Science of the Total Environment 653 (2019) 1333–1342

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Evaluation of the bioaccumulation potential of selected alternative brominated flame retardants in marine fish using *in vitro* metabolic transformation rates

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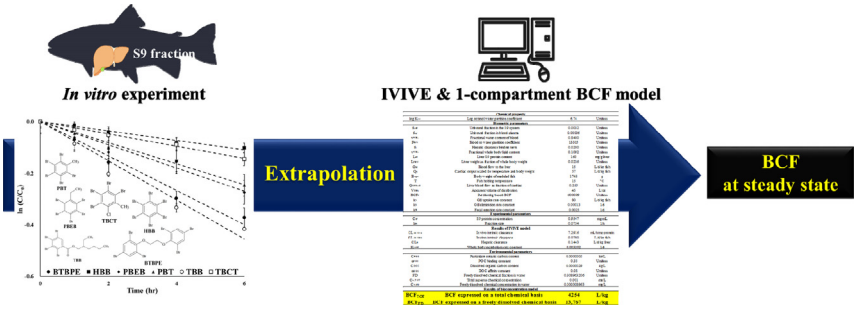
HIGHLIGHTS

- Bioaccumulation potential of 6 novel BFRs was evaluated.
- Biotransformation rates were measured using isolated S9 from marine fish.
- *In vitro* biotransformation rate was extrapolated to BCF using by an IVIVE model.
- Less hydrophobic BFRs ($6 \leq \log K_{ow} \leq 7$) would be classified as bioaccumulative.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 23 July 2018
Received in revised form 31 October 2018
Accepted 31 October 2018
Available online 05 November 2018

Editor: Adrian Covaci

Keywords:
Bioconcentration
Biotransformation
Marine fish
Alternatives

ABSTRACT

The global consumption of alternative brominated flame retardants (BFRs) has increased with the restriction of the first generation BFRs such as polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs). However, many alternative BFRs are suspected to be persistent in the environment and possibly bioaccumulative after their release into the environment because of their chemical properties, which are similar to those of the already banned BFRs. In this study, the bioaccumulation potential of selected alternative BFRs (1,2-bis (2,4,6 tribromophenoxy)ethane (BTBPE), 1,2,3,4,5,6 hexabromobenzene (HBB), pentabromoethylbenzene (PBEB), 2,3,4,5,6 pentabromotoluene (PBT), 2 ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB), and 2,3,4,5 tetrabromo-6-chlorotoluene (TBCT)) was evaluated. The *in vitro* depletion rate constants (k_{depl}) were measured for the alternative BFRs using liver S9 fractions isolated from five marine fish species (*Epinephelus septemfasciatus*, *Konosirus punctatus*, *Lateolabrax japonicus*, *Mugil cephalus*, and *Sebastes schlegelii*) that inhabit the oceans off the Korean coast. The measured k_{depl} values were converted to *in vitro* intrinsic clearance rate constants ($CL_{in\ vitro}$) to estimate whole-body metabolic rate constants (k_{MET}) using an *in vitro* to *in vivo* extrapolation (IVIVE) model. Finally, the bioconcentration factors (BCF) were determined using a one-compartment model. The transformation kinetics for obtaining k_{depl} agreed well with first-order chemical kinetics, regardless of initial BFR concentrations. The values of $CL_{in\ vitro}$ were lower in the selected marine fish species than those in freshwater fish species, implying slower metabolic transformation. The derived BCF values based on the total concentration in water (BCF_{TOT}) ranged from 16 (TBB in *M. cephalus*) to 27,000 (HBB in *K. punctatus*). The BCF values for HBB and PBT were >2000 except for those in *M. cephalus* suggesting further investigation of BCF values of BFRs whose log K_{OW} is between 6 and 7.

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https://doi.org/10.1016/j.scitotenv.2018.10.432
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1. Introduction

Flame retardants are added to various products to provide fire resistance. The global consumption of flame retardants has increased with the increasing use of plastic products (Norwegian Institute for Air Research, 2008; Shi et al., 2018; Watanabe and Sakai, 2003). One of the most commonly used groups of flame retardants are organic halogen compounds. Among them, brominated flame retardants (BFRs) have been widely used in Asia (Norwegian Institute for Air Research, 2008). The first-generation BFRs include polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) (de Wit, 2002; Norwegian Institute for Air Research, 2008). Their commercial uses in products are now restricted under the Stockholm Convention on persistent organic pollutants (POPs) because of their environmental persistence, bioaccumulation potential, and toxicity (Brown et al., 2014; Crump et al., 2008; Fernie et al., 2009; Luo et al., 2017; UNEP, 2009a, 2009b, 2013). Selected congeners of PBDEs and HBCDs have been listed for addition to Annex A of the Stockholm Convention in 2009 and 2013, respectively (UNEP, 2009a, 2009b, 2013). With this global regulation, alternative chemicals with similar chemical structures, so-called novel brominated flame retardants (NBFRs), have been introduced to the market, and their production volume has increased (Bergman et al., 2012; Covaci et al., 2011; Nguyen et al., 2017; Norwegian Institute for Air Research, 2008). It is not surprising that the alternatives share similar chemical characteristics compared to those of PBDEs and HBCDs because the bromine radicals produced from bromine-containing aromatic organic molecules are good quenchers of the hydrogen and hydroxyl radicals in flames (Rahman et al., 2001).

Unfortunately, the POP-like properties of NBFRs cannot be separated from their effectiveness as flame retardants. Aromatic bromines are not readily decomposed by aerobic microorganisms (Schwarzenbach et al., 2017), rendering their environmental half-lives sufficiently long to allow for long-range transport. In addition, their limited rate of enzymatic degradation might result in significant bioaccumulation of alternative BFRs, which could be toxic to humans and wildlife, especially *via* hormonal disruption, as has been demonstrated for many brominated aromatic compounds (Butt et al., 2011; Butt and Stapleton, 2013; Marvin et al., 2011; Palace et al., 2008; Smythe et al., 2017). Although many NBFRs are suspected to have POP-like properties (Wu et al., 2011; Zheng et al., 2018), their persistence, long-range transport potential, bioaccumulation potential, and toxicity have not been sufficiently evaluated to guide regulatory actions.

