0701

TOXICOLOGY AND APPLIED PHARMACOLOGY 58, 438–443 (1981)

Embryotoxic Effect of Cadmium on Rats upon Oral Administration

L. MACHEMER AND D. LORKE

Institute of Toxicology BAYER AG, Friedrich-Ebert-Strasse 217, Wuppertal-Elberfeld, Germany

Received July 14, 1980; accepted December 9, 1980

Embryotoxic Effect of Cadmium on Rats upon Oral Administration. MACHEMER, L., AND LORKE, D. (1981). *Toxicol. Appl. Pharmacol.* **58**, 438–443. The dose/effect relationships and/or threshold doses for embryotoxicity of cadmium in rats were studied by administering cadmium chloride both in food or by oral gavage. From the 6th to the 15th day of pregnancy, the animals were given either 1.2, 3.5, or 12.5 mg/kg body wt of Cd^{2+} in the food, or 1.8, 6.1, 18.4, or 61.3 mg/kg body wt of Cd^{2+} by oral gavage. The "no toxic-effect" doses for embryotoxic effects were 12.5 (feeding) and 6.1 mg/kg body wt of Cd^{2+} (oral gavage). These values are higher than the amount stated by WHO as safe for man (500 μg/person per week) by approx 8000 and 4000 times, respectively.

There have been numerous investigations of the embryotoxic effects of cadmium on various animals species (Ferm and Carpenter, 1967; Mulvihill *et al.*, 1970; Schroeder and Mitchener, 1971; Lucis *et al.*, 1972; Nolen *et al.*, 1972a,b; Chernoff, 1973; Barr, 1973; Dencker, 1975; Hastings *et al.*, 1978; Prigge, 1978; Samarawickrama, 1978; Gale, 1979; Layton and Layton, 1979; Rohrer *et al.*, 1979; Samarawickrama and Webb, 1979).

On the basis of available reports, the embryotoxic and teratogenic potential of cadmium in various animal species is indisputable. However, the presence of such effects alone in inadequate for the toxicologist for the purposes of risk assessment. When assessing a hazard, it is of prime importance to know the concentrations and/or doses at which toxicological effects occur. Therefore, pregnant rats were fed cadmium chloride in different concentrations, either with their daily food or by oral gavage. The following is a report on these investigations.

METHODS

Cadmium chloride made by the Merck Company, Article No. 2011, was used for the experiments. The studies were conducted with FB 30 rats (Long–Evans strain) from our own, conventionally kept, breeding station. At the beginning of the test, the females weighed 200–270 g and were 2.5–3.5 months old; the males weighed 350–500 g and were 3 to 6 months old. In each group 21 to 28 inseminated rats were used, and from this number 19 or 20 fertilized animals per group were obtained. Apart from during mating, the animals were kept individually in Makrolon[1] cages of type II under standardized conditions with 12 hr of fluorescent light per day, 20–23°C room temperature, and 40–60% relative humidity. The animals in the feeding study received a meal diet[2] and those in the gavage study received a pelletized diet[2] both received tap water *ad libitum*. For mating, one male was placed overnight with two females in a Makrolon[1] cage of type III. The following morning, a vaginal smear was taken and, if sperm were detected, the female was used for the experiment. Evidence of sperm was the criterion for Day 0 of pregnancy.

The female rats were treated from the 6th to the 15th day of pregnancy as shown in Table 1. The control group for the feeding experiment received diet with no additives. In the oral gavage experiment, the control group received 10 ml/kg body wt of demineralized water, i.e., the same volume as in the treated groups but without cadmium.

Caesarean sections were performed on the 20th day of pregnancy and the following examinations made:

[1] Makrolon cages, supplier: Presswerke AG, Essen, Germany.

[2] Altromin, supplier: Altromin GmbH, Lage, Germany.

0041-008X/81/060438-06$02.00/0

Copyright © 1981 by Academic Press, Inc.
All rights of reproduction in any form reserved.

