Polish Journal of Occupational Medicine and Environmental Health, Vol. 5, No. 2, 159–166, 1992

THE EFFECT OF MATERNAL EXPOSURE TO DIOXOLANE ON PRENATAL AND POSTNATAL DEVELOPMENT IN RATS

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Key words: Dioxolane, Prenatal and postnatal development, Rats.

Abstract. Female rats were given by gavage every other day from days 8–20 of gestation an aqueous solution of dioxolane at daily doses equal to 0.025, 0.1 and 0.2 LD_{50} (first series – prenatal development) or from days 2–20 of gestation at daily doses equal to 0.025, 0.075 and 0.15 LD_{50} (second series – postnatal development). At doses toxic or subtoxic to maternal rats (0.1 and 0.2 LD_{50}) dioxolane did not cause increased embryo or fetus intrauterine death rates or congenital defects, it did cause, however, dose-related delays in fetal development. Dioxolane does not cause impairment of physical development or behavioral disturbances. Exposure to higher doses of the compound (0.2 LD_{50}) leads to increased perinatal death rates in the offspring, without causing, however, disturbances in the maternal instinct. The exposure of pregnant rats to dioxolane decreased haemoglobin levels in 5-week-old offspring. At a dose 1.15 g/kg (0.2 LD_{50}) the chemical significantly increased exploratory motor activity of female offspring at the age of 8 weeks, but did not affect significantly locomotor activity of males and the active avoidance acquisition of adult offspring.

INTRODUCTION

Dioxolane ($C_3H_3O_2$) (1,3–dioxolane) is a colourless liquid with a characteristic smell. It is used as a solvent for cellulose esters, paints, and as a solvent in the production of FEN-1 glue (14, 19).

Dioxolane exerts slight systemic acute toxicity in animals and it does not pose a risk of acute occupational intoxication (5). Acute inhalation exposure of rats to dioxolane at conc. of 82 g/m^3 induced an irritant effect to the conjunctive and mucosa membranes, increased the number of lymphocytic infiltration in the larynx and trachea, and produced proliferation of peribronchial lymphatic tissue (8). Repeated inhalation exposures of rats to dioxolane at concentrations of 50–2500 mg/m^3 caused planoepithelial metaplasia of the bronchial epithelium, and increased the number of focal necroses of seminiferous epithelium in testes. The changes proved, evidently, to be dose-related (56). Dioxolane increased the frequency of micronucleated polichromatic erythrocytes in bone marrow of mice (16) but did not induce reverse mutations in the Salmonella/microsome test (13) or dominant lethal mutations in the germ cells of male rats (3).

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Based on the results of a chronic inhalation toxicity study in animals, tentative exposure limits of dioxolane in the air of the working environment have been introduced in Poland: time-weighted average – 10 mg/m^3, short term exposure limit – 50 mg/m^3 (7). Since no data on the effect of dioxolane on reproduction are available, this study is aimed at evaluating the effect of oral exposure of female rats on fertility and prenatal and postnatal development of their offspring.

MATERIALS AND METHODS

The dioxolane used in this study was received from the Nitrogen Plant in Tarnow (Poland) and contained 0.93% of water and ca. 0.01% of formaldehyde. Nulliparous female rats, aged approximately 14 weeks and weighing 199 ± 12 g were obtained from our Institute's breeding colony (Imp: DAK). The animals were housed in quarters with controlled temperature (approx. 22°C) and illumination (light on between 6 a.m. and 6 p.m.). Relative humidity varied between 45–55%. The animals were maintained on commercial pelleted chow (Fodder Factory, Motycz, Poland) and tap water *ad libitum*. Females were mated overnight with 17-week-old male rats of the same strain. The morning when copulation plugs or spermatozoa appeared in vaginal smears was regarded as day 1 of gestation.

The study was performed in two series. In the first series (prenatal development) the inseminated females were divided into one control and three treatment groups receiving 0.025, 0.1 and 0.2 of median lethal dose of dioxolane, which had been earlier found to be 5.8 g/kg. Dioxolane was administered orally, by gavage, every other day from day 8 do day 20 of gestation. The female rats were killed on day 21 of gestation under ethyl ether anesthesia. Assessment of teratogenicity, embryo and fetal toxicity was performed according to methods described earlier (1). The uterus was opened and the number of live fetuses, dead fetuses, early and late resorption sites were recorded. A site was judged as a late resorption when macroscopic discrimination between fetal residues and placental material was possible. When this discrimination could not be made, the site was judged as an early resorption. Total implantation was calculated as the sum of the number of fetuses and resorption sites in each female. Live fetuses were measured for body weight, crown-rump length and examined for external malformations. Approximately half of the live fetuses from each litter were preserved in 95% ethanol for subsequent skeletal examination after staining with Alizarin S. The remaining fetuses were fixed in Bouin's fluid for visceral examination.

