International Journal of Occupational Medicine and Environmental Health, 2004; 17(3): 347—353

EFFECT OF REPEATED ADMINISTRATION OF HEXABROMOBENZENE AND 1,2,4,5-TETRABROMOBENZENE ON THE LEVELS OF SELECTED CYTOCHROMES IN RAT LIVER

ELŻBIETA BRUCHAJZER, BARBARA FRYDRYCH and JADWIGA A. SZYMAŃSKA

Department of Toxicology
Faculty of Pharmacy
Medical University
Łódź, Poland

Abstract.
Objectives: Hexabromobenzene (HBB) is a flame retardant, which added to polymers, plastics, textiles, wood or paper, decreases the amount of carbon monoxide and heat release during fire. HBB is also formed as a result of decabromodiphenyl oxide pyrolysis or natural decabromobiphenyl ether debromination as the effect of photolysis. 1,2,4,5-Tetrabromobenzene (1,2,4,5-tetraBB) is a compound formed in the body as a metabolite of HBB. Both these compounds may appear in the environment and human tissue. The purpose of the study was to estimate the effect of repeated administration of HBB and 1,2,4,5-tetraBB on the levels of selected cytochromes in rat liver. **Materials and Methods:** The investigated compounds were administered intragastrically in three different doses for 7, 14, 21 or 28 days. Relative liver mass was estimated as well as total concentration of cytochromes P-450 and EROD (CYP 1A) and PROD (CYP 2B) activity in rat liver. Concentration of cytochromes P-450 was determined in microsomal fraction (using the spectrometric method). EROD and PROD were detected by fluorimetric method. **Results:** Repeated administration of 1,2,4,5-tetraBB and HBB (in the highest dose) was found to increase relative liver mass. After 1,2,4,5-tetraBB administration, total liver concentration of cytochromes P-450 increased even by several times, depending on the volume and number of doses. Less pronounced alterations were found after repeated administration of HBB. Exposure to HBB resulted in a tenfold increase in EROD activity (after 14-28 days) and a significantly lower increase in PROD activity. 1,2,4,5-TetraBB increased EROD activity by 2-3 times and PROD activity by maximum 2 times. **Conclusions:** Following the experiments, it may be stated that HBB and 1,2,4,5-tetraBB are inductors of microsomal enzymes system. 1,2,4,5-TetraBB more than HBB increases the level of total concentration of cytochromes and induces isoform CYP 2B (PROD). Administration of HBB resulted in the increase in CYP 1A (EROD) activity comparable to that after 3-methylcholanthrene.

Key words:
Hexabromobenzene, Tetrabromobenzene, Cytochromes, Rats

INTRODUCTION

Hexabromobenzene (HBB) is a flame retardant which added to polymers, plastics, textiles, wood or paper decreases the amount of carbon monoxide and heat release during fire [1]. It is mainly used in the plastics, paper and electrical industries. HBB may appear in the environment. Most frequently, it results from thermal decomposition of other flame retardants such as octabromodiphenyl ether, decabromodiphenyl ether, or hexabromobiphenyl [1,2]. HBB is also formed as a result of decabromodiphenyl oxide pyrolysis [3], or natural decabromobiphenyl ether debromination as the effect of photolysis [4].

This study was supported by the State Committe for Scientific Research, Poland (Grant No. 6 PO5A 081 21).
Received: June 29, 2004. Accepted: August 3, 2004.
Address reprint requests to E. Bruchajzer, PhD, Department of Toxicology, Faculty of Pharmacy, Medical University, Muszyńskiego 1, 90-151 Łódź, Poland (e-mail: ebruchajzer@pharm.am.lodz.pl).

Considering the amount of LD_{50} for HBB, which for rats after intragastric administration is over 10 000 mg/kg, this compound should be practically harmless. This could be confirmed by the result of experiments, in which HBB was administered to mice and rats in single high doses. In those experiments, hepato- and nephrotoxic effects of HBB were not observed [5,6]. However, the toxicity of this compound changes together with the change in exposure. Elongation of the period of HBB administration together with the decrease in the dose due to disturbances in porphyrin synthesis in rats was demonstrated, among others, by the increased elimination of porphyrins with urine [7]. Porphyrogenic activity was also found after repeated administration of benzene chlorine derivative (hexachlorobenzene) [8].

