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SUBACUTE ORAL TOXICITY OF 1,4-BUTANEDIOL IN RATS*

RYSZARD A. JĘDRYCHOWSKI[1], ROMAN GÓRNY[1], JAN STETKIEWICZ[2], AND IRENA STETKIEWICZ[2]

Department of Toxicity Evaluation[1] and Department of Pathomorphology[2], The Nofer Institute of Occupational Medicine, Lodz, Poland

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ABSTRACT. 1,4-Butanediol (BAD) was administered to male and female Wistar Imp:DAK rats by oral gavage for 28 consecutive days. Treated rats received BAD at daily doses of 5, 50 or 500 mg/kg/day. After 28 days all animals were necropsied. Blood samples were obtained and selected organs were weighed and prepared for histological examination. Subacute oral administration of BAD resulted in an overall low degree of systemic toxicity. There were no changes in body weight, food consumption, and absolute and relative organ weights. Slightly higher activities of sorbitol dehydrogenase and alanine aminotransferase were observed in male rats given BAD at the highest dose of 500 mg/kg/day. Some disturbances in hematological parameters, characterized by macrocytosis and thrombocytopenia were observed in treated rats. Mild to moderate inflammation of the liver, characterized by proliferation of bile ducts and periportal infiltrations with fibroblasts and mononuclear cells, were found in treated animals. A statistically significant difference for histopathological changes was found in animals treated with BAD at the dose of 500 mg/kg/day only in the case where both sexes were jointly taken for comparison.

INTRODUCTION

1,4-Butanediol (BAD, CAS No. 110-63-4) is a colourless viscous liquid, miscible with water and all common organic solvents. BAD is mainly used for tetrahydrofuran and 4-butyrolactone synthesis. BAD is also utilized for production of polybutyleneterephthalate resins, thermoplastics and polyuret-

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Address reprint requests to: R. A. Jędrychowski, Department of Toxicity Evaluation, The Nofer Institute of Occupational Medicine, P.O. Box 199, 90-950 Lodz, Poland.

hanes. There is a very limited data on the BAD toxicity. There is only one commonly available report regarding repeated-dose toxicity studies (4). Accordingly, the male rats were given BAD by oral gavage at doses of 0.25, 3 and 30 mg/kg/day for 6 months. Statistically significant differences were reported to occur only in rats given BAD at the highest dose of 30 mg/kg/day and included a decrease in blood cholinoesterase activity, hepatic glycogen concentration and -SH groups content in the brain, an increase in aminotransferase activities in blood serum, and morphological lesions in the brain and liver. However, there were some shortcomings in this report which makes it difficult to evaluate.

The toxicological studies of BAD have been undertaken in consideration of the limited data regarding the toxicity of the chemical. The purpose of the studies described in this paper was to evaluate the subacute toxicological effects of BAD.

MATERIALS AND METHODS

BAD, obtained from the Institute of Heavy Organic Synthesis, Kędzierzyn-Koźle, Poland, was used in the study. The purity of the material, according to the information from supplier, was above 98%.

The experimental animals were Wistar Imp:DAK male and female rats from the Nofer Institute of Occupational Medicine animal husbandry. They were identified by unique ear number and allowed to acclimatise to laboratory conditions for 2 weeks prior to initiation of the study. Test animals were approximately 6 weeks old at the start of dosing. The mean initial body weight was 118 $\pm$ 11 g and 109 $\pm$ 8 g, respectively for male and female rats. Animals were selected for the study using a computer-generated body weight sorting program. In an animal room, equipped with automatic light cycle timers (lights on from 6 a.m. to 6 p.m.), the rats were housed in polypropylene cages, with sawdust as bedding, four animals in each. Relative humidity and temperature were maintained at 40–50% and 20–21°C, respectively. Food (Murigran pelleted rodent chow, Fodder Plant, Motycz, Poland) and water were provided *ad libitum*.

Each of the four experimental groups was composed of 8 male and 8 female rats. Distilled water or diluted test material was administered in a volume of 0.5 ml/kg via oral gavage using a syringe and a stainless-steel ball-tipped feeding needle. Animals were dosed once daily for 28 consecutive days. Treated rats received BAD at doses of 5 mg/kg (low-dose), 50 mg/kg (mid-dose), and 500 mg/kg (high-dose). The control animals received an equal volume of distilled water.

Animals were observed daily for overt signs of toxicity. Body weights were recorded prior to the first dosing and twice a week thereafter during the test period. Food consumption was measured weekly.

