TERATOGENICITY, FETAL AND PLACENTAL TOXICITY OF 1,3,5-TRIOXANE ADMINISTERED TO PREGNANT FEMALE RATS

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Abstract. In the first experiment 1,3,5-trioxane at doses of 0.77, 1.55 and 3.87 g/kg was administered by gavage to female rats every other day from day 8 to 20 of gestation. In the second the pregnant rats received in the same way trioxane or formaldehyde at daily doses 0.19 g/kg and 0.02 g/kg, respectively. Trioxane aministered at doses of 1.55 and 3.87 g/kg was associated with significant maternal toxicity, embryonal and fetal lethality, congenital malformations and retarded fetal development. Administration of trioxane at a dose of 0.77 g/kg still induced fetotoxic and teratogenic effects without apparent toxicity to the dams. Formaldehyde and trioxane at the lowest dose did not affect prenatal development. Treatment with trioxane or formaldehyde induced an increase in incidence of placentas with slight histopathological changes which was in general dose dependent. The results are discussed in terms of the potential impact of occupational exposure to trioxane on human prenatal development.

INTRODUCTION

Trioxane (1,3,5-trioxane, formaldehyde trimer) is used as an intermediate product in the manufacture of synthetic resins, artificial horn and ivory. It is also used by evaporation for disinfecting sickrooms, clothing, linen and sickroom utensils. It constitutes an active ingredient of some contraceptive creams. Trioxane exhibits a low oral and inhalative toxicity having LD_{50} per os for rats above 5 g/kg and the highest nonlethal concentration for rats during 4-hour exposure — 26 g/m³ (5). In animals exposed by inhalation for 4 hours at concentrations of 6.5 and 26 g/m³ it irritates respiratory airways, mucosa and conjunctivae (7). It is not absorbed by intact skin to the extent sufficient to produce an acute intoxication in rats, neither does it produce a contact allergic dermatitis in guinea pigs (4). Trioxane was not mutagenic in the Ames Salmo-

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nella/mammalian microsome incorporation test (8). It did not increase the frequency of micronucleated polichromatic erythrocytes in bone marrow of mice (10), neither did it induce the dominant lethal mutations in germ cells of male rats (2). Based on chronic inhalation toxicity study on animals a tentative exposure limits of trioxane in the air of the working environment have been implemented in Poland: time weighted average — 15 mg/m^3, short term exposure limit — 75 mg/m^3 (6). Since no data on the effect of trioxane on reproduction are available this study aimed at evaluation of oral exposure of females on embryonal and fetal development of rats.

MATERIAL AND METHOD

Trioxane used in this study was received from the Nitrogen Plant in Tarnow (Poland) and contained 0.001% of formaldehyde and below 0.009% of water. Three-month-old female Wistar rats weighing between 205 g and 245 g were obtained from our institute breeding colony. Temperature (22 ± 2°C) and 12-h light period from 6 a.m. to 6 p.m. in the animal room were controlled automatically. Humidity was found to be in a range of 50—60%. The animals were maintained on commercial pelleted chow (Wytwornia Pasz, Motycz, Poland) and top water ad libitum. For fertilization females were mated overnight in our laboratory with 4-month-old-male rats of the same strain. The day of detection of vaginal plugs or spermatozoa in vaginal smears was assumed to indicate day 1 of gestation.

The study was performed in two series. In the first series the inseminated females were distributed into one control and three treatment groups given 0.1, 0.2 and 0.5 median lethal dose of trioxane found previously to be 7.74 g/kg. Trioxane as a 15% aqueous solution was administered orally, by gavage, every other day from day 8 to day 20 of gestation. In the second series females were given trioxane at a daily dose of 0.025 LD_{50} (3% aqueous solution) or formaldehyde (0.5% aqueous solution) at a dose of 20 mg/kg b.w. The evaluation of formaldehyde influence on prenatal rat development was included in this study since it is a known contaminant of trioxane preparations. The dose of formaldehyde was chosen assuming that the content of this aldehyde in trioxane may be as high as 0.5% w/w. Thus simultaneously with the administration of trioxane at the highest dose (3.87 g/kg) a rat was receiving approximately 20 mg/kg b.w. of formaldehyde. The control females in both experimental series were given, by gavage, an equivalent volume of water. The maternal weight gain, daily food and water intake were monitored throughout the gestation period. The female rats

