Mouse Teratology Studies with Chlorodibenzo-P-Dioxins

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Recently, much attention has been focused on the polychlorinated dibenzo-p-dioxin class of compounds. The extremely high toxicity of the 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) isomer warranted an examination of additional isomers and members of this class of compounds.

TCDD was produced as a contaminant or by-product in the manufacture of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), a herbicide which was widely used in this country. Awareness of the toxicity of TCDD occurred as the result of an industrial accident that produced chloracne in the workers (BLEIBERG et al., 1964) as well as porphyria cutanea tarda (POLAND et al., 1971). In 1968, chlorodibenzo-p-dioxins were found to be the causative agent of chick edema disease first observed in 1957 (HIGGINBOTHAM et al., 1968). More recently TCDD was found to be teratogenic and fetotoxic in mice (COURTNEY and MOORE, 1971; NEUBERT and DILLMAN, 1972) and rats (SPARSCHU, et al., 1971). The dosage levels for these effects are in the range of microgram per kilogram of body weight.

Since TCDD affected fetal development so markedly and at such low dosage levels, this compound ranks as one of the most biologically active chemicals studied in mammalian fetuses. The uniquely high degree of toxicity of TCDD has generated interest in other tetrachloro-isomers as well as other members of this class of compounds containing more or less chlorines. Two current reviews of the wide range of toxic and pathological effects has been presented by Kimbrough (1972, 1974). Possible mechanisms of actions of TCDD were suggested at a symposium on dioxins (MOORE, 1973). It was not known how many of this class of compounds shared the extremely toxic and teratogenic properties of TCDD in the mouse. Thus, this study was undertaken to evaluate the teratogenic potential in mice of other members of the class of chlorinated dibenzo-p-dioxin compounds.

MATERIALS AND METHODS

Female CD-1 mice and pregnant CD-1 mice with known insemination dates were procured from Charles River Laboratories, Wilmington, Massachusetts. Detection of a vaginal plug indicated day 1 of pregnancy. The mice were randomly selected and assigned to control or experimental groups. The various chlorinated dibenzo-p-dioxins were prepared and supplied by Dr. A. Pohland of the Food and Drug Administration (POHLAND and YANG, 1972). The abbreviation TCDD is reserved for the 2,3,7,8-tetrachlorodibenzo-p-dioxin compound.

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Compounds were administered orally or subcutaneously. Oral administration was by means of gastric intubation using a volume of 0.1 ml/mouse/day. The following solutions were used for oral administration. Dibenzo-p-dioxin was dissolved in corn oil. The octachlorodibenzo-p-dioxin was dissolved in 15% anisole in corn oil. The remaining compounds were dissolved in 5% anisole in corn oil. Compounds administered subcutaneously were dissolved in DMSO employing 0.1 mg/mouse/day.

In order to select dose levels for the teratology studies, the various chlorinated dioxins were administered by gastric intubation daily to female CD-1 mice for 14 days. They were observed for an additional 7 days.

For the teratology studies, compounds were administered from the 7th through the 16th day of gestation. These mice were sacrificed on day 18 except those receiving octachlorodioxin and their respective controls. They were sacrificed on day 17 of gestation. Upon sacrifice, the fetuses were weighed, examined and stored in Bouin's solution until necropsied. The following conventions were observed in compiling data. If a fetus was either dead or resorbed, it was regarded as a dead fetus. Only live fetuses were examined for physical abnormalities. A fetus was classified normal if it was alive and had at least one type of anomaly, regardless of type. A fetus was said to have abnormal kidneys if at least one of its kidneys were affected. In calculating the ratios of liver to body weight in the mother, maternal body weight was defined as the difference between the weight of the mother on the day it was killed and the gravid uterus weight. Maternal weight gain was defined as the difference in the corrected maternal weight on the day it was killed and its weight on day 6 of pregnancy. Averages were calculated for each litter, then across litters. Statistical analyses were performed using the Student "t" test.