At the termination of the treatment period, the rats were fasted overnight. Just prior to necropsy, blood samples were drawn from the tail vein for

hematological measurements. These included red blood cell count, hemoglobin, hematocrit, total and differential leukocyte counts, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, reticulocyte count, platelet count, and clotting time. The animals were subsequently anaesthetized with ethyl ether, killed by exsanguination from the abdominal aorta, and subjected to gross necropsy. Blood samples were collected for serum chemistry determinations. These were made for alanine aminotransferase, aspartate aminotransferase, sorbitol dehydrogenase, alkaline phosphatase, blood urea nitrogen, total protein, albumin, globulin, and glucose. Organ weights were obtained for the adrenals, kidneys, liver, spleen, and testes. The tissues and organs of 5 male and 5 female rats, which were randomly selected, were preserved in 10% neutral buffered formalin, sectined, stained with hematoxylin and eosin, and evaluated microscopically. Selected tissues for histologic evaluation were as follows: adrenals, duodenum, heart, gonads (ovaries, testes), small and large intestine, kidneys, liver, lungs, pancreas, spleen, and stomach.

Except for body weight data, other parameters from the treated groups were compared statistically to those of the control group using the following tests: Bartlett's test of homogeneity of variance was used to determine if the groups had equivalent variances at the $p < 0.01$ level (9). If the variances were not significantly different, the groups were compared using a standard one-way analysis of variance (ANOVA) (10).

If significant differences among the means were indicated, Dunnett's test was used to determine which treatment groups differed from controls (10).

If the groups did not have equivalent variances at the $p < 0.01$ level, then the Kruskal-Wallis test was used to assess differences in group means.

If the means were different, Dunn's summed rank test was used to determine which treatment group differed significantly from control (2). A dose-dependent trend was assessed by linear regression analysis (1).

Body weight data for each sex were analyzed by one-way classification analysis of covariance (ANCOVA), using preexposure (day 0) weights as the covariate.

When the group means were significantly different, comparisons to controls were made using Dunnett's test (9). All these procedures were carried out by means of the TOXSTAT program (3). Frequency of pathological lesions in the liver was evaluated by the Fisher exact test (11).

RESULTS

There were no deaths during the course of the study. For the most part, clinical observations were unremarkable. No significant differences in body weights were observed in rats exposed to BAD when compared with the controls (data not shown). A pattern of statistically significant differences in food consumption throughout the study was not apparent for either sex (data not shown).

Table 1. Effect of 1,4-butanediol on organ and terminal body weight

Parameters	Treatment group				Linear regression analysis
	Control (n = 8)	5 mg/kg (n = 8)	50 mg/kg (n = 8)	500 mg/kg (n = 8)	
Males					
Terminal body weight (g)	214 ± 12[a]	226 ± 19	226 ± 19	199 ± 19	p = 0.0137
Absolute organ weight (g)					
Liver	6.38 ± 0.68	6.54 ± 0.48	6.38 ± 0.72	6.24 ± 1.14	p = 0.0845
Spleen	0.581 ± 0.113	0.600 ± 0.100	0.581 ± 0.193	0.488 ± 0.133	p = 0.0000
Kidneys	1.58 ± 0.17	1.57 ± 0.13	1.59 ± 0.23	1.39 ± 0.12	p = 0.0000
Adrenals	0.041 ± 0.006	0.048 ± 0.003	0.041 ± 0.009	0.040 ± 0.008	p = 0.3597
Testes	2.67 ± 0.31	2.51 ± 0.21	2.71 ± 0.36	2.40 ± 0.25	p = 0.0973
Relative organ weight (g/100 g b.w.)					
Liver	2.97 ± 0.19	2.90 ± 0.22	2.82 ± 0.11	3.12 ± 0.31	p = 0.0398
Spleen	0.271 ± 0.048	0.265 ± 0.037	0.259 ± 0.091	0.242 ± 0.044	p = 0.0001
Kidneys	0.733 ± 0.046	0.695 ± 0.048	0.702 ± 0.069	0.701 ± 0.059	p = 0.6504
Adrenals	0.019 ± 0.003	0.021 ± 0.001	0.018 ± 0.003	0.020 ± 0.004	p = 0.8565
Testes	1.244 ± 0.116	1.114 ± 0.098	1.201 ± 0.131	1.218 ± 0.168	p = 0.6730
Females					
Terminal body weight (g)	171 ± 9	178 ± 14	173 ± 13	179 ± 17	p = 0.2932
Absolute organ weight (g)					
Liver	5.21 ± 0.44	5.48 ± 0.46	5.73 ± 0.46	5.49 ± 0.47	p = 0.8679
Spleen	0.550 ± 0.071	0.506 ± 0.056	0.531 ± 0.119	0.494 ± 0.068	p = 0.1762
Kidneys	1.24 ± 0.13	1.42 ± 0.13*	1.28 ± 0.10	1.19 ± 0.17	p = 0.2818
Adrenals	0.056 ± 0.008	0.057 ± 0.006	0.054 ± 0.006	0.054 ± 0.007	p = 0.2541
Relative organ weight (g/100 g b.w.)					
Liver	3.04 ± 0.22	3.08 ± 0.15	3.33 ± 0.32	3.08 ± 0.28	p = 0.8001
Spleen	0.321 ± 0.037	0.285 ± 0.029	0.308 ± 0.065	0.277 ± 0.038	p = 0.2117
Kidneys	0.721 ± 0.052	0.799 ± 0.081	0.739 ± 0.031	0.673 ± 0.113	p = 0.0905
Adrenals	0.033 ± 0.005	0.032 ± 0.003	0.032 ± 0.004	0.030 ± 0.004	p = 0.0178

