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Metabolism in the toxicokinetics and fate of brominated flame retardants—a review☆

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Abstract

Several classes of brominated flame retardants (BFRs), namely polybrominated biphenyls (PBBs), polybrominated diphenyl ethers (PBDEs), tetrabromobisphenol A (TBBPA), hexabromocyclododecane (HBCCD), bis(2,4,6-tribromophenoxy)ethane (BTBPE), and tris(2,3-dibromopropyl)phosphate (Tris), have been identified as environmental contaminants. PBDEs, TBBPA, and HBCCD are of particular concern due to increasing environmental concentrations and their ubiquitous presence in the tissues of humans and wildlife from Europe, Japan, and North America. Regardless, the toxicokinetics, in particular metabolism, of BFRs has received little attention. The present review summarizes the current state of knowledge of BFR metabolism, which is an important factor in determining the bioaccumulation, fate, toxicokinetics, and potential toxicity of BFRs in exposed organisms. Of the minimal metabolism research done, BFRs have been shown to be susceptible to several metabolic processes including oxidative debromination, reductive debromination, oxidative CYP enzyme-mediated biotransformation, and/or Phase II conjugation (glucuronidation and sulfation).

However, substantially more research on metabolism is necessary to fully assess BFR fate, uptake and elimination kinetics, metabolic pathways, inter-species differences, the influence of congener structure, and the potential health risks to exposed organisms.
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Keywords: Brominated flame retardants; Toxicokinetics; Metabolism; Elimination; Biota

1. Introduction

Brominated flame retardants (BFRs) comprise a diverse group of chemical classes, which are used or have been used in an array of commercial and industrial applications for the purpose of fire prevention. The occurrence of several classes of BFRs in the environment has become increasingly evident and presents a potential health risk to organisms exposed to these emerging classes of environmental contaminants (de Wit, 2002; McDonald, 2002).

In exposed organisms, metabolism is an important factor in determining the bioaccumulation, fate, pharmacokinetics (or toxicokinetics), and toxicity of contaminants. Contaminant exposure can result in the induction of Phase I cytochrome P450 monooxygenase (CYP) enzymes and Phase II conjugation enzymes (e.g., glucuronosyltransferases, sulfotransferases, and glutathione-S-transferases) (Nelson et al., 1996; Lewis et al., 1998). For example, the most common inducers of CYP1A are planar aromatics [e.g., polynuclear aromatic hydrocarbons (PAHs) and co-planar polychlorinated biphenyls (PCBs)], while CYP2B and CYP3A by globular molecules (e.g., *ortho*-chlorine substituted PCBs). Of the 22 CYP families known to exist in mammals, three of the 22 families (i.e., CYP1, CYP2, and CYP3) are of importance for the primary metabolism of anthropogenic compounds. Phase I metabolites (e.g., hydroxylated (OH)) are subsequently metabolized via Phase II conjugation, although competition with protective mechanisms such as protein binding may result in tissue retention (e.g., OH–PCBs). Species differences in the induction, activity, and substrate specificity of CYP1, CYP2, and CYP3 families of enzymes and Phase II enzymes will determine the patterns of contaminants and congeners within a contaminant class in tissues, as well as the fate and potential toxicity. Reductive and oxidative dehalogenation are also potential enzyme-mediated processes that can lead to dehalogenated metabolites. We presently summarize

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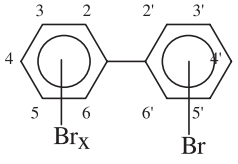
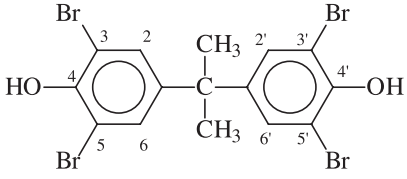
the direct and indirect evidence that demonstrates that BFR metabolism occurs in exposed laboratory organisms and wildlife species.

The present state of knowledge of the biotic fate and metabolism of BFRs is limited to a few classes of environmentally relevant BFRs. These classes are polybrominated diphenyl ethers (PBDEs), polybrominated biphenyls (PBBs), tetrabromobisphenol A (TBBPA), bis(2,4,6-tribromophenoxy)ethane (BTBPE), tris(2,3-dibromopropyl)phosphate (Tris) and hexabromocyclododecane (HBCDD) (Fig. 1). Source and chemical information on these BFR classes, on which published literature on fate and metabolism is available, are briefly described. More detailed information on these BFRs can be found in other articles found in this special issue on BFRs in the environment.

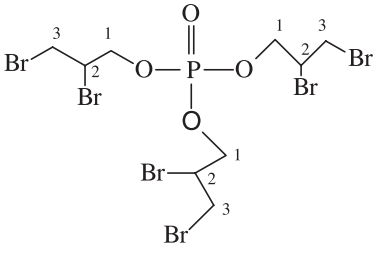
PBBs were produced throughout the 1970s as BFRs for acrylonitrile-butadiene-styrene (ABS) plastics, coatings and lacquers, and polyurethane foam (Neufeld et al., 1977; de Boer et al., 2000). PBBs achieved worldwide notoriety in 1973 when they were accidentally substituted for a feed supplement at a Michigan cattle operation. Subsequently, they were banned from the market due to their biological persistence in exposed organisms. Commercial PBBs existed as mixtures, the most common one being Firemaster BP-6, produced by Michigan Chemical. Firemaster BP-6 was a mixture of 13 congeners of PBBs, where the number of bromine atoms on the biphenyl backbone ranged from 4 to 7. However, the vast majority (85%) of the Firemaster mixture was composed of two congeners, 2,2′,4,4′,5,5′-hexabromobiphenyl (BB-153) and 2,2′,3,4,4′,5,5′-heptabromobiphenyl (BB-180). PBBs from the 1973 Michigan



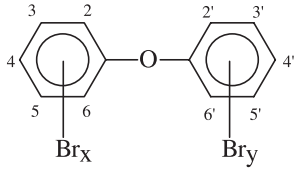
Polybrominated biphenyls
(PBBs; x + y = 6-10)



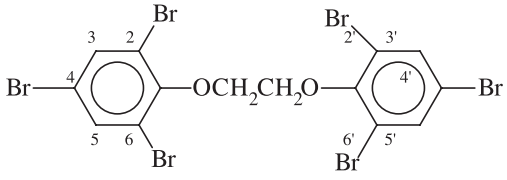
Tetrabromobisphenol-A
(TBBP-A)



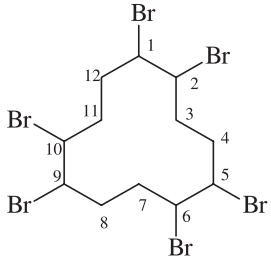
Tris(2,3-dibromopropyl) phosphate
(Tris)



Polybrominated diphenyl ethers
(PBDEs; x + y = 3-10)



Bis(2,4,6-tribromophenoxy)ethane
(BTBPE)



Hexabromocyclododecane
(HBCDD)

Fig. 1. The chemical structure of the six classes of brominated flame retardants (BFRs) discussed in this review, and for which pharmacokinetic data are available. The numbering system for bromine atom substitution is indicated for polybrominated biphenyls (PBBs), tetrabromobisphenol-A (TBBPA), tris(2,3-dibromopropyl) phosphate (Tris), polybrominated diphenyl ethers (PBDEs), bis(2,4,6-tribromophenoxy) ethane (BTBPE), and 1,2,5,6,9,10-hexabromocyclododecane (HBCDD).

incident continue to be detected in the environment in abiotic and animal tissue samples (WHO/ICPS, 1994).

TBBPA is incorporated into polymers both as a reactive (90% of total end use) or an additive flame retardant in ABS and high impact polystyrene (WHO/IPCS, 1995). TBBPA is the most widely used flame retardant worldwide. Environmental exposures to TBBPA most likely occur as a result of manufacturing industry discharges or product leakage containing the additive form. In 1992, annual worldwide production was estimated at 50,000 tons/year (de Wit, 2002). Detection of TBBPA in sediment samples indicates that it can enter the environment and has an appreciable half-live (Sellström and Jansson, 1995; Watanabe et al., 1983).

The brominated organophosphate, tris(2,3-dibromopropyl) phosphate (Tris), was a flame retardant used in children's sleepwear until 1977 when it was discontinued due to studies that showed it was a potent mutagen and carcinogen in rats (Reznik et al., 1979). Tris was replaced by the chlorinated analog tris(1,3-dichloro-2-propyl) phosphate (Fyrol FR-2). Both compounds are more mutagenic, by an order of magnitude, when activated by the S9 fraction from rat liver, indicating that hitherto unidentified metabolites are responsible for the observed mutagenicity.

HBCDD is a cyclic aliphatic BFR, which is produced commercially as a mixture of three stereoisomers. Alpha, beta, and gamma HBCDD are found in the commercial product at 6%, 8%, and 80%, respectively (American Chemistry Council, 2001). HBCDD has been produced for 20 years and worldwide usage was nearly 16,000 metric tons in 1999 (de Wit, 2002). HBCDD is added at very low levels (0.67–2.5%) to polystyrene foam, which is used as thermal insulation in the building industry and at higher levels (6–15%) to the back of upholstery textiles. Studies so far indicate that it does not appear to be acutely or chronically toxic to rats, fish, daphnia, or freshwater or marine algae (American Chemistry Council, 2001).

PBDEs are the most important class of BFR in terms of environmental occurrence and persistence in abiotic compartments and in wildlife and humans (de Boer et al., 1998a, 2000; de Wit, 2002; Pijnenburg et al., 1995). PBDEs are contaminants of increasing environmental significance, where temporal studies over approximately the last 10 years have demonstrated exponentially increasing levels in wildlife and human tissues. This increasing trend has been shown in, e.g., Swedish human milk (Norén and Meironyté, 2000), Swedish guillemot (*Uria aalge*) eggs (Sellström et al., 1993), Canadian beluga whale (*Delphinaterus leucas*) (Ikonomou et al., 2002), and Great Lakes lake trout (*Salvelinus namaycush*) (Luross et al. 2000) and herring gull (*Larus argentatus*) (Norstrom et al., 2002). The growing seriousness of PBDEs in the environment led to a voluntary ban, and a formal ban of the pentaBDE mixtures is expected on July 1, 2003, on their use and production in countries of the European Union (Renner, 2000). Like PBBs, PBDEs have a structural resemblance (Fig. 1) to PCBs and polychlorinated dibenzo-p-dioxins/furans (PCDDs/PCDFs). As with PCBs, 209 PBDE congeners are possible, but a small subset dominates as contaminant residues in the tissues of exposed organisms, that is, 2,4,4′-tribromoDE (BDE-28), 2,2′,4,4′-tetrabromoDE (BDE-47), 2,2′,4,4′,5-pentabromoDE (BDE-99), 2,2′,4,4′,6-pentabromoDE (BDE-100), 2,2′,4,4′,5,5′-hexabromoDE (BDE-153), 2,2′,4,4′,5′,6-hexabromoDE (BDE-154), 2,2′,3,4,4′,5′,6-heptabromoDE (BDE-183), and 2,2′,3,3′,4,4′,5,5′,6,6′-decabromoDE (BDE-209).

BTBPE is a BFR used in the production of plastic materials that require high manufacturing temperatures (Fig. 1). Production figures are difficult to obtain, but USA production in 1977 was between 50 and 500 tons. BTBPE is very hydrophobic, and, like many BFRs, would be expected to be persistent in the environment. However, it is possible that ether cleavage of BTBPE may yield 2,4,6-tribromophenol, or that it may serve as an alkylating agent through displacement of a bromine atom. Environmental levels of BTBPE have not been reported in the scientific literature to our knowledge. Acute toxicity appears to be low, e.g., the oral LD_{50} was over 10 g/kg for rats and dogs, and no obvious effects were seen in rats exposed to BTBPE in the diet for 14 days (Nomeir et al., 1993).

2. Polybrominated biphenyls

Despite a cessation of US production by the late 1970s and European production in 1985 (WHO, 1994), PBBs continue to be detected in tissue samples. It is therefore necessary to know how metabolic processes influence the fate of PBBs. In vitro and/or in vivo PBB metabolism has been shown for laboratory animals, domesticated agriculture species, and humans. These studies have demonstrated that in exposed animals, PBBs are susceptible to enzyme-mediated metabolic processes such as CYP enzyme-mediated Phase I oxidative metabolism, Phase II conjugation, and to a limited extent reductive debromination. PBB metabolism has been studied in captive Atlantic salmon (*Salmo salar*), which is the major salmonid species farmed in the salmon aquaculture industry. However, we are not aware of any other published reports on PBB metabolism in other captive or free-ranging wildlife species.

2.1. In vitro metabolism

Much of the study of PBB metabolism has been conducted with in vitro systems using liver microsomes from rats. Microsomes from uninduced or 3-methylcholanthrene (MC)-induced microsomes were not capable of metabolizing any congener in a Firemaster BP-6 mixture implicated in the well-known Michigan contamination incident (Dannan et al., 1978; Moore et al., 1980). The Firemaster mixture contains 13 PBB congeners ranging from penta- to heptabromobiphenyls. However, when phenobarbital (PB) or PBB-induced microsomes were used, two of the congeners

were rapidly metabolized, and identified as 2,2′,4,5,5′-pentabromobiphenyl (BB-101) and 2,2′,3,4′,5′,6-hexabromobiphenyl (BB-149) (Fig. 1). The common feature of these two congeners is a single, unsubstituted *para* position. Purification of the individual congeners was difficult and did not allow for stereochemical assignments to be made by ^{1}H-NMR. Congeners with two adjacent hydrogens were not subject to appreciable metabolism. The remaining unmetabolized congeners had neither *para* unsubstitution nor adjacent hydrogens. The conclusion of Dannan et al. (1978) was that adjacent protons are not a sufficient precondition to metabolism, but that *para* unsubstitution is a requirement. In order to test this conclusion, structure–function relationships were determined following in vitro metabolism of a series of model PBBs congeners with two or four bromine atoms (Moore et al., 1980). The rate of metabolism of 2,2′-dibromobiphenyl (a PBB congener with no *para* carbon substitution) was >2100 pmol/min/mg protein, while 4,4′-dibromobiphenyl (BB-15; a congener with bromine substitution on both *para* carbons) was not appreciably metabolized (< 0.02 pmol/min/mg protein; Fig. 2). Three tetrabromo congeners were tested in which both *para* carbons were unsubstituted, and at least one pair of adjacent hydrogens existed, and each displayed a modest

