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Tissue Distribution and Excretion of Hexabromobenzene and its Debrominated Metabolites in the Rat

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Abstract. Tissue distribution and excretion of hexabromobenzene (HBB) and some metabolites were studied in male Wistar rats administered a single oral dosage of HBB.

Most of the HBB dosage was absorbed by the intestinal tract and it was rapidly metabolized and distributed throughout the body as the debrominated metabolites, pentabromobenzene (PeBB), tetrabromobenzene (TeBB) and tribromobenzene (TrBB). The time courses of HBB, PeBB and TeBB concentrations in the tissues were roughly classified into several types, and debromination of HBB was found to take place stepwise.

The reductive debromination of HBB occurs by metabolic enzymes in the liver rather than microbes in the intestine.

Hexabromobenzene (HBB) is a commonly-used flame retardant for plastics, textiles and wood (Hutzinger *et al.* 1976). The amount of production of HBB in Japan in 1983 was 270 tons and it is used as a flame retardant in Japan.

Organobromine flame retardants are used primarily in the indoor environments, where people spend 90% of their time. (Budiansky 1980). Therefore, there is concern about the effects of organobromine flame retardants to humans. Many studies on the biological effects of the retardants have been reported (Kitagawa *et al.* 1975; Norris *et al.* 1975; Carlson 1977, 1980; Matthews *et al.* 1977; Mendoza *et al.* 1977, 1979; Wolff *et al.* 1979; Moore *et al.* 1980; Domino *et al.* 1982; Franklin *et al.* 1983).

These chemicals will be gradually dispersed into the natural environment from the indoor environment via gas and dust. In fact, some organobromine flame retardants have been detected in fish of Sweden and sediment of Japan (Andersson and Blomkvist 1981, Watanabe *et al.* 1983, 1986a, 1986b).

As a part of a study to reveal the fate of these organobromine flame retardants in the environment, our group previously reported photodegradation of HBB (Kawano *et al.* 1983) and compared the HBB and hexachlorobenzene (HCB) distribution in the rat (Yamaguchi *et al.* 1986). This paper reports the excretion and tissue distribution of HBB and its debrominated products in the rat administered a single oral dosage of HBB.

Materials and Methods

Preparation of Standard Solution

HBB was supplied by the Michigan Chemical Co. 1,2,4,5-Tetrabromobenzene (1,2,4,5-TeBB) and 1,3,5-tribromobenzene (1,3,5-TrBB) were purchased from the Wako Pure Chemical Industries, Ltd. Other bromobenzenes were prepared by photodegradation of HBB (Kawano *et al.* 1983) and their concentrations of individual bromobenzene were determined by gas chromatograph-mass spectrometer (GC-MS). Miyazaki *et al.* (1986) reported 1,2,4,5-TeBB and 1,2,3,5-TeBB were not separated by a GC-ECD equipped packed column, but 1,2,3,4-TeBB was separated into two isomers by various liquid phases on GC-ECD. Assignments of three TeBB isomers were made on the authentic 1,2,4,5-TeBB. Assignments on three TrBB isomers have been done similarly (Jan and Malnersic 1980).

Animals and Treatment

Male Wistar rats (five-weeks old; 50–100 g) were purchased and housed in individual cages. They were acclimated at 25°C and 12 hr light-dark cycle. The experiment was started after two weeks of acclimation. During the acclimation and the experiment, they were freely given a commercial diet and drinking water, but were starved one day before administration.

The rats were orally administered with 1 ml of corn oil containing 0.2 mg of HBB individually. The control rat was administered with 1 ml of corn oil only. After administration, they were sacrificed at 1, 2, 4, and 8 hr, and 1, 3, 10, 20 and 40 days respectively. Two or three rats were used at individual stage. The control rat was sacrificed at day 40. Four treated rats were transferred to a metabolic cage for collection of urine after administration.

The whole blood was taken immediately after sacrifice, hemolyzed with water, mixed with ethyl alcohol and stored at 4°C until analysis. Major tissues were dissected, weighed and frozen at −20°C until analysis. The feces were collected every day after the administration to the time of sacrifice and frozen at −20°C immediately. The urine was collected every day after administration and stored at 4°C until analysis.

Biliary Excretion

Male Wistar rats (eight-weeks old; 200–250 g) were used for the biliary excretion study.

Three rats were anesthetized by diethyl ether and were then inserted a common bile duct. Following the operation, they were placed in restraining cages. After the rats recovered from anesthesia, two rats were orally administered with 1 ml of corn oil containing 0.2 mg of HBB. The control rat was orally administered with 1 ml of corn oil only. The bile was collected in test tubes cooled with ice every hr from 1 to 24 hr after administration, and stored at 4°C until analysis.

