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THE EFFECT OF REPEATED ADMINISTRATION OF SELECTED BENZENE BROMODERIVATIVES ON THE LIVER CATALASE ACTIVITY IN RATS

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Abstract
Objectives: Hexabromobenzene (HBB) is used as a flame retardant mainly added to plastics, timber and textiles. Tetrabromobenzene (TBB) is its metabolite. Both these compounds are present in the environment and in human and animal tissues. 1,4-Dibromobenzene (1,4-dBB) has found application in agriculture, pharmaceutical and some other industries as well as in households. It can also occur in the form of an environmental product of HBB debromination. It is known from laboratory experiments that animals after repeated exposure to these compounds, show the increase in relative liver weight. These results enable us to presume that benzene bromoderivatives may prove to be potential peroxisome proliferators. **Materials and Methods**: The investigated compounds were administrated intragastrically in three different doses for 1, 3, 7, 14, 21 and 28 days. Catalase was determined according to the method of Johansson and Borg with use of Purpald. **Results:** Repeated administration of the aforesaid compounds decreased catalase activity in the rat liver. **Conclusions:** The decreased level of catalase may point to its possible inactivation by metabolites of HBB, TBB and 1,4-dBB.

Key words:
Hexabromobenzene, 1,2,4,5-Tetrabromobezene, 1,4-Dibromobenzene, Rats, Catalase

INTRODUCTION

Hexabromobenzene (HBB) is used as a flame retardant mainly added to plastics, timber and textiles [1]. Tetrabromobenzene (TBB) is its metabolite. Both these compounds are present in the environment and in human and animal tissues [2]. 1,4-Dibromobenzene (1,4-dBB) has found application in agriculture, pharmaceutical and some other industries as well as in households. It can also occur in the form of an environmental product of HBB debromination.

It is known from laboratory experiments that animals after repeated exposure to these compounds, show the increase in relative liver weight (RLW) and total liver content of cytochromes P-450 [3,4]. These results enable us to presume that benzene bromoderivatives may prove to be peroxisome proliferators (PPs), or may affect endothelial reticulum proliferation. Estimating the effects of these substances on endothelial reticulum proliferation and on peroxisome proliferation it is important to investigate the levels of cytochromes (CYP2B and CYP4A) and RLW. The increase in peroxysomal enzymes, e.g., catalase and acyl- CoA oxidase that accompany these processes should also be taken into account.

Catalase is an enzyme and the increase in its activity may be a useful indicator of peroxisomes proliferation [5,6].

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Thus, it seems that the study on the effect of selected benzene bromoderivatives on catalase activity in the rat liver is well-grounded. This is a continuation of the study on the effect of benzene bromoderivatives on antioxidative enzymes. The previous study was focused on the effect of HBB, TBB and 1,4-dBB on the level of glutathione and activity of peroxidase and glutathione transferase.

MATERIALS AND METHODS

Experiments were performed on female WISTAR rats of 180–220 g body weight, obtained from the breeding colony of the Medical University in Łódź. The animals were fed Murigram standard fodder and tap water was supplied *ad libitum.* The investigated compounds were suspended in sunflower oil and administered intragastrically in three doses: hexabromobenzene (HBB) – 15, 75, 375 mg/kg b.w; 1,2,4,5-tetrabromobenzene (1,2,4,5-tetraBB) – 8, 40, 200 mg/kg b.w.; and 1,4-dibromobenzene (1,4-dBB) – 4, 20, 100 mg/kg b.w. The particular doses were respectively: 0.15%, 0.75% or 3.75% of the approximate lethal dose (ALD) determined previously [7]. The control group comprised rats not administered any compounds ("pure control"). The dissections were performed 1, 3, 7, 14, 21 and 28 days after the exposure. The animals were sacrificed in ether narcosis by intracardiac puncture and the liver was collected to investigate the effects of these compounds on catalase.

Catalase was determined in 25% liver homogenate according to the method of Johansson and Borg [8] with use of Purpald (4-amino-3-hydrazino-5-mercapto-1,2,4-triazole) and peroxidative function of the enzyme was used to determine its activity in the liver. In this method methanol is converted into formaldehyde under the effect of catalase and H_2O_2. Formaldehyde with Purpald forms a olor complex measured spectrophotometrically and the color intensification corresponds with catalase activity in the tissue. The relationship between formaldehyde formation and increasing concentrations of purified catalase in samples was determined. The rectilinear relationship between catalase concentration in samples and the amount of formed formaldehyde (0–15 μM of formaldehyde) was obtained.

