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THE EFFECT OF ORAL EXPOSURE TO TRIOXANE ON THE OESTROUS CYCLE IN RATS

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Abstract. An aqueous solution of trioxane was administered by gavage to female rats, 5 days per week for 7 weeks, at doses of 0.19, 0.58 and 1.16 g/kg/day. A significant increase in the mean duration of the oestrous cycle, mainly due to lengthening of the dioestrus, was only noted in the 6th and 7th week of treatment in females given *per os* trioxane at a dose of 1.16 g/kg. Three weeks after cessation of treatment, no alterations of oestrous cycle were observed in all groups.
Since a dose-related decrease in body weight gain was observed in females given trioxane by gavage at doses of 0.19—1.16 g/kg and trioxane-induced behavioral changes were seen in the 1.16 g/kg group, it was concluded that exposure to trioxane did not affect the sexual cycle unless other overt signs of trioxane toxicity were induced.

INTRODUCTION

Trioxane (1,3,5-trioxane, $C_3H_6O_3$), a stable cyclic trimer of formaldehyde exerts slight systemic acute toxicity and does not pose a risk of acute occupational intoxication (2, 3). During chronic inhalation exposure, trioxane at concentrations above 50 mg/m^3 produces in animals an irritant effect to the mucous membranes of the respiratory tract as well as CNS impairment (3,5). It does not induce reverse mutations in the Salmonella/microsome test (6), micronuclei in mouse bone marrow cells (7), and dominant lethal mutations in the germ cells of male rats (1). Repeated oral administration of trioxane at doses above 0.1 LD_{50} to female rats throughout gestation has been associated with fetotoxic effects (8). Trioxane has been used as an intermediate product in the manufacture of plastic materials. Recenty, its new application as

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an insect-proofing agent is foreseen, which may result in a greater number of occupationally and non-occupationally exposed people.

This study was aimed at evaluating the relationship between the dose level of trioxane given orally and its effect on the oestrous cycle in adult female rats.

MATERIALS AND METHODS

Female Wistar rats aged 4 months weighing 214±21 g obtained from our Institute's breeding colony were housed in quarters with controlled temperature (approx. 22°C) and illumination (light on between 6 a.m and 6 p.m). Humidity was found to be in the range of 45—55%. The animals were maintained on commercial pelleted chow (Fodder Factory, Motycz, Poland) and tap water *ad libitum*. The females were assigned randomly to one control and three treatment groups. The treatment groups received by gavage, an aqueous solution of trioxane 5 days per week, for 7 weeks, at daily doses of 0.19, 0.58 and 1.16 g/kg equivalent to 0.025, 0.075 and 0.15 of the acute median lethal dose (LD_{50}) of trioxane for female rats. Concentrations of trioxane in aqueous solutions were chosen so that animals received 1 cm^3 of solution per 100 g of body weight. The control females were given by gavage an equivalent volume of distilled water amounting to 1 cm^3 per 100 g body weight.

Vaginal smears were taken daily between 8 a.m and 10 a.m in all animals for the 14 consecutive days of the first two weeks of exposure, 6th and 7th week of exposure, and for the 4th and 5th week after interruption of trioxane administration. Smears were stained by the Shorr method.

Statistical analyses of data were performed using one-way or two-way analysis of variance and Scheffe's test for multiple comparison (9).

RESULTS

Repeated administration of trioxane by gavage to female rats at doses of 0.19 and 0.58 g/kg/day for 7 weeks did not change the length of their oestrous cycle and the duration of its phases. In females given trioxane at a dose of 1.16 g/kg in 6th and 7th week of exposure, the duration of the oestrous cycle was significantly longer than in the control animals (Table 1), mainly due to lengthening of the dioestrus. No overt signs of toxicity were seen in animals given 0.19 and 0.58 g

TABLE 1. Mean length (±SD) of the oestrous cycle (in days) in female rats given *per os* trioxane

Dose	Number of females examined	1—2 weeks of exposure	6—7 weeks of exposure	4—5 weeks after cessation of exposure
CONTROL	12	4.6±1.4	4.3±0.4	4.8±1.2
TOX 0.025 DL_{50} (0.19 g/kg)	10	4.3±0.5	4.4±0.9	5.0±1.6
TOX 0.075 DL_{50} (0.58 g/kg)	10	5.1±1.6	5.4±2.8	4.5±0.6
TOX 0.15 DL_{50} (1.16 g/kg)	12	5.2±2.1	6.1±2.5[a]	5.1±2.8

a — Significantly different ($p < 0.05$) from the control group in the same period of the experiment

trioxane/kg/day, although dose — and time-dependent decrease of body weight gain was observed (Fig. 1). The animals from the 1.16 g/kg groups at the end of exposure, exhibited changes in appearance and behavior. They sat curled up in the cage corner with ruffled hair coats, they squealed when taken to hand by the experimentor and had sanquineous discharge from the nose. After cessation of exposure these disorders disappeared during the subsequent two weeks and their body weight gain levelled up to that of the control animals (Fig. 1).