9311

TABLE 1

TREATMENT OF RATS

Experiment	Dose	Equivalent (mg/kg)
	(Cd)	(Cd)
Feeding	10 ppm	approx 1.2
	30 ppm	approx 3.5
	100 ppm	approx 12.5
	($CdCl_2$)	(Cd)
Oral gavage experiment	3	1.84
	10	6.13
	30	18.39
	100	61.32

Determination of the number of implantations and of the living and dead fetuses as well as of the dead and absorbed embryos and fetuses (resorptions), the litter weight and the average fetus weight per litter, the number of stunted forms (fetuses under 3 g weight), and the average placenta weight per litter; examination of approximately one-third of all fetuses for visceral malformations using the Wilson technique (Wilson, 1965, modification according to Machemer and Stenger, 1971), exenteration of the remaining fetuses, assessment of the abdominal and thoracic organs, and subsequent clearing and staining according to Dawson (1926). The cleared fetuses were assessed according to Lorke (1963, 1965).

The results were evaluated by means of the distribution-free ranking test according to Wilcoxon (1945, 1947) in the modified version of Mann and Whitney (1947), and using the χ^2 test (correction by Yates (1934)).

RESULTS

Effects on Pregnant Rats

Administration of cadmium in food in concentrations up to 100 ppm, corresponding to roughly 22 mg cadmium chloride/kg body wt, impaired neither the appearance nor the general behavior of the animals. There were no deaths, and the food consumption of the animals was, on average, not impaired during the treatment period. Table 2 shows the average food consumption in grams per animal per day during the treatment period from the 6th to the 15 day of pregnancy.

However, the highest concentration, 100 ppm, had a negative effect on the weight gain of the females, since this group on the average gained significantly less weight than the control group during the treatment period ($p < 0.05$, Table 3). Groups receiving concentrations of up to 30 ppm in their food showed no difference from the control group in this respect. The reduced weight gain observed during the treatment period of the 100-ppm group was recovered after the treatment was stopped (Table 3).

In the oral gavage test, only the 3 mg/kg/day dose was well tolerated by the rats. After administration of 10 mg/kg/day, 3 out of 23 animals had coarse fur and ap-

TABLE 2

FOOD CONSUMPTION DURING FEEDING EXPERIMENT

Cadmium concentration (ppm)	Average food consumption (g/animal/day)
0	33.3
10	36.4
30	32.2
100	33.6

TABLE 3

WEIGHT INCREASE OF PREGNANT ANIMALS

Experiment	Concentration or dose	Average weight increase (g): Treatment period	Average weight increase (g): Pregnancy
Feeding	0 ppm	43.2	111.1
	10 ppm	47.1	127.6**
	30 ppm	45.5	120.4
	100 ppm	35.0*	112.0
Oral gavage	0 mg/kg	26.2	79.0
	3 mg/kg	27.5	87.5
	10 mg/kg	19.2**	80.7
	30 mg/kg	−0.8**	39.6**
	100 mg/kg	—[a]	—[a]

[a] Since no animal was pregnant at the time of the caesarian section, no data for the 100 mg/kg group are available.

*, ** Significantly different to control group, $p < 0.05$ or < 0.01, respectively (Wilcoxon–Mann–Whitney U test).

peared ill. Moreover, the average weight increase during the treatment period was significantly reduced in comparison with the control group, as shown in Table 3 ($p < 0.01$). When the treatment with 10 mg/kg/day was discontinued the animals compensated for the lesser weight gain, and the average increase during the entire period of pregnancy was similar to the control group. There were no death in this treatment groups.

At 30 mg/kg/day, 8 out of 28 animals had bristly fur and 2 of these animals had constipation. Another animal in this group had diarrhea and one animal died as a result of the treatment. Weight gain compared to control was clearly depressed during both the treatment period and the entire period of pregnancy ($p < 0.01$).

The 100 mg/kg/day dose was highly toxic and led to coarse fur in 14 out of 25 animals; 7 of these animals also had intestinal disorders, such as constipation or absence of defecation. Another animal in this group had diarrhea. Vaginal hemorrhages were observed in 5 animals, probably due to the embryolethal effect. Of the 25 animals in this group 15 animals died, 13 of which were pregnant. The gross necropsy findings were relatively uniform with large area haemorrhages or healed or open ulcers in the fundus and/or pylorus. The intestinal mucous membrane was generally reddened throughout its length.

The intestines were almost empty; at most, a very thin, sometimes gelatinous, yellow pap was found in the cecum. The large amount of blood found in the kidneys was striking. A number of animals from the 30 and 100 mg/kg groups, which had survived until the caesarean section, had a greenish discoloration of the kidneys.

The results of insemination and fertilization (Table 4) shows that all concentrations in the feeding experiment and doses up to 30 mg/kg/day in the oral gavage test had no effect on pregnancy. However, the few fertilized animals of the 100 mg/kg group of the oral gavage experiment which survived resorbed all of their embryos. Thus a clear embryolethal effect accompanied the distinct maternal toxicity.