Bioaccumulation potential is an important criterion that determines whether chemicals are classified as POPs. To make a regulatory decision on a weight-of-evidence basis, the observed accumulation of chemicals in aquatic and terrestrial food chains is the most important aspect to consider. However, a decision based on strong evidence from field monitoring can be made only after the target chemical has been widely disseminated and globally distributed, failing to satisfy the precautionary principle. The laboratory bioconcentration factor (BCF) is obtained following a standard method, such as the Organization for Economic Cooperation and Development (OECD)'s test guidelines for experiments with fish (OECD, 2012) as a second-tier method. However, the experimental determination of BCF requires many animals and is expensive. In addition, many NBFRs are only sparingly soluble in water, making the experimental determination of fish BCF extremely difficult. Therefore, as an alternative, various bioconcentration/bioaccumulation models may be used (Arnot and Gobas, 2004; Mackay, 1982; Meylan et al., 1999; Nichols et al., 2013; OECD, 2018a, 2018b).

The most sensitive parameter in existing bioconcentration/bioaccumulation models for hydrophobic organic chemicals is the (whole-body) biotransformation rate constant (Arnot and Gobas, 2004; Nichols et al., 2013; OECD, 2018a, 2018b). In fish, the liver is considered to be the most important organ for clearing xenobiotic chemicals from the body. Liver microsomes and protein fractions such as the S9 fraction have been isolated from various fish species and the *in vitro* biotransformation rates have been evaluated assuming pseudo-first-order kinetics (Johanning et al., 2012). In addition, standardized methods have been accepted by the OECD (OECD, 2018a, 2018b). To evaluate the whole-body BCF/BAF, extrapolation of the *in vitro* biotransformation rate to the whole-body biotransformation rate is necessary. Nichols and colleagues proposed an *in vitro* to *in vivo* extrapolation (IVIVE) model and evaluated the model performance using rainbow trout as a model fish (Nichols et al., 2013).

Although laboratory testing and models regarding the assessment of bioaccumulation potentials of hydrophobic organic chemicals have been mostly developed with freshwater organisms, bioaccumulation in marine organisms is a significant concern in marine ecosystems (McGeer et al., 2003). In addition, organic chemicals accumulated in marine biota can be biotransferred to humans through the consumption of marine products, such as fish, shellfish, and seaweed (Kim and Kang, 2017). Thus, it is important to evaluate the bioaccumulation potential of POP-like chemicals using marine organism models.

In this study, five marine fish species (*Epinephelus septemfasciatus* (convict grouper), *Konosirus punctatus* (dotted gizzard shad), *Lateolabrax japonicus* (Japanese seabass), *Mugil cephalus* (flathead grey mullet), and *Sebastes schlegelii* (Korean rockfish)) that inhabit the coastal oceans of the Korean Peninsula were chosen considering their trophic levels in the ecosystem and propensity for human consumption. Six alternative BFRs (1,2-bis(2,4,6 tribromophenoxy)ethane (BTBPE), 1,2,3,4,5,6 hexabromobenzene (HBB), pentabromoethylbenzene (PBEB), 2,3,4,5,6 pentabromotoluene (PBT), 2 ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB), and 2,3,4,5 tetrabromo-6-chlorotoluene (TBCT)) were selected for the evaluation of bioaccumulation potential because previous modeling studies have indicated that they could be as persistent (or more), traveling long distances and bioaccumulative to a similar extent as PBDE congeners already banned under the Stockholm Convention (Jin et al., 2016; Kuramochi et al., 2014; Lee and Kwon, 2016). S9 fractions were isolated from livers of the selected fish species and the *in vitro* depletion rate constants (k_{depl}) were measured using batch kinetic experiments. The measured values of k_{depl} were converted to whole-body metabolic rate constants (k_{MET}) using an IVIVE model modified with biometric parameters measured for the selected fish species. Finally, the BCF values of the selected alternative BFRs in five fish species were obtained using a one-compartment bioconcentration model to assess bioaccumulation potential.

2. Material and methods

2.1. Chemicals

Trizma hydrochloride (Tris-HCl, ≥99%; CAS Reg. no. 1185-53-1), β-nicotinamide adenine dinucleotide phosphate, reduced tetra (cyclohexylammonium) salt (NADPH, ≥93%; CAS Reg. no. 100929-71-3), glucose-6-phosphate potassium salt (G6P, 98%; CAS Reg. no. 103192-55-8), resorufin (95%; CAS Reg. no. 635-78-9), 7 ethoxyresorufin (≥95%; CAS Reg. no. 5725-91-7), ethyl 3-aminobenzoate methanesulfonate (MS-222, CAS Reg. no. 886-86-2) for anesthetization, and bovine serum albumin (BSA, ≥98%; CAS Reg. no. 9048-46-8) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Six alternative BFRs were selected to evaluate their bioaccumulation potential. Hexabromobenzene (HBB, >98%; CAS Reg. no. 87-82-1) and 2,3,4,5,6 pentabromoethylbenzene (PBEB, 99.8%; CAS Reg. no. 85-22-3) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 1,2-Bis(2,4,6 tribromophenoxy)ethane (BTBPE, 98%; CAS Reg. no. 37853-59-1), 2,3,4,5 tetrabromo-6-chlorotoluene (TBCT, CAS Reg. no. 39569-21-6), and 2 ethylhexyl 2,3,4,5 tetrabromobenzoate (TBB, >98%; CAS Reg. no. 183658-37-7) were purchased from AccuStandard (New Haven, CT, USA). 2,3,4,5,6-Pentabromotoluene (PBT, >98%; CAS Reg. no. 87-83-2) was purchased from Tokyo Chemical Industry (Tokyo, Japan). The chemical structures and log K_{OW} values of all selected alternative BFRs are shown in Fig. 1.