were sacrificed on day 21 of gestation under ethylether anesthesia. The assessment of teratogenicity, embryo- and fetal toxicity was performed according to methods described previously (1). Gross necropsy observations were made on the stomach, duodenum, liver, kidneys, adrenals, ovaries and placentas. Paraffin sections of stomach, duodenum, liver, kidney, adrenals, ovaries and placentas were stained with haematoxylin-eosin for light microscopic examination. Frozen sections of liver and kidney were stained with Fat Red 78 in 70% isopropanol for detection of neutral fat deposits. Semiquantitative analysis of the pathomorphological changes in the placentas was made assigning a score according to the following criteria: A. Fibrin deposits on the maternal surface of placenta: lack or very thin layer only on the peripheral part — 0, thin layer covering whole maternal surface — 1, thick layer covering maternal surface — 2; B. Inflammatory infiltrations within the basal zone and fibrin deposits: lack or single granulocytes — 0, multicellular small foci — 1, diffuse infiltrations — 2; C. Necrosis: lack — 0, foci restricted to one fourth of basal zone — 1, larger foci occupying basal zone labirynth — 2. For each evaluated feature the index of morphological alterations (IMA) was calculated according to the formula:

$$IMA = \frac{2a + 1b + 0c}{(a + b + c)}$$

where: 2, 1, 0 — score of given feature in the individual placenta;
a, b, c — number of placentas with given score.

The significance of differences between means was assessed using Student t-test. Frequency data were analyzed with Fisher's exact probability test (12).

RESULTS

Treatment with 1,3,5-trioxane did not affect the survival of female rats. Trioxane given orally at daily doses of 1.55 g/kg and 3.87 g/kg induced a dose-dependent decrease of body weight gain of dams during gestation and a reduction of their daily food intake (Fig. 1, Table 1). At the highest dose trioxane caused a decrease of maternal liver and placenta absolute weights and an increase of kidney and adrenal weights in ratio to female body weight (Table 1 and 2). The relative weight of kidneys was also increased in the 1.55 g/kg group (Table 2). Histopathological examinations revealed an increase of binuclear hepatocytes and hepatocytes at the mitosis phase in female rats exposed to trioxane at doses of 1.55 g/kg and 3.87 g/kg. In addition, hydropic liver degeneration was found in rats administered trioxane at a dose of

TABLE 1. Effect of Trioxane (TOX) and Formaldehyde Administered within 8—10 Days of Gestation on Pregnancy and Fetal Development in Rats [a]

	Experiment I				Experiment II		
	Control	TOX 0.77 g/kg	TOX 1.55 g/kg	TOX 3.87 g/kg	Control	TOX 0.19 g/kg	Formaldehyde 0.020 g/kg
Females inseminated	24	23	22	23	17	17	15
Females pregnant [b]	22(92)	20(87)	19(86)	16(70)	14(82)	17(100)	15(100)
Live fetuses per litter [c]	10.3±3.7	9.7±3.6	7.4±3.5 [e]	0.6±2.0 [e]	11.8±2.2	11.3±2.2	9.7±2.8
Litters with resorptions	9	12	15 [e]	16 [e]	3	5	7
Litters with late resorptions	2	8 [e]	8 [e]	15 [e]	2	1	4
Resorption per litter with resorptions [c]	1.7±1.3	0.6±5.9	3.2±1.7 [e]	9.9±3.0 [e]	1.0±0	1.6±1.3	2.0±0.8
Food intake on day 21 of gestation (g) [c]	20.8±4.8	21.7±5.3	18.0±4.0 [e]	15.9±3.0 [e]	22.2±2.5	21.5±4.2	20.3±1.6 [e]
Fetal crown-rump length (cm) [d]	4.1±0.16	3.9±0.15 [e]	3.7±0.14 [e]	2.9±0.71 [e]	4.2±0.11	4.1±0.07	4.2±0.16
Fetal body weight (g) [d]	3.4±0.21	3.0±0.26 [e]	2.6±0.20 [e]	1.8±13 [e]	3.4±0.21	3.5±0.13	3.4±0.22
Weight of placenta (g) [d]	0.55±0.15	0.54±0.11	0.55±0.11	0.33±0.04 [e]	0.48±0.04	0.52±0.05	0.52±0.10