In the second series (postnatal development) the inseminated females were given by gavage, every other day from day 2 to 20 of gestation, aqueous solution of dioxolane at daily doses equal to 0.025, 0.075 and 0.15 LD_{50}. Concentrations of dioxolane in aqueous solution were adjusted such that the animals received 1 cm^3 of solution per 100 g of body weight. The female rats were allowed to deliver and nurse their progeny until they were weaned at 28 days of age. The assessment of physical development, viability and behavioral tests were performed according to methods described earlier (2, 18). Performance of all pups was assessed in the following tests: surface righting at the age of 3–6 days; negative geotaxis at the age of 6–12 days; forepaw suspension at 6–16 days; and air righting at 14–19 days of life. In all pups

age at appearance of eye opening, incisor eruption and pinna unfolding was determined. Randomly chosen offspring from each litter were assessed for exploratory motor activity at the age 8, 11 and 14 weeks, and avoidance acquisition of the adult offspring at age ca. 14 weeks.

In both experimental series the control animals were given, by gavage, an equivalent volume of distilled water. The maternal weight gain, daily food and water consumption intake were monitored throughout the gestation period.

Statistical evaluation. In the case of homogeneity of variance, one-way analysis of variance and Dunnett's test were used: in the case of heterogeneity the Kruskal-Wallis analysis of variance was followed by non-parametric tests (20). Frequency data were analyzed with Fisher's exact probability test. The effect of dioxolane on behavioral performance, food and water consumption and body weight gain of the offspring was evaluated with two-way analysis of variance and Scheffe's test for multiple comparison (20).

RESULTS

Evaluation of female rat fertility and of the prenatal development of their offspring

The toxic effect of dioxolane on the organism of the pregnant female rats was manifested by significantly smaller body weight gain during their pregnancy and a significantly greater relative mass of the adrenals in the animals receiving the compound at the highest dose, i.e. 1.15 g/kg (Tables 1 and 2). The daily intake of the fodder and water, and the general appearance and behaviour of the exposed and control animals did not differ significantly. Female rat fertility in terms of the percentage of the inseminated female rats found to be pregnant, and female rat

Table 1. Absolute and relative organ weights of female rats receiving daily by gevage dioxolane on days 8–20 of gestation.

	Control	Dioxolane 0.14 g/kg	Dioxolane 0.58 g/kg	Dioxolane 1.15 g/kg
Liver	14	18	18	17
(g)	11.69 ± 1.5 [a]	11.68 ± 1.27	11.62 ± 1.08	11.21 ± 1.02
(g%)	3.91 ± 0.24	3.86 ± 0.24	3.87 ± 0.24	3.85 ± 0.23
Kidneys	14	18	18	17
(g)	1.36 ± 0.15	1.40 ± 0.11	1.37 ± 0.13	1.37 ± 0.12
(g%)	0.46 ± 0.03	0.47 ± 0.04	0.46 ± 0.03	0.47 ± 0.04
Adrenals	14	18	18	17
(mg)	71.4 ± 6.3	72.9 ± 9.0	74.4 ± 8.2	80.1 ± 13.8
(mg%)	24.2 ± 2.6	24.3 ± 3.8	24.9 ± 2.7	27.7 ± 5.9 [b]

a – Mean ± SD.
b – Significantly different ($p < 0.05$) from the control group.

Table 2. Effect of dioxolane administered within 8 – 20 days of gestation on pregnancy and development in rats.

	Control	Dioxolane 0.14 g/kg	Dioxolane 0.58 g/kg	Dioxolane 1.15 g/kg
Females inseminated	17 (82.4)[a]	19 (94.7)	18 (100)	19 (89.5)
Live fetuses per litter	11.8 ± 2.2[b]	11.3 ± 2.2	11.1 ± 1.6	12.0 ± 2.0
Dead fetuses per litter	0	0	0.06 ± 0.24	0.12 ± 0.33
Litters with resorptions	3	10	9	7
Litters with late resorptions	2	6	4	4
Resorption per litter with resorptions	1.1 ± 0[b]	1.7 ± 1.1	1.2 ± 0.4	1.1 ± 0.4
Body weight gain of dams (g)	102.1 ± 13.5[b]	104.7 ± 17.6	101.1 ± 11.8	86.8 ± 20.5[d]
Fetal crown-rump length (cm)	4.2 ± 0.1[c]	4.1 ± 0.1	4.1 ± 0.08	3.8 ± 0.2[d]
Fetal body weight (g)	3.4 ± 0.2[c]	3.3 ± 0.3	3.4 ± 0.2	3.4 ± 0.2[d]
Weight of placenta (g)	0.48 ± 0.4[c]	0.58 ± 0.18[d]	0.53 ± 0.04[d]	0.51 ± 0.04[d]

a – Percentage of pregnant females given in parentheses.
b – Mean ± SD.
c – Mean of litter means ± SD.
d – Significantly different ($p < 0.05$) from control group.

