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# EFFECTS OF MATERNAL EXPOSURE TO TRIOXANE ON POSTNATAL DEVELOPMENT IN RATS*

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**Abstract.** Female rats were given by gavage every other day from days 2 to 20 of gestation an aqueous solution of trioxane at daily doses equal to 0.025; 0.075 and 0.15 $LD_{50}$ (0.19: 0.58 and 1.16 g/kg). Maternal exposure to trioxane at the highest dose resulted in death of most neonates a few days after birth. Viability and postnatal growth of the offspring of dams that were given 0.19 and 0.58 g/kg were not affected. Transitional depression of exploratory locomotor activity in female offspring and a decrease of active avoidance acquisition were observed in adult male and female offspring from the 0.58 g/kg group, whereas a dose of 0.19 g trioxane /kg did not affect the behavioral performance of progeny.

## INTRODUCTION

Trioxane (1,3,5-trioxane. $C_3H_6O_3$) is a stable cyclic trimer of formaldehyde used as an intermediate product in the manufacture of plastic materials. The number of people occupationally and non-occupationally exposed to trioxane may be expected to increase due to the chemical's new application as an insect-proofing agent. Trioxane exerts slight systemic acute toxicity in animals but it does not pose a risk of acute occupational intoxication (4,5). Repeated inhalation exposure of rats (6 hrs a day, 5 days a week for 12 months to trioxane concentrations of 500 and 2500 mg/m$^3$) impaired performance in the rotarod test; decreased plasma acetylocholinesterase activity; increased the number of lymphocytic infiltrations in the larynx and trachea; produced proliferation of peribronchial lymphatic tissue; and caused planoepithelial

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metaplasia of the bronchial epithelium. Electron microscopy revealed partial or complete loss of cilia in the tracheal epithelium. Locomotor coordination on a rotating rod was also depressed slightly in animals exposed for 12 months to trioxane at 50 mg/m$^3$; however, among irritant effects produced at higher concentrations, planoepithelial metaplasia was found in only 1 out of 20 rats exposed to trioxane at 50 mg/m$^3$ (5). There is evidence that trioxane does not have genetic activity since it does not increase the number of polychromatic erythrocytes with micronuclei in mouse bone marrow (9), nor does it induce dominant lethal mutations in the germ cells of male rats (3) or reverse mutations in the Salmonella/microsome test (7).

Trioxane administered *per os* every other day from days 8 to 20 of gestation at doses of 0.2 and 0.5 $LD_{50}$ (1.55 g/kg and 3.87 g/kg) has been 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, but not at 0.19 g/kg, still induced fetotoxic effects, but without apparent toxicity to the dams (10). Based on data from animal studies a permissible exposure limit of 15 mg/m$^3$ of air was established in Poland for occupational environments (6).

This study was aimed at assessing the influence of trioxane exposure of female rats during gestation on postnatal growth, viability and behavior of offspring.

## MATERIALS AND METHODS

Trioxane (TOX) used in this study was obtained from the Zakłady Azotowe (Nitrogen Plants) in Tarnow (Poland) and contained 0,001% of formaldehyde and below 0,009% of water.

### Animals and treatment

Four-month old female Wistar rats weighing between 190 g and 210 g were obtained from our Institute breeding colony. The animals were maintained on commercial pelleted chow (Fodder Factory, Motycz, Poland) and tap water *ad libitum*. Females were mated overnight with 4-month old male rats of the same strain. The morning when copulation plugs or spermatozoa in vaginal smears appeared was regarded as day 1 of gestation.

The study was performed in two series. The inseminated females were given by gavage, every other day from day 2 to 20 of gestation, aqueous solutions of trioxane at daily doses equal to 0.025 and 0.15 acute median $LD_{50}$ (first experimental series) or at daily doses of 0.075 $LD_{50}$ (second experimental series). $LD_{50}$ of trioxane given *per os* to female rats was found by us previously to be 7.74 g/kg. Concentrations of trioxane in aqueous solutions were adjusted such that the animals received 1 cm$^3$ of solution per 100 g of body weight. In both experimental series the control females were given, by gavage, an equivalent volume of distilled water. The females were allowed to deliver and nurse their progeny until they were weaned at 28 days of age.

## Physical development and viability

The number of live and dead pups and body weight at birth were recorded. The age (in days) at which pinna detachment, incisor eruption and eye opening occured, was noted for each pup in order to assess morphological development. The body weight gain of offspring was recorded once per week for 19 weeks after birth. Daily food and water consumption were determined from week 8 to week 19 *post partum*. The indices of fertility, viability, lactation and mortality were counted. At the age of 4 and 8 weeks the number of erythrocytes, hematocrit and hemoglobin level were measured in the offsprings' blood.