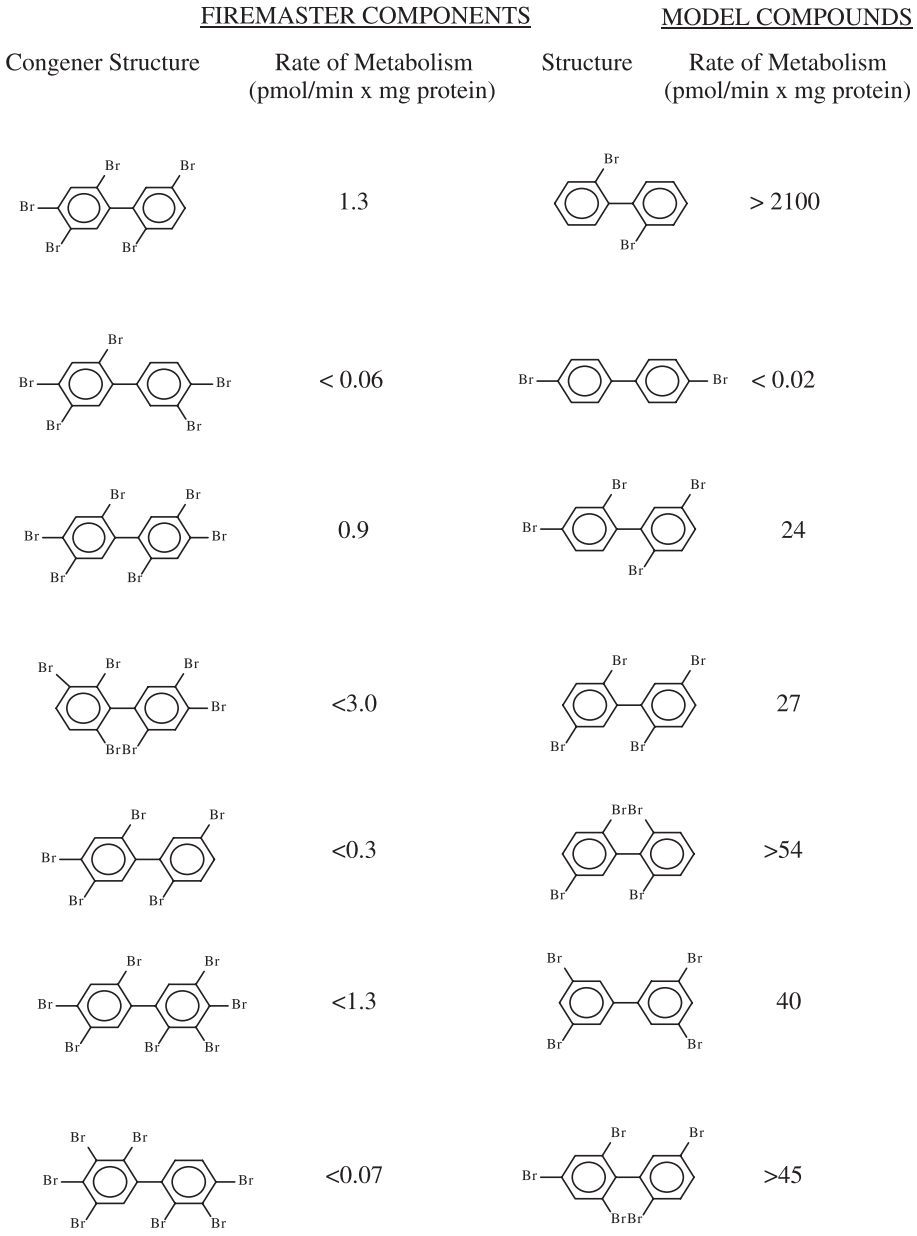


Fig. 2. The in vitro metabolism of PBB congeners in phenobarbital-induced microsomes demonstrating the influence of the substitution pattern of bromine atoms on the biphenyl ring. The compounds in the left column are congeners present in Firemaster BP-6 commercial flame retardant, while compounds in the right column are model congeners synthesized in-house. The data are from Moore et al. (1980).

level of in vitro metabolism (Fig. 2). A fourth PBB congener, 3,3′,5,5′-tetrabromobiphenyl (BB-80), which lacked adjacent protons but possessed two unsubstituted *para* carbons, was also metabolized. These data confirmed the requirement that at least one *para* carbon remain free of bromine substitution for PBB metabolism to occur, demonstrated a reduced importance of adjacent hydrogens, and showed that phenobarbital (PB)-type induction was required for metabolism of the components present in Firemaster BP-6.

Dannan et al. (1978) showed that a Firemaster mixture, 85% of which was comprised of two PBB congeners, BB-153 and BB-180, was not metabolically activated by control microsomes to reactive metabolites, which could bind to microsomal macromolecules (<0.05% of the substrate; incubation conditions: 50 μg PBB, 10 mg microsomal protein, 1 h, 37 °C, final volume 10 ml). Binding to microsomal macromolecules could be doubled by using 3-MC-induced microsomes, and increased 5-fold with PB or PBB-induced microsomes. Most of the binding to macromolecules was identified as protein rather than DNA. Binding to DNA molecules is a necessary precondition to the onset of cancer, as has been demonstrated for benzo[a]pyrene binding to DNA via a diol-epoxide intermediate (Sims and Grover, 1974). Therefore, the most persistent and common PBBs, BB-153 and BB-180 would probably be poor mutagens or carcinogens due to their low level of metabolism even in induced systems.

In vitro metabolism of radiolabeled BB-153 for 60 min was significantly enhanced when incubated with induced liver microsomes from Firemaster BP-6 or BB-153 exposed rats compared with control microsomes (Purdy and Safe, 1980). The result of these in vitro incubations was the formation of three metabolite fractions: a polar, lipophilic fraction; an acidic, conjugated fraction; and a macromolecular adduct fraction. Firemaster BP-6 induced microsomes were more effective in metabolizing BB-153 than BB-153-induced microsomes and resulted in 30–300% more product for each of the three metabolite fractions. No individual metabolites were characterized, but the polar metabolite fraction contained three metabolites as determined by TLC. They were presumed to be monohydroxylated (OH) metabolites of BB-153.

In another study, Mills et al. (1985) showed that liver microsomes from PB and 3-MC induced rats both increased the rate of metabolism of model PBB congeners, ranging from two to six bromine atoms. PB-induced microsomes increased the metabolism rate of brominated biphenyls that possessed adjacent nonhalogenated *meta* and *para* carbons, while 3-MC-induced microsomes increased the metabolism rate of PBBs, which possessed adjacent nonhalogenated *ortho* and *meta* carbons, respectively, on at least one ring. Apparently, highly brominated biphenyls are inherently resistant to metabolism because 2,3′,4,4′,5-pentabromobiphenyl (BB-118), 2,2′,3,4,4′,5′-hexabromobiphenyl (BB-138) and 2,3,3′,4,4′,5-hexabromobiphenyl (BB-156), compounds which possess adjacent nonhalogenated *ortho* and *meta* carbons, were not metabolized by 3-MC-induced microsomes. These data indicated that the metabolism of PBBs is dependent on the substitution pattern of bromines and the particular CYP isoform induced. Furthermore, the CYP1A and CYP2B preference for PBB congeners is the same in rats as for PCBs.

The structure of metabolites has been determined for model PBB congeners, which have not necessarily been found in the environment. The most common biotransformation is monohydroxylation, but dihydroxylation also occurs for several congeners, and debromination has been shown to be a rare occurrence. Monohydroxylation appears to proceed according to CYP enzyme-mediated formation of an arene oxide intermediate. Kohli et al. (1978) showed that 4-bromo-4′-deuterobiphenyl displayed deuterium migration during metabolism. Thus, 1,2-shift of a bromine atom occurs during epoxide hydrolase-mediated ring opening of the epoxide, which is analogous to CYP enzyme-mediated formation of a PCB arene oxide (Letcher et al., 2000). The 3′,4′-dihydroxylation of 4-bromo-4′-deuterobiphenyl was shown to proceed by both an arene oxide and via direct hydroxylation (Kohli et al., 1978). The formation of PBB arene oxides suggests that metabolic activation to these intermediates is necessary for subsequent binding to subcellular macromolecules. Borlakoglu and Wilkins (1993) examined the metabolism of 2-, 3-and 4-bromobiphenyls (i.e., BB-1, BB-2, and BB-3, respectively) by hepatic microsomes isolated from control and Aroclor 1254-treated rats and pigeons. *Meta*- and *para*-OH, as well as di-OH, metabolites were detected, but *para* hydroxylation was the preferred route of metabolism for all three monobrominated PBBs. *Ortho*- and *meta*-bromine substituted PBBs were more slowly metabolized than the *para*-substituted PBB. The overall rates of hydroxylation were greater for rat rather than pigeon microsomes and indicated species differences in PBB metabolism. Aroclor 1254 treatment, a potent inducer of hepatic CYP enzymes, resulted in an increase in the rates of metabolism and enhanced formation of the diol metabolites.

2.2. Whole organism metabolism of model PBBs

2.2.1. Rats

Trace amounts of hexabromobiphenyl metabolites were isolated, but not characterized, from rat urine and feces when administered purified BB-153 (>95%), the major congener of Firemaster BP-6 (Safe et al., 1978). Metabolism of BB-153 was studied in male Sprague–Dawley rats injected intravenously with 1 mg/kg (Matthews et al., 1977). Relatively high levels of BB-153 were recovered in fat (26% of dose), skin (18%), muscle (40%), liver (12%), and blood (1.5%) at 1 day. At 7 and 42 days, the muscle and liver levels had largely repartitioned into the fat, while skin concentrations did not change over time. Greater than 95% of the tissue levels of BB-153 could be extracted, but no

metabolites were detected by thin layer chromatography. Excretion of BB-153 proceeded largely via the feces, but was very slow over the first 7 days and was almost negligible thereafter. Cumulative fecal excretion at 1, 7, and 42 days was 0.96%, 3.3%, and 6.6%, respectively, and <0.1% cumulative urinary excretion was detected at 7 days. Estimates were that only 9.5% of the dose would ever be excreted in the lifetime of the rat. These results confirm what was determined in the metabolism of the model PBBs, in that *para* bromination, i.e., 4, 4′-dibromination, and a lack of adjacent hydrogens inhibits metabolism. Pharmacokinetics was also studied in bile duct cannulated rats and less than 4% of the biliary radioactivity was present as metabolites. Approximately, 93% intestinal absorption was determined by comparing the excretion of a single oral dose (1 mg/kg) with an i.v. dose. In addition, the data demonstrated that the route of administration or the size of dose did not affect tissue retention.

Firemaster BP-6 was administered i.p. in corn oil (10 mg/kg) to a group of male Wistar rats that were killed at 6, 12, 24, and 36 weeks (Miceli and Marks 1981). Serum and tissue levels of total PBBs were quantified with time and half-lives were determined. Fat and adrenals contained the highest concentrations of PBBs at all time points, and fat stores continued to increase with time, reflecting a redistribution of BB-153 away from other tissues. Liver, lung, and pituitary gland initially contained high concentrations, which declined quickly between 6 and 12 weeks. Brain, kidney, and spleen contained concentrations of PBBs that were several orders of magnitude lower than the fat and adrenals. The apparent half-life of BB-153 in tissues ranged from 9.0 weeks in the spleen to over 69 weeks in the fat. These results are in agreement with the observation that BB-153 is highly bioaccumulative, and would likely persist at greater than 1 μg/g in the fat at the end of the 2-year life span in the rat.

Koss et al. (1994) studied the toxicokinetics of BB-153 in female Wistar rats by oral dosing (112 mg/kg) every other day for 6 weeks, and following the rats for an additional 22.5 months. Intestinal absorption was calculated to be 95%. At the end of the dosing period (6 weeks), adipose tissue contained the highest concentration of the dose (5600 μg/g), and was 17- to 30-fold less in the GI tract, central nervous system, kidney, muscle, lung, spleen, and liver. The maximum concentration in the adipose tissue was achieved at 10.5 months, and the concentration in all other tissues declined slowly after receiving the last dose. The half-life of BB-153 in the adipose tissue was 12 months. The identified compound in the feces was parent (95%), with 5% being converted into four metabolites. Three metabolites were unidentified pentabromobiphenyls, while the fourth was a hydroxylated hexabromobiphenyl.

No marked differences were noted for the distribution of PBBs in pregnant or lactating rats. Three groups of female Sprague–Dawley rats were fed Firemaster BP-6 in the diet (50 mg/kg); from gestation day 8 to 21; from day 1 postpartum to day 14 postpartum; and from day 8 postpartum to day 14 postpartum (Rickert et al., 1978). Very low concentrations of PBBs (<5 μg/g) were located in the brain, heart, lung, liver, small intestine, placenta, or uterus. Higher concentrations were observed in the most lipophilic tissues, i.e., kidneys, skin, mammary tissue, and fat. Lactation did not reduce the PBB levels in the tissues except for the mammary tissue. The conclusion was that PBBs were transferred from mother to young primarily through milk transfer and not via the placenta.

A series of monobrominated biphenyls, BB-1, BB-2, and BB-3, resulted in the metabolism to laterally substituted mono- and di-OH (o-catechol) biphenyls excreted in the urine of rats (Kohli et al., 1978). Utilizing tritiated substrates, these data demonstrated that PBB metabolism proceeded via arene oxide and followed the same metabolic pathway, as has been described for PCBs. It was also demonstrated that these reactive arene oxide intermediates could alkylate cellular macromolecules, a precursor to the induction of cancer by aromatic carcinogens. In fact, BB-3 was shown to be highly mutagenic to *S. typhimurium* mutant TA 1538, which is sensitive to frame-shift mutations.

Cumulative excretion over 16 days in three male and female rats receiving an oral 1.0 mg/kg dose of octabromobiphenyl (OBBP) was less than 1% in the urine or respired air (Norris et al., 1975) with no sex differences noted. Sixty-two percent of the dose was excreted in the feces at 24 h, but a very slow rate of elimination of the remaining dose was observed. From day 2 to 16, only 11% cumulative excretion into the feces was observed, the majority (7%) occurring at day 2. Thus, it appeared that OBBP displayed a biphasic disappearance pattern. The first phase displayed a half-life of less than 24 h, while the second phase exceeded 16 days. The single oral dose of octabromobiphenyl in both male and female rats resulted in over 26% OBBP remaining in the body at 16 days. The conclusion was that OBBP, like BB-153, had a significant potential to bioaccummulate in rats. The major tissues in which OBBP persisted on a concentration basis were the adrenals, adipose tissue, heart, and skin (0.14–0.25%/g tissue), while lesser concentrations were observed in the liver, pancreas, and spleen. Rats fed 0.1 mg OBBP/kg/day for 180 days did not differ from controls in terms of mean bromine content either in kidney, muscle, serum, or testes. However, beginning at 90 days and continuing through 180 days, there was a significant increase in bromine content in both liver and adipose tissue. Rats, fed 90 days on an OBBP-spiked diet and followed by 90 days of a clean diet, demonstrated that the adipose tissue concentrations of bromine were no lower than at the time when clean feed was first given. OBBP levels in liver were only slightly diminished over the same period of time.

2.2.2. Humans

In order to determine the half-lives of PBBs as a class in human blood, cohorts were selected from Michigan residents who either lived on quarantined farms from the 1973

PBB incident or received tainted farm products from the quarantined farms (Rosen et al., 1995). Inclusion in the study required an initial serum PBB level of at least 20 ppb. After nearly 20 years of data collection, the half-life for PBB in human serum was estimated at 11 years, with no differences between sexes noted. It was estimated that a person with an initial body burden of 45.5 ppb of PBB would require more than 60 years to reduce those levels to below 1 ppb. In a preliminary study with the cohort, a median half-life estimate of 12 years (range 4.6–94.7 years) was obtained (Lambert et al., 1990), therefore, PBBs appear to be a highly persistent class of BFRs in humans. In another study with women of the Michigan cohort, who had initial PBB levels <10 ppb, a half-life of 12.9 years was calculated, but this rose to 28.7 years in women whose initial PBB levels were >10 ppb (Blanck et al., 2000). Breastfeeding was not associated with a body burden decline, but half-lives rose with an increasing body mass index. The Michigan cohort was also used to determine the distribution of PBBs in the human (Eyster and Kimbrough, 1983). A good correlation of PBB in human serum to adipose tissue was observed in the cohort, ranging from 1:140 to 1:260 and did not change in pregnant or nonpregnant women or male chemical workers. Potential placental or milk transfer to a developing fetus or newborn was demonstrated in that PBBs were found in cord-blood (one-tenth the serum level) and in human milk (107–119 times the serum levels). A minor amount of the total body burden was detected in the feces and bile, which demonstrated that PBBs had a slow rate of metabolism and excretion in humans.

A pharmacokinetic model prediction for humans was developed for BB-153 based on studies from single i.v. and multiple oral doses in rats (Tuey and Matthews 1980). A blood flow-limited physiological compartmental model from rats was scaled to man with the appropriate adjustments and comparisons were made using the best available human data. The model gave remarkably close agreement with the available fat/serum ratios from humans involved in the Michigan cohorts and resulted in a calculated body burden half-life of 6.5 years. According to the model, the half-life of BB-153 was most influenced by fat reserves. Therefore, obese or actively growing individuals would be expected to have the highest body burdens. However, the model also predicted that reduction of fat reserves in exposed individuals would result in mobilization to tissues that may be more sensitive to BB-153 toxicity.