[a] Mean ± SD.

*Significantly different from control ($p \leq 0.05$).

Measurement of selected organs weights indicated no difference between treated and control animals of both sexes (Table 1).

There were some statistically significant differences in mean hematology values between the treated and control groups (Table 2). They included a decrease of red blood cell counts in males from all treated groups and in females from the low- and high-dose groups, elevated hemoglobin concentration in males from low- and high-dose groups, and lower hematocrit value in males from the high-dose group. The erythrocytic MCV, MCH, and MCHC values were also changed. Reticulocyte counts, total and differential white blood cell counts, and clotting time in treated rats were comparable to those in control groups (data not shown). Some differences in platelet counts

Table 2. Effect of butanediol-1,4 on hematological parameters

Parameters[a]	Treatment group				Linear regression analysis
	Control (n = 8)	5 mg/kg (n = 8)	50 mg/kg (n = 8)	500 mg/kg (n = 8)	
Red blood cells ($x10^6/mm^3$)					
Males	12.0 ± 0.6[b]	10.5 ± 0.8**	10.4 ± 0.7**	10.3 ± 0.4**	p = 0.4578
Females	11.8 ± 1.1	9.8 ± 0.7**	10.5 ± 1.3	9.8 ± 1.1**	p = 0.4058
Hematocrit (%)					
Males	53.0 ± 1.5	51.6 ± 2.3	51.6 ± 1.9	50.6 ± 1.1*	p = 0.0891
Females	49.5 ± 0.8	50.0 ± 1.5	47.6 ± 3.0	48.1 ± 2.4	p = 0.4351
Hemoglobin (g/dl)					
Males	17.3 ± 1.1	19.0 ± 0.8*	18.2 ± 1.1	19.4 ± 1.1**	p = 0.1982
Females	17.4 ± 0.8	16.9 ± 1.2	16.9 ± 1.3	17.5 ± 0.9	p = 0.2188
Mean corpuscular volume (μm^3)					
Males	44.2 ± 1.9	49.5 ± 3.2**	49.6 ± 3.3**	49.3 ± 1.7**	p = 0.6166
Females	42.4 ± 4.4	51.3 ± 3.8**	45.8 ± 4.9	49.6 ± 4.1**	p = 0.5507
Mean corpuscular hemoglobin (pg)					
Males	14.4 ± 0.8	18.2 ± 1.7**	17.5 ± 1.1**	18.9 ± 0.8**	p = 0.3236
Females	14.8 ± 0.8	17.4 ± 2.0*	16.3 ± 1.9	18.2 ± 2.6**	p = 0.1690
Mean corpuscular hemoglobin concentration (%)					
Males	32.7 ± 1.9	36.8 ± 1.8**	35.3 ± 2.5	38.3 ± 2.4**	p = 0.1513
Females	35.1 ± 1.8	33.9 ± 2.9	35.7 ± 3.1	36.5 ± 3.5	p = 0.0950
Platelets ($x10^3/mm^3$)					
Males	338 ± 73	375 ± 94	313 ± 65	212 ± 51**	p = 0.0002
Females	251 ± 69	189 ± 42*	188 ± 35*	242 ± 35	p = 0.4949

[a] Other parameters not included in the Table were not significantly different from control.
[b] Mean ± SD.
*Significantly different from control ($p \leq 0.05$).
**Significantly different from control ($p \leq 0.01$).

between treated and control rats were revealed in both male and female rats. A statistically significant, as compared to controls, decrease in thrombocytes was observed in males from the high-dose group and in females from the low-dose and mid-dose group.

Among the various clinical chemistry values measured, a statistically significant increase of alanine aminotransferase and sorbitol dehydrogenase activities, and a decrease of total protein level was observed in the high-dose males (Table 3). Other instances of statistically significant differences in clinical chemistry values were judged to be toxicologically insignificant.