2.2. Animals

The subject fish species were chosen based on those consumed by Koreans considering the trophic levels in the marine food web: *E. septemfasciatus* (convict grouper), *K. punctatus* (dotted gizzard shad), *L. japonicus* (Japanese seabass), *M. cephalus* (flathead grey mullet), and *S. schlegelii* (Korean rockfish). According to the FishBase database (FishBase, 2018), the range of the trophic levels for the five selected fish species are reported to range from 2.5 ± 0.17 (*K. punctatus*) to 4.0 ± 0.66 (*E. septemfasciatus*) and have hepatosomatic index (HSI) values ranging from 0.010 ± 0.0014 to 0.024 ± 0.0036. Physiological parameters such as the body and liver weights of the subject fish were measured and are listed in Table 1. All fish were purchased from the Noryangjin fish market in Seoul as live fish. The fish were caught near the coast of the Korean Peninsula, *i.e.*, Tongyeong (*E. septemfasciatus* and *K. punctatus*, November 2017), Seosan (*L. japonicus*; November 2017), and Boryeong (*S. schlegelii* and *M. cephalus*; June and August 2017) areas. Three samples each of *E. septemfasciatus* and *L. japonicus*, five each of *M. cephalus* and *S. schlegelii*, and eleven *K. punctatus* samples were pooled for the preparation of the liver S9 fraction.

2.3. Preparation of the liver S9 fractions

Anesthetic (MS-222; 300 mg mL^{-1}) was added to a bucket containing the fish, which were subsequently euthanized. After the fish were euthanized, they were removed from the bucket, and their lengths and weights were measured. The liver was excised from the subject fish and placed in an ice-chilled Petri dish. After measuring the liver weight, the liver tissue was rinsed with 0.15 M KCl solution. The rinsed liver tissue was then transferred to a 0.15 M KCl solution and homogenized using an HS-30E tissue homogenizer (Daihan Scientific Co., Seoul, Korea). For *E. septemfasciatus*, *L. japonicus*, *M. cephalus*, and *S. schlegelii*, the liver tissue was cut into 2 g pieces and combined for homogenization. Whole livers of *K. punctatus* (approximately 1.1 g per fish) were used for homogenization because of the smaller liver size. One milliliter of the KCl solution was added per gram of homogenate. The minced liver tissue solution was centrifuged at 10,000*g* at 4 °C for 20 min, and the supernatant (S9 fraction) was carefully taken and stored at −80 °C until use (Lo et al., 2015). The yields of the S9 fraction obtained were between 0.4 (*S. schlegelii*) and 1.2 mL g_{liver}^{-1} (*K. punctatus*).

2.4. Protein concentration in the S9 fractions and whole-body lipid contents

The Bradford reagent was diluted 5-fold in distilled water. The BSA stock solution was prepared at a concentration of 1 mg mL^{-1} and diluted to make standard solutions containing a protein concentration between 0.05 and 0.3 mg mL^{-1}. The protein concentration of the S9 fraction was determined after 4-fold dilution with distilled water. After preparing all solutions and samples, 20 μL of the BSA standard solution and 20 μL of the diluted S9 fraction were added to 1 mL of the Bradford working solution, and the mixed solution was maintained at room temperature for 5 min. The protein concentration was determined using the absorbance at 595 nm using a DR/4000 U UV/Vis spectrophotometer (Hach Co., Loveland, CO, USA) (Bradford, 1976).

Whole-body lipid content was measured using the Rose-Gottlieb method (Richardson, 1985) for homogenized tissues after separating bones and heads. Briefly, 6 mL of concentrated hydrochloric acid was added to the homogenized tissues, followed by heating at 70 °C in a water bath for 90 min. After transferring the acid-extracted solution to a Mojonnier tube, deionized water was added to a final sample volume of 20 mL. Then, 7 mL of ethanol and 25 mL of petroleum ether were added, and the tube was shaken vigorously. After the upper ether layer clarified, it was filtered through a filter paper and the mass of dried residue was measured as the dry lipid content.

2.5. Assay conditions for EROD activity analysis

The ethoxyresorufin-*o*-deethylase (EROD) method was used to determine the activity of CYP1A1, involved in xenobiotic metabolism, as an indicator of S9 activity. The reaction cocktail was comprised of 2 mM NADPH (100 μL; 20% v/v), 4 μM 7 ethoxyresorufin (5 μL; 1% v/v), S9 fraction (100 μL; 20% v/v), and 50 mM Tris buffer containing 10 mM $MgCl_2$ (295 μL; 59% v/v) (Pikkarainen, 2006; Zamaratskaia and Zlabek, 2009). The reaction was initiated by adding NADPH and 30 min after the initiation of the reaction at 15 °C, 150 μL of the reaction mixture was collected from the experimental vial, and the reaction was terminated by adding the reaction mixture to 500 μL of ice-cold methanol. EROD activity was expressed in the units of picomoles per milligram of protein per minute (pmol mg $protein^{-1}$ min^{-1}). Resorufin, used for EROD activity, was analyzed using a high-pressure liquid chromatography-fluorescence detector (HPLC-FLD). The HPLC-FLD system was composed of a Waters 515 pump, 717 autosampler, and 2475 multi λ fluorescence detector (Waters, Milford, MA, USA). The samples (10 μL) were injected onto a Fortis C18

