

62176

LANL Ref 9/8/1985

Toxicology Letters, 26 (1985) 85-88

85

Elsevier
TOXICOL 1985

TERATOGENESIS STUDY OF DIOXANE IN RATS

(Dioxane; rat; embryotoxicity; teratogenicity)

ERMINIO GIAVINI, CLAUDIO VISMARA and MARIA LUISA BROCCIA

University of Milan, Department of Biology, Via Celoria 26, 20133 Milan (Italy).

Received January 14th, 1985

Revised Received May 6th, 1985

Accepted May 8th, 1985

SUMMARY

The industrial solvent dioxane (1,4-diethylene dioxide) was evaluated for teratogenic potential in Sprague-Dawley rats. The compound was administered on days 6-15 of gestation by gavage (0, 0.25, 5 and 1.0 ml/kg/day). A slight maternal toxicity, as evidenced by reduced weight gain, was observed with 1.0 ml/kg. Animals were killed and subjected to uterine examination on day 21 of pregnancy. There were no differences between control and dioxane-treated groups in implantation numbers, live fetuses, postimplantation loss or major malformations. Embryotoxicity, manifested by reduced fetal weight, occurred only at the highest dose level.

INTRODUCTION

Dioxane is an industrial solvent for cellulose acetate, ethyl cellulose, oils, resins and other compounds. Long-term studies have shown it to be carcinogenic in rats, inducing nasal and hepatocellular carcinomas when administered in drinking water [2] but not when administered by inhalation [3]. Slight teratogenic effects were observed by Salzgeber and Salaun [4] in chick embryos after administering dioxane to the eggs, whereas Franceschini [5] observed developmental arrest of chick embryo buds cultured in vitro in the presence of the solvent.

Since we could find no data on the possible embryotoxic effects of this product in mammals, we decided to study this.

MATERIALS AND METHODS

Female Sprague-Dawley rats (Charles River, Calco, Italy) were used, weighing initially 180 ± 20 g. After 2 weeks of acclimatization at constant room temperature



9401

and humidity ($20 \pm 2^\circ\text{C}$, 60% relative humidity) with alternating 12-h periods of light and dark, the females were caged overnight with males of proven fertility (2 ♀:1 ♂). The day on which sperm was found in the vaginal smear was considered day 1 of gestation. The females were divided into 4 experimental groups. From day 6-15 of pregnancy, 0, 0.25, 0.5 or 1.0 ml/kg/day of dioxane (99% purity; major impurity acetal 0.7%; SIO, Ospiate, Italy), diluted with water, was administered daily by gavage at constant volume (3 ml/kg).

All dams had free access to water and food (Standard diet 4RF21, Italian Mangimi, Settimo Milanese, Italy). Food consumption was determined daily and the rats were weighed every 3 days. All were killed by chloroform on day 21 of gestation. During laparotomy, the number of corpora lutea, implantations, resorptions and live fetuses was recorded. The latter were immediately weighed and inspected for external malformations. 50% Were subjected to examination of the viscera, ac

TABLE I
REPRODUCTIVE PERFORMANCES

	Dioxane dose (ml/kg/day)			
	0	0.25	0.5	1
Pregnant females	17/18	17/18	19/19	20/20
Maternal weightgain (g)				
Days 6-15 (mean \pm SD)	33.5 \pm 11.7	36.8 \pm 9.4	41.1 \pm 14.4	27.5 \pm 10.8
Days 15-21 (mean \pm SD)	66.0 \pm 12.2	65.0 \pm 8.8	67.9 \pm 10.9	60.4 \pm 14.2
Days 1-21 (mean \pm SD)	120.2 \pm 21.9	124.2 \pm 19.6	124.3 \pm 16.0	107.8 \pm 16.2
Food consumption (g/rat/day)				
Days 6-15 (mean \pm SD)	24.9 \pm 1.8	25.7 \pm 1.6	25.3 \pm 3.1	23.7 \pm 1.8 ^b
Days 16-20 (mean \pm SD)	26.9 \pm 1.4	28.6 \pm 2.4 ^b	29.7 \pm 1.7 ^a	28.2 \pm 1.0 ^a
Corpora lutea	270	262	294	303
(mean \pm SD)	15.8 \pm 2.1	15.4 \pm 1.7	15.4 \pm 2.1	15.1 \pm 1.7
Implantations	252	241	278	275
(mean \pm SD)	14.8 \pm 2.3	14.2 \pm 2.1	14.6 \pm 1.8	13.7 \pm 2.5
Live fetuses	230	229	263	252
(mean \pm SD)	13.5 \pm 2.7	13.4 \pm 2.2	13.8 \pm 2.1	12.6 \pm 2.9
Resorptions and dead fetuses	22	12	15	23
Preimplantation loss (%)	6.6	8.0	5.4	9.2
Postimplantation loss (%)	8.7	4.9	5.3	8.3
Dams with resorptions	10	9	8	8
Mean fetal weight (g)				
(mean \pm SD)	3.8 \pm 0.2	4.0 \pm 0.2	3.9 \pm 0.2	3.6 \pm 0.2 ^a

^a $P < 0.01$

^b $P < 0.05$

according to Wilson's free-hand section method [6], and the other 50% were used for skeletal examination after staining with Alizarin red S [7]. The degree of ossification reached was evaluated using the parameters indicated by Aliverti et al. [8].