DISCUSSION

The data presented in this report provide evidence of dose-dependent effects of repeated oral exposure to trioxane on the oestrous cycle in female rats. Furthermore, they support the conclusion that alterations of the oestrous cycle evoked by trioxane treatment appear only in females exhibiting other severe signs of trioxane intoxication. No change in oestrous cycle duration was observed in the rats given 0.19 and 0.58 g trioxane/kg which exhibited diminished body weight gain. Since relatively high doses — approximately 15% of LD_{50} — of trioxane given repeatedly by gavage are required to affect the oestrous cycle, it is likely that under such treatment trioxane can exert both indirect and direct toxic effects on the ovary.

The lowest observed adverse effect level (LOAEL) of trioxane for rats under 12-months' inhalation exposure was found to be 50 mg/m^3 (3, 4). Under such treatment trioxane induced slight histopathological changes in respiratory system (irritant effects) and depression of the quadruped locomotor coordination in exposed rats (3). Since distinct and wide spread of histopatological changes in the respiratory system were seen only in rats exposed to trioxane at concentrations of 500 and

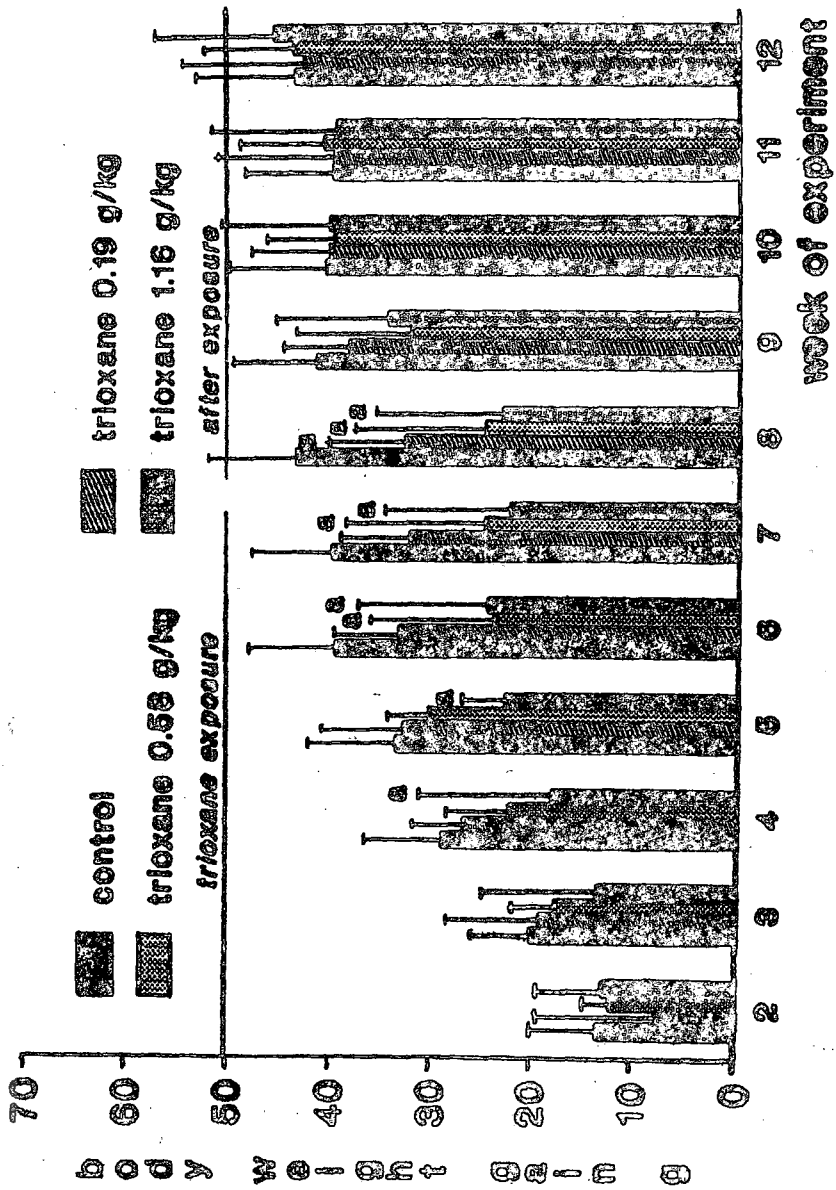

Fig. 1. Body weight gain in g (mean ±SD) of female rats during and after treatment by gavage with trioxane at doses of 0.19, 0.58 and 1.16 g/kg/day
a — significantly different ($p < 0.05$) from the control group.

2500 mg/m³ for 12 months the trioxane concentration of 15 mg/m³ was accepted in Poland as the permissible exposure limit in the air at occupational settings (4). In the air of work-places at industrial installations for the production of the plastic material Tranoform, concentrations of trioxane were found to be about 20 mg/m³ (4). So far, available data suggest that under occupational or environmental inhalation exposure to trioxane, the first most probable adverse effects in exposed humans will appear in the respiratory system before damage of other systems becomes possible. Thus, based on the findings of this study it seems unlikely that the occupational exposure of women to trioxane, at concentrations not inducing systemic intoxication, can produce alteration in their ovarian functions.

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