Table 3. Effect of dioxolane in the incidence of fetuses retarded in development.

	Control	Dioxolane 0.14 g/kg	Dioxolane 0.58 g/kg	Dioxolane 1.15 g/kg
No. litters examined	14	18	18	17
No. fetuses external exam	157	203	199	204
No. fetuses visceral exam	80	99	98	101
No. fetuses skeletal exam	77	104	101	103
Findings: number (% incidence of finding)				
Incomplete development of skull bones	1 (1.3)	8 (7.7)	12 (12.1)[a]	52 (50.5)[a]
Absence of one or several sternum ossifications	1 (1.3)	7 (6.7)	3 (3.0)	13 (12.6)[a]
Shortening of 13th ribs	0	0	3 (3.0)	1 (1.0)

a – Significantly different ($p < 0.05$) from the control group.

fecundity in terms of the number of live fetuses in a litter were similar in the control group and in the groups exposed *per os* to dioxolane (Table 2).

Dioxolane administered to the maternal rats at the dose of 1.15 g/kg (0.2 LD_{50}) showed fetotoxic effect, causing a reduction of fetus body weights and lengths (Table 2). The weight of the placenta in the female rats poisoned with 0.14 g/kg – 1.15 g/kg of this compound was significantly higher than that in the controls. The

dose-effect relationship, however, was not detected (Table 2). There were no evident malformations in any of the examined groups of the offspring of the control and dioxolane-exposed female rats. The frequency of occurrence of the delays in epicranial bones and sternum ossification was significantly higher in the groups which were prenatally exposed to higher doses of the compound. Macro evaluation of the internal organs of the fetuses did not reveal any macro pathology within the organs in any of the evaluated groups of the animals (Table 3).

Evaluation of female rat fecundity and of the postnatal development of their offsprings

The fecundity, in terms of the number of live fetuses in a litter, was similar in all control and dioxolane-exposed female rat groups. The viability of the offspring of the female rats receiving dioxolane at the highest dose (1.15 g/kg) was significantly lower than that for the control group, and the death rate among the young animals was significantly higher than in the controls (Table 4). The pups died chiefly within the first 2–4 days after their birth. It is, therefore, reasonable to suppose that their prenatal exposure led to pathological changes in the animals, as no disturbances in the maternal instinct of the rats were observed.

Table 4. Effect of dioxolane administered within 2–20 days of gestation on the reproductive performance of female rats and the viability of their progeny.

	No. of pregnant females examined	No. of pups per litter [a]		Indices		
		born alive	born dead	Viability [b]	Lactation [c]	Mortality [d]
Control	10	11.3 ± 2.2	0.22 ± 0.44	78.4	96.3	25.5
Dioxolane 0.58 g/kg	14	11.0 ± 1.5	0.07 ± 0.27	78.5	95.9	25.3
Dioxolane 1.15 g/kg	12	9.5 ± 3.3	1.2 ± 2.1	47.4 [e]	98.1	55.3 [e]

a – Mean ± SD.
b – % of pups born alive that survived to 4 days,
c – % of pups alive at 4 days that survived to 21 days,
d – % of pups born alive that did not survive to 60 days,
e – Significantly different ($p < 0.05$) from the control value.

Maternal exposure to dioxolane at doses 0.58 and 1.15 g/kg neither changes the age of pups at the time of pinna unfolding, incisor eruption, and eye opening, nor altered the performance of righting reflex, negative geotaxis and forepaw suspension. At the dose of 1.15 g/kg, the chemical significantly increased exploratory motor activity of the female offspring at the age of 8 weeks (to 144% of the respective value for the control group). Prenatal exposure to dioxolane at dose 0.58 g/kg and 1.15 g/kg did not affect significantly the locomotor activity of males and the active avoidance acquisition of the adult offspring.

Observations of the physical development of the offspring revealed that, after reaching sexual maturity (14th-15th week), the male offspring of the female rats receiving the highest dose of dioxolane had significantly lower body weight gain than