The data were analysed using Student's *t*-test or analysis of variance, except for pre- and postimplantation loss and frequency of malformations, which were analysed using 2 × 2 contingency tables.

RESULTS AND DISCUSSION

The females in all groups stood up well to dioxane treatment. Those in the group treated with 1 ml/kg showed a slightly smaller gain in weight compared to control

TABLE II
INCIDENCE OF FETAL MALFORMATIONS AND ANOMALIES

	Dioxane dose (ml/kg/day)			
	0	0.25	0.5	1
Major malformations				
External	1/230	0/229	0/263	0/252
Runted	1	0	0	0
Visceral	1/112	0/113	1/128	0/123
Undescended testis	1	0	0	0
Adrenals ectopia	0	0	1	0
Skeletal	0/102	1/116	0/135	0/129
Sacro-caudal agenesis	0	1	0	0
Minor anomalies				
Visceral	1/112	2/113	2/128	4/123
Enlarged ureter	1	1	0	3
Renal pelvis dilatation	0	1	2	1
Skeletal	19/102	24/116	23/135	36/129
Sternebrae bipartite	1	0	0	0
Asymmetrical sternebrae	3	2	3	2
Emisternebrae	6	4	13	19
Extra ribs	9	16	6	11
Wavy ribs	0	0	1	0
Vertebrae bipartite	1	2	0	4
Emivertebrae	0	1	0	0
Ossified (mean ± SD)				
Sternebrae	5.74 ± 0.26	5.87 ± 0.27	5.54 ± 0.50	5.40 ± 0.48
Metacarpal	3.67 ± 0.27	3.89 ± 0.17	3.74 ± 0.86	3.64 ± 0.31
Caudal vertebrae	3.44 ± 0.45	3.88 ± 0.51	3.52 ± 0.36	3.36 ± 0.52

$P < 0.05$.

(data not shown). This effect occurred not only during the period of treatment, but continued into the second stage of gestation (data not shown). This could be due to reduced consumption of food, which was especially evident in the first 2 days of treatment (see also Table I). However, a toxic effect of the solvent cannot be excluded, particularly in the last stage of gestation, when a significant increase in food consumption (Table I) was not matched by a corresponding gain in weight.

Compared with controls, dioxane did not induce variations in the number of implantations, live fetuses and resorptions, whereas the average weight of live fetuses from dams treated with 1 ml/kg was significantly less than control (Table I).

The frequency of major malformations (Table II) remained within normal limits for all groups, and no deviations were found regarding minor anomalies and variants when compared with the control group. On the other hand, with the highest dioxane dose a significant retardation was found in the area of the sternum. Together with the reduced fetal weight, this leads to the assumption that developmental retardation had occurred at term.

Dioxane at the dosage used was not teratogenic in our strain of rats. Nevertheless, an embryotoxic effect (developmental retardation) has been observed with 1 ml/kg/day dioxane. At this dosage, the females showed a reduced weight gain, mainly evident in the first 3 days of treatment. Kociba et al. [2] observed the same effects in rats drinking water containing 1% dioxane, within 2 days after initiating the treatment. Because no other treatment-related effects on the litters have been observed, it is likely that the fetal retardation can be ascribed to maternal toxicity.

REFERENCES

- 1 M.F. Argus, J.C. Arcos and C. Hoc-Ligeti, Studies on carcinogenic activity of protein-denaturing agents: hepatocarcinogenicity of dioxane, *J. Natl. Cancer Inst.*, 35 (1965) 949-958.
- 2 R.J. Kociba, S.B. McCollister, C. Park, T.R. Torkelson and P.J. Gehring, 1,4-Dioxane, I. Results of 2-year ingestion study in rats, *Toxicol. Appl. Pharmacol.*, 30 (1974) 275-286.
- 3 T.R. Torkelson, B.J.K. Leong, R.J. Kociba, W.A. Richter and P.J. Gehring, 1,4-Dioxane, II. Results of 2-year inhalation study in rats, *Toxicol. Appl. Pharmacol.*, 30 (1974) 287-298.
- 4 B. Salzgeber and J. Salaun, The action of thalidomide on the fowl embryo, *J. Embryol. Exp. Morphol.*, 13 (1965) 159-170.
- 5 M. Franceschini, The influence of dioxane and thalidomide on the growth of chick embryotibial buds in organotropic cultures, *Sperimentale*, 114 (1964) 1-17.
- 6 J.G. Wilson, Methods for administering agents and detecting malformations in experimental animals, in J.G. Wilson and J. Warkany (Eds.), *Teratology: Principles and Techniques*. University of Chicago Press, Chicago, 1965.
- 7 R.E. Staples and V.L. Schnell, Refinements in rapid technique in the KOH alizarin red S method for fetal bone, *Stain Technol.*, 39 (1964) 61-63.
- 8 V. Aliverti, L. Bonanomi, E. Giavini, V.G. Leone and L. Mariani, The extent of fetal ossification as an index of delayed development in teratogenic studies on the rat, *Teratology*, 20 (1979) 237-242.