In the liver, proliferation of bile ducts and periportal infiltrations composed of fibroblasts and mononuclear cells were observed most often in treated animals. Frequencies of such changes were 0/5, 3/5, 1/5, and 3/5 in males, and 1/5, 1/5, 3/5, and 4/5 in females, respectively for control, low-, mid-, and high-dose groups. Proliferation of bile ducts was statistically significantly

Table 3. Effect of 1,4-butanediol on clinical chemistry values

Parameters[a]	Treatment group				Linear regression analysis
	Control (n = 8)	5 mg/kg (n = 8)	50 mg/kg (n = 8)	500 mg/kg (n = 8)	
Alanine aminotransferase (U/l)					
Males	32.2 ± 3.0[b]	30.4 ± 4.4	28.8 ± 3.7	38.2 ± 5.1*	p = 0.0048
Females	34.1 ± 5.7	28.5 ± 5.5	27.9 ± 4.6	34.1 ± 4.7	p = 0.3936
Sorbitol dehydrogenase (U/l)					
Males	1.98 ± 0.34	1.76 ± 0.30	1.78 ± 0.30	3.22 ± 1.89*	p = 0.0000
Females	2.29 ± 0.58	2.05 ± 0.59	2.15 ± 0.25	2.50 ± 1.57	p = 0.0311
Total protein (g/dl)					
Males	7.06 ± 0.17	7.01 ± 0.19	7.05 ± 0.31	6.72 ± 0.20*	p = 0.0000
Females	7.44 ± 0.20	7.44 ± 0.29	7.34 ± 0.27	7.62 ± 0.29	p = 0.3207
Glucose (mg/dl)					
Males	128 ± 26	128 ± 33	138 ± 36	151 ± 23	p = 0.0005
Females	111 ± 20	144 ± 26*	123 ± 18	117 ± 11	p = 0.6219

[a]Other parameters not included in the Table were not significantly different from control.
[b]Mean ± SD.
*Significantly different from control ($p \leq 0.05$).

more pronounced in animals from the high-dose group when male and female rats were jointly taken for comparison (Fisher exact test, $p < 0.01$). Most of the histopathological changes observed in the array of other tissues examined were limited to naturally occurring lesions which were present in approximately equal frequency in all groups, including controls.

DISCUSSION

Subacute oral administration of BAD resulted in an overall low degree of systemic toxicity. For some parameters tested – body weight, food consumption, and absolute and relative organ weights the highest dose of 500 mg/kg/day was the no-observed-effect-level (NOEL) for both sexes. Slightly higher activities of sorbitol dehydrogenase and alanine aminotransferase were observed in male rats given BAD at the highest dose of 500 mg/kg/day. Both these enzyme activities were found to be the most sensitive biochemical indices of liver damage also in the case of other hepatotoxic chemical compounds (7).

For clinical chemistry parameters a dose of 500 mg/kg/day was NOEL in females, and lowest-observed-effect-level (LOEL) in males, while a dose of 50 mg/kg/day was a NOEL in males.

Disturbances in hematological parameters observed in treated rats were of questionable toxicological significance since the changes were not generally

dose-related and the values remained within normal physiological limits. Nevertheless, a pattern of disturbances was similar both in male and female animals and suggested macrocytic anemia, accompanied by thrombocytopenia. This type of hematological injury may be caused by several factors including deficiencies of vitamin B_{12}, folic acid, cobalt, protein, as well as hepatic diseases (8). However, an elevated erythrocytic mean corpuscular volume (MCV), accompanied by thrombocytopenia, is much more frequently seen in liver disease associated with alcoholism than as a result of other causes (6). Thus, the hematological disturbances along with a slight elevation of sorbitol dehydrogenase and alanine aminotransferase activities may reflect the changes found in the liver of treated rats.

Mild to moderate inflammation of the liver, characterized by proliferation of bile ducts and periportal infiltrations with fibroblasts and mononuclear cells, was found in treated animals. Such lesions are reported to occur in hepatic inflammation caused by several agents, e.g. alcohol, viruses, cirrhosis, cholestasis, and in cases of chronic liver diseases (5). Although we did not observe by light microscopy evident lesions of the parenchymatous cells in the liver, the proliferation of bile ducts and periportal mononuclear cell infiltrations were considered to be indices of chronic toxic inflammation of the liver. A frequency of liver changes was higher in treated animals, although statistical significance occurred in rats from the high-dose group only in the case when both sexes were taken together for comparison. On this basis a dose of 500 mg/kg/day may be considered as LOEL for histopathological changes, and a dose of 50 mg/kg/day as a NOEL.

On the basis of our subacute oral studies performed on rats BAD is not considered to be a danger of serious damage to health by prolonged exposure.

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