During the acclimation and the experiment, the rats were given a commercial diet and drinking water freely.

Debromination by Intestinal Microbes

Male Wistar rats (seven weeks old; 320–340 g) were used for the debromination of HBB by intestinal microbes.

A commercial diet contained bacitracin, neomycin sulfate and polymixin B sulfate in amounts of 56 mg, 0.031 mg and 4.1 mg/30 g diet, respectively (Chawla *et al.* 1976). Rats were given 30 g of this diet with or without antibiotics per day for 10 days. Drinking water was given freely. 0.2 mg of HBB in corn oil was given to rats orally at 11 days after administration of antibiotics.

Rats were divided into three groups. In group I, rats were given a crushed diet without antibiotics and corn oil only. In another group, two rats were given a crushed diet with antibiotics and HBB in corn oil. In another group, two rats were given a crushed diet with antibiotics. The feces were collected every two hours from 2 to 48 hr after administration and stored at −20°C until analysis.

Chemical Analysis

The hemolytic blood (5–10 g) was shaken with ethyl alcohol and acetone, then water and hexane were added. After thorough partitioning the hexane phase was collected carefully and concentrated in a Kuderna-Danish concentrator after treatment with fuming sulfuric acid.

The feces (4–8 g) and tissues (1–5 g) were extracted by homogenizing with acetone and acetone + hexane (1 + 1), then water and hexane were added. The organobromines were transferred from the acetone and water mixture to hexane by shaking.

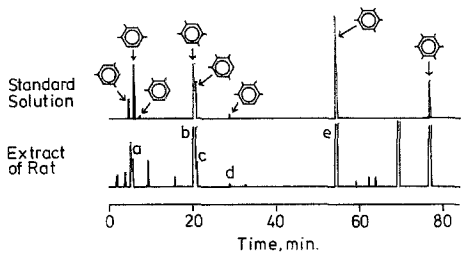

Fig. 1. Gas chromatograms of standard solutions prepared by photodegradation and extracts of whole rat's body after oral administration of HBB. a:1,2,4- b:1,2,4,5- c:1,2,3,5- d:1,2,3,4- e:1,2,3,4,5-

A Florisil® dry column was used to remove the fat from the extracts (Tatsukawa and Wakimoto 1972). Further interfering substances were eliminated by silica-gel (activated at 130°C, 8 hr, 1.5 g) column chromatography. HBB, PeBB, TeBB and TrBB were eluted by hexane (60 ml).

The carcass was homogenized and the organobromines were analyzed by the procedure mentioned above.

The urine and bile samples were diluted with water. After hexane extraction, silica-gel column chromatography was used for clean-up.

The organobromines in the final extracts were determined by a Shimadzu GC-7A gas chromatograph equipped with a ^{63}Ni electron capture detector (GC-ECD). The column consisted of a 30 m length × 0.3 mm inside diameter glass capillary-SCOT-OV17. The temperature of the column was programmed from 140°C to 220°C at a rate of 2°C/min with an initial 32 min and final 8 min hold. Both injector and detector temperatures were kept at 250°C. Nitrogen was used both as the carrier and make-up gas. Bromine compounds were identified by a Shimadzu Auto GC-MS 9020 DF gas chromatograph-mass spectrometer equipped with a multi-ion detector (GC-MS-MID); GC conditions were the same as mentioned above. The fragment ions used for HBB, PeBB, TeBB and TrBB were 552, 474, 394 and 316 m/z, respectively.

Results

Identification of Debrominated Products in the Rat

Referring to the chart, peaks, a, b, c, d and e in rat (Figure 1) were assigned to be 1,2,4-TrBB, 1,2,4,5-, 1,2,3,5-, 1,2,3,4-TeBB, and PeBB, respectively and further confirmation was made by GC-MS (m/z 316 for TrBB, m/z 394 for TeBB and m/z 474 for PeBB, respectively).