Effects on Embryonic and Fetal Development

Table 5 provides information on the reproduction parameters. Since the 100

TABLE 4

INSEMINATION AND FERTILIZATION RESULTS

Experiment	Concentration or dose	Number of inseminated animals[a]	Number of fertilized animals: Total	Number of fertilized animals: As % of inseminated animals	Number of pregnant animals: Total	Number of pregnant animals: As % of fertilized animals
Feeding	0 ppm	21	20	95.2	20	100
	10 ppm	21	20	95.2	20	100
	30 ppm	24	19	79.1	19	100
	100 ppm	21	20	95.2	20	100
Oral gavage	0 mg/kg	22	20	90.9	20	100
	3 mg/kg	22	20	90.9	19	95
	10 mg/kg	23	20	86.9	20	100
	30 mg/kg	27	20	74.0	18	90
	100 mg/kg	10	5*	50.0	0**	0

[a] The data do not take into consideration the dead animals of the 30 or 100 mg/kg groups.

*, ** Significantly different from the control group, $p < 0.05$ or < 0.01, respectively (χ^2 test).

TABLE 5

REPRODUCTION DATA (AVERAGE PER LITTER)

Experiment	Concentration or dose	Number of: Implantations	Number of: Fetuses	Number of: Resorptions	Average weight (g): Fetuses	Average weight (g): Placentas	Number of fetuses assessed: By Wilson method	Number of fetuses assessed: By staining method	Fetuses with: Slight osseous changes	Fetuses with: Malformations	Stunted forms (less than 3 g)
Feeding	0 ppm	11.7	11.0	0.6	4.00	0.57	3.2	7.8	4.25	0.00	0.05
	10	12.7	12.2	0.5	4.05	0.55	3.7	8.5	4.60	0.05	0.10
	30	12.4	11.5	0.8	4.02	0.55	3.4	8.1	3.53	0.05	0.00
	100	11.4	10.4	1.0	4.04	0.58	3.3	7.1	3.90	0.25	0.10
Oral gavage	0 mg/kg	10.0	9.1	0.9	3.54	0.49	2.7	6.3	4.65	0.05	0.40
	3	10.6	10.1	0.5	3.54	0.50	3.3	7.4	6.16	0.05	0.05*
	10	10.9	10.2	0.7	3.51	0.49	3.0	7.2	5.95	0.10	0.45
	30	10.1	8.9	1.2	3.28*	0.47*	2.9	6.9	5.11	0.56*	1.89**

*, ** Significantly different to the control group, $p < 0.05$ or < 0.01, respectively.

mg/kg group of the oral gavage experiment had no fetuses at the time of the caesarean section, this group is not included in the table.

The administration of cadmium in food in concentrations up to 100 ppm or of cadmium chloride by oral gavage in doses up to 10 mg/kg body wt, daily from the 6th to the 15th day of pregnancy, had no recognizable adverse effects on the following parameters: number of implantations, number of live fetuses, number of dead and absorbed embryos and/or fetuses, average fetus weight, average placental weight, number of underdeveloped fetuses (under 3 g), number of fetuses with skeletal retardations and malformations. Administration of 30 mg cadmium chloride/kg body wt in the oral gavage experiment resulted in the following: significantly reduced fetal and/or placenta weight ($p < 0.05$), significant increase in stunted forms ($p < 0.01$) as well

TABLE 6

FREQUENCY AND TYPE OF MALFORMATIONS

Experiment	Concentration dose	Number of fetuses examined	Number of malformed fetuses: n	Number of malformed fetuses: %	Number of pregnant animals	Number of pregnant animals with malformed fetuses	Malformations
Feeding	0 ppm	221	0	0	20	0	Telencephalic hypoplasia
	10 ppm	245	1	0.41	20	1	
	30 ppm	219	1	0.46	19	1	Microphthalmia
	100 ppm	209	5	2.39	20	3	Telencephalic hypoplasia (4),[a] wavy ribs (1), microphthalmia (1)
Oral gavage	0 mg/kg	182	1	0.55	20	1	Dysplasia of the facial bones, hypoplasia of the long bones of the rear limbs
	3 mg/kg	203	1	0.49	19	1	Dysplasia of the facial bones
	10 mg/kg	205	2	0.98	20	2	Costal fusion (1), anophthalmia (1)
	30 mg/kg	178	10	5.62	18	4	Dysplasia of the facial bones (6), dysplasia of the rear limbs (6), general edema (6), palatoschisis (2), cryptorchism (1), exenteration (1)
Control groups of other experiments carried out during the same period	0 mg/kg	236	4	1.69	20	2	Telencephalic hypoplasia (3), wavy ribs (1)
	0 mg/kg	222	4	1.80	20	4	Telencephalic hypoplasia (3), costal fusion, and vertebral dysplasia (1)

