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ASSESSMENT OF THE EFFECT OF N-BUTANOL GIVEN TO FEMALE RATS IN DRINKING WATER ON FERTILITY AND PRENATAL DEVELOPMENT OF THEIR OFFSPRING

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Abstract. Female rats were given aqueous solutions of n-butanol containing 0.24, 0.8 and 4% n-butanol (0.3; 1.0 and 5.0 g/kg/day) for 8 weeks before and during gestation. The control animals received tap water. The experiment was performed in two stages. The first comprised of the assessment of the oestrous cycle before exposure and then during 4–5 and 7–8 weeks of exposure, and the second stage of the fertility of female rats and their foetal development. The duration of the cycle and its individual stages in the control and the exposed females were similar. It was found that n-butanol alcohol is a foetotoxic agent and produces developmental anomalies in a foetus's skeleton and central nervous system.

INTRODUCTION

Aliphatic alcohols belong to a group of pollutant chemicals capable of affecting human procreation. Ethanol, the best known representative of that alcohol group, is known to be capable of disturbing prenatal development in humans and its intake by pregnant women results in the disturbed psychical development of their progeny (6, 12). Employees in the pulp and paper industry, shipbuilding, the automotive industry, leather manufacture and the printing industries are exposed to n-butyl alcohol. No data is available in the literature on the effects of n-butanol on human or animal procreation (5, 7, 11). In view of the fact that the exposure of pregnant women to ethanol results in the disturbed prenatal and postnatal development of their progeny, investigations were undertaken in order to assess the effect of n-butanol on the sexual cycle and fertility of female rats and on the development of their offspring.

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MATERIALS AND METHODS

N-butyl alcohol purchased from POCh Gliwice, Poland was used. The animals were exposed to n-butyl alcohol in drinking water. The stability of the aqueous n-butyl alcohol solutions was assessed on several consecutive days after their preparation using a Varian Aerograph 2800 gas chromatograph equipped with FID. The column was glass (2 m × 2 mm id.) packed with Porapak Q. The operating conditions were; carrier gas flow (nitrogen) 30 cm^3/min; hydrogen 30 cm^3/min; air 300 cm^3/min; the temperature of the column, injection part and detector were 200°C, 200°C, 230°C, respectively. It was determined that the aqueous n-butyl alcohol solutions were stable within the concentration range used in the experiment (0.24-4%).

Nulliparous female rats, aged approximately 10 weeks and weighting 180—200 g were obtained from our own 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). Three treatment groups consisting of 11-17 females were given aqueous solutions of n-butanol containing 0.24; 0.8 and 4% n-butanol (0.3; 1.0 and 5.0 gkg/day), respectively; 16 control animals received tap water.

The experiment was performed in two stages. The first stage comprised of the assessment of oestrus cycle, the second — fertility and foetal development. Vaginal smears to assess the oestrous cycle were taken daily between 8 a.m. and 10 a.m. in all animals for 14 consecutive days before exposure and then during the 4-5 and the 7-8 the weeks of exposure. Smears were stained by the Shorr method.

After 8 weeks of treatment all the females were mated with untreated 17-week-old male rats for a maximum of 3 weeks. The day of detection of spermatozoa in the vaginal smears was assumed to be day 0 of gestation. The administration of n-butanol was continued throughout the mating and gestation period. The general behaviour of the animals was observed throughout the experiment. Weight gain and the daily intake of food and water or n-butanol solutions was monitored every week in the nonpregnant females and on days 3, 7, 10 and 17 of gestation in the pregnant animals.

The female rats were killed on day 20 of gestation under ethyl ether anaesthesia. The assessment of teratogenicity, embryo- and foetotoxicity was performed as described earlier (2). The uterus was opened and the number of live foetuses, dead foetuses, early and late resorptions sites were recorded. Total implantation values were calculated as the sum of the number of foetuses and resorption sites in each female rat. Live foetuses were measured for body weight, crown-rump length and examined for external malformations. Approximately half of the live foetuses from each litter were preserved in 95% ethanol for subsequent skeletal examination after staining with Alizarin S. The remaining foetuses were preserved in Bouin fluid for visceral examination.

Statistical evaluation. In the case of variance homogeneity, one-way variance analysis and Dunnett tests were used; in the case of heterogeneity Kruskal-Wallis variance analysis was followed by non-parametric tests (13). Frequency data was analyzed with a Fisher exact probability test. The consumption of food and water

or n-butanol solution, and body weight gain of dams were evaluated with two-way variance analysis and Scheffe test for multiple comparison (13).