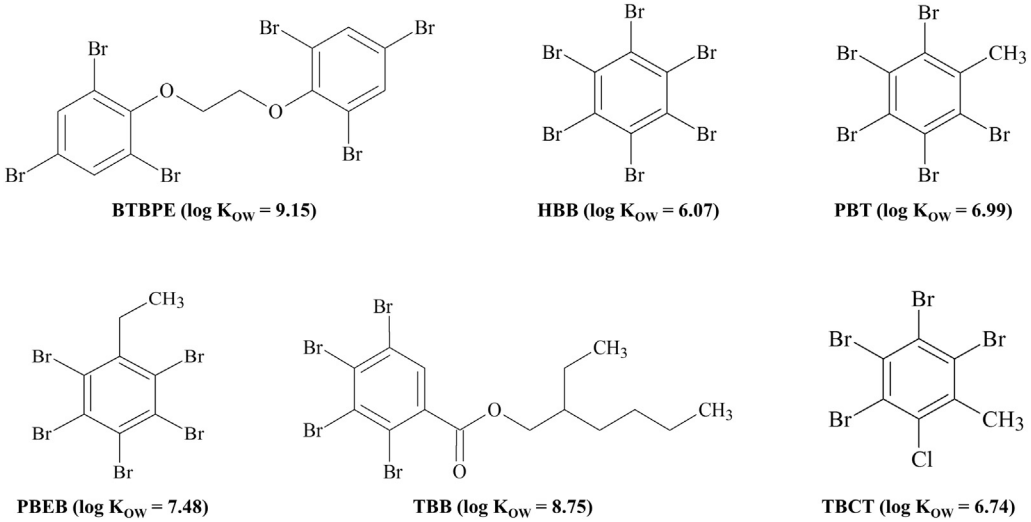

Fig. 1. Structure of the selected chemicals: 1,2-Bis(2,4,6 tribromophenoxy)ethane (BTBPE), hexabromobenzene (HBB), 2,3,4,5,6 pentabromoethylbenzene (PBEB), 2,3,4,5,6 pentabromotoluene (PBT), 2,3,4,5 tetrabromo-6-chlorotoluene (TBCT), and 2 ethylhexyl 2,3,4,5 tetrabromobenzoate (TBB).

Table 1
Physiological parameters for the studied fish.

	E. septemfasciatus (n = 3)	*K. punctatus* (n = 11)	*L. japonicas* (n = 3)	*M. cephalus* (n = 5)	*S. schlegelii* (n = 5)
Trophic level[a]	4.0 ± 0.66	2.5 ± 0.17	2.9 ± 0.22	3.4 ± 0.43	3.8 ± 0.70
Fish weight (g)[b]	1743.3 ± 58.6	74.2 ± 5.5	1868.0 ± 211.6	1395.0 ± 139.3	539.4 ± 53.9
Liver weight (g)[b]	41.1 ± 8.5	1.1 ± 0.2	18.0 ± 3.5	24.2 ± 5.5	11.6 ± 1.0
Lipid contents (%)[b]	11 ± 2	19 ± 4	2.1 ± 0.7	2.5 ± 1.0	2.4 ± 0.4
Length (cm)[b]	48.3 ± 1.5	20.9 ± 0.7	62.8 ± 3.0	54.8 ± 2.2	32.4 ± 0.5
HSI (LW/FW)[c]	0.024 ± 0.005	0.016 ± 0.002	0.010 ± 0.002	0.017 ± 0.002	0.022 ± 0.002

[a] FishBase (http://www.fishbase.org).
[b] Measured value.
[c] Calculated value using fish weight and liver weight.

column (5 μm, 150 × 4.6 mm, Fortis Technologies Ltd., Cheshire, UK) at a flow rate of 1.0 mL min^{-1}. The mobile phases were 20 mM phosphate buffer (A, pH 6.8) and methanol (B) with a gradient program (Rowland et al., 1973), starting at 30% B, followed by a 3.5 min hold, then an increase to 80% B over 2.5 min, then a 3.1 min hold, a reduction back to 30% B over 5 min, and finally a hold for 3 min.

2.6. Measurement of in vitro intrinsic clearance rate ($CL_{in\ vitro}$)

The assay for determination of *in vitro* depletion rate constant was performed in 50 mM Tris buffer (pH 7.8) containing 10 mM $MgCl_2$ at 15 °C up to 6 h to observe sufficient depletion. The reaction mixture consisted of 1 mM NADPH (120 μL; 10% *v*/v), 4 mM G6P (120 μL; 10% v/v), S9 fraction (120–360 μL; 10–30% v/v), 1 μM or 0.1 μM alternative BFR solution in dimethylsulfoxide (DMSO, 6 μL; 0.5% v/v), and the 50 mM Tris buffer containing 10 mM $MgCl_2$ solution (594–834 μL; 49.5–69.5% v/v). NADPH and G6P were freshly prepared before the assay. The initial concentration of all alternative BFRs was either 1 or 0.1 μM. To stop the reaction, a 150-μL aliquot of the reaction cocktail was taken and transferred to 1350 μL of ice-cold methanol. After centrifugation (12,000 *g* at room temperature for 20 min) of the methanol solution containing the sample aliquot, the supernatant (1000 μL) was taken and mixed with 0.5 mL of Tris buffer solution for chemical analysis. The supernatant solution was extracted by a liquid–liquid extraction method three times using 1 mL of diisopropyl ether (DIPE) and 50 μL of hydrochloric acid (1 mol L^{-1}) according to Harju et al. (2007). The extracts were combined and transferred to a vial, and the DIPE was evaporated under a gentle nitrogen stream. The evaporated residue was then dissolved in 750 μL *n*-hexane for gas chromatography (GC) analysis.

Experiments using the denatured S9 fractions were also performed to confirm that the concentration changes occurred due to biotransformation and not due to other processes such as volatilization or absorption to the reaction vial. This control experiment was performed according to the same experimental procedure described above. The denaturation of the S9 fraction was conducted by heating at 80 °C for 15 min.

For determination of the *in vitro* intrinsic clearance rate ($CL_{in\ vitro}$; mL h^{-1} $mg_{protein}^{-1}$), the measured natural log concentration of the alternative BFRs was plotted against reaction time. The obtained slope corresponds to the *in vitro* depletion rate constants (k_{depl}; h^{-1}). The k_{depl} was divided by the protein concentration of the liver S9 fraction used in the experiment (C_{S9}) to calculate $CL_{in\ vitro}$.

