Bull. Environ. Contam. Toxicol. (1986) 37:164–168
© 1986 Springer-Verlag New York Inc.

Bulletin of Environmental Contamination and Toxicology

Teratologic Evaluation of *p*-Dichlorobenzene in the Rat

E. Giavini, M. L. Broccia, M. Prati, and C. Vismara

University of Milan, Department of Biology, Via Celoria 26, 20133 Milan, Italy

p-Dichlorobenzene (p-DCB) is a significant enviromnmental chemical largely used as a moth repellent, space deodorant and fungicide. Long term rodents studies did not demonstrate carcinogenic potential after inhalation exposure levels up to 500 ppm (Loeser and Litchfield 1983). In vivo and in vitro, mutagenicity tests demonstrated no effect suggestive of p-DCB mutagenicity (Loeser and Litchfielf 1983). Teratogenic study in rats exposed to atmospheric concentrations of 75,200 or 500 ppm did not reveal embryotoxic, fetotoxic or teratogenic effects (Hodge et al. 1977, unpublished work); furthermore p-DCB was not teratogenic or fetotoxic in rabbits at exposure levels up to 800 ppm by inhalation (Hayes et al. 1985).

The purpose of this study was to assess the teratogenic potential of p-DCB by a different route from that of inhalation, allowing higher levels of exposition.

MATERIALS AND METHODS

Virgin CD-rats (Charles River, Calco,Italy) weighing 190 ± 20g, after two weeks of acclimatization, were caged overnight with mature males of the same strain (2 females:1 male) in a controlled environment (12 hours light/darkness, temperature 22 ± 2°C, 60% relative humidity, and food and water ad libitum). Mating was confirmed by detection of spermatozoa in the vaginal smear the following morning (day 1 of gestation). Pregnant females were caged individually, divided into five groups (Tab.1) and treated on days 6 through 15 of gestation with 0, 250, 500, 750 or 1000 mg/kg/day of p-DCB (99% purity,Fluka,Switzerland) dissolved in corn oil and administered by gavage at constant volume (2.5 ml/kg).

All females were observed daily for possible clinical signs of toxicity; the food consumption was determined daily and the body weight was recorded on days 1,6,9,12,15,18 and 21 of pregnancy, on the last of which, day 21, they were sacrificed to undergo necroscopy to identify major organ related pathological signs. The livers were removed and weighed. The uteri were exposed and

the following data recorded: number of corpora lutea, number and position of live, dead, and reabsorbed fetuses, and weight of the live fetuses. Half of them were subjected to examination of the viscera according to Wilson's free-hand section method (1965), whereas the rest were used for skeletal examination after staining with Alizarin red S (Staples and Schnell,1964). The degree of ossification reached was assessed according to Aliverti et al.(1979).

The data were analysed by analysis of variance or a 2X2 contingency chi square test. In all the analysis a probability of $p < 0.05$ was accepted as significant.

RESULTS AND DISCUSSION

The exposure to p-DCB induced maternal weight gain retardation only during the period of treatment at 500 mg/kg and higher. This effect was correlated to a significant reduction in food consumption (Tab.1). The maternal deaths observed at 500 and 1000 mg/kg were due to a technical error. No differences were observed in liver weight of treated females compared with the controls. The mean fetal weight was significantly reduced only at the highest dose level (Tab.1).

The occurence of visceral and skeletal alterations is reported in Tab.2. The incidence of major malformations observed in fetuses of dams treated with p-DCB was not different from that among fetuses from control dams; however, a significant increase in the number of skeletal variations was observed at 750 and 1000 mg/kg. A dose-related increase in the frequency of extra ribs was found starting from 500 mg/kg level. No differences were observed in comparison with control values in the degree of ossification of selected districts.

Hawkins et al.(1977) reported that, after repeated doses, the tissue concentrations of p-DCB following 1000 ppm inhalation exposures are similar to those after 250 mg/Kg doses by gavage. Although the embryonic levels of p-DCB in our trial are probably much higher than those obtained by Hodge et al.(1977), signs of embryotoxicity were limited to an increase in skeletal variations (500 mg/kg and higher) and to a reduction in fetal weight at 1000 mg/kg dose level. Since these embryotoxic effects were associated with a reduction in food consumption and weight gain of the exposed dams, they could be a consequence of maternal suffering rather than of a direct effect of the chemical on the embryonic development. We must conclude, therefore, that p-DCB, also by oral exposure, is not teratogenic in the rat.