HBB undergoes alterations in the body, they proceed in two ways: debromination and hydroxylation [9,10]. However, the process of debromination seems to be the main metabolic pathway, and 1,2,4,5-tetrabromobenzene (1,2,4,5-tetraBB) is one of its products found i.e. in the human fatty tissue [11].

Little is known about the toxicity of 1,2,4,5-tetraBB. After a single administration of its high doses to mice, a decreased concentration of reduced glutathione (GSH) and an increased level of malondialdehyde (MDA) were found in the liver. After exposure extended to 7 days with simultaneous decrease in doses, the liver mass increased in relation to the body mass of animals and GSH level decreased, while delta-aminolevulinate synthase (ALA-S, EC 2.3.1.37) raised in the liver [12,13]. The effect of repeated administration of 1,2,4,5-tetraBB on cytochromes P-450 level is still to be elucidated. In experiments carried out to date, only alterations in total concentration of cytochromes P-450 have been estimated [13]. Therefore, the aim of our study was to assess the effect of repeated administration of HBB and its metabolite, 1,2,4,5-tetraBB, not only on the total concentration of cytochromes P-450 but also on the activity of its two isoforms CYP 1A (EROD) and CYP 2B (PROD).

MATERIALS AND METHODS

Experiments were carried out on female WISTAR rats of 180–220 g body weight from the breeding colony of the Medical University in Łódź. Our earlier experiments on benzene bromoderivatives toxicity were performed on female rats, that is why the effect of the investigated compounds on the levels of selected cytochromes were assessed on the same gender. The animals were fed a standard Murigram diet (Agripol, Motycz, Poland), with water supplied *ad libitum*. Two weeks prior to the exposure, the animals were acclimatized in laboratory conditions. All investigations were performed in accordance with the Polish law on the protection of animals [14].

In this experiment, the rats were divided into groups, four animals each. HBB and 1,2,4,5-tetraBB were administered in sunflower oil intragastrically through 7, 14, 21 and 28 days. The doses of HBB were 15, 75 or 375 mg/kg/day, and of 1,2,4,5-tetraBB 8, 40 or 200 mg/kg/day. These doses made up a comparable percentage of approximate lethal dose (ALD) determined according to the method of Deichman and LeBlanc [15]. Two types of control groups were used: (a) pure control, not administered any compounds and (b) oil control, administered sunflower oil at a volume of 2.5 cm^3 per 1 kg body weight. To compare alterations taking place in the activity of cytochrome P-450 isoforms CYP 1A and CYP 2B, additional groups of animals ("positive controls") were administered intraperitoneally for 4 successive days; inductors of these cytochromes were 3-methylcholanthrene (3-MCh, in a dose of 20 mg/kg/day) or phenobarbital (PB, in a dose of 60 mg/kg/day), respectively. The doses were given according to the literature data [16].

On the day of dissection, the rats were sacrificed by bleeding in ether narcosis and liver was collected for determination of total concentration of cytochromes P-450. The activity of adequate monooxidases associated with cytochromes P-450, CYP 1A (EROD – ethoxyresorufin O-deethylase) or CYP 2B (PROD – pentoxyresorufin O-deethylase) were considered as the markers of CYP 1A or CYP 2B isoforms. Relative liver mass, which determined the percentage contribution of liver mass was also calculated.

Total concentration of cytochromes P-450 was determined in the liver microsomal fraction according to Cinti et al. [17], and Guengerich [18]. Cytochrome P-450 was reduced

through the reaction with sodium dithionite, and after saturation with carbon monoxide absorbance the maximum was measured at λ = 450 nm.

Activity of EROD and PROD (isoforms CYP 1A and CYP 2B) was determined by measuring O-dealkylation of 7-ethoxy- and 7-pentoxyresorufin, respectively, the substrates of monooxygenases associated with these cytochromes [19,20]. Fluorescence of the formed resorufin was measured with a Jensen 2100 spectrofluorimeter at the wave length of 512 nm (excitation) and 621 nm (emission).

Total concentration of cytochromes P-450 as well as EROD and PROD activity were presented in terms of microsomal protein. The protein was determined with Lowry method [21], where the reaction of peptide bonds and aromatic aminoacids with phenolic reagent Folin-Ciocalteu was used. Absorbance of the obtained color was measured at the wave length λ = 750 nm.