2.7. Quantitative analysis of alternative BFRs

Alternative BFRs were quantified using a Hewlett-Packard 5890 GC equipped with an electron capture detector (ECD). One microliter of the extract was injected in a splitless mode and was separated on a 30-m HP-5 column (i.d. 0.25 mm, 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA) using nitrogen as the carrier gas. The temperature program for the alternative BFRs (BTBPE, HBB, PBEB, PBT, TBB, and TBCT) was as follows: initial temperature of 100 °C was held for 2 min, followed by an increase at the rate of 25 °C min^{-1} to 250 °C, at 2 °C min^{-1} to 280 °C, and at 25 °C min^{-1} to 300 °C, followed by a hold for 6 min. The injector temperature was 240 °C and the detector temperature was 300 °C. The method detection limits for BTBPE, HBB, PBEB, PBT, TBB, and TBCT were 8.1, 0.50, 0.38, 1.0, 0.48, and 0.28 nM, respectively. The recoveries at an initial concentration of 1 μM were 88.1–103.3% (BTBPE), 94.8–112.5% (HBB), 89.9–100.6% (PBEB), 94.1–98.7% (PBT), 94.0–103.3% (TBB), and 89.9–102.4% (TBCT), respectively (Table S1, Supplementary Material).

2.8. In vitro to in vivo extrapolation and estimation of BCF

The IVIVE model (Nichols et al., 2013) and a one-compartment BCF model (Arnot and Gobas, 2004) were used to obtain BCF values for the fish. Because these models were originally developed for rainbow trout, fish-specific input parameters were measured and estimated using parameters applied to the selected marine fish species.

The input parameters of the IVIVE model are divided into the chemical properties (log K_{OW}) and biometric parameters (*e.g.*, liver weight as a fraction of whole-body weight (L_{FBW}), fractional whole-body lipid content (f_{lip}), blood flow to the liver (Q_H)), and detailed values for selected marine fish species are shown in Tables S2–S6 (Supplementary Material) with the calculated dependent variables. To extrapolate the $CL_{in\ vitro}$ value to the k_{MET} value, a two-step procedure is required. The value of the *in vivo* intrinsic clearance ($CL_{in\ vivo}$; L d^{-1} kg_{fish}^{-1}) is obtained using Eq. (1).

$$CL_{invivo} = CL_{invitro} \times L_{S9} \times HSI \times 24\frac{h}{d} \quad (1)$$

where L_{S9} and HSI (hepatosomatic index) are the liver S9 protein content [mg g^{-1}] and the liver weight as a fraction of whole-body weight [unitless], respectively. The value of HSI was measured when preparing the S9 fraction (Table 1), and the default value of L_{S9} for rainbow trout (163 mg g_{liver}^{-1}) (Nichols et al., 2013) was used because the S9 content was not measured for marine fish. Subsequently, the hepatic clearance rate (CL_H) was calculated using $CL_{in\ vivo}$, following a well-stirred liver model (Eq. (2)) (Rowland et al., 1973; Wilkinson and Shand, 1975).

$$CL_H = \frac{Q_H \times f_U \times CL_{invivo}}{(Q_H + f_U \times CL_{invivo})} \quad (2)$$

where Q_H is the hepatic flow rate [L d^{-1} kg_{fish}^{-1}], estimated by multiplying the value of cardiac output (Q_C) and the liver blood flow as a fraction of cardiac output (Q_{HFRAC}). The value of Q_{HAFRAC} was estimated to be 0.259 for rainbow trout (Nichols et al., 1990) and used as a default. The Q_C was calculated using an empirical equation by Erickson and McKim, as (Erickson and McKim, 1990)

$$Q_C = \left[(0.023T - 0.78) \times \left(\frac{Bwg_M}{500}\right)^{-0.1}\right] \times 24 \quad (3)$$

where Bwg_M is the weight of the subject fish [g] and T is the temperature [°C]. In Eq. (2), f_U is the hepatic clearance binding term, defined as the

ratio of the unbound chemical fraction in the *in vitro* system ($f_{U,S9}$) to that in the blood plasma ($f_{U,P}$). The $f_{U,S9}$ is related to the liver S9 fraction used in the experiment (C_{S9}) and log K_{OW}. The $f_{U,P}$ is determined by dividing fractional water content of the blood (V_{WBL}) by blood-to-water partition coefficient (P_{BW}) (Nichols et al., 1990; Han et al., 2009).

$$f_{U,S9} = \frac{1}{\left(C_{S9} \times 10^{(0.694\times\ logK_{OW}-2.158)} + 1\right)} \quad (4)$$

$$f_{U,P} = \frac{V_{WBL}}{P_{BW}} \quad (5)$$

$$f_U = \frac{f_{U,P}}{f_{U,S9}} \quad (6)$$

The value of V_{WBL} was assumed to be 0.84 based on previous measurements in rainbow trout (Bertelsen et al., 1998), and the values of P_{BW} were calculated using the empirical equation proposed by Fitzsimmons et al. (Eq. (7)) (Fitzsimmons et al., 2001).

$$P_{BW} = \left(10^{0.73\times\ logK_{OW}} \times 0.16\right) + 0.84 \quad (7)$$

The whole-body first-order metabolic rate constant (k_{MET}) was obtained by dividing CL_H by the apparent volume of distribution (V_{DBL}), which was estimated by dividing the partitioning-based bioconcentration factor (BCF_P), obtained by multiplying K_{ow} and whole-body lipid content (f_{lip}), by P_{BW}.

