigs to 115 μg/kg in rabbits. Dogs appear to be less sensitive than rabbits. Others have reported 100% mortality in rabbits treated with 10 μg/kg (*15*) and chick embryos treated with 0.05 μg/egg (*5*).

Death following treatment with a lethal dose of 2,3,7,8-TCDD is often delayed for several weeks. Among the animals which died following treatment, approximately half the deaths occurred between 13 and 18 days after treatment, with one animal dying as late as 43 days after a single oral dose. In mice and rabbits, there is a marked individual difference in susceptibility to this compound which makes it difficult to conduct acute lethality studies.

If the results of the rabbit eye irritation test can be extrapolated to man, accidental contact of these chlorodibenzodioxins with the eyes should not present a serious threat to vision. However, repeated contact with the skin of small amounts of either 2,3,7,8-TCDD or HCDD may be expected to produce chloracne. Sensitivity to 2,3,7,8-TCDD was recognized by industry years ago, and precautions have been taken to minimize its occurrence and prevent contamination of worker's skin. HCDD is apparently a less potent acnegen than 2,3,7,8-TCDD.

As previously reported, 2,3,7,8-TCDD is highly embryotoxic (*6*). The no-effect level for embryotoxicity was 0.03 μg/kg-day of 2,3,7,8-TCDD. In contrast to the high embryotoxicity of the symmetrical 2,3,7,8-TCDD, 1,2,3,4-TCDD was not embryotoxic at doses as high as 800 μg/kg-day (*16*).

By previously described definitions of teratogenicity and embryotoxicity (*17*), HCDD is teratogenic in the rat at a 100 μg/kg-day dose level, given orally on days 6 through 15 of gestation. Treatment of pregnant rats with HCDD caused embryotoxicity evidenced by a dose-related decrease in fetal body weight and crown–rump length and an increase in the incidence of fetal resorptions (Table 4). Likewise, the incidence of certain soft tissue and skeletal anomalies increased in a manner related to the dose level of HCDD (Table 5). A 0.1 μg/kg-day dosage of HCDD had no effect on embryonal or fetal development.

OCDD caused embryotoxicity but was not teratogenic at 500 mg/kg-day. OCDD and 2,7-DCDD caused neither teratogenicity nor embryotoxicity at 100 mg/kg-day. Khera and Ruddick (*16*) reported that the administration of 2 mg 2,7-DCDD/kg-day was associated with microscopic myocardial and pericardial lesions in rat fetuses. However, examination of sections of myocardium and pericardium from fetuses of dams treated with 100 mg doses in this study revealed no morphological differences from controls.

Both 2,3,7,8-TCDD and HCDD give positive results in chick edema bioassays (Table 6). This HCDD result is consistent with a previous report that the HCDD isolated

from pentachlorophenol produced chick edema (*5*). These same authors reported that 2,3,7,8-TCDD was extremely toxic in the chick embryo assay but did not report that it produced chick edema.

Pathological changes observed in animals treated with chlorodibenzodioxins were inconsistent from animal to animal and species to species. Hepatic lesions were observed consistently, but the nature, degree, and distribution of the lesions were variable. Changes in organs other than the liver were sporadic and unpredictable. Gross and microscopic examination of tissues after chlorodibenzodioxin treatment did not reveal the cause of death. An in-depth evaluation of the toxicity associated with chronic exposure to the chlorobenzodioxins is needed.

Isomers of a chlorodibenzodioxin can produce different degrees of toxicity; 2,3,7, 8-TCDD is highly embryotoxic and a potent acnegen, but 1,2,3,4-TCDD is neither embryotoxic nor acnegenic.

The toxicity of chlorodibenzodioxins other than those evaluated in this study has not been reported. Purified samples of trichloro-, pentachloro-, and heptachlorodibenzo-*p*-dioxin which are free of tetrachloro- and hexachlorodibenzo-*p*-dioxin need to be synthesized for study. However, heptachlorodibenzo-*p*-dioxin cannot be highly toxic, since studies on octachlorodibenzo-*p*-dioxin containing several per cent of heptachlorodibenzo-*p*-dioxin have tested the same as the pure product.