The concentration of individual PBB congeners in sera may vary in different mammalian species. Wolff and Aubrey (1978) showed that sera concentrations of a few PBB congeners in Firemaster BP-6, relative to the major congener BB-153, were different between Michigan Chemical workers, dairy farmers directly affected by the Michigan PBB incident and rats 4 days after receiving a 80 mg/kg oral dose (Table 1). In particular, the rats had a serum profile, which resembled Firemaster BP-6, except for the absence of an unidentified hexabromobiphenyl congener in rat sera, probably BB-149. In humans, the serum levels of the other major Firemaster BP-6 congener, BB-180 and three hexabromobiphenyl congeners, but not BB-153, were also low or absent when compared to Firemaster BP-6. Metabolism or a lack of accumulation may have been responsible.

2.2.3. Dog

Reported studies of PBB metabolism in dog are limited. A single metabolite was found in the feces of 1-year-old beagles after receiving a 1 mg/kg/day oral dose of Firemaster BP-6 for 6 weeks (Gardner et al., 1979). The metabolite was identified as 6-OH-BB-153 (Fig. 1) by comparison with a synthetic standard. A hydroxylation at the 6-position is rather unusual in highly halogenated systems in mammals, and therefore, the authors concluded that it had formed microbially in the gut. The concentration

Table 1
Relative area of PBB homolog gas chromatographic peaks (Peak D = 100)

Peak	Component	Firemaster BP-6	Peak area ratios[a]		
			Serum from Michigan Chemical employees	Serum from dairy farmers	Serum from rats (n = 4) (4 days after 80 mg/kg dose)
A	Pentabromobiphenyl	7	5 ± 3	1 ± 1	< 1
B	Pentabromobiphenyl	12	13 ± 6	3 ± 1	9 ± < 1
C	Hexabromobiphenyl	2	0.2	–	–
D	2,4,5,2′,4′,5′-Hexabromobiphenyl	100	100	100	100
E	Hexabromobiphenyl	24	13 ± 6	13 ± 4	24 ± 1
F	Hexabromobiphenyl	7	5 ± 4	6 ± 4	9 ± 2
G	Heptabromobiphenyl	4	0–3	< 1–5	5 ± 6
H	2,3,4,5,2′,4′,5′-Heptabromobiphenyl	19	2 ± 3	2 ± 2	22 ± 2
I	Heptabromobiphenyl	1	< 1	< 1	< 1

[a] Peak areas ratios, expressed as mean ± standard deviation, were calculated for 24 chemical workers (serum PBB >10 ppb) and 37 farmers (>15 ppb). The means of the two human groups were significantly different (p < 0.001) for peaks A and B (from Wolff and Aubrey, 1978).

of the metabolite in the feces was 10-fold less than the PBBs.

2.2.4. Cattle

In cattle, the pharmacokinetic behavior of a given PBB congener has been shown to vary depending on the extent of metabolism, fat deposition or mobilization, lactation, and quantity of the exposure. The distribution and elimination of the congeners present in Firemaster BP-6 were studied in 60 dairy cows under seven experimental conditions (Willet and Durst, 1978). Daily oral doses ranged from 0.25 to 25,000 mg, and exposure length ranged from 1 to 202 days. The major congeners of Firemaster BP-6 were readily absorbed from the gastrointestinal tract of cattle and could be detected in the blood within 4 h by GC analysis. Fecal excretion in non-lactating animals was the major route of elimination of PBBs and urine contained only minor amounts of conjugated metabolites. At steady state conditions, fecal excretion represented approximately 50% of the administered dose, but declined rapidly to 1–2% following withdrawal of the dose. In lactating animals, BB-153 could be detected in milk 13 h post-exposure, and milk became the major excretory route, three times higher than feces on a mass basis. As with feces, milk excretion declined rapidly upon withdrawal of the dose. A 71% decline in PBB milk excretion in the first 15 days following withdrawal was observed in four lactating cows fed 10 mg/day Firemaster BP-6, although the decline was 5-fold less for BB-153 than BB-180 (Fries and Marrow, 1975). PBBs distributed primarily to highly lipophilic tissues in cattle, except for the nervous system (Willet and Durst, 1978). However, bile, liver, and mammary tissue contained disproportionately high levels of PBBs.

Approximately, 50% of an intraruminal dose of Firemaster BP-6 (5 mg/kg) in lactating cows was eliminated in the feces in 168 h (Willett and Irving, 1976), while urinary elimination of PBBs in cattle was insufficient to quantify by GC analysis. At 95 days, approximately 23% of the dose was excreted in the milk. The concentrations of PBBs in different tissues were variable, but in general, were very similar to each other when expressed on a lipid-adjusted basis.

2.2.5. Pig

A pig administered two model PBB substrates, BB-3 and BB-15, at 100 mg/kg i.p. produced OH-metabolites, which were excreted in the urine, via a 1,2-shift mechanism and oxidative debromination (Kohli and Safe, 1976). The major component produced from BB-3 was 4′-bromo-4-OH-biphenyl (4′-OH-BB3; 3% yield). Minor amounts of 4′-bromo-3-methoxy (MeO) biphenyl, and an uncharacterized bromo-OH-biphenyl were also detected. The major metabolites of BB-15 in the pig were 4, 4′-dibromo-3-OH-biphenyl (3-OH-BB15, 5% yield) and 3, 4′-dibromo-4-OH-biphenyl (4-OH-BB13, 1% yield). In addition, two minor methoxylated metabolites were observed, i.e., 4-bromo-3-MeO-4-OH-biphenyl and a dibromomethoxyhydroxybiphenyl for which stereochemistry could not be determined. When Firemaster BP-6 was administered to a pig (100 mg/kg i.p.), the major metabolite identified was a pentabromo-OH-biphenyl by GC/mass spectrometry (MS) (Kohli and Safe, 1976). Two possible mechanisms for the formation of this metabolite are possible. It may either have formed from direct hydroxylation of a pentabromobiphenyl or the oxidative debromination of the major Firemaster BP-6 component, BB-153.

2.2.6. Rabbit

Rabbits administered BB-15 by intraperitoneal injection (75 mg/kg) formed the same two monohydroxylated species, as was observed in the pig, and an additional debrominated, hydroxylated metabolite (Safe et al., 1976). The metabolites were identified by ^{1}H-NMR and mass spectrometry as 3-OH-BB15 (10% yield); 4-OH-BB13 (1%); and 4-OH-BB3 (2%). Minor metabolites of BB-15 in rabbit were characterized as 4′-bromo-3-MeO-4-OH-biphenyl, and an uncharacterized dibromo-MeO-biphenyl and dibromo-MeO-OH-biphenyl. These results contrasted with the in vitro results from rat microsomes where no metabolism was detected with BB-15 (Dannan et al., 1978).

2.2.7. Fish

PBB uptake and elimination studies in aquatic species have been limited to one species of fish, the Atlantic salmon (*Salmo salar*). The accumulation from water (18–23 μg/l) and feed (100 μg/g) of PBBs present in commercial mixtures, such as OB (Dow Chemical) and Firemaster BP-6 was investigated (Zitko, 1977). OB consists of more highly brominated PBB congeners than BP-6, i.e., one hepta-, two octa-, and one nonabromobiphenyl congener. Atlantic salmon, after a 48-h exposure, rapidly accumulated PBBs from the BP-6 mixture but not the OB mixture. Furthermore, it was shown that the lower brominated congeners in BP-6 were more readily absorbed from water by salmon than the higher brominated congeners, e.g., only a pentabromobiphenyl and BB-153 were detected in the fish, but no BB-180. The PBB absorption was compared to absorption of a commercial polychlorinated biphenyl (PCB) mixture, Aroclor 1254, and found to be less than the chlorinated biphenyls. However, the absorption of PBBs in food contaminated with BP-6 or OB was equal to or greater than Aroclor 1254 from food. The recovery of BP-6 and OB in salmon from contaminated food was 81% and 71%, respectively. The congener pattern in salmon was virtually the same as the BP-6 commercial mixture, but the salmon receiving the OB mixture contained only small amounts of the original PBBs. Metabolic debromination was indicated in the OB-treated salmon since the majority of the detected PBBs were of lower bromination, where gas chromatographic (GC) retention time suggested that the most abundant component was BB-153, the major congener in BP-6. No accumulation differences in salmon were observed between chlorinated and brominated biphenyl congeners ranging from 2 to 4 halogens (Zitko and Hutzinger, 1976),

although bioaccumulation was higher from water (46–58 μg/L) than feed (8 μg/g). Bioaccumulation decreased with increasing halogenation from water, but increased in feed.

3. Tetrabromobisphenol-A

TBBPA (Fig. 1) is the most abundant BFR currently in use, and previous pharmacokinetic studies have demonstrated that it is readily absorbed and eliminated primarily from organisms as unchanged parent. Therefore, it is imperative to know the ultimate environmental fate of the parent molecule. Several studies with rats, aquatic organisms, and/or microorganisms have indicated that metabolic Phase II conjugation and debromination of TBBPA does occur in biota.

3.1. Rat

In an early study, Brady (1979) administered a single oral dose (6.5–7.5 mg/kg) of ^{14}C-labeled TBBPA to Sprague–Dawley rats and concluded that it was poorly absorbed from the gastrointestinal tract. Greater than 95% of the dose was found in the feces as parent TBBPA, while 1.1% was eliminated in the urine at 72 h (Table 2), although metabolites were not determined. However, because the dose was administered orally, it was unclear whether the compound was poorly absorbed from the intestines or TBBPA was excreted unchanged in the bile.

Table 2
Recovery of [^{14}C]TBBPA in dosed rats from available metabolism studies

Excreta and tissues	Percent of dose			
	Hakk et al. (2000)		Szymanska et al. (2001)	
	Conventional	Bile duct cannulated	250 mg/kg	1000 mg/kg
0–24 h urine	0.1	0.4	0.11	0.13
24–48 h urine	0.2	0.3	0.18	0.22
48–72 h urine	0.02	0.03	0.27	0.31
0–24 h bile	–	48.4	–	–
24–48 h bile	–	21.0	–	–
48–72 h bile	–	1.9	–	–
0–24 h feces	6.6	6.0	37.0	25.3
24–48 h feces	65.6	15.3	24.5	17.5
48–72 h feces	19.5	5.0	4.0	8.1
Adipose (epididymal)	<0.0005[a]	<0.0005[a]	2.78	6.06
Blood	<0.0005[a]	<0.0005[a]	3.83	n.d.
Carcass	0.2	0.3	–	–
Intestines				
Large	1.0	0.4	–	–
Small	0.7	0.2	–	–
Heart	<0.0005[a]	<0.0005[a]	–	–
Kidney	0.003	0.001	–	–
Liver	0.06	0.05	0.06	0.09
Lungs	0.2	0.07	–	–
Muscle	–	–	3.81	14.26
Spleen	<0.0005[a]	<0.0005[a]	–	–
Testes	<0.0005[a]	<0.0005[a]	–	–
Thymus	<0.0005[a]	<0.0005[a]	–	–
Other organs	–	–	0.04	0.04

[a] Limit of quantification was 0.0005% of dose.

An intraperitoneal (i.p.) dose of TBBPA was administered to female Wistar rats at a pair of high doses (250 and 1000 mg/kg) (Szymanska et al., 2001). Urine and fecal excretion of TBBPA derived ^{14}C was followed for 72 h. Cumulative urine excretion was very low (<0.3%), and fecal excretion was 51–65% of the administered dose (Table 2). Although the i.p. study design was able to eliminate the lack of intestinal absorption as a reason for the high level of parent in feces (>90% of fecal ^{14}C), it remained uncertain whether hepatic metabolism/biliary elimination or intestinal microflora were responsible for the single tribromobisphenol-A metabolite observed in feces (10% of fecal ^{14}C).

In a recent study using both conventional and bile duct-cannulated male Sprague–Dawley rats, it was concluded that ^{14}C-labeled TBBPA was readily absorbed from the GI tract (Hakk et al., 2000). Nearly 92% of a single oral dose of TBBPA (2.0 mg/kg) was excreted in the feces of conventional rats, which was similar to the levels previously reported (Brady, 1979). The majority of the fecal radioactivity (>90%) was TBBPA parent. However, administration of the same dose to bile–duct cannulated rats demonstrated that 71.3% of the dose was excreted in the bile at 72 h (Table 2). The biliary radioactivity was composed entirely of conjugated metabolites. Urine was again a minor route for the excretion of TBBPA in conventional or bile duct-cannulated rats. The cumulative urine excretion was considerably less than 1% of the dose, i.e., 0.32% and 0.73%, respectively. Therefore, the results demonstrated that TBBPA could be readily absorbed from the GI tract of rats, metabolized in the liver, and excreted via the bile back to the gut. The high level of parent observed in the feces was the result of deconjugation of TBBPA metabolites by the intestinal microflora, which would regenerate the parent TBBPA. In the absence of abiotic degradation mechanisms, TBBPA would be expected to be environmentally persistent despite the demonstrated ability of the compound to be absorbed from the intestines.

The half-life of TBBPA in all rat tissues was estimated to be less than 3 days (Brady, 1979). The tissues with the longest half-life were fat and testes (70.8 and 60.5 h, respectively), and the shortest half-life was observed in the liver and kidney (10.8 and 17.1 h, respectively). TBBPA was distributed to most tissues in rat, but cumulative residues were only 2.1% of the administered dose (2.0 mg/kg), and no tissue contained more than 1% of the dose (Hakk et al., 2000). Tissues with TBBPA at the limit of quantification (0.0005% of the dose) were fat, blood, heart, spleen, testes, and thymus (Table 2). Except for the large and small intestines, which were not flushed of fecal contents, the lung and remaining carcass contained the bulk of the tissue radioactivity at 72 h (0.2% of the dose each). A

very different result was obtained in a study where considerably larger doses were used, as well as different routes of administration. Intraperitoneal doses of 250 and 1000 mg/kg of TBBPA were administered to male Sprague–Dawley rats (Szymanska et al., 2001). Each sampled tissue peaked in TBBPA concentration at 1 h. A biphasic decline in tissue concentrations was observed, relatively rapid between 1 and 4 h, but was followed by a very slow period of decline through 72 h. In both doses, blood, fat, and muscle contained the bulk of the tissue radioactivity at 72 h. The blood contained nearly 4% of the 250 mg/kg dose at 72 h, while the fat and muscles contained 2.8% and 3.8%, respectively. No statistically significant dose dependency for tissue disposition was observed at any time before 72 h.

Almost 18% of TBBPA and/or its metabolites excreted in the urine were associated with carrier proteins following a 2.0 mg/kg oral dose (Hakk et al., 2000). Characterization of the carrier protein(s) was not possible due to insufficient mass. However, other polyhalogenated aromatic hydrocarbons have been shown to bind to mammalian urinary albumin and α_{2u}-globulin, which may serve as a route to facilitate excretion of these compounds. In 0–24 h bile samples, essentially no measurable level of protein binding of TBBPA could be demonstrated. However, in 24–48 h bile samples, 2.0% of the TBBPA derived ^{14}C associated with protein, which increased to 6.6% at 48–72 h. The protein was characterized as a 79-kDa protein by SDS-PAGE. The majority of the TBBPA in bile did not associate with carrier proteins.