[a] Females were sacrificed on Day 21 of gestation
[b] Percentage of pregnant females given in parentheses
[c] Mean ± SD
[d] Mean of litter means ± SD
[e] Significantly different ($p < 0.05$) from control group

3.87 g/kg. No apparent signs of trioxane maternal toxicity were found in females given this compound at doses of 0.19 g/kg and 0.77 g/kg.

Trioxane at doses of 0.77—3.87 g/kg induced a dose-dependent increase in the number of resorptions and a decrease of fetal body weight and length (Table 1). At two higher doses (1.55 g/kg and 3.87 g/kg) trioxane decreased a mean number of live fetuses per litter (Table 1). The results of external, skeletal and visceral examinations of the fetuses are presented in Table 3. Due to high intrauterinal mortality and the extremely low body size of several survived fetuses from the 3.87 g/kg group it was impossible to determine whether trioxane at this dose pro-

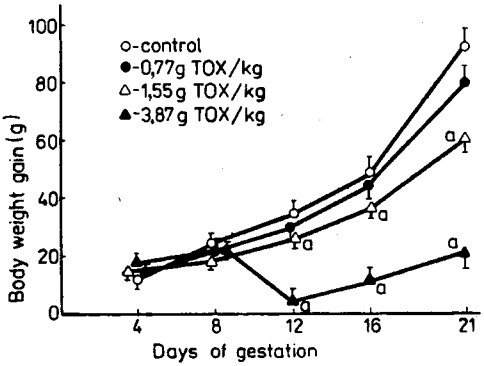

Fig. 1. Body weight gain of female rats receiving by gavage trioxane (TOX) at different doses every other day from day 8 to day 20 of gestation. **a** — significantly different ($p < 0.05$) from control group.

duced the congenital malformations. However, the tested compound at doses of 0.77 g/kg and 1.55 g/kg evoked malformations of brain, kidneys and/or skeletal system and a significant increase in fetuses with delayed ossifications. No significant effect of trioxane at a dose of 0.19 g/kg and of formaldehyde at a dose of 0.02 g/kg on prenatal rat development was observed.

Histopathological examinations revealed the increased frequency of the placentas with all pathological changes: fibrin deposits on the maternal surface, inflammatory infiltrations and focal necrosis — only in females given trioxane at a dose of 1.55 g/kg. In general a dose-dependent response was observed in the first experiment. However, in the second experiment, formaldehyde and trioxane at the lowest dose still induced some frequency increase of placentas with pathological changes in comparison to a relatively low control level (Table 4).

The differences in frequency of placentas with histopathological changes between experimental groups were similar to differences in values of the index of morphological alterations between the same groups. This finding indicates that the wide spread and degree of inten-

TABLE 2. Absolute and Relative Organ Weights of Female Rats Receiving Daily by Gavage Trioxane (TOX) and Formaldehyde on Days 8 — 20 of Gestation [a]