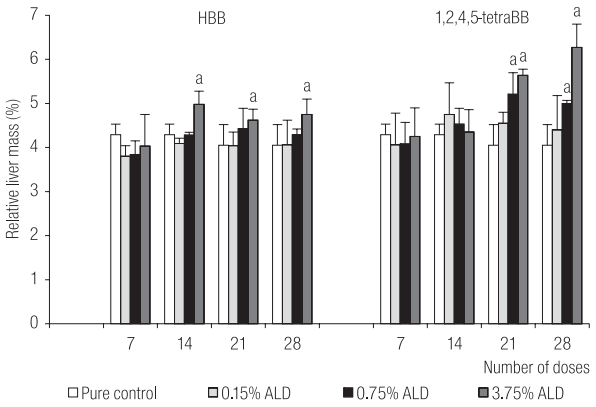
Statistical analysis

The statistical analysis was based on SYSTAT 5.30 program [22]. The Bartlett test for homogeneity of group variances was used. The differences between related parameters were evaluated with Tuckey HSD multiple comparison test.

RESULTS

To estimate the effect of repeated administration of HBB and 1,2,4,5-tetraBB on selected liver cytochromes in rat, three doses were applied: 15, 75 or 375 mg/kg/day for HBB and 8, 40 or 200 mg/kg/day for 1,2,4,5-tetraBB (0.15%, 0.75% or 3.75% of ALD, respectively). The results obtained in the study groups were compared statistically with those in controls (pure control and oil control).

Assessment of alterations in relative liver mass is a useful parameter pointing to xenobiotics action in the liver. An increase in relative liver mass to 116% of pure control values was found after administration of the highest dose (375 mg/kg/day) of HBB for 14 days (Fig. 1). After this dose the level of the indicator remained significantly elevated in relation to both pure and oil controls until termination of HBB exposure. More pronounced alterations of relative liver mass were observed after repeated administration of 1,2,4,5-tetraBB (Fig. 1). Statistically significant increase in this parameter was found only after 21 and 28 days of exposure. However, it was higher than after HBB administration and reached 125–130% of the pure control values after the dose of 40 mg/kg/day and to 140-155% after the highest dose (200 mg/kg/day).



a – Significantly different from pure control, α = 0.05.

Fig. 1. Change in relative liver mass (%) in rats following HBB and 1,2,4,5-tetraBB administration. The results are the mean ± SD.

Repeated administration of 1,2,4,5-tetraBB resulted in significantly increased total concentration of cytochromes P-450 in rat liver (Fig. 2). After administration of the lowest dose, the increase reached 175% and after the highest dose (200 mg/kg/day) – 300% of the control values. At all measurement points, these changes depended on the ap-

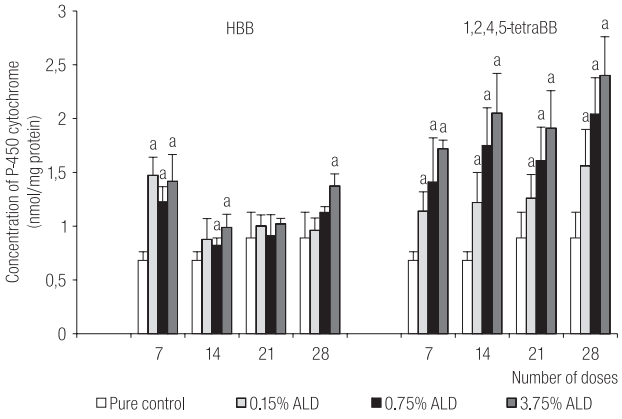


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Fig. 2. Total concentration of P-450 cytochrome in rat liver (nmol/mg protein) following HBB and 1,2,4,5-tetraBB administration. The results are the mean ± SD.

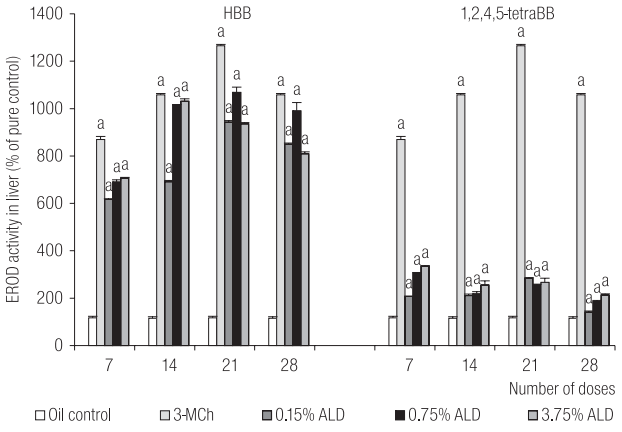
plied dose of the compound. Less pronounced alterations were found after repeated administration of HBB; they were statistically significant after 7 days and after exposure to the highest dose (375 mg/kg/day). After a 28-day exposure, the observed increase in the level of total concentration of cytochromes P-450 depended on the administered dose of the compound.