Excretion

The time courses of cumulative amounts of HBB and its metabolites excreted in feces are shown in Figure 2. Only about 10% of the dosage (20.6 μg) was excreted through the feces during the first day

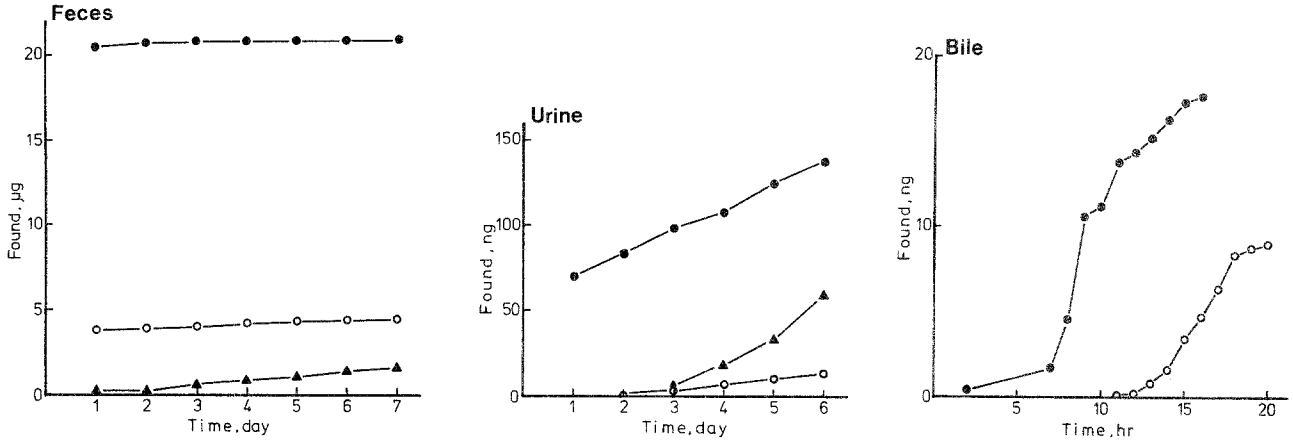

Fig. 2. Cumulative excretions of HBB (●), PeBB (○) and TeBB (▲) in feces, urine and bile of rat after oral administration of HBB

after administration, although the excretion amount increased gradually up to 20.8 μg by the seventh day after administration. This indicates that the major portion of the dose was absorbed by the intestinal tract in the rat.

Minor amounts of PeBB and TeBB, which were debrominated from HBB, were detected in the feces, and they increased gradually after administration.

The cumulative urinary excretions are shown in Figure 2. Similar excretory patterns were also found with HBB, PeBB and TeBB in urine as noted in the feces. However, the amount of the cumulative urinary excretion of TeBB was larger than that of PeBB, being different from the case of feces, which might be due to the higher polarity of TeBB than that of PeBB.

Only 0.0085% of the dosage was excreted by bile until the 16th hr after administration (Figure 2). The biliary excretion of PeBB began after the 11th hr of administration, and its cumulative amount was 0.0045% of dosage. Tetrabromobenzenes (TeBBs) in bile could not be determined by GC-ECD, because of the presence of interfering materials.

Tissue Distribution and Body Burden

The time courses of HBB, PeBB and TeBB concentrations in several tissues are shown in Figure 3.

The time of attaining maximum concentration of the debrominated products of various bromobenzenes in the brain depends on the number of bromine atoms in the benzene ring. The compound with the higher number of bromine atoms attain the maximum concentration earlier than those having a lower number of bromine atoms. The tissues, in which a same pattern of change in the concentrations of HBB and its metabolites were observed, were the lung, spleen, muscle, heart, testis, and blood.

In the liver, HBB concentration began to decrease an hour after administration. Concentrations of PeBB and TeBB increased until about 10 hr after administration, and thereafter decreased, indicating a biphasic excretion pattern.

In the kidney, the time course of HBB and PeBB were similar to the liver, but TeBB was maintained at the same concentration (100 ng/wet g) from 4 hr through 20 days after administration.

In the adipose tissue, the time course of the patterns of HBB, PeBB and TeBB concentrations were similar to the brain, but the time of maximum concentrations of these compounds were later than the brain. The concentrations of HBB and its debrominated metabolites in the adipose tissue is about ten-fold larger than the other tissues.

As shown in Figure 3, the accumulation patterns of bromobenzenes in individual tissue were roughly classified into several types.

The time courses of HBB, PeBB, TeBBs and tribromobenzenes (TrBBs) burdens in the rat body are shown in Figure 4. HBB and PeBB showed a biphasic course, but the TeBBs and TrBBs did not. The time course of the total bromobenzene (HBB + PeBB + TeBBs + TrBBs) burden was biphasic.

Discussion

HBB administered orally to the rat was absorbed by the intestine, transferred rapidly to the tissues by the blood, and metabolized and excreted.