[a] Numbers in parentheses indicate number of fetuses affected.

as a significant increase in malformations compared to the control group ($p < 0.05$). The type of malformations occurring in the 30 mg/kg group were varied and affected very different organ systems. Table 6 summarizes the information on the malformations occurring in these investigations.

DISCUSSION

The results of the feeding experiment demonstrate that a concentration of 100 ppm of cadmium in the food, corresponding to roughly 22 mg cadmium chloride/kg body wt, shows no observable adverse effects with respect to the development of the embryos. The corresponding dose in oral gavage experiment was 10 mg cadmium chloride/kg body wt, corresponding to 6.1 mg cadmium/kg body wt. Higher doses, which were severely maternally toxic, inhibited fetal and placenta growth, and had teratogenic effects (30 mg cadmium chloride/kg body wt, corresponding to 18.4 mg cadmium/kg body wt) and/or they were not at all compatible with embryo development (100 mg cadmium chloride/kg body wt, corresponding to 61.3 mg cadmium/kg body wt). Embryotoxic effects in maternally toxic doses are not particularly surprising and provide no clues as to a specific embryotoxic property of the examined agent.

The fact that individual malformations occurred in all groups treated in the feeding experiment does not necessarily mean that they were caused by the treatment, whereas this was not the case in the control group. These malformations also occur spontaneously in the same types and frequencies, and without recognizable external cause, in the fetuses of untreated dams. A wavy rib malformation and hypoplasia of the telencephalon are the most frequent spontaneous malformations occurring in the rat strain used. Thus we have included for comparison the results of two further control groups from two investigations carried out at approximately the same time as our investigation with cadmium. The same types and frequencies of malformations occurred in these control groups as in the cadmium chloride groups of the feeding experiment. Fetuses with telencephalic hypoplasia were generally associated with individual dams, indicating an endogenous cause. On the other hand, the malformations in the 30 mg/kg group of the oral gavage experiment are unusual in type and frequency, and must be attributed to the treatment. Our results in rats basically confirm the findings of other authors. In the clearly toxic dose for the dams embryolethal damage was also present and in some cases teratogenic effects as well. In contrast to Nolen *et al.* (1972a), who reported primarily abnormalities of the urogenital system, several organs were affected in our studies. Our findings, however, are similar to those of Chernoff (1973) who also reported abnormalities of head region and limbs.

Our investigations demonstrated threshold doses for embryotoxic effects of cadmium chloride in rats of at least 100 ppm by feeding and 10 mg/kg by oral gavage. Relationships can be established between these so-called "no toxic-effect" doses and any cadmium residue, e.g., in food, etc., thus permitting the calculation of safety factors for the human intake of cadmium. Taking as a basis the WHO (1972) value of a safe intake of 500 μg/person per week, the calculation for a person of 50 kg weight would be 10 μg/kg per week or 1.5 μg/kg/day. Compared to the no toxic-effect level for embryonic development found in our studies, a safety factor of more than 8000 for the feeding experiment (100 ppm $\hat{=}$ 12.5 mg Cd/kg), and of over 4000 for the oral gavage experiment (10 mg $CdCl_2$ $\hat{=}$ 6.1 mg Cd/kg) can be calculated.

REFERENCES

BARR, M. (1973). The teratogenicity of cadmium chloride in two stocks of Wistar rats. *Teratology* **7**, 237–242.

CHERNOFF, N. (1973). Teratogenic effects of cadmium in rats. *Teratology* **8**, 29–32.

DAWSON, A. B. (1926). A note on the staining of the skeleton of cleared specimens with Alizarin red S. *Stain Technol.* **1**, 123–124.

DENCKER, L. (1975). Possible mechanisms of cadmium fetotoxicity in golden hamsters and mice: Uptake, by the embryo, placenta and ovary. *J. Reprod. Fert.* **44**, 461–471.