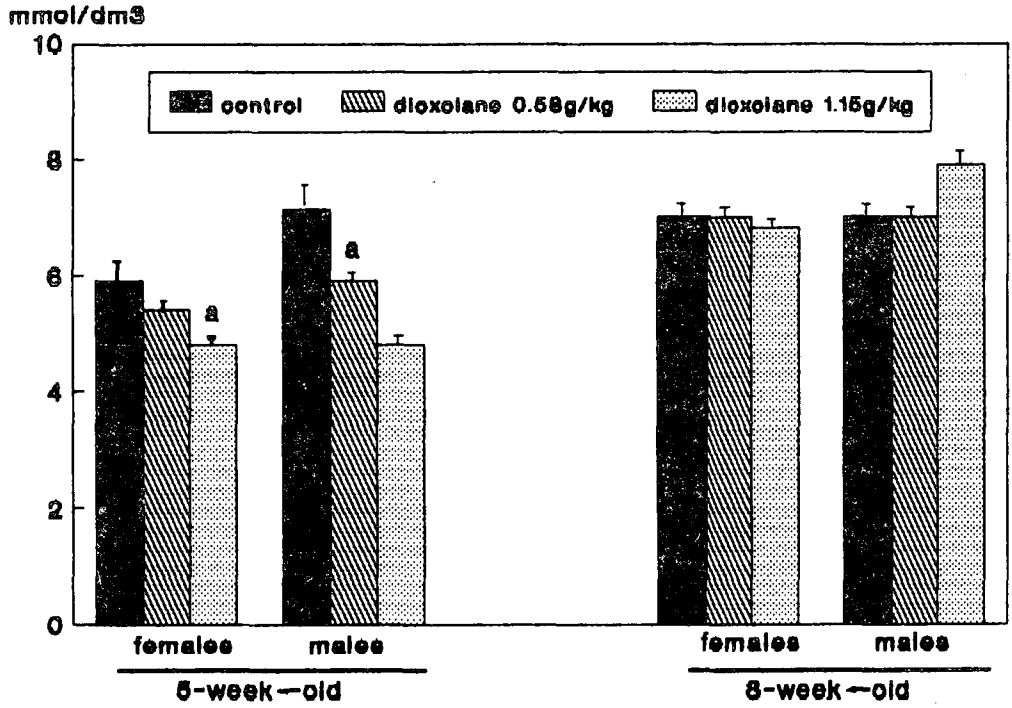

Fig. 1. Haemoglobin levels of 5 and 8-week-old female and male rats born to female given by gavage during gestation dioxolane at a daily doses 0.58 and 1.15 g/kg. The bars represent means ± SE with 8–10 animals in each group. a – significantly different ($p < 0.05$) from the control group.

the control male offspring of the same age (57.6 ± 18.4 g vs. 48.2 ± 15.1 g). Exposure of the pregnant females to dioxolane at dose 1.15 g/kg decreased hemoglobin level in 5-week old female offspring and at a dose of 0.58 g/kg in young males (Fig. 1) but did not change hematocrit value in 5- and 8-week old male or female offspring.

DISCUSSION AND CONCLUSIONS

Disturbances in the prenatal development of the offspring as the result of exposure to a chemical are often due to the toxic damage to the pregnant female organism (10, 11, 12). For that reason, in studying the effects of chemical factors on the development of the offspring of the exposed animals, it is essential to identify chemicals which are toxic selectively to the embryo or fetus, i.e. chemicals causing congenital defects, delays in fetal development and/or increased intrauterine death rate without inducing toxic effect in the mother's organism (9).

At doses toxic or subtoxic to maternal organism dioxolane did not cause increased embryo or fetus intrauterine death rates or congenital defects, it did cause, however, dose-related delays in fetal development. Dioxolane can be classified, therefore, as a weak fetotoxic agent.

That compound, like other chemicals, administered to pregnant females at moderately high doses ($0.1LD_{50}$ and lower), does not cause impairment of the physical development or behavioral disturbances during the postnatal period of the life of the offspring (4, 15, 17, 18). Exposure to higher doses of that compound ($0.2LD_{50}$) leads to increased perinatal death rates in the offspring without causing, however, disturbances in the maternal instinct. Data on transplacental transport and distribution of dioxolane and/or its metabolites in the fetal tissues are, unfortunately, not available. Considering lower prenatal toxicity of dioxolane as compared with trioxane, it seems quite probable that dioxolane or its metabolites permeate the placenta to a lesser degree than trioxane and/or its metabolites (17, 18).

The relevance of the present findings to possible health effects in humans cannot be fully assessed due to the lack of adequate epidemiological data. Assuming for women a daily dose of dioxolane equal to 1.2 mg/kg due to an 8-hour occupational inhalation exposure to this compound, at a concentration of 10 mg/m^3, it maybe concluded that such a dose is 480 times lower than the dioxolane dose which produced a weak fetotoxic effect without affecting postnatal development in rats.

Thus, based on the experimental data obtained in this study, it seems highly improbable that occupational exposure of a woman to dioxolane at or below 10 mg/m^3 can effect her fertility and the development of her child. However, it may become dangerous for progeny development if exposures at considerably higher concentrations are involved.

ACKNOWLEDGMENTS

The study was supported by the CPBR project 11.11.77 Occupational Medicine.

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Received for publication: March 2, 1992
Accepted for publication: April 30, 1992