Following the oral dose in the male rat, the overwhelming majority (>90%) of the extractable radioactivity in feces was found to be TBBPA (Hakk et al., 2000). No metabolites of TBBPA could be detected or characterized in any of the fecal extracts. Approximately, 28–54% of the TBBPA radiolabel was nonextractable from rat feces, which is thought to be a covalent attachment to proteins and/or lipids through phenolic groups. TBBPA would be well suited to such a mechanism, since it possesses two phenolic groups. Three major metabolites of TBBPA have been identified as polar conjugates in rat bile. The glucuronide ether, the diglucuronide ether, and the glucuronide ether–sulfate ester diconjugate of TBBPA were identified by negative ion fast atom bombardment MS (Hakk et al., 2000). Parent TBBPA was the only compound detected in feces of conventional rats. These conjugated metabolites were apparently deconjugated back to parent by intestinal microorganisms, and subsequently eliminated in the feces as the unchanged parent (Fig. 3). An additional metabolite was observed when a much higher i.p. dose of TBBPA (250 and 1000 mg/kg) was administered to adult female Wistar rats (Szymanska et al., 2001). Again, the majority of the fecal radioactivity was identified as unchanged TBBPA (>90%), however, approximately 10% of the fecal ^{14}C was the debrominated tribromobisphenolA. The formation of tribromobisphenolA (TriBBPA) was concluded to have occurred in the rat liver and excreted into the feces via the bile, but the alternate possibility that TriBBPA was formed by microbial action in the gut could not be rigorously excluded. Urine metabolites have not been characterized in any of the studies due to insufficient mass.

One human study examined the half-life of TBBPA in occupationally exposed Swedish workers (Hagmar et al., 2000). TBBPA was found to have an estimated half-life in blood serum of 2.2 days, and was thus rapidly depleted.

3.2. Aquatic organisms

The absorption of TBBPA has also been investigated in a limited number of aquatic species, but almost exclusively fish. The bluegill sunfish (*Lepomis macrochirus*) rapidly absorbed TBBPA, present at a concentration of 0.0098 mg/L (Nye, 1978), and equilibrium was achieved in less than 3 days. Upon removal from dosed water, TBBPA was rapidly eliminated from all tissues of the bluegill sunfish. The whole body half-life of TBBPA in bluegill sunfish was calculated to be less than 24 h. TBBPA was not found in mussels in Osaka Bay, Japan, but the di-MeO derivative of TBBPA was detected at concentrations estimated to be 5 μg/kg wet weight (Watanabe et al., 1983). It was suggested to have formed bacterially, which has also been observed for pentachlorophenol (Rott et al., 1979). If methylation of TBBPA turns out to be a common degradation pathway, then a serious environmental concern may be the result, since di-MeO-TBBPA would be expected to bioaccumulate more than the parent due the increased hydrophobicity as a consequence of the OH groups being converted to MeO groups. The di-MeO-TBBPA metabolite has also been associated with TBBPA in sediments taken both upstream and downstream from a plastics manufacturer in Sweden (Sellström and Jansson, 1995). The levels of both compounds were similar upstream (24–34 ppb), but increased dramatically downstream. The di-MeO-TBBPA metabolite was present at 1500 ppb, while the TBBPA was present at 270 ppb. No conclusions were made as to whether the metabolite formed bacterially or by metabolism in an aquatic species.

3.3. Microorganisms

At neutral pH, TBBPA is virtually insoluble in water, therefore, soil mobility would be expected to be minimal. However, at higher pH, the solubility of TBBPA increases significantly. High pH soils can be found in arid areas, and soil mobility and groundwater contamination may be a consideration in these parts of the world. Mineralization of polyhalogenated aromatic hydrocarbons requires reductive halogenation as a key first step. Subsequent aerobic degradation of the remaining aromatic rings proceeds readily. The biodegradation of TBBPA was measured on various soil matrices under aerobic conditions (WHO/ICPS, 1995). The soils were a Massachusetts sandy loam (sand 83%, silt 13%, and clay 4%), a California sandy loam (16%, 58%, and

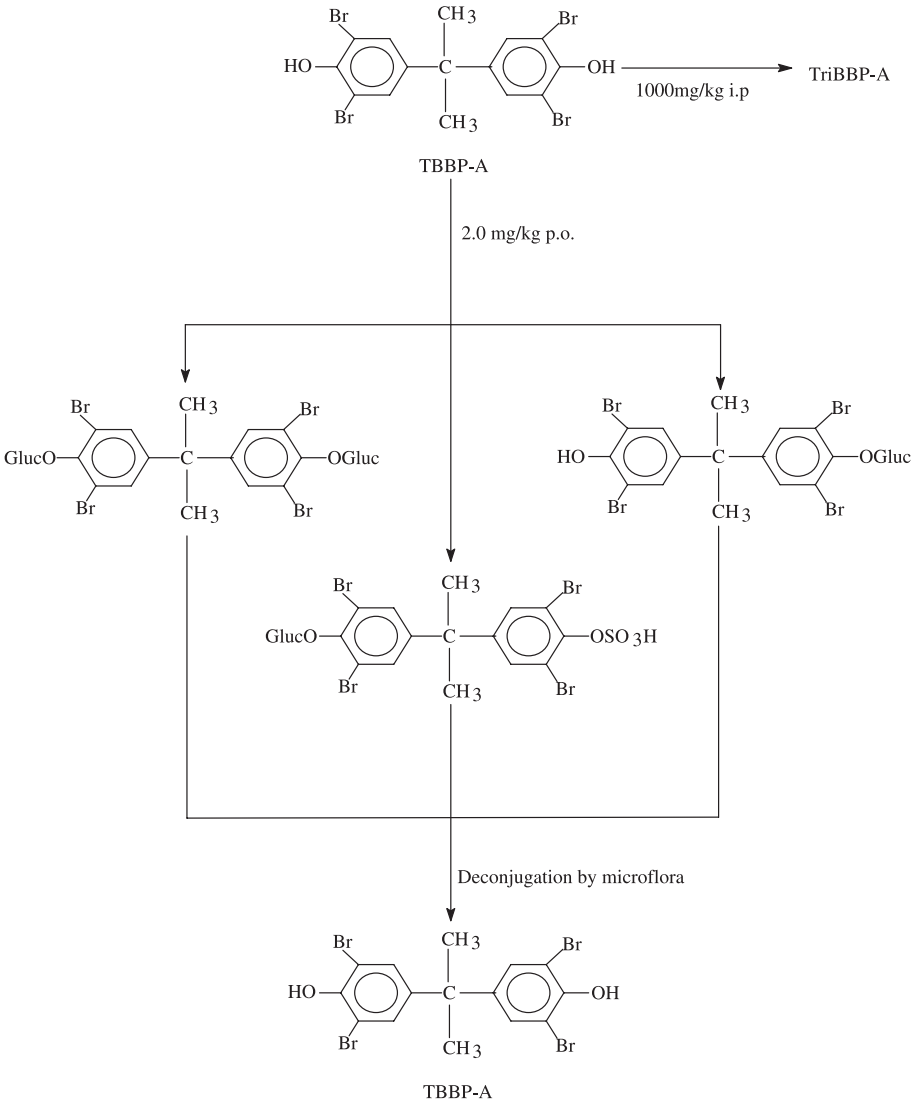


Fig. 3. Proposed pathway for the metabolism of TBBPA in the rat based on mass spectral evidence of biliary and fecal metabolites. The data are from Hakk et al. (2000) and Szymanska et al. (2001).

26%, respectively), and an Arkansas silty loam (43%, 24%, and 33%, respectively). Variable degradation of TBBPA in the various soil types was observed by thin layer chromatography (TLC) at 64 days. Mineralization to CO_2 was minimal because only 6% of the total dose was found in volatile traps. Between 36% and 82% of the TBBPA remained in the soils, the most in the California sandy loam, the least in the Arkansas silty loam. Anaerobic biodegradation for 64 days with the same soils yielded less than 0.5% volatilization (Fackler, 1989a). Variable biodegradation was observed in all soils. In the Massachusetts sandy loam, 43–57% of the TBBPA remained, in the Arkansas sandy loam, between 53% and 65% remained, while in the California loam, nearly 90% of the applied TBBPA remained. Oxidation of TBBPA to water-soluble metabolites under these anaerobic conditions was only 0.5–2.5%.

Aerobic biodegradation was performed in a water/sediment microbial test system, which used native river sediments and water, and TBBPA concentrations of 10, 100, and 1000 μg/l (Fackler, 1989b). A concentration and microbial dependence for biodegradation was observed. At 10 μg/l, the half-life was 48 days, and at 1000 μg/l, increased to 84 days. In sterile sediment, the half-life was estimated to be 1300 days. Very little biodegradation of TBBPA to CO_2 was observed under these conditions (<8%) and only 5% remained in the water, the remainder partitioned into the sediment. In another sediment study, an 80% reduction of TBBPA was observed over 45 days of anaerobic degradation of a highly contaminated Israeli soil (Ronen and Abeliovitch, 2000). TBBPA was found to be converted into a single metabolite, which was identified as bisphenol-A by GC/MS, by reductive debromination under anaerobic conditions. This may have significant environmental ramifications in that bisphenol-A is a known endocrine disruptor (Tollefsen, 2002; Yamasaki et al., 2002) although TBBPA apparently is not (Berg et al., 2001; Garber et al., 2001).

Upon switching to aerobic conditions, further degradation was observed. A gram-negative bacteria (genus *Sphingomonas*, strain WH1) was identified, which could utilize bisphenol-A as the sole source of carbon and energy. During the aerobic phase of degradation, the bacteria yielded two metabolites of bisphenol-A. These were identified as 4-hydroxybenzoic acid and 4-hydroxyacetophenone. The authors concluded that sequential application of anaerobic and aerobic microbial degradation may be successful in breaking down environmental TBBPA. Voordeckers et al. (2002) examined biodebromination of TBBPA in anoxic esturine sediments. Complete debromination of TBBPA to bisphenol-A was observed under both methanogenic and sulfate-reducing conditions.

4. Brominated organophosphates

Published information on the metabolism of brominated organophosphates exists only for tris(2,3-dibromopropyl) phosphate (Tris) (Fig. 1). Tris has been shown to be a potent mutagen and carcinogen in rats, which increased by an order of magnitude when activated by the S9 fraction from rat liver, indicating that hitherto unidentified metabolites are responsible for the observed mutagenicity (Reznik et al., 1979). Mutagenicity is thought to be mediated by covalent binding of the mutagen metabolite to DNA (Devoret, 1985). In a study by Lynn et al. (1980), ^{14}C-labeled Tris was administered to male Sprague–Dawley rats via intravenous administration (25 mg/rat). The cumulative urine excretion after 120 h was 57.2%, and the major metabolite (7.8% of the urinary ^{14}C) was identified as the de-esterified Tris, bis(2,3-dibromopropyl) phosphate. The mechanism of bis(2,3-dibromopropyl) phosphate formation was unknown, but phosphate ester bond hydrolysis in mammals is known to proceed by three enzymatic mechanisms: mixed function oxidase reactions, hydrolase reactions, and glutathione-S-alkyl transferase reactions (Dauterman, 1971).

[^{14}C] Tris was administered intravenously to male rats in another study (5.8 mg/kg) and was metabolized rapidly (Lynn et al., 1982). At 120 h, 58% of Tris was excreted in urine, 9% in feces, and 19% in respired air as CO_2. No parent was detected in the urine or feces, and 9% of the dose remained in the body at 120 h. Bis(2,3-dibromopropyl) phosphate and 2,3-dibromopropanol were the major metabolites characterized in the urine. Tris was also metabolized to 2,3-dibromopropanol in studies with rat (St. John et al., 1976) and human (Blum et al., 1978). However, in these rat and human studies, Tris was initially present in all sampled tissues, but by 8 h was not detected in any tissue (Lynn et al., 1982). Concomitant with the tissue disappearance of Tris was the appearance of the metabolite bis(2,3-dibromopropyl) phosphate. The bis(2,3-dibromopropyl) phosphate metabolite also had a half-life several folds greater than Tris, and in lung, muscle, fat, and blood the metabolite comprised >90% of the ^{14}C radiolabel activity. The highest concentration of radiolabel was detected in the kidney, and was 11-fold higher than the average body concentration. Liver, lung, and spleen contained intermediate concentrations of Tris at 120 h, while fat, brain, and muscle were exceedingly low (Fig. 4). Bile duct-cannulated rats excreted 33.92% of the dose in the bile, the majority in the first hour (20.44%). Bis(2,3-dibromopropyl) phosphate was the major metabolite and accounted for 21.5% of the biliary radioactivity. Therefore, enterohepatic circulation is an important factor in the pharmacokinetic behavior of Tris in rats.

Nomeir and Matthews (1983) compared the metabolism and tissue distribution of Tris in oral vs. intravenously dosed rats. Tris (1.38 mg/kg) was readily absorbed from the gastrointestinal tract and distributed throughout the entire organism. Tissue distribution of Tris was the same regardless of the route of administration, except for a slightly higher percent of the dose in liver for orally dosed rats (3.4% orally dosed vs. 1.9% i.v. dosed), and slightly more in lung after an i.v. dose (0.2% vs. 0.8%, respectively). In bile duct-cannulated rats receiving an i.v. dose, 25% of the dose was eliminated in bile at 3 h, which exceeds fecal elimination over the same period of time. Therefore, it was concluded that enterohepatic circulation played an important role in mediating the elimination kinetics of Tris. The half-life of Tris was determined to be 2.5 days, but in individual tissues, such as liver and kidney, it was slightly longer (3.8 days). The excretion of Tris in the urine, feces, and respired air proceeded, as previously described (Lynn et al., 1980,

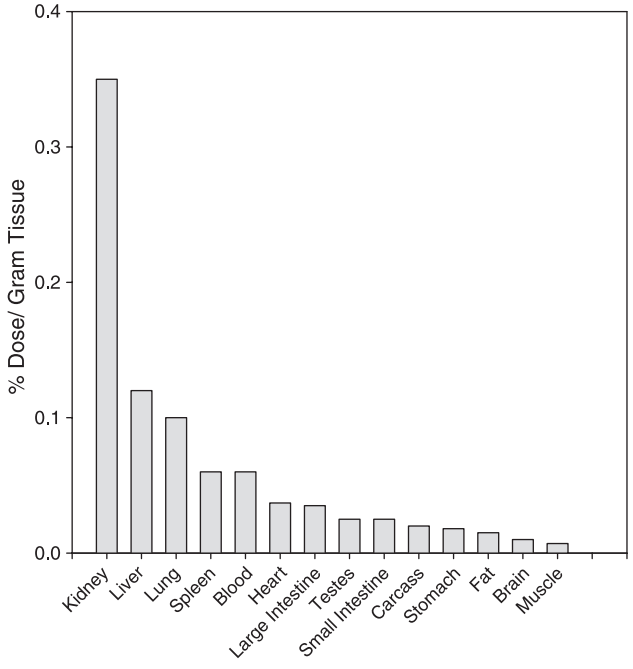


Fig. 4. Tissue distribution pattern of Tris in the rat 5 days after receiving an intravenous dose (5.8 mg/kg body weight dose). Tris was detected in all tissues, however, kidney contained the highest concentration at 5 days. The data are from Lynn et al. (1982).

1982). In these studies, Tris was readily metabolized, and the amount of Tris metabolites in tissues at 2 h exceeded the amount of parent. Six metabolites, which represented 33.3% of the urinary radioactivity, were identified, while the remainder of the urinary metabolites could not be identified. The same metabolites were detected in bile but at different levels, which indicated that Tris undergoes dealkylation, dehydrobromination, and glutathione conjugation (Fig. 5).

In the metabolism of Tris in other species, dermal absorption was measured for [^{14}C] Tris in rabbits after an exposure period of 96 h, and urine and feces elimination was compared to an i.v. dose (Ulsamer et al., 1978). Almost 17% was absorbed when the cloth containing the dose was wetted with urine. Only 6% of the dose was absorbed when the cloth was wetted with simulated sweat, which was slightly more than the absorption from a dry cloth (3.6%). The majority of the percutaneously absorbed Tris was recovered in the urine after 96 h. Dermal absorption of Tris resulted in the highest tissue levels in the kidney, which is the target tissue for toxicity (Mason Research Laboratories, 1997). In the Ulsamer et al. (1978) study, liver contained less than half the concentration of kidney, and there was little observed deposition of Tris into fatty tissues. Intravenous administration of Tris into rabbits resulted in excretion of >75% of the dose into the urine by 72 h, while 8.4% was eliminated in the feces, and mineralization to CO_2 accounted for nearly 11%. The majority of the Tris (>85%) was excreted in the first 24 h after i.v. administration. The half-life of Tris in rabbits was estimated to be less than 6 h.