## Neurodevelopmental and behavioral assessment

The performance of all pups was determined in the following tests (1,8): surface righting reflex at the age of 3–6 days, negative geotaxis at 6–12 days, forepaw suspension at 6–15 days, and air righting reflex at 14–19 days. Randomly chosen offspring from each litter (10 animals per treatment group) were assessed for exploratory motor activity at the age of 8, 11 and 14 weeks and for active avoidance acquisition through weeks 18 and 19 of life according to the methods previously described (2).

## 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 (12). Frequency data were analyzed with Fisher's exact probability test. The effect of trioxane on behavioral performance, food and water consumption and body weight gain of offspring was evaluated with two-way analysis of variance and Scheffe's test for multiple comparison (12).

# RESULTS

Trioxane at any dose did not affect the survival and behavior of the dams. However, the body weight gain during gestation was lowered in animals receiving trioxane at a daily dose of 0.58 g/kg (0,075 $LD_{50}$; $74.6 \pm 16.3$ g vs $89 \pm 18.8$ g in the control group) and at a dose of 1.16 g/kg (0.15 $LD_{50}$; $78.7 \pm 13.7$ g vs $90.7 \pm 98$ g in the appropriate control group). Food and water consumption was similar in all experimental groups. Litter size was reduced in the highest exposure group (Table 1).

Trioxane administered at a dose of 0.15 $LD_{50}$ (1.16 g/kg) led to reduction of maternal instinct. The dams from this group did not build a nest; they had small, pale nipples and their neonates were spread all over the cage without signs of feeding. Viability of their offspring over the first 4 days was markedly

**TABLE 1.** Effect of trioxane exposure on the reproductive performance of female rats and viability of their progeny

| Dose | Number of inseminated females | Number of pregnant females (%)[a] | Number of litters examined | Mean no. of pups per litter ± SD | Indices | | |
|---|---|---|---|---|---|---|---|
| | | | | | Viability[b] | Lactation[c] | Mortality[d] |
| Control I | 15 | 14 (93.3) | 14 | 11.6±2.1 | 98.5 | 93.9 | 9.0 |
| TOX 0.025 $DL_{50}$ 0.19 g/kg | 15 | 12 (80.0) | 12 | 11.0 ± 2.7 | 99.5 | 88.8 | 16.6 |
| TOX 0.15 $DL_{50}$ 1.16 g/kg | 14 | 12 (85.7) | 12 | 8.6 ± 3.2[e] | 12.7[e] | 7.6[e] | 92.3[a] |
| Control II | 17 | 15 (88.2) | 15 | 11.5 ± 3.0 | 99.5 | 87.0 | 7.4 |
| TOX 0.075 $DL_{50}$ 0.58 g/kg | 17 | 14 (82.4) | 14 | 10.1 ± 1.3 | 92.1 | 95.4 | 15.5 |

a – % of inseminated females delivering live pups
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 control value

reduced (Table 1). Due to high mortality (over 90%) it was not possible to score postnatal physical and behavioral development in that group. Exposure of pregnant rats to trioxane at doses of 0.19 g/kg and 0.58 g/kg (0.025 $LD_{50}$ and 0.075 $LD_{50}$) neither changed the age of pups at the time of pinna unfolding, incisor eruption and eye opening, nor altered the performance of pups in tests of surface and air righting reflex, negative geotaxis and forepaw suspension. Maternal exposure to trioxane at a daily dose of 0.19 g/kg and 0.58 g/kg did not affect body weight gain, viability or daily food and water consumption. At a dose of 0.58 g/kg the chemical significantly decreased exploratory motor activity of female offspring at the age of 8 and 14 weeks (Fig. 1) and of male offspring at the age of 8 weeks (to 70% of respective control group value). No maternal exposure-related changes in hemoglobin level, hematocrit value or number of erythrocytes were found in offspring at