RESULTS AND DISCUSSION

The results from treating female CD-1 mice with various doses of the different dibenzo-p-dioxins are presented in Table 1. Administration of the compounds for 14 days at the doses indicated produced no lethal dose values. The dosage for the dibenzo-p-dioxin, dichlorodibenzo-p-dioxin, and octachlorodibenzo-p-dioxin are expressed as milligrams per kilogram of body weight per day while the others are expressed as micrograms per kilogram of body weight per day. The change in body weight reflects the difference in body weight on the first day of the study to the last, which was seven days after the last dose. The only unusual value was from those mice which received 50 mg/kg of dibenzo-p-dioxin. Further study is needed to determine if this increase of almost seven grams was real or not. Limitations of the supply of these compounds precluded further exploration of dose studies at this time. In general, none of the dibenzo-p-dioxins studied

Table 1. Oral Administration of Dioxins to Female CD-1 Mice for 14 Days

Compound	No. of Mice	Dose/ Day	No. Dead/ Doses	Change Body Wt. (gms)
Dibenzo-p-dioxin	6	1 mg/kg	0	+0.6
	6	10	0	-0.3
	6	50	1/14	+6.8
2,7-Dichlorodibenzo-p-dioxin	6	0.5 mg/kg	0	-0.1
	6	1.0	1/8	+1.5
Combination of:				
40% 2,7-Dichlorodibenzo-p-dioxin	6	10 µg/kg	1/9	+1.2
60% 2,3,7-Trichlorodibenzo-p-dioxin	6	50	0	+0.8
	6	100	1/13	+1.6
1,2,3,4-Tetrachlorodibenzo-p-dioxin	6	5 µg/kg	0	0
	6	10	0	-0.2
	6	50	0	+0.8
	6	100	0	-0.9
2,3,7,8-Tetrachlorodibenzo-p-dioxin	10	10 µg/kg	0	+0.1
Octachlorodibenzo-p-dioxin	6	0.5 mg/kg	1/11	+0.1
	6	0.75	0	+0.7
	6	1.0	0	-0.4

Table 2. Toxicologic Evaluation of Chlorinated Dibenzo-p-dioxin Compounds in Pregnant CD-1 Mice (Compounds Administered from Day 7 to 16 of Gestation)

Dibenzo-p-dioxin Compound	Route	Dose/kg/day	No. of Litters	Av. % Fetal Mortality/ Litter	Fetal Weight (gms)		Maternal Weight Gain (g)		Maternal Liver/Body Weight x 100	
					$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
5% anisole:corn oil	oral	0.1 ml/mouse	15	6	1.02	0.16	3.5	1.93	7.8	0.59
2/3 mixture[a]	oral	100 µg	6	3	1.36	0.13***	4.6	1.10	8.0	0.84
	oral	200 µg	5	5	1.25	0.19**	4.4	1.59	7.5	0.46
1,2,3,4-tetrachloro	oral	100 µg	4	10	1.09	0.09	4.8	0.51*	7.8	0.47
	oral	250 µg	4	20	1.10	0.12	4.8	1.44	7.7	0.37
	oral	500 µg	5	5	1.08	0.08	4.5	1.56	7.8	0.43
	oral	1000 µg	5	10	1.03	0.09	3.7	1.31	8.3	0.58
	subc	500 µg	5	7	1.24	0.07	3.4	0.82	7.6	0.40
	subc	1000 µg	6	2	1.31	0.07	2.3	0.57	8.1	0.44
DMSO[b]	subc	0.1 ml/mouse	6	14	1.19	0.17	3.1	1.96	7.5	0.94
2,3,7,8-tetrachloro	oral	25 µg	7	6	1.13	0.13	3.4	2.20	8.6	0.68**
	oral	50 µg	7	13	1.01	0.13	3.4	1.07	9.4	0.44***
	oral	100 µg	6	14	0.95	0.12	2.1	3.57	7.8	1.00
	oral	200 µg	6	87	d		d		d	
	oral	400 µg	5	97	d		d		d	
	subc	25 µg	5	36	1.25	0.07	3.0	0.86	9.7	0.42***
	subc	50 µg	6	56	1.20	0.15	2.3	1.17	9.0	1.09*
	subc	100 µg	6	72	d		d		d	
	subc	200 µg	6	76	d		d		d	
15% anisole:corn oil[c]	oral	0.1 ml/mouse	5	8	0.61	0.05	0.8	1.47	6.2	0.22
octachloro[c]	oral	5 mg	6	14	0.60	0.06	0.3	1.40	6.6	0.22
	oral	20 mg	6	12	0.53	0.05	-0.1	2.31	7.2	0.60*