Statistical analysis

The statistical analysis was based on the SYSTAT 5.30 program [9]. The Bartlett test for homogeneity of group variances was used. The differences between related parameters were evaluated with the Tukey HSD multiple comparison test.

Determinations in the control groups were performed parallely to the determinations in the investigated groups. The obtained results did not show statistically significant difference, that is why all the results from the controls were combined into one control group for comparison.

All investigations were performed with the consent of the local Ethic Committee in Łódź.

RESULTS

After single exposure to HBB (two lower doses), catalase activity below the level noted in control animals was observed and it remained at the same level throughout the whole experiment. After single exposure to 375 mg/kg and exposure to 75 mg/kg, repeated for 21 days, the level of this parameter reached the level of the pure control, but did not exceed it. The lowest activity of catalase was observed after administration of 15 mg/kg on days 7–21 (Fig. 1).

The exposure to TBB at all the doses decreased the catalase activity throughout the experiment, except the 21st day of exposure, when about a 10% increase for a dose of 8 mg/kg compared to controls was observed. The lowest activity level was noted on the 14th day for the highest dose. There was about 35% difference in the levels compared with the control group (Fig. 2).

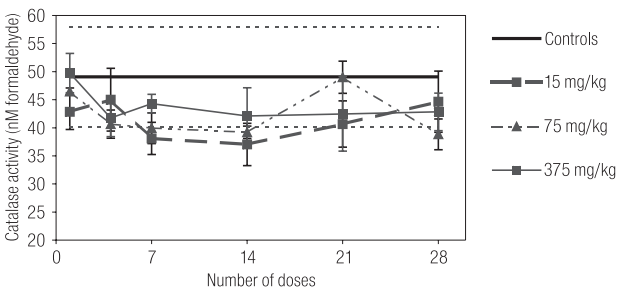


Fig. 1. Activity of catalase in the rat liver after HBB administration (nM formaldehyde). The figure presents mean values and standard deviations.

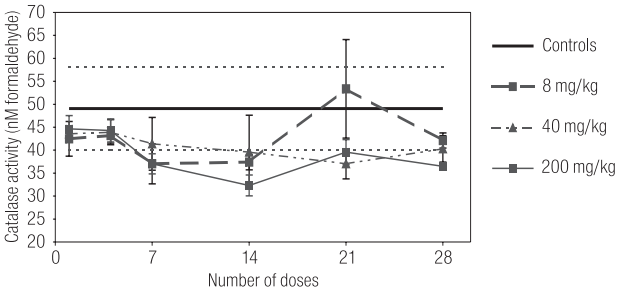
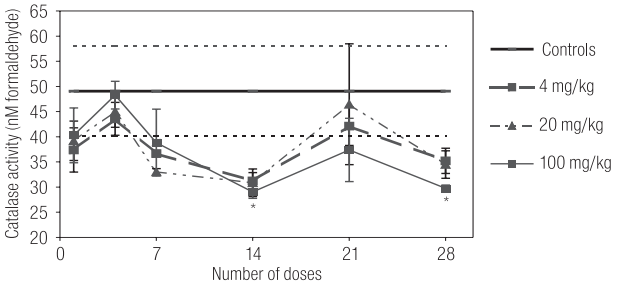


Fig. 2. Activity of catalase in the rat liver after 1,2,4,5-tetraBB administration (nM formaldehyde). The figure presents mean values and standard deviations.



***** Significantly different as compared to pure controls ($\alpha = 0.05$)

Fig. 3. Activity of catalase in the rat liver after 1,4-dBB administration (nM formaldehyde). The figure presents mean values and standard deviations.

The lowest levels of catalase activity were observed after exposure to 1,4-dBB. Statistically significant decrease was found for the highest dose after the 14th and 28th days of exposure. During the whole experiment no increase in catalase activity above the values obtaind for pure control after exposure to 1,4-dBB was observed (Fig. 3).

DISCUSSION AND CONCLUSIONS

Several methods are used to determine cell proliferation: morphological and histopathological (increase in the liver weight, the number of cells in various stages of mitotic division), immunohistological (BrdU incorporation), radiohistological (tritiated thymidine incorporation), and those defining the levels of enzymes associated with peroxisomes (e.g., catalase and acyl-CoA oxidase).