RESULTS

The general appearance and behaviour of the animals exposed to n-butyl alcohol given in drinking water during the 8 weeks, as well as body weight gain, food and liquid intake were similar to that of the control animals. There were no cases of mortality in either group. The duration of the cycle in the control rats and the exposed female rats was similar, 4 days on average. The duration of the individual stages of the oestrous cycle was not dependent on exposure to n-butanol and was similar to that observed in the control animals.

Integral toxicity indexes observed in the female rats, such as body weight gain during gestation, food and liquid (water or butanol solutions) intake, absolute and relative organ weights hemoglobin concentration and haematocrit values did not differ between the exposed and control groups.

N-butyl alcohol given to the female rats in drinking water during the 8 weeks before fertilization and until day 20 of gestation did not adversely affect body weight of foetuses. The intrauterine mortality of the foetuses in the control, which received tap drinking water, was similar to that in the animals exposed to n-butanol at

Table 1. Effect of n-butanol administered in drinking water for eight weeks before and during gestation on pregnancy and foetal development in rats

	Control	n-butanol dose (g/kg/day)		
		0.3	1.0	5.0
Females examined	16	17	17	11
Females inseminated	16	16	15	11
Females pregnant	12	14	12	9
Females nonpregnant	4	2	3	2
Live foetuses per litter[a]	10.5 ± 3.1	11.0 ± 1.4	12.2 ± 1.9	11.3 ± 2.2
Litters with resorptions	12	9	7	9
Litters with early resorptions	10	9	7	9
Litters with late resorptions	5	2	3	2
Early resorptions per litter[a]	0.8 ± 0.4	1.1 ± 1.0	1.2 ± 1.5	1.8 ± 1.3
Late resorptions per litter[a]	0.7 ± 1.2	0.1 ± 0.4	0.3 ± 0.5	0.2 ± 0.4
Corpora lutea[a]	14.8 ± 2.6	14.6 ± 1.8	14.5 ± 2.1	15.1 ± 2.6
Total implants[a]	12.0 ± 3.2	12.3 ± 2.1	13.6 ± 2.0	13.3 ± 2.3
Preimplantation losses[a]	2.8 ± 2.1	2.4 ± 0.9	1.5 ± 1.6	2.0 ± 1.9
Postimplantation losses[a]	1.5 ± 1.2	1.3 ± 1.3	1.4 ± 1.8	2.0 ± 1.6
Mean daily food intake[b] (g)	21.2 ± 5.4	19.4 ± 4.6	19.4 ± 3.4	18.8 ± 1.5
Mean daily water intake[b] (ml)	30.3 ± 7.9	30.5 ± 6.9	35.1 ± 9.3	27.7 ± 4.2
Body weight gain of dams[a] (g)	89.6 ± 18.9	90.0 ± 18.5	94.3 ± 16.9	93.9 ± 12.7
Foetal body weight[c] (g)	3.2 ± 0.2	3.2 ± 0.3	3.2 ± 0.2	3.2 ± 0.3
Foetal crown-rump length[c] (cm)	4.0 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	3.8 ± 0.1[d]
Weight of placenta[c] (g)	0.55 ± 0.07	0.48 ± 0.07	0.53 ± 0.05	0.60 ± 0.13

a – Mean ± SD;
b – Total mean ± SD calculated through 20 days of gestation;
c – Mean of litters means ± SD;
d – Sigificantly different from control ($p < 0.05$)

0.3 – 5.0 g/kg daily doses. It was found that the foetuses of the animals receiving n-butanol at dose ca. 5 g/kg were significantly smaller than those of the control (Table 1). This sole manifestation of the foetotoxic effect of n-butanol, however, resulted from the exposure to very high doses of the chemical.

N-butanol given to the female rats before fertilization and during gestation at daily doses of 0.3 – 5.0 g/kg body weight produced developmental anomalies in the foetus skeleton, which involved wavy in the 13th rib pair and the presence of an extra rib in the 14th pair and central nervous system defects (Table 2). Internal hydrocephalus was the most frequent anomaly found in the foetuses of female rats exposed to n-butanol (Table 2). N-butanol given to female rats at doses of 0.3 – 5.0 g/kg is a foetotoxic agent which induced pathologic changes in the central nervous system and kidneys of their foetuses. The changes involve a dilation of subarachnoid space, cerebral ventricles and renal pelvis, and a retarded ossification of the sternum (Table 2). It should be noted, however, that those changes were detected only in the animals exposed to high n-butanol doses.