Tris and its chlorinated analog tris(1,3-dichloro-2-propyl) phosphate (Fyrol FR-2) are bacterial mutagens and this is thought to be due to covalent binding of these compounds to subcellular macromolecules (Nakamura et al., 1979). The S9 fractions isolated from rat livers lead to greater mutagenic activity, implicating Tris and Fyrol FR-2 metabolites as the actual mutagens through putative binding to subcellular macromolecules. Morales and Matthews (1980) compared the covalent binding of both flame retardants to mouse macromolecules following an intravenous dose (65.3 mg/kg). At 6 h, the highest concentration of Tris was in the kidney, while Fyrol FR-2 was highest in the liver. The highest level of covalent binding to subcellular macromolecules of liver, kidney, and muscle was to the low molecular weight RNA (lmw RNA), followed by protein, ribosomal RNA (rRNA), and DNA. Significantly, Tris binding to kidney DNA was far greater than Fyrol-DNA binding (11.5 pmol/mg vs. <1 pmol/mg). Kidney is the target organ for Tris and Fyrol tumorigenesis, but Tris is the more potent mutagen.

5. Polybrominated diphenyl ethers

Knowledge of PBDE uptake, enzyme induction, elimination, and metabolism is restricted largely to experimental rodents (rats and mice) in vitro and in vivo. However, recent studies indicate that PBDE metabolism via oxygen insertion and reductive debromination pathways may be more common, in consideration of evidence in other mammals and

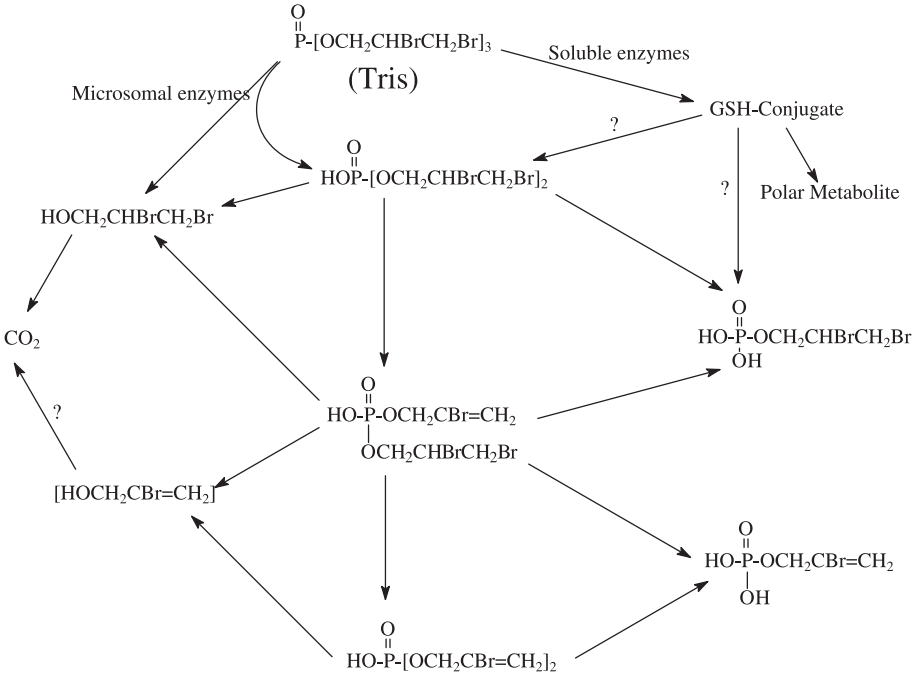


Fig. 5. Proposed scheme for the metabolism of the flame retardant tris(2,3-dibromopropyl) phosphate (Tris) in the rat from in vitro and in vivo metabolism studies. Tris metabolism apparently proceeds via dealkylation, glutathione conjugation, and dehydrobromination (Nomeir and Matthews, 1983).

aquatic wildlife, such as fish and mollusks. Darnerud et al. (2001) recently summarized PBDE toxicokinetics (i.e., absorption, elimination, and enzyme induction and metabolism) in microsomal and whole organism dosing studies with experimental animals. de Wit (2002) has also summarized PBDE toxicokinetics in mammals, fish, and shellfish. In this review, we expand on our understanding of PBDE metabolism in wildlife from several perspectives.

5.1. Microsomal enzyme induction

Given that PBDEs are a major BFR class of emerging environmental importance, this review also examines what is known about their microsomal enzyme-inducing potential. Darnerud et al. (2001) and de Wit (2002) recently summarized known studies on hepatic microsomal enzyme induction by PBDEs and associated toxicologies. Therefore, only the highlights of these reviews are mentioned, but more recent reports are included.

Technical PBDE mixtures and selected individual congeners are capable of inducing both Phase I and II enzymes, which mediate xenobiotic metabolism, in some species. Individual PBDE congeners and PBDE technical mixtures have been shown to induce CYP1A1 and CYP1A2 enzymes. As indicated by increased ethoxyresorufin-*O*-deethylase (EROD) activity, Bromkal 70 induced liver microsomal CYP1A activity in Wistar rats at a threshold level between 3 and 10 mg/kg (von Meyerinck et al., 1990), and EROD was similarly induced in exposed H4IIE cells (Hanberg et al., 1991). Relative to a number of other congeners, EROD was also weakly induced by 3,3′,4,4′-tetrabromoDE (BDE-77), 2,3′,4,4′,6-pentabromoDE (BDE-119) and 3,3′,4,4′,5-pentabromoDE (BDE-126) at concentrations of approximately 25–50 nM in primary chicken and rat liver hepatocytes, human (HepG2), rat (H4IIE) and rainbow trout (*Oncorhynchus mykiss*, RTL-W1) liver cell lines, and the human intestinal Caco-2 cell line (Chen et al., 2001). At approximately 30 mg/kg/day (× 4 days) dose regime, 2,3′,4′,6-tetrabromoDE (BDE-71) and 3,3′,4,5′-tetrabromoDE (BDE-79) were shown to induce hepatic EROD activity 10- to 20-fold, and pentoxyresorufin-*O*-dependentylase (PROD, CYP2B1) activity 30- to 40-fold, and Phase II uridinediphosphate-glucuronosyltransferase (UDPGT) activity 3- to 4-fold in weanling Long Evans rats (female) (Zhou et al., 2001). Sustained hepatic UDPGT induction was also shown in rats by oral administrations of 0.1 mmol/kg/day (for 14 days) with low brominated (24% tetra, 50% penta) and high brominated (45% hepta, 30% octa) PBDE mixtures (Carlson, 1980).

Injection exposure of rainbow trout larvae and dietary exposure of three-spined stickleback (*Gasterosteus aculeatus*) with Bromkal 70-5DE resulted in weakly induced hepatic EROD activity (Norrgren et al., 1993; Holm et al., 1994). In a rainbow trout feeding study, fish fed food containing BDE-47 (i.e., total dose of 21 mg/kg bw) after 22 days had significantly lower hepatic EROD activity, as well as glutathione reductase activity, compared to fish fed BDE-99 (Tjärnlund et al., 1998). Juvenile Atlantic salmon feed 10 and 50 mg/kg body weight of the Penta-BDE and Octa-BDE commercial mixtures, and sacrificed after 7 days, showed no significant induction of hepatic CYP1A activity (EROD) or protein levels (ELISA) in comparison to positive (β-naphthaflavone) and negative controls (Boon et al., 2002). However, in brain, filet, and especially liver tissue of the dosed fish, chemical analysis confirmed the uptake and accumulation of PBDEs congeners. In the Penta-BDE dosed fish, BDE-47 and BDE-99 were the most concentrated residues followed by BDE-100, BDE-153, and BDE-154. In the Octa-BDE-dosed fish BDE-183 was the most significant residue in comparison to BDE-153 and BDE-154.

5.2. In vitro metabolism

de Boer et al. (1998b, 2000) recently summarized in vitro PBDE and PBB metabolism studies using liver microsomes from single individuals of two species of marine mammals from the North Sea (i.e., harbour seal (*Phoca vitulina*) and sperm whale (*Physeter catodon*)). Microsomal depletion of the parent PBDEs and PBBs would be indicative of CYP enzyme-mediated metabolism, however no detectable depletion was observed for the environmentally relevant congeners in the Bromkal 70 mixture (i.e., BDE-47 and BDE-99) (1.7 μg/ml treatment concentration) or the fully brominated BDE-209 (31 μg/ml). In comparison, using the same microsomes, approximately 80% of BB-15 (25 μg/ml) was metabolized under the replicate assay conditions, but no detectable metabolism (1–4 μg/ml treatment concentrations) occurred for 2,2′,4,5′-tetrabromobiphenyl (BB-49), 2,2′-5,5′-tetrabromobiphenyl (BB-52), BB-101, BB-153, 3,3′,4,4′,5,5′-hexabromobiphenyl (BB-169), or 2,2′,3,3′,4,4′,5,5′,6,6′-decabromobiphenyl (BB-209). It was concluded from this study that the rate of microsomal PBDE and PBB metabolism was too slow to measure an observable depletion of the parent substrate. It was also concluded that the lack of microsomal depletion was consistent with the persistent and accumulative nature of these congeners in the environment. However, this evidence is not entirely conclusive of a lack of metabolism in vitro or in vivo. Firstly, the timeframe (90 min) of microsomal metabolism assay was short when compared to conditions in environmentally exposed organisms. Second, PBDE and PBB concentrations under enzyme-saturated conditions in the microsomal assays were too high to measure a significant depletion. An alternate approach would be to determine the formation of OH-PBDE and OH-PBB metabolites in vitro.

Thyroid hormone-like OH-PCB, TBBPA and OH-PBDE congeners have been shown to bind competitively with human transthyretin (TTR), a transport protein for thyroid hormones (e.g., thyroxine (T4) and thyronine (T3)), in the presence of ^{125}I-labeled T4 (Brouwer et al., 1998; Meerts et

al., 2000). Ghosh et al. (2000) demonstrated using X-ray diffraction that bromophenols bind through the OH-group in the central channel of the TTR molecule.

Meerts et al. (2000) reported on the metabolism of PBDEs in vitro in liver microsomes from enzyme-induced rats (Wistar WU). Seventeen PBDE congeners (BDE-15, -28, -30, -32, -47, -51, -71, -75, -77, -85, -99, -100, -119, -138, -153,-166, and -190) were individually incubated with liver microsomes from control and PB-(CYP2B), β-napthaflavone-(CYP1A), and clofibrate-(CYP4A3) induced rats. Metabolite-containing extracts from the incubation mixtures were then screened in a competitive T4-TTR (human) binding assay. No competition of T4-TTR was observed for the control assays, but considerable competition was observed in PB microsomes for BDE-15, -28, -30, -47, -51, -71, -75, -77, -85, -99, -100, and -119. PBDE extracts from β-napthaflavone and clofibrate induced microsomes were much less active than for PB induced microsomes. Extracts for highly brominated BDE-138, -153, -166, and -190 after microsomal incubation did not show any competitive binding in the T4-TTR assay indicating slow or negligible metabolism in vitro in rat. These results indicated that CYP2B-mediated, and to a lesser extent CYP1A- and CYP4A3-mediated PBDE metabolism occur in rats, although the apparent OH-PBDE metabolites were not identified in the extracts.

5.3. Whole organism metabolism of model PBDEs

5.3.1. Rat and mouse

Facile intestinal absorption of PBDEs has been observed in rats. However, elimination may depend on the molecular weight or degree of bromination. A kinetic study designed to investigate the half-lives of various PBDE components in a Bromkal 70 (a commercial penta mixture) using male and female Wistar rats demonstrated that tetra-, penta-, and hexabromo-DE congeners were slowly eliminated following a single oral dose (300 mg/kg) (von Meyerinck et al., 1990). The majority of the bioaccumulation after 4 days was in the adipose tissue. Five congeners were investigated, but were not rigorously identified, due to a lack of available standards. They included a tetraBDE (probably BDE-47, based on rigorous GC/MS analysis of Bromkal-70 performed by Sjödin et al. (1998), two pentaBDEs (probably BDE-99 and 100), as well as two hexaBDEs (probably BDE-153 and 154). Results from the study indicated that very little, if any, PBDE congeners remained in extra-adipose tissue after 4 days. The half-life in the adipose tissue was subsequently assumed to be a measure of the terminal elimination for each congener. Half-lives of the congeners were not significantly different between male and female rats, and ranged from 25 to 91 days in female rats vs. 19–119 days in male rats (Table 3). In both sexes, the half-life generally increased with increasing bromination. No statistically significant half-life differences for the pairs of pentabromo- and hexabromo-DE congeners were observed between male and female rats. These results would seemingly contradict observations in environmental samples where the lower brominated BDE-47 is presumably the most persistent PBDE congener. However, these data were obtained following a large dose of 300 mg/kg, which was in great excess of the threshold necessary for minimal cytochrome P450 induction (3–10 mg/kg). An altered rate of metabolism for individual PBDE congeners may occur at lower environmental exposures.

Table 3
Half-lives of individual components of pentabromo diphenyl ether (PBDE) in male Wistar rats (single oral dose of 300 mg/kg Bromkal 70 dissolved in peanut oil). Groups of four rats were sacrificed consecutively until the 10th week

PBDE HPLC Peak	Half-lives in female rats in days	Confidence interval $P=0.05$	Half-lives in male rats in days	Confidence interval $P=0.05$
Br_4DE	29.9	26.8–33.1	19.1*	16.5–21.7
Br_5DE1	47.4	42.5–52.4	36.8	33.7–40.0
Br_5DE2	25.4	22.6–28.4	24.9	22.6–27.1
Br_6DE1	44.6	37.4–51.9	55.1	48.4–61.7
Br_6DE2	90.9	78.7–103.6	119.1	102.8–136.1

Concentrations of PBDE components in the perirenal fat were determined by HPLC and data were corrected for the body weight of the rats.
Abbreviations: Br_4DE = tetrabromo diphenyl ether, Br_5DE1 = pentabromo diphenyl ether, Br_5DE2 = pentabromo diphenyl ether, Br_6DE1 = penta- and hexabromo diphenyl ether, Br_6DE2 = hexabromo diphenyl ether (von Meyerinck et al., 1990).
* $P=0.01$.

Two absorption studies were conducted that attempted to measure the absorption and bioconcentration of the various PBDE congeners present in Bromkal 70 and DE-79 (a commercial octa mixture) at environmentally relevant levels. Male Sprague–Dawley rats were administered the technical mixtures Bromkal 70 (dominated by BDE-47 and 99) or DE-79 (dominated by hexabromo-through nonabromo-DE congeners) in the feed for 21 days at 33 ng/day/rat. The liver, carcass, and feces were analyzed for individual PBDEs by a modification of EPA Method 1613 used for environmental dioxin/furan analyses. The minimum amount of absorption of DE-71 (36.7% of the dose) could be derived from liver and carcass retention at 21 days, i.e., 0.8% in the liver, and 35.9% in the carcass (Hakk et al., 2001a). The minimum amount of absorption of DE-79 was 32.3% of the total dose (1.2% and 31.1%, respectively) (Huwe et al., 2002). In general, there were no bioconcentration differences for the lower brominated Bromkal 70 congeners, i.e., the tissue congener distribution patterns resembled that of the commercial mixture. However, a decrease in bioavailability was observed with increasing bromination with DE-79. The data indicated that higher brominated congeners were more highly metabolized, and no evidence was obtained to suggest that lower brominated congeners were formed. The recovery of BDE-153, a congener common to both studies, was very similar in each compartment, which justified bioavailability conclusions of each study.