a mixture = 40% 2,7 dichlorodibenzo-p-dioxin and 60% 2,3,7 trichlorodibenzo-p-dioxin
b DMSO = dimethylsulfoxide
c sacrificed day 17 of gestation: all others sacrificed day 18
d marked edema p values: * = 0.05; ** = 0.01; *** = 0.001

were as toxic as TCDD, the 2,3,7,8 tetrachlorodibenzo-p-dioxin isomer, and some of the compounds could be considered relatively non-toxic.

Toxicologic results of administering the various dibenzo-p-dioxins to pregnant CD-1 mice are presented in Table 2. The diluent control solutions of 5% anisole in corn oil, DMSO or 15% anisole in corn oil did not adversely affect fetal mortality or fetal weight.

The mixture of the dichloro- and trichlorodibenzo-p-dioxin administered orally at doses of 100 and 200 mg/kg/day had no adverse effects on the fetal and maternal parameters. The fetal weights of these two groups are higher than those of their respective controls. They are also higher than values which might be anticipated for a gestational day 18 mouse fetus. It was assumed that these mice were very near term because the weights reflect a gestational day 19 mouse and one member of the experimental groups littered (omitted from these data). The maternal weight gains of the experimental groups were slightly higher than the values of the control groups, but these differences were not statistically significant. This slight increase in maternal weight gain was also seen in mice treated with the 1,2,3,4 tetrachlorodibenzo-p-dixoin isomer administered orally at doses ranging from 100 to 1000 mg/kg/day. Whether or not this increased weight gain was a true increase in weight or a manifestation of edema needs to be explored since studies with TCDD produced maternal edema. In contrast, the subcutaneous administration of the 1,2,3,4 tetrachloro-isomer produced a slight decrease in maternal weight gain compared to controls. The difference in these values was not statistically significant.

In marked contrast, TCDD adversely affected fetal weight and survival and maternal weight gain at a dose of 100 mg/kg/day. Dose levels of 200 and 400 µg/kg/day produced generalized edema in the mothers and vaginal bleeding after the sixth dose with some mice aborting shortly thereafter.

The increase in the ratio of liver to body weight seen in the mice receiving the lowest dose either orally or subcutaneously was primarily due to an increase in the liver weight. The dose of 50 µg/kg/day produced a decrease in maternal weight gain, and at the two highest doses the obvious edema made it difficult to interpret maternal weight changes. The subcutaneous administration of TCDD produced more fetal mortality at lower doses than oral administration. This suggests that the bioavailability of a subcutaneous dose is greater than that of an orally administered dose.

Treatment of pregnant mice with octachlorodibenzo-p-dioxin at 5 mg/kg/day had no adverse effects on fetal or maternal parameters. At a dose of 20 mg/kg/day, there was a slight

Table 3. Teratogenic Evaluation of Chlorinated Dibenzo-p-dioxin Compounds in CD-1 Mice