$$k_{MET} = \frac{CL_H}{V_{DBL}} \quad (8)$$

$$BCF_P = \frac{f_{lip}}{K_{OW}} \quad (9)$$

$$V_{DBL} = \frac{BCF_P}{P_{BW}} \quad (10)$$

Finally, a steady-state one-compartment bioaccumulation model including gill uptake and elimination, fecal egestion, and metabolic transformation (Arnot and Gobas, 2006) was used to estimate whole-body BCF:

$$\frac{dC_{fish}}{dt} = (k_1 C_{W,FD}) - (k_2 + k_E + k_{MET})C_{fish} \quad (11)$$

$$C_{W,TOT} = (1 + \alpha_{DOC} C_{DOC} K_{OW} + \alpha_{POC} C_{POC} K_{OW}) C_{W,FD} \quad (12)$$

$$BCF_{FD} = \frac{C_{fish}}{C_{W,FD}} = \frac{k_1}{(k_2 + k_E + k_{MET})} \quad (13)$$

$$BCF_{TOT} = \frac{C_{fish}}{C_{W,TOT}} \quad (14)$$

where $C_{W,FD}$ and $C_{W,TOT}$ are the freely dissolved and total chemical concentrations in the water and C_{fish} is the chemical concentration in fish, k_1 and k_2 are the gill uptake and elimination rate constants, k_E is the fecal egestion constant, α_{DOC} and α_{POC} are affinity constants for dissolved organic carbon (DOC) and particulate organic carbon (POC), and C_{DOC} and C_{POC} are the DOC and POC concentrations in water. Values of α_{DOC} and α_{POC} were assumed as 0.08 (Burkhard, 2000) and 0.35 (Seth et al., 1999), respectively. The values of C_{DOC} and C_{POC} were assumed to be 2.9 and 0.5 mg L^{-1} using the default values by the US EPA (US EPA, 2003). The values of k_1, k_2, and k_E were obtained using empirical equations, and were related to Bwg_M, T, and K_{OW} (Arnot and Gobas, 2003) and expressed as:

$$k_1 = \frac{1}{\left(\left(0.01 + \frac{1}{K_{OW}}\right) \times {Bwg_M}^{0.4}\right)} \quad (15)$$

$$k_2 = \frac{k_1}{BCF_P} \quad (16)$$

$$k_E = 0.125 \times \left(\frac{0.02 \times {Bwg_M}^{-0.15} \times e^{0.06T}}{5.1 \times 10^{-8} K_{OW} + 2}\right) \quad (17)$$

3. Results and discussion

3.1. Protein content and S9 enzyme activity in S9 fractions

The measured protein concentrations and EROD activities of the isolated S9 fractions are shown in Table 2. The highest protein concentration was measured in the *L. japonicus* S9 fraction (5.06 ± 0.06 mg mL^{-1}), followed by *M. cephalus*, *E. septemfasciatus*, *K. punctatus*, and *S. schlegelii* in order. The EROD activity was measured at 15 °C, which was chosen based on the average temperature of seawater near the Korean Peninsula (Flammarion et al., 1996). The EROD activity was highest in *L. japonicus* (3.18 ± 0.04 pmol $mg_{protein}^{-1}$ min^{-1}) and the lowest in *S. schlegelii* (0.52 ± 0.15 pmol $mg_{protein}^{-1}$ min^{-1}).

The EROD activities obtained in this study were slightly lower than those previously reported in S9 fractions of many freshwater and marine fish (Della Torre et al., 2014; Flammarion et al., 1996; Pikkarainen, 2006; Strobel et al., 2015; Webb and Gagnon, 2002). We could only find EROD activities for the fish species belonging to the same orders as *M. cephalus* and *L. japonicus* in the literature (Della Torre et al., 2014; Pikkarainen, 2006; Webb and Gagnon, 2002). The reported EROD activities were 2.7 ± 3.9 pmol mg $protein^{-1}$ min^{-1} (Baltic sea perch, *Perca fluviatilis*) (Pikkarainen, 2006), 7 to 15 pmol mg $protein^{-1}$ min^{-1} (sea bass, *Dicentrarchus labrax*) (Della Torre et al., 2014), and 9.2 ± 1.2 pmol mg $protein^{-1}$ min^{-1} (sea mullet, *Mugil cephalus*) (Webb and Gagnon, 2002). These values were higher than those of *M. cephalus* and *L. japonicus* (2.61 ± 0.19 and 3.18 ± 0.04 pmol mg $protein^{-1}$ min^{-1}, respectively) obtained in this study by a factor of 2.2–4.7. The EROD activity is related to the sexual maturity and environmental temperature. It has been acknowledged that the EROD activity is higher in immature fish and at lower temperature (Sleiderink et al., 1995). Higher EROD activities in the earlier studies (Della Torre et al., 2014; Pikkarainen, 2006; Webb and Gagnon, 2002) were obtained for immature fish, whereas adult fish were used in this study. It is also known that EROD activities are greater at lower temperature (Ferreira et al., 2006). Because *M. cephalus* were caught in August when seawater temperature was warmer, the EROD activity may be underestimated.

The EROD activities in the S9 fraction of rainbow trout, a representative model freshwater fish species, were found to be between 11 and

Table 2
The protein concentration and EROD activity of the S9 fractions of the fish species tested.

Species	Protein concentration (mg mL^{-1})	EROD activity (pmol mg $protein^{-1}$ min^{-1})
E. septemfasciatus	4.81 ± 0.04	1.76 ± 0.19
K. punctatus	3.88 ± 0.03	2.28 ± 0.42
L. japonicas	5.06 ± 0.06	3.18 ± 0.04
M. cephalus	4.91 ± 0.17	2.61 ± 0.19
S. schlegelii	3.72 ± 0.16	0.52 ± 0.15

129 pmol mg protein^{-1} min^{-1} (Connors et al., 2013; Flammarion et al., 1996; Smith and Wilson, 2010; Strobel et al., 2015). Although the EROD activities depend on age and sex, the approximately one order-of-magnitude lower values imply that CYP1A-related metabolic activity would be lower in these selected marine fish species than in rainbow trout. These results suggest that the CYP-related xenobiotic transformation rates of hydrophobic organic chemicals in marine fish species might be low, leading to higher bioaccumulation factors when biotransformation is dominated by CYP-related enzymatic processes.