Table 2. Skeletal and visceral observations in foetuses of female rats exposed of n-butanol supplied in drinking water for eight weeks before and during pregnancy

	Control	n-butanol dose (g/kg/day)		
		0.3	1.0	5.0
No. of foetuses (litters) examined:	126 (12)	154 (14)	146 (12)	102 (9)
Visceral observations				
No. of foetuses (litters) examined:	61 (12)	73 (14)	71 (12)	51 (9)
Percentage of foetuses (litters) with dilation of:	2 (8)	25[a] (64)[a]	32[a] (83)[a]	41[a] (100)[a]
– subarachnoid space	0	3 (14)[a]	10[a] (25)[a]	20[a] (78)[a]
– lateral ventricle and/or third ventricle of the brain	2 (8)	23[a] (57)[a]	17[a] (67)[a]	25[a] (78)[a]
– unilateral renal pelvis	0	0	7[a] (42)[a]	0
– bilateral renal pelvis	0	0	4 (25)[a]	0
Percentage of foetuses (litters) with congenital defects:	0	0	7[a] (33)[a]	4 (22)[a]
– external hydrocephalus	0	0	3[a] (17)[a]	0
– internal hydrocephalus	0	0	7[a] (25)[a]	4 (22)[a]
Skeletal observation				
No. of foetuses (litters) examined	65 (12)	81 (14)	75 (12)	51 (9)
Percentage of foetuses (litters) with delayed ossification:	15 (67)	16 (50)	24 (58)	33[a] (67)
Percentage of foetuses (litters) with congenital defect:	0	1 (7)[a]	0	2 (11)[a]
– 14th rib (L_1)	0	0	0	2 (11)[a]
– wavy ribs	0	1 (7)[a]	0	0

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

DISCUSSION

The sub-acute continuous (round-the clock) exposure of female rats in our study to n-butanol at daily doses twice as high as LD_{50} of that chemical given *per os*, equal to 2.1 g/kg body weight does not produce detectable toxic effect in the organism of pregnant female rats; it does, however, produce congenital defects in the skeleton and in the central nervous system of their offspring.

The results of this study can be compared with those of Nelson et al. (1989) although the route and duration of exposure were different. Inhalation exposure to a high concentration 1-butanol (8000 ppm) was teratogenic to rats. 1-butanol is not a selective developmental toxicant in rats because teratogenic effects were observed only in the presence of maternal toxicity (10). The relatively low teratogenic potential of n-butanol contrasts with the results seen with methanol (8) and propanols (9). This may be due to differences in such properties as the physiochemical nature of the alcohols or to metabolic considerations.

Such surprisingly high resistance of pregnant female rats to the toxic action of n-butanol is probably attributable to its very rapid metabolism. In rats, n-butanol is enzyme-oxidated by alcohol dehydrogenase to aldehyde, and then to carbon dioxide and water (1, 3, 4). Experiments demonstrate also that n-butanol is rapidly eliminated from the organism and discharged mainly through the lungs, and to some extent also with urine (3, 4). In spite of its rapid elimination from the organism and the resultant high resistance of the adult animals to the chemical, n-butanol exerts obvious adverse effect on embryonal and foetal development, which means that toxic doses are several times lower for the offspring during their prenatal development than for their mothers. It is quite reasonable to suppose that maintaining workplace n-butanol concentrations at levels which are sufficiently low to ensure good health to female employees may prove insufficient to preserve the good health of their progeny.

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International Information

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The Declaration calls for the establishment of effective measures to prevent environmental conditions which will harm human health, including the use of early warning systems and appropriate public health countermeasures. It also calls for a common research strategy so that the relationship beteen human health and environmental conditions is better understood. In order for close co-operation to be assured among national governments and the various bodies involved in environmental and health management in the European Region, including WHO, EU, ECE and the Council of Europe, the Declaration lays down that a new Committee should be set up, which will inter alia:

* coordinate and evaluate the implementation of Action Plans
* when requested by countries, facilitate and support the development of national action plans
* provide advice on environmental health issues to organizations and donors to support countries in economic transition or recovering from the effect of armed conflicts.

The Third European Conference on Environment and Health in 1999 will be held in London.

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