Dibenzo-p-dioxin Compound	Route	Dose/kg/day	No. of Litters	Av. No. Live Fetuses/ Litter	Av. No. Abnormal Fetuses/ Litter	% Anomalies/Total Fetuses		
						Cleft Palate	Kidney	Clubfoot
5% anisole:corn oil	oral	0.1 ml/mouse	15	11.0	0.8	0	1	4
2/3 mixture[a]	oral	100 µg	6	12.3	3.2	0	10[d]	9
	oral	200 µg	5	12.8	3.8	0	1	22
1,2,3,4 tetrachloro-	oral	100 µg	4	11.8	0.8	2	0	2
	oral	250 µg	4	11.5	0.5	0	1	3
	oral	500 µg	5	11.6	0.2	0	0	0
	oral	1000 µg	5	11.8	1.0	0	0	0
	subc	500 µg	5	11.6	5.4	0	0	2
	subc	1000 µg	6	12.2	1.0	1	4	3
DMSO[b]	subc	0.1 ml/mouse	6	11.3	0.2	0	0	1
2,3,7,8 tetrachloro-	oral	25 µg	7	10.9	4.6	3	34	3
	oral	50 µg	7	11.0	8.1	19	72	7
	oral	100 µg	6	9.7	8.3	66	71	13
	oral	200 µg	6	1.5	1.5	100	100	14
	oral	400 µg	5	0.4	0.4	100	50	50
	subc	25 µg	5	7.7	6.7	82	53	11
	subc	50 µg	6	5.3	5.0	79	58	17
	subc	100 µg	6	3.5	3.5	85	95	0
	subc	200 µg	6	3.3	3.1	100	38	18
15% anisole:corn oil[c]	oral	0.1 ml/mouse	5	11.2	0	0	0	0
octachloro-[c]	oral	5 mg	6	11.2	0.2	1	0	0
	oral	20 mg	6	11.6	0	0	0	0

a mixture = 40% 2,7 dichlorodibenzo-p-dioxin and 60% 2,3,7 trichlorodibenzo-p-dioxin
b DMSO = dimethylsulfoxide
c sacrificed day 17 of gestation: all others sacrificed day 18
d 9/10 from one litter

reduction of fetal weight which was not statistically significant. Also, the reduction in maternal weight gain was not statistically significant due to a very large standard deviation. The increase in the ratio of liver to body weight was primarily due to a loss in body weight. Fetal mortality was not affected at either dose.

All of the live fetuses were examined for malformations. These results are shown in Table 3.

The mixture of dichloro- and trichlorodibenzo-p-dioxin produced a slight increase in the number of abnormal fetuses. At the lower dose this was partly due to an increase in kidney malformations which were a mild form of hydronephrosis. Since most of these fetuses (9/10) were from one litter and kidney malformations were not observed at the higher dose, it is doubtful that this malformation was produced by the compound under study. At both dose levels there was an increase in the incidence of clubfoot. This may reflect both a natural incidence of the malformation and uterine crowding, since these fetuses weighted slightly heavier and the litters were slightly larger than the controls. However, this does not negate a possible compound effect.

The 1,2,3,4 tetrachloro-isomer did not increase the incidence of malformation at any dose level by either oral or subcutaneous administration. Since this strain of mouse has a tendency to display clubfoot, the 8% incidence of this anomaly observed at the 1000 mg/kg/day dose level needs further substantiation before being accepted as a compound effect.

In contrast TCDD produced many abnormal fetuses at all doses studied and by both routes of administration. The majority of the malformations were cleft palates and hydronephrotic kidneys, both unilateral and bilaterial. A few other anomalies such as hydrocephalus and open eye were occasionally seen. TCDD administered subcutaneously produced a greater teratogenic response at a lower dose than administration by the oral route. Administration by the subcutaneous route at the lowest dose produced about 87% abnormal fetuses per litter. This made it difficult to demonstrate a dose related response since this was close to being a maximum response. At the higher dose with both routes of administration many fetuses were observed with marked edema and petechiae.

The oral administration of 5 or 20 mg/kg/day of octachlorodibenzo-p-dioxin to pregnant CD-1 mice did not affect fetal development morphologically. The only malformation detected in this group of fetuses was a single cleft palate at the low dose.

In conclusion, TCDD, the 2,3,7,8 tetrachlorodibenzo-p-dioxin member of this class of compounds was the most fetotoxic and teratogenic of the compounds studied. The related compounds were relatively non-toxic and were not teratogenic at the doses studied.

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