Ten % of the total dosage of HBB was excreted via the feces within 7 days after administration without any metabolism, *i.e.*, the parent compound

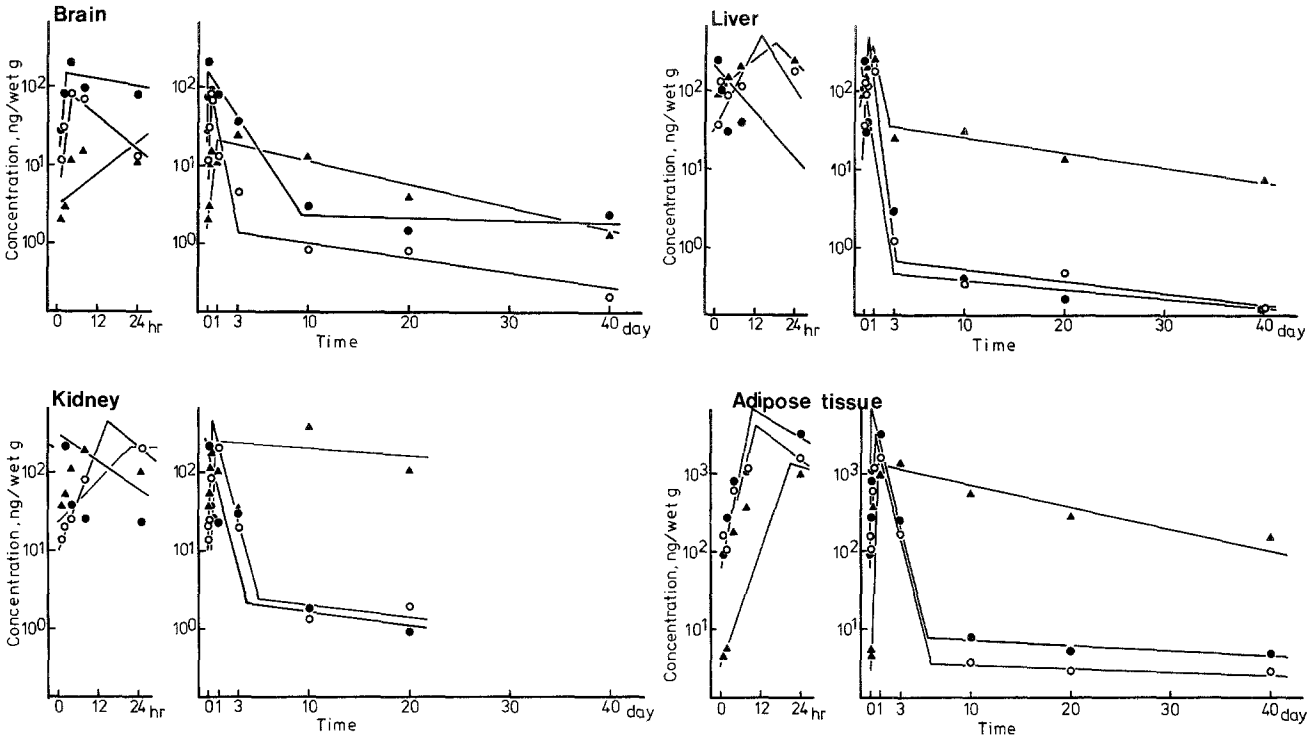

Fig. 3. Time courses of HBB (●), PeBB (○) and TeBB (▲) concentrations in brain, liver, kidney and adipose tissue of rat after oral administration of HBB

itself. Tanabe *et al.* (1981) reported that the absorption rates via intestine decreased with increase of molecular weight in rats administered the PCB mixture. In this report, the absorption rate of octachlorobiphenyl (molecular weight 430) was about 75%. However, HBB has a higher molecular weight (552) than PCBs and 90% of the dosage was absorbed by the rat intestine. Similarly, in 2,4,5,2′,4′, 5′-hexabromobiphenyl (MW 628) and [^{13}C]-hexabromonaphthalene (MW 602), absorption rates in rat were 92.1% (Matthews *et al.* 1977) and 76% (Birnbaum *et al.* 1983), respectively. Therefore, the absorption via the intestine seems to depend upon parameters (*e.g.* molecular size and shape) other than the molecular weight.

In rats, HBB was reductively debrominated to PeBB, TeBB and TrBB. To our knowledge, the studies on the reductive dehalogenation of halogenated aromatic hydrocarbon in organisms are scarce (Mehendale *et al.* 1975; Wolff *et al.* 1979; Koss *et al.* 1982; Ogino 1984).