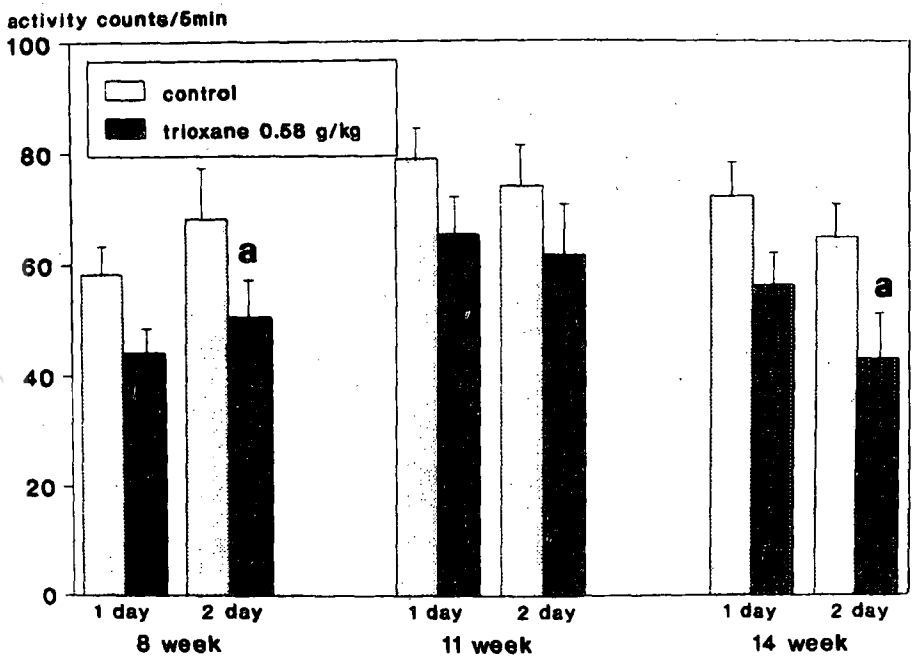


Fig. 1. Exploratory locomotor activity of 8,11 and 14-week-old female rats born to females given by gavage during gestation trioxane at a daily dose of 0.58 g/kg. The bars represent means ± SD with 8 animals in each group.
a — Significantly different ($p < 0.05$) from the control group.

the age of 4 and 8 weeks. Active avoidance acquisition of 5-month old male and female offspring from dams treated with trioxane at 0.58 g/kg was significantly depressed (Fig. 2). Maternal exposure to trioxane at a dose of 0.19 g/kg did not affect significantly the active avoidance acquisition of adult offspring. The reduction in avoidance acquisition appeared to be apparently dose-related.