3.2. Determination of in vitro intrinsic clearance

The metabolic transformation rate is often described by Michaelis–Menten kinetics, in which the apparent reaction rate is described by pseudo-first-order reaction kinetics at sufficiently low substrate concentrations. Because the concentration of hydrophobic organic contaminants, such as BFRs in this study, is much lower than the typical half-saturation constant (K_M) in Michaelis–Menten kinetics, k_{depl} should be determined in the range where the Michaelis–Menten kinetics is approximated to first-order transformation kinetics to accurately reflect the environmental levels of BFRs. Because it is difficult to determine Michaelis–Menten parameters for the alternative BFRs for which major transformation products have not been identified and the solubility in water is very low, k_{depl} values were measured at two different initial BFR concentrations to ensure that the obtained k_{depl} is independent of initial concentration. Fig. 2 shows representative examples determining k_{depl} for TBB and TBCT in *E. septemfasciatus* and *K. punctatus*. All experimental results are shown in Figs. S1 to S5 (Supplementary Material). As shown in Figs. 2, S1–S5 and Table S7 (Supplementary Material), the measured values of k_{depl} at two different initial concentrations did not significantly differ from each other. Paired *t*-test for k_{depl} values at two different initial concentrations showed that all *p* values were >0.05 except for TBB in *Epinephelus septemfasciatus* ($p = 0.022$). However, k_{depl} values at two different initial concentration did not differ much (0.26 and 0.27 h^{-1}; Table S7, Supplementary Material). In many cases, the remaining concentration after 2 h was not significantly different ($p = 0.05$) from the initial concentration at 0 h. In addition, k_{depl} values between 0 and 6 h were not significantly different from those between 0 and 2 h for most cases at $p = 0.05$. Thus, the slopes of the entire time range (0–6 h) were used to obtain k_{depl} although it is expected that some enzymatic activity would be lost with time (Johanning et al., 2012). Furthermore, no concentration changes of all BFRs were observed using the heat-denatured S9 fraction for five marine fish species, suggesting that the depletion of BFRs obtained using the activated S9 fraction is the result of biotransformation.

The values of $CL_{in\ vitro}$ at the two different initial BFR concentrations are shown in Table 3. The obtained values were within a factor of 17, ranging from 0.034 to 0.57 mL h^{-1} mg$_{protein}^{-1}$, with chemical-to-chemical and species-to-species variations in $CL_{in\ vitro}$. The $CL_{in\ vitro}$ of TBB was the highest of all the tested compounds in the five fish species, except for that of the *M. cephalus* S9 fraction. TBCT was the most rapidly depleted BFR in the *M. cephalus* S9 fraction. The $CL_{in\ vitro}$ values for all

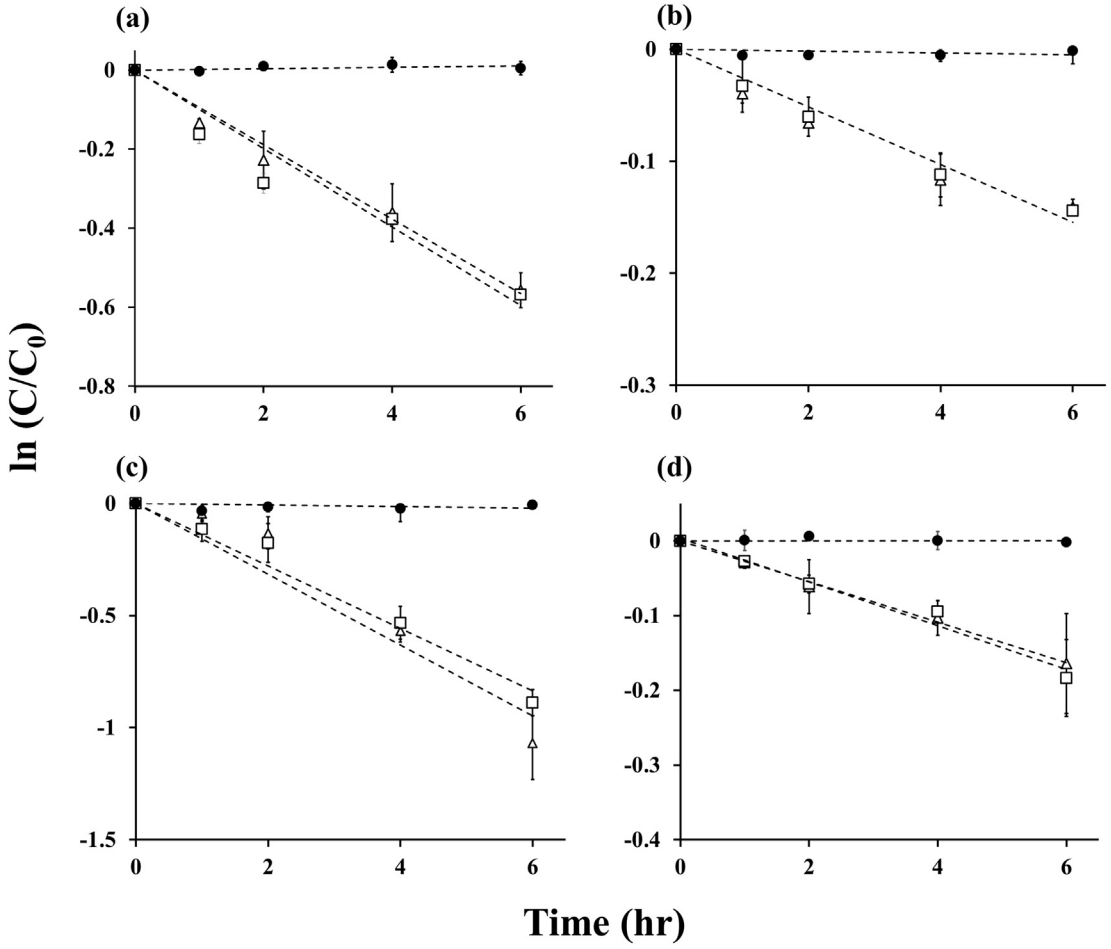

Fig. 2. Decline of the natural logarithm of the concentration of selected alternative BFRs: (a) 2-ethylhexyl-2,3,4,5 tetrabromobenzoate (TBB) in *Epinephelus septemfasciatus*, (b) 2,3,4,5 tetrabromo-6-chlorotoluene (TBCT) in *Epinephelus septemfasciatus*, (c) TBB in *Konosirus punctatus*, and (d) TBCT in *Konosirus punctatus*. Open squares and triangles represent results obtained at initial concentrations of 0.1 and 1 μM, respectively. Closed circles present concentration changes using denatured S9 fractions at 1 μM.