In an earlier experiment, the highest level of total concentration of cytochromes P-450 in rat liver was observed after 21 and 28 days of HBB administration [7].

Estimating the effect of HBB and 1,2,4,5-tetraBB on EROD and PROD activity, an additional group, positive control, was included in the study. Rats receiving 3-methylcholanthrene were used to determine EROD and rats on phenobarbital to determine PROD.

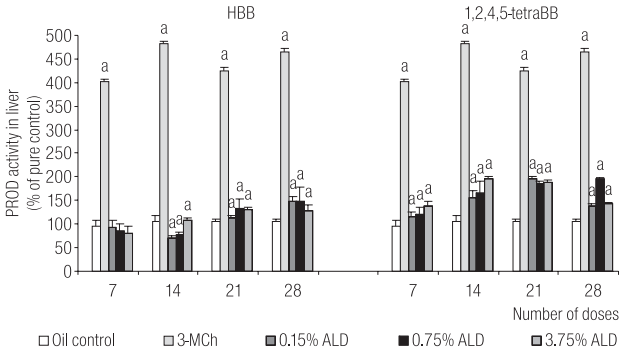
After repeated HBB administration a significant increase in EROD activity was observed in rat liver (Fig. 3). At the analyzed points, EROD was 10-12 times higher than in the control groups (pure and oil) of animals. This activity was similar to that found after administration of EROD inductor – 3-MCh. Repeated administration of 1,2,4,5-tetraBB also caused an increase in EROD activity in rat liver (Fig. 3). However, these alterations, although statistically significant, reached only 250-300% of control values after 21 days and 165-250% after 28 days of exposure.

Somewhat different tendency was observed in the case of the increased PROD activity in rat liver (Fig. 4). Statistically significant increase in the activity of this parameter was noted only after 21 and 28 days of HBB administration. The increase reached 130–150% of the control values. More pronounced alterations were observed after repeated administration of 1,2,4,5-tetraBB (Fig. 4). The lowest increase in PROD activity, reaching 116–140% of the control values, was found after sevenfold administration of this compound. The highest, twofold increase in the analyzed indicator of activity was detected after 21 days of exposure.



a – Significantly different from pure control, $\alpha = 0.05$.

Fig. 3. Activity of EROD (CYP 1A) in rat liver (% of pure control) following HBB and 1,2,4,5-tetraBB administration. The results are the mean ± SD.



a – Significantly different from pure control, $\alpha = 0.05$.

Fig. 4. Activity of PROD (CYP 2B) in rat liver (% of pure control) following HBB and 1,2,4,5-tetraBB administration. The results are the mean ± SD.

DISCUSSION

Based on the observations made in the course of the experiment concerning alterations in relative liver mass, it may be assumed that HBB administered in the highest dose and 1,2,4,5-tetraBB administered in two higher doses may increase activity of hepatocytes. Among others, it is manifested by the liver hypertrophy and hyperplasia at stages when cells are forced to a more extensive labor [23]. A more pronounced increase in relative liver mass was found after 1,2,4,5-tetraBB administration, particularly after exposure of 21 and 28 days.

Administration of 1,2,4,5-tetraBB also resulted in significant elevation of total concentration of cytochromes P-450, which at all measurement points depended on the administered dose of the compound (Fig. 5). Significantly weaker effects were noted after administration of HBB; alterations of similar intensity were found only 7 days after the exposure. It is known from earlier experiments that

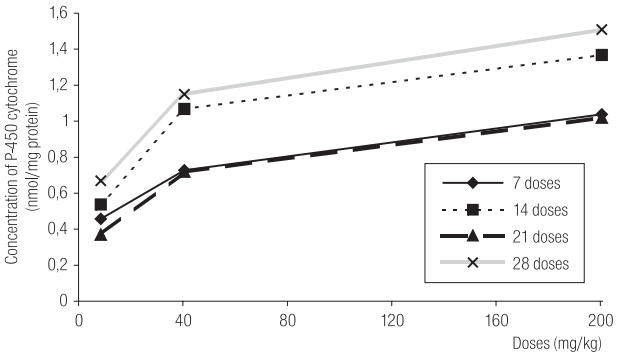


Fig. 5. Relationship between total concentration of cytochromes P-450 (nmol/mg protein) and 1,2,4,5-tetraBB dose (dose-effect dependence).