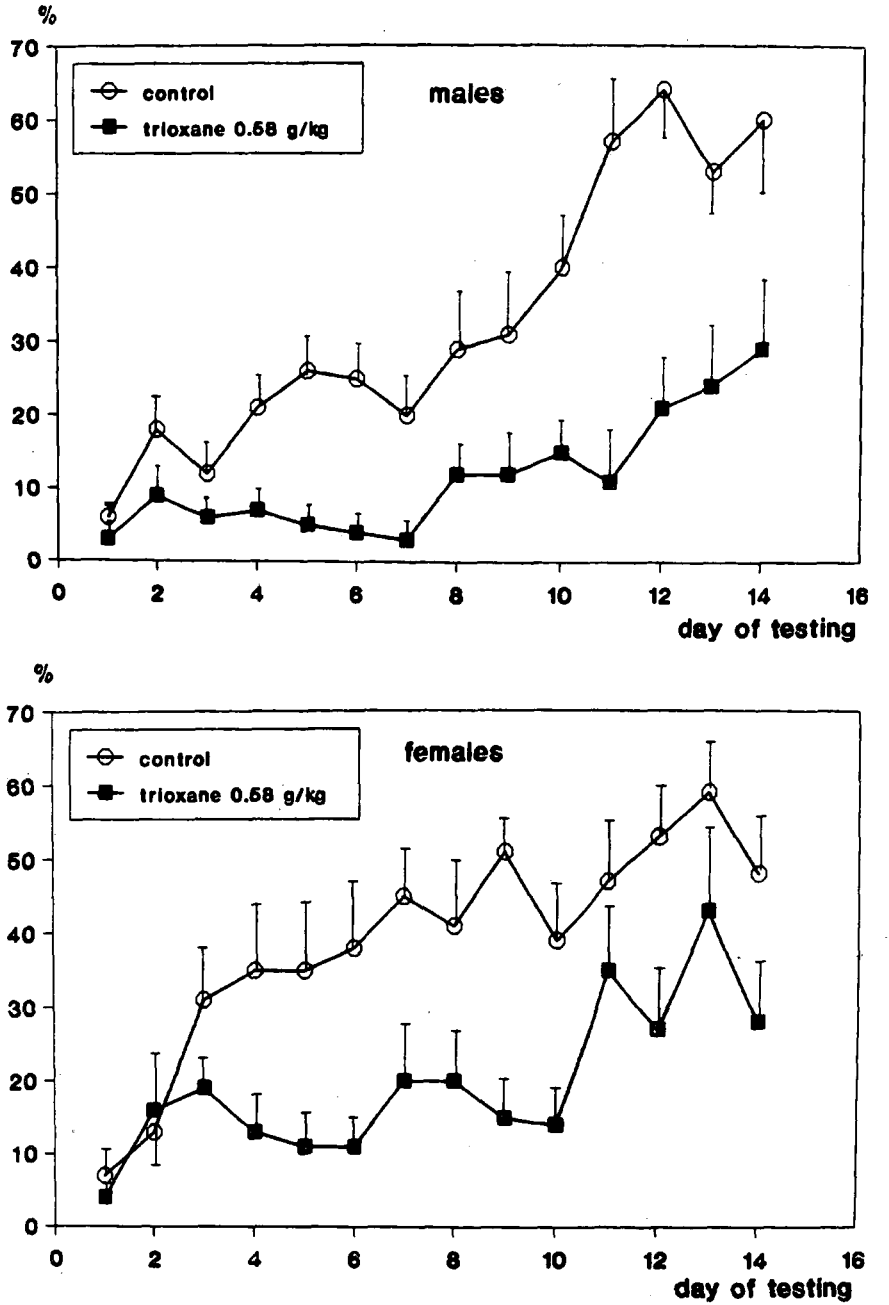


**Fig. 2.** Performance in avoidance acquisition test of 18-week-old female and male rats born to females given by gavage during gestation trioxane at a daily dose of 0.58 g/kg. Data points represent means ± SD with 8 animals in each group. Two-way analysis of variance indicated significant ($p < 0.05$) differences between control and exposed animals.

## DISCUSSION

Maternal treatment with trioxane may result in increased mortality and alterations of behavioral development of progeny. The high mortality of offspring, mainly during their first few days of life, in the 1.16 g/kg group was most probably due both to lowered maternal care of the dams (as a result of intoxication with trioxane) and to the retarded fetal development of offspring shown in the previous report (10). At half that dose administered repeatedly to the dams during gestation (0.58 g/kg) there was no trioxane-related mortality of offspring. Transitional reduction in exploratory motor activity and impairment in the acquisition of active avoidance responses in both male and female offspring of dams treated with trioxane at a daily dose of 0.58 g/kg points to central nervous system (CNS) dysfunction.

Neither the mechanism nor the critical period of trioxane-induced CNS dysfunction in adult offspring due to prenatal exposure is known. The experiments using 1.3.5[U$^{14}$C]-trioxane have shown that trioxane and/or its metabolites are transferred, shortly after oral administration to pregnant rats, from maternal blood to the fetus to a comparable or greater extent than to most organs and tissues of the mother herself. The decline of $^{14}$C radioactivity content between 3 and 48 hrs after oral administration of 1.3.5[U$^{14}$C]-trioxane to pregnant rats is much lower in fetal organs (brain, liver, kidneys) than in the respective maternal organs, suggesting that upon repeated administration, trioxane and/or its metabolites may accumulate in fetal tissue before birth (11).

Neither reduction of viability nor alterations of neuromuscular development were observed in the period between birth and weaning in offspring from rats exposed to trioxane at doses of 0.58 g/kg and 0.19 g/kg. Lack of overt signs of toxicity and alterations of physical and neuromuscular development in suckling offspring of females that had received trioxane during gestation at a dose of 0.58 g/kg does not exclude the possibility of subtle damage in their CNS, which later on resulted in deficit of learning and memory capacities in adult offspring. The dose-dependent depression of avoidance acquisition in offspring is not apparently a function of decreased motor activity, since it was also found in males from the 0.58 g/kg group, who did not exhibit reduced exploratory locomotor activity, at least since week 11 of life.

The relevance of the present findings to possible health effects in man cannot be fully assessed due to the lack of adequate epidemiological data. Assuming for women a daily dose of trioxane equal to 1.2 mg/kg, due to an 8-hour occupational inhalation exposure to this compound at a concentration of 15 mg/m$^3$, it may by concluded that such a dose is 480 times lower than a dose of trioxane inducing impairment of memory and learning in rat offspring. Furthermore, one should take into account that alterations in behavioral development were only found in offspring of dams which exhibited signs of trioxane intoxication. However, taking into account the common interspecies differences in susceptibility to toxic action of chemicals, it would be prudent not to allow pregnant women to be occupationally exposed to this compound.

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