	Experiment I				Experiment II		
	Control	TOX 0.77 g/kg	TOX 1.55 g/kg	TOX 3.87 g/kg	Control	TOX 0.19 g/kg	Formaldehyde 0.020 g/kg
Liver [b]	22	20	19	16	14	17	15
(g)	11.5±1.56	10.8±1.17	10.9±1.25	9.6±1.17 [c]	11.7±1.50	11.1±1.42	11.1±1.18
(g%)	3.7±0.44	3.5±0.19	3.8±0.36	3.9±0.45	3.9±0.29	3.7±0.27	3.7±0.35
Kidneys [b]	22	20	19	16	14	17	15
(g)	1.54±0.12	1.52±0.17	1.53±0.16	1.57±0.12	1.36±0.15	1.40±0.15	1.41±0.17
(g%)	0.49±0.05	0.48±0.06	0.52±0.05 [c]	0.64±0.06 [c]	0.46±0.03	0.47±0.03	0.47±0.05
Adrenals [b]	22	20	19	16	14	17	15
(mg)	68.2±8.4	72.8±7.9	67.5±7.8	67.1±8.0	71.7±6.3	73.1±9.0	69.3±7.8
(mg%)	21.9±3.0	23.6±3.7	23.4±4.9	27.2±3.1 [c]	24.2±2.6	24.7±2.6	23.1±3.0

[a] Animals were sacrificed on day 21 of gestation

[b] Mean ± SD

[c] Significantly different ($p < 0.05$) from the control group

TABLE 3. Effect of Trioxane (TOX) and Formaldehyde on the Incidence of Fetuses Retarded in Development or Malformed

	Experiment I			Experiment II		
	Control	TOX 0.77 g/kg	TOX 1.55 g/kg	Control	TOX 0.19 g/kg	Formaldehyde 0.020 g/kg
No. litters examined	21	20	18	13	17	15
No. fetuses external exam	226	194	139	157	192	145
No. fetuses visceral exam	113	97	68	80	95	70
No. fetuses skeletal exam	113	97	71	77	97	75
Findings: number (% incidence of finding)						
Hydrocephalus internal	0	3 (3.1)	12 (17.6) [a]	0	0	0
Hydrocephalus external	0	1 (1.0)	0	0	0	0
Hydronephrosis	0	0	3 (4.4)	0	0	0
Incomplete development of skull bones	3 (2.7)	16 (16.5) [a]	19 (26.8) [a]	1 (1.3)	4 (4.1)	0
Wavy ribs	0	0	2 (2.8)	0	0	0
Absence of one or several sternum ossification	1 (0.9)	5 (5.2)	24 (33.8) [a]	1 (1.3)	2 (2.1)	2 (2.7)

[a] — Significantly different ($p < 0.05$) from the control group

TABLE 4. Frequency of Pathomorphological Changes in Placentas of Rats Administered Trioxane and Formaldehyde on Days 8 — 20 of Gestation

	Experiment I			Experiment II		
	Control	TOX 0.77 g/kg	TOX 1.55 g/kg	Control	TOX 0.19 g/kg	Formaldehyde 0.020 g/kg
No. of placentas	81	68	55	79	87	101
No. of placentas with:						
— histopathological changes	26 (32.1) [a]	27 (39.7)	37 (67.3) [b]	7 (8.9)	21 (24.1) [b]	42 (41.6) [b]
— fibrin deposits	17 (21.0)	25 (36.8) [b]	36 (65.5) [b]	4 (5.1)	11 (12.6)	24 (23.8) [b]
— inflammatory infiltrations	11 (13.6)	17 (25.0)	15 (27.3) [b]	4 (5.1)	5 (5.8)	8 (7.9)
— focal necrosis	1 (1.2)	4 (5.9)	13 (23.6) [b]	0	6 (6.9) [b]	13 (12.9) [b]

[a] Percentage of placentas with changes is shown in parentheses
[b] Significantly different ($p < 0.05$) from control group

TABLE 5. Index of Morphological Alterations (IMA) in Placentas of Rats Administered Trioxane (TOX) and Formaldehyde

	Experiment I			Experiment II		
	Control	TOX 0.77 g/kg	TOX 1.55 g/kg	Control	TOX 0.19 g/kg	Formaldehyde 0.020 g/kg
— fibrin deposits	0.23	0.39	0.76	0.06	0.14	0.23
— inflammatory infiltrations	0.13	0.25	0.31	0.02	0.06	0.08
— focal necrosis	0.04	0.07	0.27	0.04	0.07	0.13

sity of these changes were not increased significantly by treatment, which caused mainly an increase of their incidence (Table 5).