repeated administration of HBB may cause alterations in the level of total concentration of cytochromes P-450 and in the activity of cytochrome C reductase [7,24,25]. Distinct increase in total concentration of cytochromes P-450 allows to suppose that HBB, and to a greater degree 1,2,4,5-tetraBB, are compounds able to induce the system of microsomal enzymes. It is known from the literature data that induction of microsomal cytochromes and monooxygenases was observed after administration of chloroderivative – hexachlorobenzene (HCB) – to mice and rats [8,26].

Isoform CYP 1A – EROD could be considered as the biomarker of CYP 1A activity. It plays a significant role in tranformation of numerous environmental contaminations (e.g., polycyclic aromatic hydrocarbons). In phase I of metabolism, it is responsible for epoxidation reactions, in which unstable epoxides are formed. They are considered to be genotoxic compounds, responsible for polycyclic aromatic hydrocarbons cancerogenic action. CYP 1A induction may thus intensify formation of toxic metabolites. In our experiment, an increase in CYP 1A (EROD) activity was obtained through intraperitoneal administration of 3-methylcholanthrene for 4 successive days. It resulted in a 11–12-time induction of this indicator. Only a slightly weaker (about 10-times) increase in CYP 1A (EROD) activity was observed after 14, 21 and 28 days of exposure to HBB. This may speak for relatively strong inductive properties of this compound. It is known from the literature data that CYP 1A induction is also observed after administration of hexachlorobenzene (0.1%) in rat fodder for 3 months [27].

It is evident from the analysis of the results that 1,2,4,5-tetraBB also affects CYP 1A activity, but its induction of this isoform is weaker than that of HBB.

Isoenzyme CYP 1A2 is known to catalyse, e.g., uroporphirynogen metabolism [28]. Liver microsomes CYP 1A take part in uroporphyrinogen oxidation to porphyrin [29]. This would point to the relation between markedly elevated CYP 1A activity in the liver and disturbances in metabolism of porphyrins observed earlier after repeated HBB administration to rats. Increased elimination of porphyrins (e.g., coproporphyrins and uroporphyrins) with urine was found in the experiment, in which rats were administered HBB in the doses of 15, 75 or 375 mg/kg for 28 days. At the same time total liver concentration of cytochromes P-450 also increased [7].

The analysis of CYP 2B (PROD) activity shows that the increase in this parameter was more pronounced after 1,2,4,5-tetraBB administration, which would point to slightly stronger inductive properties of this compound as compared to HBB. However, these alterations were markedly weaker than those after administration of model CYP 2B inductor phenobarbital. It may be concluded (from the performed experiment) that HBB is stronger in inducing CYP 1A than 1,2,4,5-tetraBB, which in turn is a stronger inductor of CYP 2B.

No findings have been noted in the available literature that could confirm the results of our study. There is no information on the effect of HBB and 1,2,4,5-tetraBB on CYP 1A and CYP 2B activity, and the data on the total concentration of cytochromes P-450 are very limited.

It is known that chlorine derivative hexachlorobenzene affects the level of liver cytochromes. It is determined as an inductor of mixed type: phenobarbituric and methylcholanthene. Hexachlorobenzene (HCB) binds to aromatic hydrocarbons (Ah) receptors, which may eventually lead to the increased risk of cancer development in rodents. In *in vitro* and *in vivo* studies, some authors applied the measurement of CYP 1A activity (ethoxyresorufin-O-deethylase, EROD) as an indicator of Ah receptor induction [30, 31]. HCB also demonstrates weak properties for direct induction of gene mutations and damage to chromosomes, although its mutagenic activity is rather weak. *In vitro* and

in vivo studies also demonstrated the possibility of HCB binding to DNA. However, its level was too low to induce genotoxic cancerogene [32].

There is lack of data on similar effect of HBB and 1,2,4,5-tetraBB activity. However, due to their distinct inductive activity, particularly in the case of CYP 1A after exposure to HBB, the effects similar to those which were observed after animals exposure to HCB cannot be totally excluded. More thorough investigations ought to be carried out to explain a possible mechanism of HBB effect on Ah receptor and probable formation of this compound metabolites.

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