Studies on the chlorodibenzodioxins have led to the following conclusions: (1) 2,7-dichlorodibenzo-*p*-dioxin and octachlorodibenzo-*p*-dioxin have a low acute toxicity; (2) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin has an unusually high toxicity; (3) hexachlorodibenzo-*p*-dioxin is highly toxic but less toxic than 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; (4) all chlorodibenzodioxins are not alike in their toxicological properties. Isomers of the same dibenzo-*p*-dioxin vary in toxicological properties, making it important to identify them specifically.

Acknowledgement

The authors are grateful to J. E. Bourne, P. A. Keeler and R. W. Lisowe for their assistance in all aspects of this study.

REFERENCES

1. Herxheimer, K. Über chlorakne. Münch. Med. Wochenschr. 46: 278 (1899).
2. Hofman, H. Th. New experiences with highly toxic chloro hydrocarbons. Archiv Exper. Pathol. Pharmacol. 232: 228 (1957).
3. Kimmig, J., and Schulz, K. H. Berufliche Akne (sog. Chlorakne) durch chlorierte aromatische zyklische Äther. Dermatologica 115: 540 (1957).
4. Jones, E. L., and Krizek, H. A technic for testing acnegenic potency in rabbits, applied to the potent acnegen, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. J. Invest. Derm. 39: 511 (1962).
5. Higginbotham, G. R., et al. Chemical and toxicological evaluations of isolated and synthetic chloroderivatives of dibenzo-*p*-dioxin. Nature 220: 802 (1968).
6. Sparschu, G. L., et al. Study of the teratogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the rat. Food Cosmet. Toxicol. 9: 405 (1971).
7. Anonymous. Search for chick edema factor. Chem. Eng. News 45: 10 (Jan. 30, 1967).
8. Thompson, W. R. Use of moving averages and interpolation to estimate median effective dose. Part 1. Fundamental formulas, etc. Bacteriol. Rev. 11: 115 (1947).
9. Weil, C. S. Tables for convenient calculation of median effective dose (LD50 or ED50) and instructions in their use. Biometrics 8: 249 (1952).
10. Litchfield, J. T., and Wilcoxon, F. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Therap. 96: 99. (1949).
11. Wilson, J. G. Methods for administering agents and detecting malformations in experimental animals. In: Teratology Principles and Techniques. J. G. Wilson and T. Warkany, (Eds.), University of Chicago Press, Chicago, 1965, p. 262.
12. Dawson, A. B. A note on the staining of the skeleton of cleared specimens with Alizarin Red-S. Stain Technol. 1: 123 (1926).
13. Steel, R. G. D. and Torrie, H. H. Principles and Procedures of Statistics. McGraw-Hill, New York, 1960, pp. 73, 81, 347, 349, 366.
14. Horwitz, W., Ed., Official Methods of Analysis. 10th ed. Association of Official Agricultural Chemists. Washington, D.C., 1965, Sections 26.087–26.091.
15. Milnes, M. H. Formation of 2,3,7,8-tetrachlorodibenzodioxin by thermal decomposition of sodium 2,4,5-trichlorophenate. Nature 232: 395 (1971).

16. Khera, K. S., and Ruddick, J. A. Polychlorodibenzo-*p*-dioxins: Perinatal effects and dominant lethal test in Wistar rats. Advan. Chem. Ser. 121, R. F. Gould, Ed., American Chemical Society, Washington, D.C., in press.

17. Schwetz, B. A., Sparschu, G. L., and Gehring, P. J. The effect of 2,4-L and esters of 2,4-D on rat embryonal, foetal and neonatal growth and development. Food Cosmet. Toxicol. 9: 801 (1971).