Table 4
Excretion results from metabolism studies in rats administered various PBDE congeners (data are in percent of administered dose)

	Male Sprague–Dawley rat 14.46 mg/kg po, 5 days BDE-47	Male Sprague–Dawley rat 8.1 mg/kg po, 72 h BDE-99	Male Sprague–Dawley rat 9.2 mg/kg po, 72 h BDE-99	Male Sprague–Dawley rat 1.09 mg/kg po, 16 days BDE-209	Male Fischer 344 rat 0.0277% diet, days 1–7, 9–11 cold BDE-209, at 8 days ^{14}C BDE-209	Male Fischer 344 rat 4.8% diet, days 1–7, 9–11 cold BDE-209, at 8 days ^{14}C BDE-209	Male Fischer 344 rat 1.07 mg/kg iv, 72 h BDE-209	Male Sprague–Dawley 3.0 mg/kg po, 72 h BDE-209
Urine	<0.05	0.9	0.35	<1.0	0.012	0.008	0.129	<0.05
Feces								
Day 1	5.7	22.3	52.5	90.6				
Day 2	5.4	14.8	30.4	>8.4				
Day 3	1.2	6.0	3.6	–				
Day 4	0.9	–	–	–				
Day 5	0.5	–	–	–				
Total	13.7	43.1	86.5	>99	82.5 (0–72 h)	85.1 (0–72 h)	70.0	>90
Bile								
Day 1			0.7					
Day 2			1.8					
Day 3			1.4					
Total			3.9					9.5
References	Örn and Klasson-Wehler (1998)	Hakk et al. (2002)	Hakk et al. (2002)	Norris et al. (1975)	El-Dareer et al. (1987)	El-Dareer et al. (1987)	El-Dareer et al. (1987)	Mörck and Klasson-Wehler (2001)

Congener-specific PBDE metabolism in rat and mouse has been restricted to mainly BDE-47, BDE-99 and BDE-209. Tables 4 and 5 summarize the results of several excretion, metabolism, and tissue distribution studies in BDE-47 exposed rats. Örn and Klasson-Wehler (1998) showed that absorption of a 14.5 mg/kg (30 μmol/kg) oral dose of ^{14}C-labeled BDE-47 by male Sprague–Dawley rats was high, although metabolism was slow since only 14% and <0.5% of the dose was excreted in the feces and urine by 5 days, respectively. Because extremely low levels of ^{14}C were detected in rat urine at 5 days no further analysis was attempted. Approximately, 87% of the BDE-47 dose

Table 5
Tissue recoveries of PBDE congeners administered to rats

	Male Sprague–Dawley rat 8.1 mg/kg po, 72 h BDE-99	Male Sprague–Dawley rat 9.2 mg/kg po, 72 h BDE-99	Male Sprague–Dawley rat 1.09 mg/kg po, 16 days BDE-209	Male Fischer 344 rat 0.0277% diet, days 1–7, 9–11 cold BDE-209, at 8 days ^{14}C BDE-209	Male Fischer 344 rat 4.8% diet, days 1–7, 9–11 cold BDE-209, at 8 days ^{14}C BDE-209	Male Fischer 344 rat 1.07 mg/kg iv, 72 h BDE-209	Male Sprague–Dawley 3.0 mg/kg po, 72 h BDE-209
Liver	0.9	0.3		0.109	0.016	4.27	0.9
Kidney	0.1	0.03		0.013	<0.001	0.697	0.05
Lungs	0.1	0.04		0.004	0.001	0.361	<0.1
Spleen	<0.1	–	0.06	0.001	<0.001	0.027	<0.1
Pancreas	–	–	–	–	–	–	–
Adrenals	0.1	0.01	0.01	–	–	–	<0.1
Heart	0.03	0.01		–	–	–	<0.1
Brain	–	–		<0.001	<0.001	0.047	–
GI	6.1	1.5		0.09	0.60	5.063	3.5
Muscle	0.7	–		0.248	0.008	12.9	0.7
Skin	0.4	–		0.136	0.036	7.25	0.4
Fat	3.8	0.8		0.048	0.012	2.99	0.3
Blood	0.03	0.007		0.026	0.006	0.763	0.05
References	Hakk et al. (2002)	Hakk et al. (2002)	Norris et al. (1975)	El-Dareer et al. (1987)	El-Dareer et al. (1987)	El-Dareer et al. (1987)	Mörck and Klasson-Wehler (2001)

remained in the tissues at 5 days and deposited with the highest concentration in the adipose tissue on both a fresh weight basis and a lipid weight basis (Table 5). The BDE-47 adipose tissue concentration was 70-fold (fresh weight) and 3.5-fold (lipid weight) greater than the lung, which contained the next highest concentration. The lung, in turn, was twice as high as liver and kidney on a lipid weight basis. Smaller amounts of BDE-47 deposited in brain. The only form of BDE-47 detected in the adipose tissue, kidney, and brain was parent. However, trace amounts (<1% of the extractable ^{14}C radiolabel) of OH-metabolites were detected in the liver and lung. Gel permeation chromatography fractionation of fecal extracts resulted in lipid-bound, parent compound/metabolite, and nonextractable fractions (Table 6). The majority of the fecal ^{14}C recovered daily was contained in the parent compound/metabolite fraction (45–94% of the dose). GC/MS analyses demonstrated that the major compound in rat feces was parent (>85% of the parent compound/metabolite fraction). Six metabolites in rat feces were characterized by mass spectral ion intensities of the methylated PBDE metabolites. These metabolites were tentatively assigned to be two *ortho*-OH-tetrabromoDE metabolites, one *meta*-OH-tetrabromoDE metabolite, and two *para*-OH-tetrabromoDE metabolites (Fig. 6). One of the *ortho*- and both of the *para*-OH-metabolites must have been the result of an NIH-shift, evidence for arene oxide as an intermediate. A trace amount of a thiol-tetrabromo diphenyl ether metabolite was also characterized in rat feces, however, in the absence of reference compounds this assignment remained tentative.

In contrast to rats, mouse is more capable of metabolizing BDE-47. In one study, approximately 33% of a 14.5 mg/kg dose of ^{14}C-labeled BDE-47 in mice was excreted in urine by 5 days (Örn and Klasson-Wehler, 1998). Approximately, 20% of the urinary mouse ^{14}C was characterized as parent BDE-47, but the remainder could not be characterized. Detected BDE-47 in the mouse urine was speculated to arise from decomposition of a labile metabolite(s). Over 20% of the dose was excreted in 5 days in the feces, and the major portion of the fecal extract was the metabolite fraction by GPC (70%). The metabolite fraction contained the same six metabolites characterized in the rat feces. The relative amount of nonextractable and lipid-bound BDE-47 derived ^{14}C increased with time in mouse feces. Nearly 47% of the administered dose remained in the tissues at 5 days. Adipose tissue contained the highest concentration of BDE-47 on a fresh weight basis, over 10-fold higher than any other tissue. However, the concentrations in adipose tissue, liver, lung, and kidney were nearly the same on a lipid-adjusted basis. Activation of BDE-47 to reactive intermediates was suggested by nonextractable radioactivity in liver (12% of total tissue ^{14}C), lung (19%), and kidney (4%). The vast majority (>99%) of the tissue radioactivity was parent, however, five mono-OH-tetrabromo- and two mono-OH-tribromo-DE metabolites were detected in the liver.

Table 6
Relative amount (%) of a BDE-47 dose excreted in the feces of the rat and mouse, and distribution of ^{14}C in different fractions (percent of total ^{14}C in each sample) (Örn and Klasson-Wehler, 1998)

Feces	Percent of dose	Non-extractable	Water-soluble ^{14}C	GPC-lipid	GPC-metabolite
Rat					
Day 1	5.7	1.9	1.0	2.8	94
Day 2	5.4	8.6	2.9	11	78
Day 3	1.2	15	9.9	28	47
Day 4	0.9	13	7.0	31	45
Day 5	0.5	17	8.5	11	79
Mouse					
Day 1	7.6	4.3	3.0	5.9	87
Day 2	6.4	16	7.0	16	61
Day 3	2.2	16	7.4	14	62
Day 4	2.2	20	7.1	17	56
Day 5	1.7	20	9.4	16	57

A 2.2 mg/kg single, oral dose of radiolabelled BDE-99 was administered to both conventional and bile duct-cannulated male rats (Hakk et al., 2002). The metabolism of BDE-99 to water-soluble metabolites or conjugates was low, and cumulative recovery of the radiolabel in urine was less than 1% in conventional rats at 72 h, and only 0.3% in bile duct cannulated rats (Table 4). Cumulative excretion of BDE-99 in bile was only 3.9% at 72 h. The urinary radioactivity was composed exclusively of metabolites, which were not characterized, in both the conventional and bile duct-cannulated rat. Fecal excretion was the major route of elimination of BDE-99. In the conventional rat feces, 43% of the dose was excreted, and >86% was excreted in bile duct-cannulated rat feces in 72 h (Table 4). Circumstantial evidence of labile metabolites was obtained in the fecal extracts, where reversion of purified metabolites back to parent was observed on several occasions. In addition, evidence for reactive intermediates in the feces of conventional rats was indicated by high nonextractable fractions ranging from 18% to 52%. BDE-99 was preferentially distributed to lipophilic tissues at 72 h, i.e., adipose tissue, skin, and GI tract. No other tissues in conventional or bile duct-cannulated rats contained more than 1% of the ^{14}C at 72 h. Trace amounts of OH-metabolites in the liver extracts were detected, but the remaining tissues contained only parent. The feces from BDE-99 conventional rats contained minor amounts (<10% of extracted radioactivity) of metabolites, while the remainder was the parent compound. Fecal metabolites consisted of two mono-OH-pentabromoDE metabolites, and two debrominated mono-OH-tetrabromoDE metabolites (Fig. 6) Absolute stereochemical assignments could not be made due to insufficient mass. Two mono-OH-pentabromoDE metabolites were identified in rat bile, as well as three di-OH-pentabromoDEs. Mass spectral evidence also suggested the presence of two thiol-substituted pentabromoDEs.

Greater than 99% of a single oral dose of decabromoDE (BDE-209; 1.0 mg/kg suspended in corn oil) administered to male and female rats was excreted in the feces at 48 h

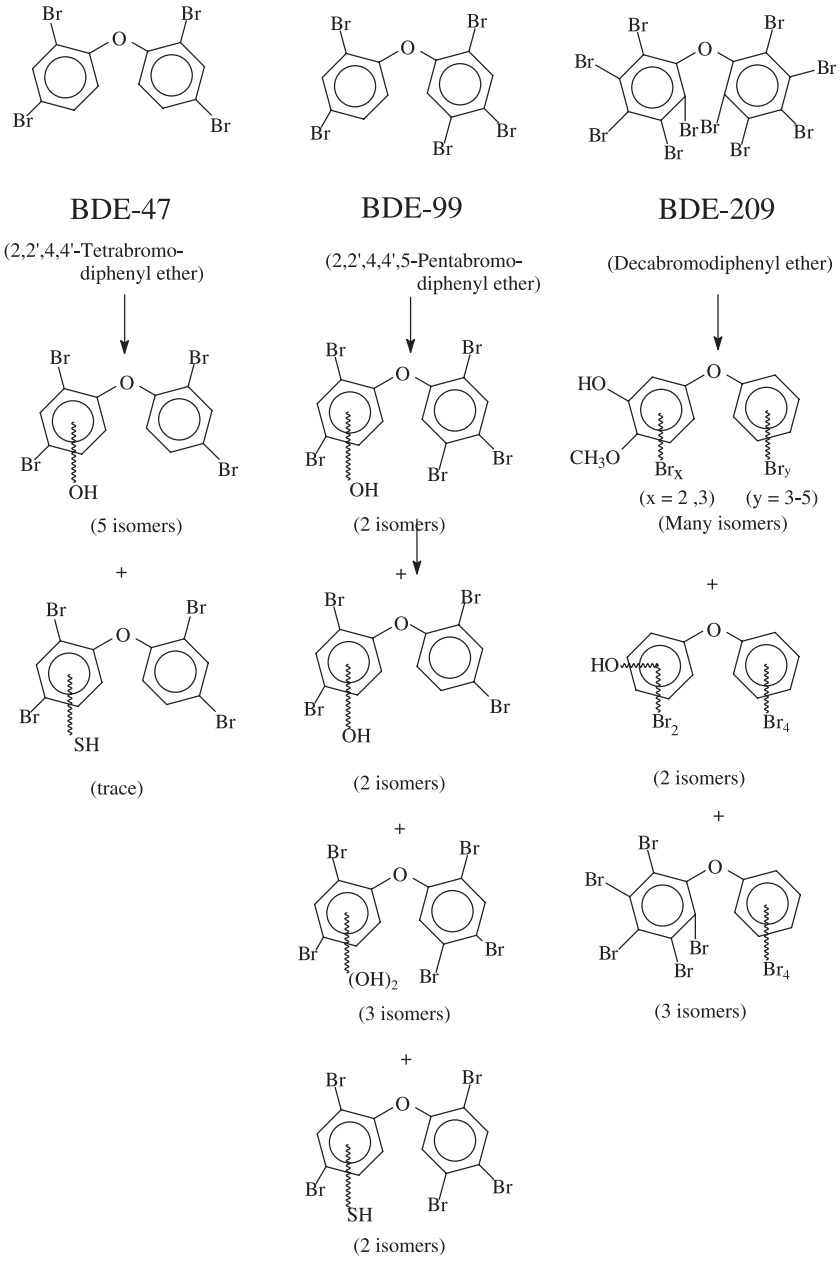


Fig. 6. Fecal and biliary metabolites formed and identified by mass spectrometry following doses of BDE-47, -99, and -209 in the rat. Single oral doses were administered to male Sprague–Dawley rats at 14.5, 2.2, and 3.0 mg/kg, respectively. Each congener underwent oxidative debromination, but BDE-47 and 99 also underwent oxidation and thiol formation. Only BDE-209 was metabolized to guiacols. The data are from Örn and Klasson-Wehler (1998), Hakk et al. (2002), and Mörck and Klasson-Wehler (2001).

(Table 4). The level of BDE-209 excretion in urine and respired air was less than 1% at 16 days (Norris et al., 1975). Animals sacrificed at 1, 3, and 16 days showed that limited absorption of BDE-209 from the GI tract had occurred. At 16 days, only the adrenals and spleen contained measurable radioactivity (0.01% of dose/g and 0.06%, respectively; Table 5). When rats were maintained for 180 days on a diet spiked with BDE-209 at 0.1 mg/kg/day, no differences in bromine content of kidney, muscle, serum, testes, or liver were observed. Only the adipose tissue displayed a slight, statistically significant increase in bromine content over controls. Similarly, when ^{14}C doses of BDE-209 ranging from 250 to 50,000 mg/kg were administered, all rats excreted >99% of the dose in the feces in 72 h (NTP Toxicology Study, 1986) and only 0.01% of the dose was excreted in the urine.

El Dareer et al. (1987) observed that intestinal absorption of BDE-209 in male Fischer 344 rats was minimal when administered in feed, and did not depend on the BDE-209 concentration (0.025–5.0% of the diet). Greater than 99% of the dose was excreted in the feces at 72 h, while only 0.012% was observed in the urine (Table 4). The feces

contained three uncharacterized metabolites and these ranged from 1.5% to 27.9% of the fecal radioactivity. The total amount of BDE-209 found in tissues was less than 1% of the dose. The liver contained the largest percentage of the administered dose at 24 h, i.e., 0.449%, and declined to 0.016% of the dose at 72 h (Table 5). Following an intravenous dose (1.07 mg/kg), 74% of BDE-209 was excreted in feces at 72 h, while 0.129% was eliminated via the urine. The majority of the i.v.-derived fecal radioactivity (63%) was present as metabolites of BDE-209. The muscle and skin both contained large portions of the dose, 12.9% and 7.25%, respectively. In bile duct, cannulated rats administered the same i.v. dose, over 7% was eliminated via the bile in 4 h, indicating that the bile was the major excretion route for i.v. administered BDE-209. Greater than 99% of the BDE-209 in bile was present as metabolites. These data indicate that BDE-209 can be readily metabolized in rats if absorption can be achieved.