DISCUSSION AND CONCLUSIONS

The results indicate that trioxane at sufficiently high doses induces fetal lethality, retarded fetal development and congenital malformations in rats. The fetotoxic effects show a dose-response or a dose-effect relationship. The data from this study also show a relationship between fetal and maternal toxicity. Trioxane administered per os at two higher daily doses of 1.55 g/kg and 3.87 g/kg exerted a clear-cut toxic action on pregnant females reflected in their reduced body weight gain and daily food consumption, altered organ weight and morphological changes in the liver. At lower doses the tested compound did not induce apparent maternal toxicity. However, trioxane at a daily dose of 0.77 g/kg apparently nontoxic to the mother, still evokes retarded fetal development, malformations of the brain and increased incidence of litters with late resorptions. Thus, 1,3,5-trioxane administered orally appears to be more harmful to a fetal rat than to its mother. This conclusion is further supported by the extremely high death rate among embryos and fetuses in the 3.87 g/kg group while no lethality was observed among their mothers.

The fetotoxicity and teratogenicity of trioxane in the light of this study is not related to formaldehyde, a contaminant of the technical trioxane preparations. Formaldehyde at a dose much higher than the ones which could be administered to females simultaneously with trioxane doses did not affect prenatal rat development. This result coincides with other data on formaldehyde fetotoxicity. No congenital malformation nor fetotoxicity was induced by formaldehyde given to female mice from day 6 to day 15 of gestation at a dose of 185 mg/kg., although this dose killed 22 of 34 dams so treated (9). No visible fetal malformations were found in female rats exposed continuously during pregnancy to formaldehyde vapours at a concentration of 1 mg/m^3 (11).

The data on toxicokinetics of trioxane are not available, but the distinct fetotoxic effects of trioxane at daily dose not inducing maternal toxicity (0.1 LD_{50}) suggest that it can cross the placental barrier.

The wide spread and intensity of morphological alterations in placentas were similar in all groups, and treatment with trioxane or formaldehyde only increased their incidence. The increased incidence of placentas with such changes was found also in experimental groups within which no fetotoxicity of trioxane or formaldehyde was shown. Thus, it is hard to state to what extent the trioxane-induced histo-

pathological changes in placentas are involved in the induction of its fetotoxicity.

The fetotoxic and teratogenic effects are multicellular threshold phenomena, their incidence and severity increase with the dose (3). This means that trioxane, which is at high doses a teratogenic and fetotoxic agent, at substantially lower doses may have no effect on prenatal development of mammals.

The exposure limits (time weighted average and short term exposure limit) of trioxane in the air of the working environment in Poland were established at a relatively low level in order to protect workers from irritation of respiratory airways mucosa. Assuming an 8-hour workday, lung ventilation during a hard job — 25 dm^3/min, body weight of worker — 70 kg and 100% absorption of trioxane in lung — a daily dose absorbed into the body of a man exposed at a concentration of 15 mg/m^3 would be equal to 2.6 mg/kg. This dose is about 73 times lower than the dose of 0.19 g/kg at which trioxane does not affect prenatal development of rats and over 270 times lower than the dose of 0.77 g/kg at which trioxane induces fetotoxic and teratogenic effects. Thus, based on experimental data obtained in this study it seems highly improbable that occupational exposure of a woman to trioxane at or below 15 mg/m^3 can affect prenatal development of her child. However, it may become dangerous for progeny development if exposure takes place at considerably higher concentrations. The risk assessment of trioxane influence on human prenatal development derived from the presented experimental data is subject to several uncertainties such as interspecies differences in susceptibility.

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