FERM, V. H. AND CARPENTER, S. J. (1967). Teratogenic effect of cadmium and its inhibition by zinc. *Nature (London)* **216**, 1123.

GALE, T. F. (1979). Toxic effects of cadmium and amaranth on the developing hamster embryo. *Bull. Environ. Contam. Toxicol.* **22**, 175–181.

HASTINGS, L., CHOUDHURY, H., PETERING, H. G., AND COOPER, G. P. (1978). Behavioral and biochemical effects of low-level prenatal cadmium exposure in rats. *Bull. Environ. Contam. Toxicol.* **20**, 96–101.

LAYTON, W. M., AND LAYTON, M. W. (1979). Cadmium induced limb defects in mice; Strain associated differences in sensitivity. *Teratology* **19**, 229–236.

LORKE, D. (1963). Zur Methodik der Untersuchungen embryotoxischer und teratogener Wirkungen an der Ratte. *Arch. Exp. Pathol. Pharmakol.* **246**, 147–151.

LORKE, D. (1965). Embryotoxische Wirkungen an der Ratte. *Arch Exp. Pathol. Pharmakol.* **250**, 360–382.

LUCIS, O. J. LUCIS, R., AND SHAIKH, Z. A. (1972). Cadmium and zinc in pregnancy and lactation. *Arch. Environ. Health* **25**, 14–22.

MACHEMER, L., AND STENGER, E. G. (1971). Zur Beurteilung der Foeten im teratologischen Experiment. Modifikation der "Wilson-Technik". *Arzneimittel-Forschung [Drug Res.]* **21**, 144–145.

MANN, H. B., AND WHITNEY, D. R. (1947). On a test of whether one of two random variables is stochastically larger than the other. *Ann. Math. Stat.* **18**, 50–60.

MULVIHILL, J. E., GAMM, S. H., AND FERM, V. H. (1970). Facial formation in normal and cadmium-treated golden hamsters. *J. Embryol. Exp. Morphol.* **24**, 393–403.

NOLEN, G. A., BUEHLER, E. V., GEIL, R. G., AND GOLDENTHAL, E. I. (1972a). Effects of trisodium nitrilotriacetate on cadmium and methyl mercury toxicity and teratogenicity in rats. *Toxicol. Appl. Pharmacol.* **23**, 222–237.

NOLEN, G. A., BOHNE, R. L., AND BUEHLER, E. V. (1972b). Effects of trisodium nitrilotriacetate, trisodium citrate and a trisodium nitrilotriacetate-ferric chloride mixture on cadmium and methyl mercury toxicity and teratogenesis in rats. *Toxicol. Appl. Pharmacol.* **23**, 238–250.

PRIGGE, E. (1978). Inhalative cadmium effects in pregnant and fetal rats. *Toxicology* **10**, 297–309.

ROHRER, S. R., SHAW, S. M., AND LAMAR, C. H. (1979). Cadmium fetotoxicity in rats following prenatal exposure. *Bull. Environ. Contam. Toxicol.* **23**, 23–29.

SAMARAWICKRAMA, G. P. (1978). Acute effects of cadmium during pregnancy and embryo-foetal development. In *Society of Chemical Industry, Pesticides Group Meeting, Medical Research Council Laboratories, Carshalton, Sept. 28, 1978*, Abstract

SAMARAWICKRAMA, G. P., AND WEBB, M. (1979). Acute effects of cadmium on the pregnant rat and embryo-fetal development. *Environ. Health Perspect.* **28**, 245–249.

SCHROEDER, H. A., AND MITCHENER, M. (1971). Toxic effects of trace elements on the reproduction of mice and rats. *Arch. Environ. Health* **23**, 102–106.

WHO (1972). Evaluation of certain food additives and the contaminants mercury, lead, and cadmium. *World Health Organ. Tech. Rep. Ser.*, No. 505.

WILCOXON, F. (1945). Individual comparisons by ranking methods. *Biometrics* **1**, 80–83.

WILCOXON, F. (1947). Probability tables for individual comparisons by ranking methods. *Biometrics* **3**, 119–122.

WILSON, J. G. (1965). In *Teratology, Principles and Techniques* (J. G. Wilson and J. Warkany, eds.), pp. 262–277. Univ. Chicago Press, Chicago/London.

YATES, I. (1934). Contingency tables involving small numbers and the χ^2 test. *J. Roy. Stat. Soc.* (*B*) **1**, 227–235.