In the studies on polybromobenzenes toxicity carried out to date, we observed the increase in RLW and numerous cell divisions on microscopical examination [3,10]. After exposure to HBB, the increase in the liver weight and total liver content of cytochromes P-450 was seen [3,11]. The increased liver weight and cytochromes concentration in hepatocytes were found after exposure to TBB [4]. After exposure to both these compounds, the increase in CYP1A and CYP2B concentrations was observed [12–14]. In order to complete these investigations, it was decided to determine one of the peroxisomal enzymes. Catalase was selected from this group because it catalizes the reaction of H_2O_2 decomposition and indirectly protects the cell against hydroxyl radicals activity. This enzyme was used as peroxisome proliferation marker, when investigating among others the toxicity of clofibrate [15], some herbicides [5,6] and many other compounds [16].

The decrease in catalase activity in the rat liver may be associated with its inactivation by some superoxide anions, e.g., alkyl, which is associated with the decreased resistance of the organism to destructive activity of free radicals [17]. It is also associated with ultrastructural damage to hepatocytes, excluding a significant decrease in the number and size of peroxisomes. This decrease is typical of the liver because it was not observed in kidneys and erythrocytes [18]. The studies carried out by Frydrych et al. [19] on the effect of benzene bromoderivatives on the change in glutathione levels showed the decrease in GSH concentration after 3 days of rats exposure to 1,4-dBB, which was followed by its increase above control values that maintained throughout the experiment. Moreover, the effect of this xenobiotic on glutathione peroxidase and transferase activity was also observed in these studies. After seven-time repeated exposure to 1,4-dBB, the decrease in glutathione peroxidase activity by about 50% below the control values, followed by the increase to the level obtained for the pure control animals, was detected [19]. Furthermore, exposure to TBB and HBB caused significant increase in glutathione peroxidase activity in the rat liver after 7 days of exposure, which with extended time of the experiment decreased to the control values [20].

It results from the studies on bromobenzene biotransformation that this compound undergoes conversion to bromophenol in the first phase [21]. Studies on the toxicity of other benzene halogen derivatives also confirm the formation of hydroxyl derivatives in the process of biotransformation [22].

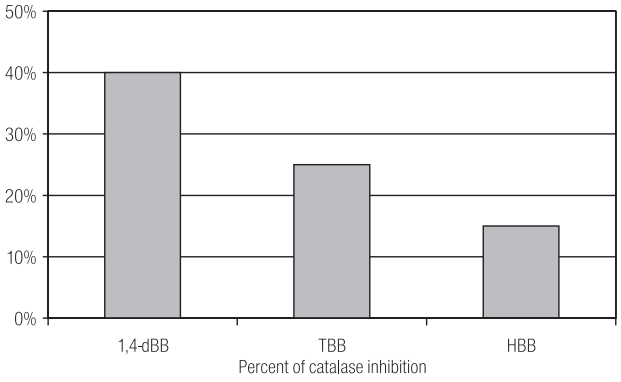


Fig. 4. The percent of catalase inhibiton after a 28-time repeated exposure to HBB, TBB and 1,4-dBB in a dose of 3.75% ALD.

The investigations on HBB metabolism showed that 70% of the administered dose is excreted with feces in the form of metabolites containing sulfur or oxygen and the ratio of oxygen and sulfur containg metabolites was 15:1 [23]. Based on these data, it may be suggested that the decrease in the level of catalase, (natural antioxidant) caused by the investigated compounds may indicate a possible disactivation by their hydroxyl metabolites being able to form alkoxyl radicals.

To compare the results, the tested compounds were administered in doses corresponding with 0.15%, 0.75% and 3.75% ALD, which for the highest doses (3.75% ALD) was 0.45; 0.50; 0.70 mM, respectively. From these investigations it also appears that higher brominated compounds cause a lower decrease in catalase activity than compounds of a fewer number of bromine atoms in the element (Fig. 4). Thus, after exposure to 1,4-dBB the liver is devoid of catalase to a higher degree than after exposure to HBB (at least for short time). In normal conditions, in animals not exposed to these compounds the decrease in catalase will result in taking over by selene-dependent glutathione peroxidase the responsibility for H_2O_2 decomposition.

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