REFERENCES

Aliverti A, Bonanomi L, Giavini E, Leone V G, Mariani L (1979)

TABLE 1. Reproductive performances

	p-DCB dose (mg/kg/day)				
	0	250	500	750	1000
Females					
Mated	16	16	17	16	13
Dead	0	0	1	0	1
Pregnant at term	16	12	14	16	11
Maternal weight gain (g)					
Days 6-15 (mean±SD)	47.1±10.0	38.5±12.4	37.5±9.3a	36.6±11.4a	21.2±10.3b
Days 15-21 (mean±SD)	75.0±14.1	82.4±13.2	70.2±17.7	77.5±10.4	82.3±11.0
Food consumption (g/rat/day)					
Days 6-15 (mean±SD)	27.0±1.9	24.0±1.7b	24.0±1.5b	23.2±1.3b	21.6±2.7b
Days 16-20 (mean±SD)	34.1±5.8	31.1±1.5	31.0±1.5	31.8±2.0	31.7±3.4
Liver weight (g)					
(mean±SD)	15.9±1.5	15.1±1.5	14.8±1.9	15.1±1.8	14.9±1.5
Corpora lutea	288	197	237	303	181
(mean±SD)	18.0±2.5	16.4±2.5	16.9±2.3	18.9±2.4	16.4±1.6
Implantations	253	186	193	265	176
(mean±SD)	15.8±1.8	15.5±1.9	13.7±4.1	16.5±2.1	16.0±1.4
Live fetuses	236	176	182	248	168
(mean±SD)	14.7±2.7	14.6±2.2	13.0±3.9	15.5±2.4	15.2±2.6
Resorptions	17	10	11	17	8
% preimplantation loss	12.1	5.6	18.5	12.5	2.7
% postimplantation loss	6.7	5.3	5.7	6.4	4.5
Dams with resorptions (%)	9(56.2)	6(50.0)	7(50.0)	10(62.5)	6(54.5)
Fetal weight (g)					
(mean±SD)	3.7±0.3	3.8±0.2	3.7±0.3	3.6±0.2	3.4±0.2b

a $p < 0.05$ b $p < 0.01$

TABLE 2. Incidence of fetal malformations and anomalies

	p-DCB dose (mg/kg/day)				
	0	250	500	750	1000
VISCERAL MALFORMATIONS					
Affected fetuses	2/121	0/91	1/94	0/126	0/85
Affected litters	1/16	0/12	1/14	0/16	0/11
Monolateral microphthalmia	-	-	1	-	-
Hydronephrosis	2	-	-	-	-
SKELETAL MALFORMATIONS					
Affected fetuses	0/115	0/85	0/88	0/122	0/83
Affected litters	0/16	0/12	0/14	0/16	0/11
VISCERAL ANOMALIES					
Affected fetuses	5/121	8/91	12/94	7/126	3/85
Affected litters	4/16	4/12	8/14	4/16	3/11
Cerebral hemorrage	-	-	-	2	-
Enlarged ureter	3	7	4	2	1
Renal ectopy	-	1	-	-	-
Renal pelvis dilatation	1	-	4	1	-
Urinary bladder enlarged	1	-	5	3	2
SKELETAL ANOMALIES					
Affected fetuses	41/115	29/85	37/88	61/122a	42/83a
Affected litters	15/16	12/12	12/13	14/16	11/11
Asymmetrical sternebrae	11	17	11	12	10
Emisternebrae	4	-	3	13	9
Extra ribs	7	8	15a	35b	26b
Reduced pelvic girdle ossification	2	-	-	-	-
Reduced skull ossification	10	-	3	1	1
Sternebrae bipartite	1	1	-	1	-
Vertebrae bipartite	14	6	10	9	7
Wavy ribs	6	1	1	-	-

a $p < 0.05$ b $p < 0.01$

The extent of fetal ossification as an index of delayed development in teratogenic studies on the rat. Teratology 20:237-242

Hawkins D R, Chasseud L F, Woodhouse R N, Cresswell D G (1980) The distribution, excretion and biotransformation of p-dichloro C benzene in rats after repeated inhalation, oral and subcutaneous doses. Xenobiotica 10:81-85

Hayes W C, Hanley T R, Gushow T S, Johnson K A, John J A (1985) Teratogenic potential of inhaled dichlorobenzenes in rats and rabbits. Fund Appl Toxicol 5:190-202

Loeser E and Litchfield M H (1983) Review of recent toxicology studies on p-dichlorobenzene. Fd Chem Toxicol 21:825-832

Staples R E and Schnell V L (1964) Refiniments in rapid technique in the KOH alizarin red S method for fetal bone.Stain Technol 39:61-63

Wilson J G (1965) Methods for administering agents and detecting malformations in experimental animals. In: Wilson J G and Warkany J (eds) Teratology: principles and techniques. University of Chicago Press, Chicago, p 262.

Received October 31,1985;accepted November 15,1985.