Together, the existing data suggest that intestinal uptake of BDE-209 is not efficient and it therefore has a very low bioaccumulation potential. However, the absorption of BDE-209 from the intestines following an oral exposure may display a dosing vehicle dependency. The majority of a single, oral 3.2 umol/kg dose of BDE-209 (3.0 mg/kg) in soya phospholipid-Lutrol F127 vehicle in male Sprague–Dawley rats was excreted in the feces (>90%), and less than 0.05% was excreted in the urine at 3 days (Mörck and Klasson-Wehler, 2001). However, 9.5% of the dose was excreted in the bile of bile duct-cannulated rats within 3 days. The tissue distribution of BDE-209 ranged from slightly more than 0.9% of the dose in liver to 0.7% in muscle, 0.4% in skin, 0.3% in adipose tissue, 0.25% in colon wall, and all other tissues sampled were less than 0.1% of the dose. On a lipid adjusted basis, the liver contained the highest concentration of BDE-209 (14 nmol/g). The kidney (2 nmol/g), lung (2 nmol/g), adrenals (4.4 nmol/g), skin, and muscle all contained higher levels of BDE-209 than the fat (< 1 nmol/g). There was evidence for the formation of reactive intermediates, since 60% of the jejunum wall ^{14}C and 30% of the liver ^{14}C were nonextractable. Rats metabolized BDE-209 to fecal metabolites via oxidative debromination, and these included debrominated mono-OH- and *ortho*-MeO-OH-BDEs (Fig. 6). The MeO group was probably introduced by a catechol-O-methyl transferase of an *ortho* catechol substrate, although the route of formation of the putative catechol is not known. Since arene oxide formation appears unlikely when all carbons are brominated, debromination probably preceded the oxidation in BDE-209. Trace levels of three debrominated nonabromodiphenyl ethers were observed, but no diphenyl ethers with less bromination were detected. The *ortho* catechol metabolites may have the potential to oxidize to quinones, which are known to be very reactive intermediates (Amaro et al., 1996), and may explain the nonextractable radioactivity observed in the jejunum wall, liver, and feces.

5.3.2. Fish

PBDE accumulation and metabolism in fish is restricted to a few species. In an experiment, where a dietary uptake of 12 halogenated, diaromatic compounds, including PCBs, polychlorinated naphthalenes, and PBDEs, were investigated in captive northern pike (*Esox lucius*) (Burreau et al., 1997), the calculated uptake efficiency of BDE-47 was 90%, while the pentabrominated BDE-99 was 60%, and the hexabrominated BDE-153 was 42%. No correlation was found for the PBDEs between uptake rates, and octanol/water partition coefficient, molecular weight, or effective cross-sectional area, but a negative correlation with degree of halogenation was observed. Halogenated aromatic compounds with cross-sectional areas >9.5 Å are thought to be impervious to uptake by cell membranes (Opperhuizen et al., 1985). However, maximal uptake efficiency occurred with compounds of molecular weight 450. When comparing experimentally derived data with those from an empirical model based exclusively on diffusion, these results suggested a mediated uptake, perhaps via a membrane protein in the gastrointestinal tract mucosol lining (Stremmel 1988).

In a metabolism study in northern pike, a single dose of ^{14}C-labeled BDE-47 was administered into a natural food matrix, i.e., a brown trout (*Salmo trutta*) (Burreau et al., 2000). BDE-47 was absorbed by pike with a calculated uptake efficiency of 96%. The tissue distribution of radiolabeled BDE-47 in pike was determined by whole body autoradiography at 9, 18, 36, and 65 days. The entire carcass, except the backbone, contained radioactivity at 9 days. In general, storage of BDE-47 was in the most lipophilic tissues. At 18 days, the tissue distribution pattern was identical to that found at 9 days, the highest levels were in the liver, adipose tissue, vertebrae-surrounding tissue, eye, and gall bladder. Intermediate levels were observed in the brain, spinal chord, heart, and kidney, while the lowest levels were in the muscle, spleen, and gills. At 36 days, most tissues contained decreased levels of BDE-47, except the liver, adipose tissue, and vertebrae-surrounding tissue. Gall bladder and eye were nearly void of radioactivity at 36 days. At 65 days, a marked drop in radioactivity was observed in liver, however, levels in adipose and vertebrae-surrounding tissues remained high. In a repeat experiment intended to characterize tissue metabolites, six metabolites were detected (Kierkegaard et al., 2001). Selective retention of the six metabolites was observed for each tissue. 2-OH-2′,4,4′,6-tetraBDE and 2-OH-2′,3,4,4′-tetraBDE were characterized by comparison with synthesized standards. In pike, BDE-47 was not readily metabolized into metabolites that could be easily eliminated, since the concentration in highly lipophilic tissues did not diminish significantly with time. The results also suggested that tissue distribution differences might occur based on the leanness of the species. In lean species, lipid-rich tissues, such as the brain, can become more important depots for hydrophobic compounds, such as PCBs and PCDDs (Ingebrigtsen et al., 1990; Hektoen et al., 1992).

As mentioned earlier, differential metabolic capacities between the rat and mouse have been demonstrated for BDE-47 (Örn and Klasson-Wehler, 1998). Similar observations have been found for differential PBDE metabolism among fish species from Washington State (USA). Johnson and Olson (2001) found that extremely low accumulations of PBDEs were routinely observed in white suckers (*Catostomus commersoni*) and common carp (*Cyprinus carpio*) when compared to rainbow trout and mountain whitefish (*Prosopium cylindraceum*) obtained from the same location and time. A possible explanation may reside in diet differences, since suckers are benthic omnivores, while trout are invertivores/piscivores. However, the large magnitude of the difference (1–2 orders) between these fish species was more likely due to a higher inherent metabolic activity in suckers towards PBDEs than in rainbow trout. The analytical methodology did not make provisions for metabolite characterization in these fish samples. Similar observations (unpublished) were communicated to the authors from work performed in Maine (USA) where smallmouth bass had a 10-fold higher total PBDE concentration than suckers obtained from the same waters. These results justify more work in this area to determine the nature of any metabolic differences between species.

Several benthic fish residing close to suspected PBDE point sources showed elevated concentrations of highly brominated PBDEs (Dodder et al., 2002; Hale et al., 2001). Of the fish in these studies, common carp contained an unusual pattern of accumulated PBDE congeners, in which BDE-99 was significantly depleted in tissues relative to the tissues of other fish species from the same geographical area. In a series of experiments by Stapleton et al. (2002), the uptake, metabolism, and depuration of mixtures of environmentally relevant PBDE congeners in common carp was reported. In a preliminary experiment, carp were exposed to a diet of blood worm pellets containing BDE-47, -99, -100, -153, -154, and -180 (100 ng/g wet weight for each congener) for a period of 25 days. Rapid uptake of BDE-47, -100 and -154, but low uptake of BDE-99, -153, and -183 was observed, which was similar to the PBDE congener pattern in carp from the wild (Hale et al., 2001). In a separate carp uptake experiment with BDE-183 alone, debromination of BDE-183 to BDE-154 was suggested. Since BDE-99 and BDE-153 uptake was expected, the rapid accumulation of BDE-47, and not BDE-99 and BDE-153, further suggested rapid metabolism of the latter two congeners. Separate experiments with BDE-209 showed poor uptake by carp (<1%), but the presence of several hexa- through octa-brominated DE congeners suggested debromination of BDE-209 by carp.

Further studies investigating possible debromination of BDE-209 in vivo have suggested that this metabolic pathway may occur in fish. Kierkegaard et al. (1999a) studied the intestinal absorption of BDE-209 in rainbow trout, which were fed cod-chips treated with BDE-209 (7.5–10 mg/kg/day) for 16, 49, or 120 days. Depuration of BDE-209 was studied in an additional group, which received control feed after 49 days of treated feed. The calculated uptake efficiency of BDE-209 was extremely low, 0.005%, with elevated concentrations in both liver and muscle with increased time of exposure compared with controls. Concentrations in liver were 20–40 times greater than muscle on a fresh weight basis, and decreased rapidly in both tissues upon depuration. Lower brominated BDE congeners were also detected in trout muscle by 16 days, some of which were not present as contaminants in the original BDE-209 dose. The concentration of these congeners also increased with length of exposure, but no decrease was observed for BDE-153, -154, two heptas, or an octa congener during depuration (Fig. 7). No increase in muscle concentrations of BDE-47, -99, or -100 was observed, therefore, the study found no evidence for the debromination of BDE-209 to these environmentally persistent congeners, although the BDE-153, 154, two heptas, and the octa congener may arise from this mechanism. Possible degradation of BDE-209 with UV light, and subsequent uptake of UV-debrominated products was not considered in the experimental design probably because UV degradation of BDE-209 does not lead to lower brominated congeners in water (Norris et al., 1973), only in organic solvents (Watanabe and Tatsukawa, 1987).

5.3.3. Aquatic organisms

Blue mussels (*Mytilus edulis*) from the Baltic sea were exposed to BDE-47, -99, and -153 for 44 days in a flow-through experimental design, and then allowed to depurate for 26 days (Gustafsson et al., 1999), a method that allowed uptake clearance rate coefficients and bioaccumulation factors for each PBDE congener to be determined. Each congener was readily absorbed, however, BDE-47 and -99 had the highest uptake clearance rate coefficients. These values, as well as the bioaccumulation factors, were six to 10 times higher than PCBs of high environmental interest, PCB-77 and 118. Despite the fact that commercial Bromkal 70 has nearly equal concentrations of BDE-47 and 99, sediment samples from numerous locations around Denmark contained one to three times as much BDE-99 as BDE-47 (Christiansen and Platz, 2001). However, at higher trophic levels, the concentration of BDE-47 increased dramatically when compared to BDE-99. In addition, the ratio of the two common pentaBDEs, BDE-99:BDE-100 also decreased at higher trophic levels. This suggests that BDE-99 has a higher rate of metabolism than BDE-47 or 100 in higher organisms, which leads to lower bioaccumulation for BDE-99 relative to BDE-47 and BDE-100.

5.3.4. Bacteria

The metabolism of monohalogenated DEs and their ultimate utilization as a sole carbon source has been observed in the bacterium *Sphigomonas* sp. strain SS3 (Schmidt et al., 1992). The metabolism decreased with increasing atomic weight (F>Cl>Br), and stereochemistry

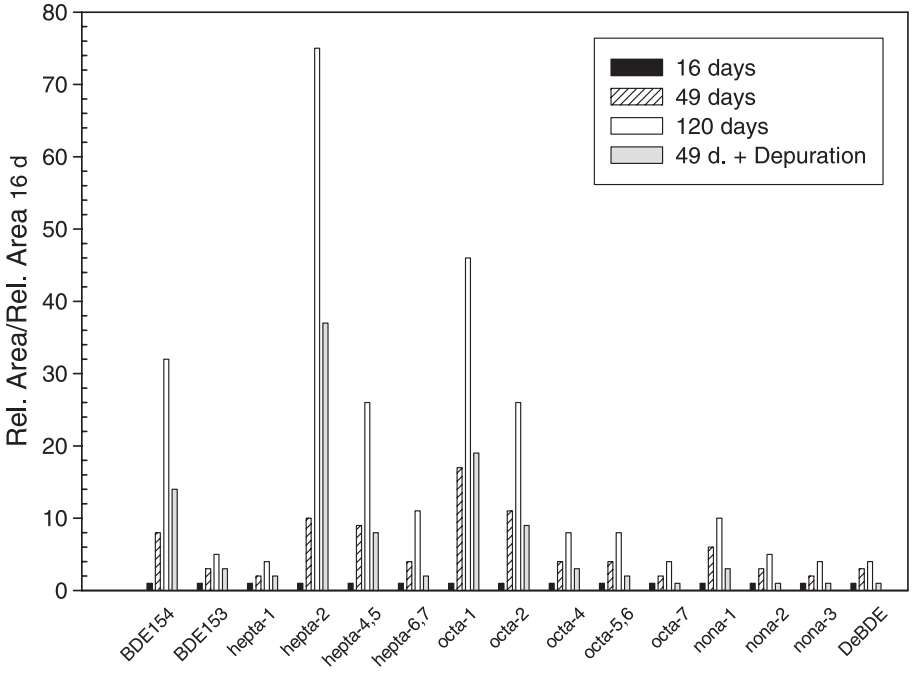


Fig. 7. Congener pattern of PBDEs in rainbow trout muscle after receiving BDE-209 for 16, 49 (with and without depuration), or 120 days in the diet (7.5–10 mg/kg bw/day). Results are on a fresh weight basis and are normalized to 16 days of exposure. The data are from Kierkegaard (1999a).

(*para>meta>ortho*). The first step in the metabolism was dioxygenolytic attack yielding an unstable intermediate, which decomposed to phenols and *ortho*-catechols. However, the addition of a second bromine atom prevented 4,4′-dibromoDE (BDE-15) from being used as the sole carbon source, presumably due to the bulkiness of the bromine atom and the steric hindrance produced. Therefore, by extension, the available data would indicate that more highly brominated diphenyl ethers, i.e., BDE-47, -99, -100, -153, or -154, would not be subject to appreciable metabolism by bacteria in soil or sediment.

5.4. Residual congener patterns and levels of PBDEs and PBDE metabolites in tissue

As previously mentioned, OH-PBDE metabolites were suggested to form metabolically in hepatic microsomes from PB-induced rats (Meerts et al., 2000) and OH-PBDE metabolites of BDE-47 form in exposed northern pike (Burreau et al., 2000). The presence of detectable residues of OH-PBDEs in the blood of PBDE exposed wildlife is a likely reflection of oxidative PBDE metabolism. However, other biological sources cannot be excluded, for instance, marine sponges can also produce *ortho*-OH-PBDEs. *Dysidea heracea* produces tetra-through hexabromo-*ortho*-OH-BDEs, and other sponge species can as well (Carté and Faulkner 1981; Capon et al., 1981; Sharma and Vig, 1972).

Recent studies have reported the presence of retained hydroxylated and methoxylated PBDEs in the blood plasma from several wildlife species from the Baltic Sea. Haglund et al. (1997) identified a number of MeO-PBDEs in ringed seals (*Phoca hispida botnica*), grey seals (*Halichoerus grypus*), Altantic salmon (*Salmo salar*), herring (*Clupea harengus*), and commercial fish oils. These MeO-PBDEs were present at slightly lower levels than the parent PBDEs in fat, muscle, liver, and fish oils. The MeO-PBDE concentrations generally increased with trophic level in the Baltic biota samples. This may suggest OH-PBDE biomagnification, an increase in the rate of CYP enzyme-mediated PBDE metabolism and/or OH-PBDE retention and half-life in higher trophic level species. A difference in metabolic capacity to form OH-PBDEs is a more likely explanation. For example, tissue concentrations of persistent and bioccumulative methyl sulfone PCB metabolites in biota, which are formed via enzyme-mediated metabolism of PCBs, have been shown to be more significant in seals relative to cetaceans and a few fish species, which appear to be due to relatively greater activities of Phase I and Phase II enzymes in seals (Letcher et al., 2000). In the Haglund et al. (1997) study, tentative assignments included *ortho* methylation of three tetra- and one penta-OH-PBDEs. Since MeO-PBDEs are not produced commercially, it was suggested that they could be formed in two steps by hepatic hydroxylation and methylation by intestinal microflora or in a single step directly in sediments.