Reductive dehalogenation seems to occur by metabolic enzymes in the liver and/or by intestinal microbes. The time courses of HBB and its debrominated metabolites concentrations in liver suggest that HBB is debrominated in this organ (Figure 3). It is known that reductive dehalogenation is catalyzed by metabolic enzymes located in the microsomal fraction of the liver (Mehendale *et al.* 1975). In order to know the participation of intestinal microbes in the debromination of HBB, antibiotics such as bacitracin, neomycin sulfate, and polymixin B sulfate were administered to rats together with HBB. But debromination processes of HBB by the rat, administered antibiotics were not different from the results without antibiotics. Therefore, it is safe to say that the debromination of HBB occurs in the liver rather than by intestinal microbes.

There are some reports that HBB is also metabolized to hydroxylated compounds (Koss *et al.* 1982, Ogino 1984). In the present study, 10% of the dosage was excreted as HBB and its debrominated metabolites and 20% remained in rats 10 days after administration. Therefore, 70% of the dosage seemed to be metabolized to other compounds such as hydroxylated compounds. In previous reports, we showed that 10% of HCB dosage was excreted intact and 75% of dosage remained in rat at 10 days after administration (Yamaguchi *et al.* 1986), but dechlorinated metabolites were not detected. Therefore, HBB is more easily metabolized to dehalogenated products than HCB.

The postulated metabolic pathway of HBB debromination in the rat is HBB → PeBB → 1,2,4,5-TeBB → 1,2,4-TrBB (Figure 5), which is similar to

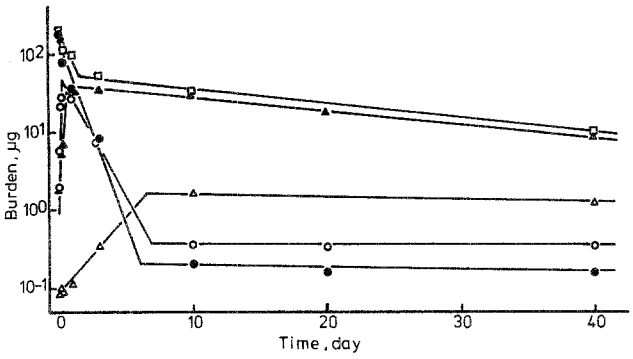

Fig. 4. Time courses of HBB (●), PeBB (○), TeBB (▲), TrBB (△) and total BB (□) burdens in whole rat's body after oral administration of HBB

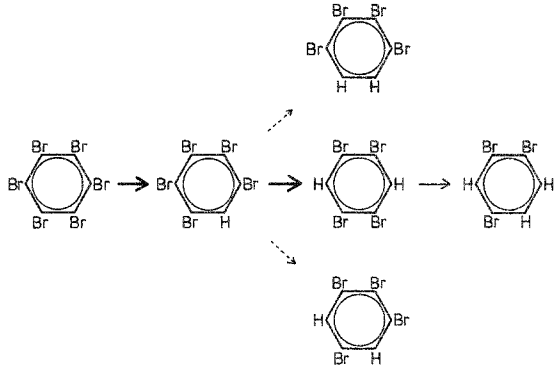

Fig. 5. Proposed metabolic pathway of HBB in rat after oral administration

the pathways suggested for photodegradation and microbial degradation in soil (Kawano *et al.* 1983; Yamaguchi 1984). But compared with degradation by light and in soil, the number of isomers produced in the rat was less. The burden of 1,2,4-TrBB was much less than that of TeBB. Ruzo *et al.* (1976) reported metabolites in the rabbit administered 1,2,4,5-TeBB and 1,2,4-TrBB, individually. In this study, the phenolic metabolite of 1,2,4,5-TeBB was not found, but 1,2,4-TrBB was metabolized to 2,4,5- and 2,4,6-tribromophenol in the rabbit. The burden of TeBBs in this study seems to be larger than that of 1,2,4-TrBB, because 1,2,4-TrBB is more easily metabolized than TeBBs.

The time course of total bromobenzene (HBB + PeBB + TeBB + TrBB) burden was biphasic. In phase II, the half-life of total bromobenzenes was 16 days. The half-life of HCB was 20 days (Yamaguchi *et al.* 1986) and dechlorinated compounds were not found. Additionally, 1,2,4-TrBB which is one of the debrominated metabolites is a stronger inducer of drug metabolizing enzymes than its parent compound, HBB (Carlson 1977). Therefore, we must pay attention to not only the parent compound but also to its metabolites.

HBB was metabolized to debrominated compounds rapidly, and accumulated as 1,2,4,5-TeBB. This is not usually found in the study of organochlorines and is a singularity of organobromine compounds. Ecotoxicological studies of debrominated compounds of HBB are very few. Therefore, studies on residue levels in the environment and their toxicological effects on the ecosystem are necessary.

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