The presence of OH- and/or MeO-PBDEs has also been observed in other species. Kierkegaard et al. (1999b) reported MeO-BDE-47 in the blood plasma of pike from a freshwater Swedish lake (Lake Bolmen), which is consistent with OH-PBDE metabolites found for pike dosed with BDE-47 (Burreau et al., 2000). Blood plasma of Baltic white-tailed sea eagle (Olsson et al., 2000), Atlantic salmon (Marsh et al.,

2001), and blue mussels and red algae (Asplund et al., 2001) were also found to contain OH-PBDEs. More than 100 phenol-type contaminants were detected in the blood plasma of healthy salmon and individuals suffering from thiamine deficiency (M74 syndrome) (Asplund et al., 1999). A number of the phenol-type compounds were identified as OH-and MeO-PBDEs, but many of the phenolic compounds remained unidentified. OH-PBDEs have also been reported in Swedish human plasma (Hovander et al., 2002). The OH-PBDEs present in the blood plasma of the Baltic species are likely the result of PBDE metabolism. Marine sponges are not native to the Baltic Sea, and natural production in invertebrates or algae is unlikely due the lack of CYP enzyme activity necessary to metabolize PBDE to PBDE arene oxides. However, invertebrates and algae as sources of OH-PBDEs to higher trophic level organisms cannot be ruled out due to the lack of experimental evidence.

There are indications of brominated phenol-type compounds, perhaps including OH-PBDEs, and mixed brominated-chlorinated phenol-types as residues in the blood of other wildlife and humans outside the Baltic region. These studies are restricted to Canada and the US. The blood plasma of blue marlin (*Makaira nigricans*) was found to contain OH-PBDEs (Greeves and Harvey, 2000). The blood plasma from killer whale fed a diet of wild Pacific herring was found to contain OH-PCBs, but also a dominant but unidentified mixed bromo-chloro-phenolic compound, as well as other unidentified, halogenated phenolics (Bennett et al., 2002). In a survey of blood plasma of 14 species of pelagic and benthic feeding fish from the Detroit River, OH-PCBs as well as an unknown number of brominated phenolics were detected in all the fish studied (Li et al., 2002a,b, 2003).

6. Bis(2,4,6-tribromophenoxy)ethane

A study in which BTBPE was administered in the diet of rats for 28 days at a rate of either 100 or 1000 mg/kg, demonstrated accumulation in the fat, liver, and muscle. During the withdrawal period, however, BTBPE levels decreased steadily until background levels were observed (NTP, 1987). The manufacturer of BTBPE reported that 80% of a single, oral dose of unspecified size was excreted in the feces at 96 h, and 5% was eliminated in the urine, as measured by radiolabelled ^{14}C (Great Lakes Chemical, 1981). Poor absorption from the gut was concluded from the data. Maximum plasma concentrations occurred at 24 h (0.58 mg/kg) and by 96 h had decreased to 0.15 mg/kg.

Nomeir et al. (1993) administered [^{14}C]BTBPE in the diet to five groups of male Fischer 344 rats. Three groups received different amounts of BTBPE in the diet for 1 day (0.05%, 0.5%, and 5.0% of the diet). A fourth group received 0.05% BTBPE daily for 10 days. A fifth group was bile duct cannulated and received a single oral dose of 200 mg/kg. The excretion results were the same for the first three groups of rats at 96 h. Greater than 99% of the dose was excreted in the feces, and less than 1% was excreted in the urine (Table 7). Urine contained only metabolites of BTBPE, while feces contained only parent. The fat, skin, and thymus contained measurable radioactivity in some of the rats. Rats that received a continuous dose for 10 days contained trace levels of BTBPE in all tissues examined except the brain. In addition to the gastrointestinal tract, which would be expected to have relatively high BTBPE concentrations, the fat, kidney, skin, and thymus contained the highest concentrations (3.19–0.95 nmol/g; Table 7). The liver, blood, heart, lungs, and muscle were of intermediate concentration (0.52–0.38 nmol/g), and brain, testes, and spleen were the lowest concentration (0.02–0.17 nmol/g). Total tissue recovery of BTBPE in the 10 days feeding study was less than 0.2% of the dose. Approximately, 75% of the 10 days cumulative dose was excreted in the feces of rats, and greater than 99% of the fecal ^{14}C was identified as parent. Less than 0.04% of the dose in bile duct-cannulated rats was eliminated via the bile, and insufficient amounts of chemical mass were available for mass spectral characterization. No respired [^{14}C]CO_2 was detected in any of the experiments. The conclusion from the experiments was that BTBPE was poorly absorbed from the gastrointestinal tract and not a preferred route for exposure. However, the consequences of other routes of exposure need to be investigated.

Less than 1.6% of a 8.0 mg/kg oral dose was excreted by 72 h in the urine of male Sprague–Dawley rats (Table 7) and approximately, 0.03% was excreted in the urine of bile duct-cannulated rats (Hakk et al., 2001a,b). Fecal excretion was high at 24 h for conventional and bile duct-cannulated rats, 93% and 58%, respectively, and declined rapidly with time. Biliary excretion of BTBPE was exceedingly low, approximately, 0.2% of the dose. Based on these excretion data, it was concluded that intestinal absorption of BTBPE was very poor. As a result, low tissue levels of BTBPE were also observed. In conventional rats, only the gastrointestinal tract and remaining carcass contained greater than 0.1% of the dose at 72 h in conventional rats (Table 7). Skin from the carcass contained 0.005% of the dose/g, and therefore, the bulk of the carcass ^{14}C was presumably associated with muscle, abdominal fat, or bone. Less than 0.03% of the BTBPE deposited in the liver or fat. Despite greater than 98% of the extractable BTBPE existing as the parent, at least eight metabolites were characterized in feces. These metabolites were formed by debromination, oxidation via arene oxide, and loss of a tribromophenoxy moiety. The metabolites were mono-OH-BTBPE, tetrabromo di-OH-bisphenoxyethane, two isomers of pentabromo-di-OH-bisphenoxyethane, two isomers of pentabromo-mono-OH-bisphenoxyethane, 2,4,6-tribromophenoxy ethanol, and 2,4,6-tribromophenol. 2,4,6-tribromophenol is a known carcinogen, however, the remaining metabolites possess an unknown toxicity.

In pharmacokinetic studies, it was observed that BTBPE was poorly soluble in all common vehicles used for oral dose preparations. Peanut and corn oil have been used for dosing (Nomeir et al., 1993; Hakk et al., 2001b), but heating was

Table 7
Recovery of BTBPE from available metabolism studies in the rat (data are percent of administered dose)

Tissue/Excreta		Hakk et al. (2001b) Conventional	Hakk et al. (2001b) Cannulated	Nomeir et al. (1993)			
				1-day feed study		10-day feed study	
				0.05% in diet	0.5% in diet	5.0% in diet	0.05% in diet
Urine	0–24 h	1.1	0.01				
	24–48 h	0.4	0.005				
	48–72 h	0.09	0.02				
	72–96 h	–	–	0.73	0.28	0.26	0.4
Bile	0–24 h	–	0.2				
	24–48 h	–	0.02				
	48–72 h	–	0				
Feces	0–24 h	92.8	58.1				
	24–48 h	20.5	30.8				
	48–72 h	0.7	5.8				
	72–96 h			99.5	84.7	91.3	74.9
Adrenals		0.002	0.0002				–
Adipose (epididymal)		0.01	0.0001	0.38	–	1.76	3.19
Blood plasma		0	0	–	–	–	0.44
Carcass		1.5	0.6				–
GI tract		0.4	1.5	n.d.	n.d.	n.d.	19.8
Heart		0.03	0.0001	–	–	–	0.4
Kidney		0.008	0.0005	–	–	–	1.64
Liver		0.03	0.06	–	–	–	0.52
Lungs		0.009	0.0006	–	–	–	0.4
Spleen		0.003	0.0003	–	–	–	0.17
Testes		0.003	0.0001	–	–	–	0.13
Thymus		0.02	0.00001	0.44	0.36	–	0.95
Brain				–	–	–	0.02
Skin				0.15	–	–	1.09
Skeletal muscle				–	–	–	0.3

needed to keep BTBPE in solution (Hakk et al., 2001a,b). Toluene and anisole are perhaps the best solvents for BTBPE, but are toxic to the organism receiving the dose. Lutrol-soya phospholipid has been tried as a vehicle, but addition of any water resulted in an immediate precipitation of BTBPE from solution. As a result of these limited pharmacokinetic and solubility studies, it can be concluded that mammalian absorption of BTBPE via ingestion would be minimal. However, among the highest BFR concentrations detected in the dismantling hall air of a Swedish electronics recycling plant was BTBPE, i.e., 29 pmol/m^3 (Sjödin et al., 2001), a level that rivaled those of much higher production BFRs, e.g., TPPBA and BDE-209 (55 and 38 pmol/m^3, respectively). Quite possibly, the major route for human and wildlife exposure to BTBPE is via inhalation, and, therefore, additional experiments designed to expose an organism via this route, as well as other routes, may be needed to fully assess the risk of exposure to BTBPE.

7. Hexabromocyclododecane

Little is known about the metabolism of HBCDD, based on the existence of no peer-reviewed pharmacokinetic studies. One study conducted by a manufacturer showed that [^{14}C]HBCDD was readily absorbed in rats that received a single oral dose (Yu and Atallah, 1980). The half-life was exceedingly short, i.e., approximately 2 h. Within 72 h, 16% of the dose was excreted in the urine and 72% in the feces. Four metabolites of unknown structure were found. HBCDD had deposited in every sampled tissue in the rat, and the tissues with the highest levels were the adipose tissue, liver, kidney, lung, and gonads. In another study conducted by a manufacturer, Dean and Leong (1977) administered 7–9 mg/kg to rats as a single oral dose, and discovered that gastrointestinal absorption was facile. Approximately, 86% was eliminated in 72 h, the majority in the feces (70%) and a lesser amount in the urine (16%). The half-life was again estimated to be less than 2 h. Conflicting data was obtained in an unpublished study in which ^{14}C-labeled HBCDD was administered daily for 5 days at 500 mg/kg (Ryuich et al., 1983). In that study design, there was no detectable urinary excretion over 96 h, and the average daily fecal excretion ranged from 29% to 37%. In yet another unpublished study (Commission on Life Sciences, 2000), HBCDD (500 mg/kg/day for 5 days) was found to accumulate exclusively in the adipose tissue with none detected in spleen, pancreas, liver, kidney, or heart of male Wistar rats. HBCDD was excreted in the feces (32–35%

of the administered dose), however, no HBCDD was detected in the urine.

8. Summary and research directions

PBDEs, PBBs, TBBPA, BTBPE, Tris, and HBCDD are classes of BFRs that are known environmental contaminants. Of the minimal research done so far, BFRs are susceptible to several metabolic processes including oxidative debromination, reductive debromination, oxidative CYP enzyme-mediated biotransformation, and/or Phase II conjugation (glucuronosylation and sulfation). Among the BFR classes, the role of metabolism is best understood for PBBs and increasingly for PBDEs. For selected PBB congeners, oxidative debromination and/or CYP enzyme-mediated OH-PBB formation has been shown for rat, mouse, dog, cattle, pigs, and Atlantic salmon. The ease in which a PBB congener is metabolized in vitro or in vivo is dependent on at least one *para* carbon being bromine-unsubstituted and/or the presence of adjacent, bromine-unsubstituted carbons. Hydroxylated penta- and hexabromobiphenyl metabolites have also been detected in BB-153-exposed rats. Based on the study of human subjects from the Michigan PBB incident, the half-lives PBBs in humans appear to be long, which suggests PBBs in human are slowly metabolized.

Studies on TBBPA metabolism have been limited to laboratory rats and several fish species. Further species-specific research on TBBPA metabolism is necessary. In rats, TBBPA undergoes both debromination and metabolism to TBBPA conjugates. However, the TBBPA conjugates are readily deconjugated to regenerate TBBPA by enzymes in the intestinal microflora. In rat bile, the TBBPA conjugates, glucuronide ether, diglucuronide ether, and glucuronide ether-sulfate ester diconjugate were identified. Although not confirmed as forming metabolically, several species of fish appear to be able to methylate TBBPA. This process could have considerable environmental consequences. Methylation would likely render TBBPA to be more lipophilic and bioaccumulative.

Tris is metabolized to bis(2,3-dibromopropyl) phosphate in Tris-exposed rats. Although not confirmed, phosphate ester bond hydrolysis in mammals is known to proceed by three enzymatic mechanisms: mixed function oxidase reactions, hydrolase reactions, and glutathione-S-alkyl transferase reactions.

PBDE metabolism has been directly indicated or strongly suggested in in vitro liver microsomal studies, in dosing studies with captive animals, and by the presence of retained OH-PBDE metabolites in blood in a few wildlife species. PBDE exposure studies are restricted to a few species of aquatic organisms, i.e., fish, saltwater mussels, and bacteria. Most data comes from metabolism studies with technical mixtures, with few congener-specific studies. Unlike structurally analogous PBBs, the connection between PBDE metabolism and OH-PBDE metabolite formation has only been conclusively shown for BDE-47 in laboratory rats and mice, and not for terrestrial or marine wildlife species. Moreover, only in the BDE-47 dosing study with pike have metabolically formed OH-PBDE ever been identified. In rats and fish, such as common carp and northern pike, oxidative debromination of BDE-209 and/or BDE-99 occurs. In addition, methylation of ortho-catechol metabolites from BDE-209 dosed rats to ortho-MeO-OH-DEs was a novel biotransformation.

The growing body of evidence from measurable blood residues of OH-PBDEs suggests that PBDE metabolic capacity is possible across various taxa and by numerous species. However, the importance of oxidative PBDE metabolism, i.e., the rate of metabolic elimination and the generation of OH- and/or -MeO-PBDE metabolites, is unclear among species, etc. Several OH- and MeO-PBDEs (tetra and penta) have been found in Atlantic salmon, herring, ringed seal, grey seal from the Baltic Sea, and MeO-BDE-47 in freshwater pike. The origin of these is not clear, although metabolic formation from PBDEs is a likely source. However, pure OH-PBDE and MeO-PBDE standards are lacking, both radiolabeled and unlabeled, which makes it difficult to identify and quantify residues in tissue and for toxicokinetic and toxicological assessments. OH-PBDE and TBBPA are potent competitors for TTR, the plasma protein responsible for the transport of thyroid hormones. Brominated structural analogues of T4 and T3 also interact with thyroid hormone receptors. BFRs, especially PBDEs and TBBPA, are capable of affecting hormonal systems via formation of metabolites. OH-PBDEs formed from PBDEs, as well as TBBPA, possess the necessary structural properties to interact with TTR and thyroid receptors, which can lead to a cascade of thyroid effects.

Metabolism studies on BTBPE have shown that both debromination and CYP enzyme-mediated OH-metabolite formations are biotransformation pathways in rats. These metabolites were formed by debromination, oxidation via arene oxide, and loss of a tribromophenoxy moiety.

There are exceedingly minimal reports on the uptake and elimination, and virtually nothing on the metabolism of HBCDD in humans and wildlife, although HBCCD has been shown to be rapidly absorbed from the gut in rats, and have a short whole-body half-life (2 h). Conflicting data exists whether HBCDD can be excreted in the urine or whether accumulation can occur in organs other than adipose tissue. No human toxicokinetic data are available for HCBDD, nor are any available studies for other routes of exposure, i.e., inhalation or dermal.

Many of the references regarding early studies on TBBPA, HCBDD, and BTBPE are not available in peer-reviewed journals, and therefore, not available to the general researcher. They are commonly papers compiled by the industry and submitted to regulatory bodies. Toxicokinetic data is very limited for BFRs. There are limited data especially for PBDEs (BDE-47, -99, -100, and -209),

HBCDD and TBBPA. Species-specific differences are seen in metabolic capacity. However, in humans and in more ecotoxicologically relevant animals, i.e., mammals, birds, and fish, more toxicokinetics study is required. It is a clear conclusion from this summary that more research is necessary on BFR metabolism with respect to, e.g., uptake and elimination kinetics, metabolic pathways, inter-species differences and the influence of congener and BFR structure on metabolic susceptibility, and the presence of BFR metabolite residues in exposed species.

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