Table 3
Calculated *in vitro* intrinsic clearance rate ($CL_{in\ vitro}$; mL h^{-1} $mg_{protein}^{-1}$) for the BFR alternatives.

BFR abbreviation	BFR concentration (μM)	Species				
		E. septemfasciatus	*K. punctatus*	*L. japonicus*	*M. cephalus*	*S. schlegelii*
BTBPE	1	0.16 ± 0.011	0.16 ± 0.008	0.13 ± 0.02	0.20 ± 0.01	0.18 ± 0.02
	0.1	0.15 ± 0.010	0.16 ± 0.003	0.13 ± 0.02	0.19 ± 0.01	0.18 ± 0.01
HBB	1	0.094 ± 0.008	0.061 ± 0.004	0.058 ± 0.015	0.13 ± 0.01	0.048 ± 0.009
	0.1	0.087 ± 0.005	0.063 ± 0.003	0.061 ± 0.005	0.12 ± 0.01	0.052 ± 0.004
PBEB	1	0.11 ± 0.01	0.052 ± 0.006	0.14 ± 0.02	0.40 ± 0.01	0.12 ± 0.01
	0.1	0.10 ± 0.01	0.052 ± 0.006	0.13 ± 0.01	0.40 ± 0.02	0.13 ± 0.01
PBT	1	0.14 ± 0.01	0.05 ± 0.003	0.06 ± 0.003	0.28 ± 0.01	0.11 ± 0.01
	0.1	0.14 ± 0.01	0.05 ± 0.01	0.06 ± 0.003	0.27 ± 0.01	0.11 ± 0.01
TBB	1	0.27 ± 0.01	0.20 ± 0.02	0.19 ± 0.01	0.50 ± 0.00	0.20 ± 0.05
	0.1	0.29 ± 0.01	0.18 ± 0.01	0.20 ± 0.004	0.50 ± 0.01	0.19 ± 0.02
TBCT	1	0.079 ± 0.003	0.035 ± 0.011	0.034 ± 0.002	0.63 ± 0.09	0.064 ± 0.022
	0.1	0.076 ± 0.001	0.037 ± 0.007	0.051 ± 0.002	0. 62 ± 0.06	0.076 ± 0.012

BFRs were greatest in the *M. cephalus* S9 fraction. For the other fish species, no trend in metabolic capacity was observed.

The measured $CL_{in\ vitro}$ values did not show a clear correlation with EROD activity (Fig. S6, Supplementary Material). BFRs are thought to be metabolized *via* several pathways including oxidative debromination, reductive debromination, and oxidative CYP enzyme-mediated pathways (phase I and II) (Hakk and Letcher, 2003). Because the experimental conditions of the *in vitro* biotransformation favor oxidative pathways, reductive debromination and phase II biotransformation would not be represented in the incubation conditions of the present study. The structural similarity of alternative BFRs in this study with polybrominated benzenes (PBBs) and PBDEs implies that other oxidative enzymes could be also important. Cytochrome enzymes such as CYP2B and CYP4A also play significant roles in transforming PBBs and PBDEs (Hakk and Letcher, 2003). Activities of other oxidative enzymes are not well represented by the EROD activity used for estimating CYP1A activity. It needs further investigations to know the major biotransformation pathways.

No studies have reported the *in vitro* intrinsic clearance rates using fish S9 fractions for the alternative BFRs chosen in this study. Only one study has reported the *in vitro* clearance rate constants for TBB using rat and human liver microsomes and cytosols (Roberts et al., 2012). The *in vitro* clearance rate constants in the rat liver cytosol and microsomes are higher than those in human liver cytosol and microsomes. The *in vitro* clearance rate constant of TBB in human liver cytosol and microsomes were 12.42 ± 0.020 and 15.54 ± 0.033 μM h^{-1} $mg_{protein}^{-1}$ and those in rat liver cytosol and microsomes were 25.32 ± 0.093 and 375 ± 0.058 μM h^{-1} $mg_{protein}^{-1}$ (Roberts et al., 2012). The corresponding range for TBB at an initial concentration of 1 μM in the S9 fractions of the five marine fish species (0.19 ± 0.010 to 0.45 ± 0.001 μM h^{-1} $mg_{protein}^{-1}$, Table 3) are orders of magnitude lower than those in rat and human liver cytosols and microsomes. Although further investigations are needed, these results suggest slower metabolic transformation rates in marine fish compared to those in mammals, implying a higher bioaccumulation potential of alternative BFRs for these marine fish.

3.3. Extrapolation to the whole-body metabolic rate constant (k_{MET})

As shown in Table 3, the values of $CL_{in\ vitro}$ did not depend on the initial concentration of the BFR alternatives. Thus, the results obtained for the initial concentration of 1 μM were used to derive the whole-body metabolic rate constants (k_{MET}; d^{-1}), which are listed in Table 4. The k_{MET} values in *E. septemfasciatus*, *K. punctatus*, and *L. japonicus* S9 fractions were the lowest for BTBPE and the highest for HBB. For S9 fractions from *S. schlegelii*, PBT had the greatest k_{MET} values, followed by HBB, PBEB, TBCT, TBB, and BTBPE in decreasing order. The values of k_{MET} among different fish species decreased in the order *M. cephalus* > *E. septemfasciatus* > *L. japonicus* > *S. schlegelii* > *K. punctatus*, except for those for PBEB and PBT.

The k_{MET} value for a given compound not only depends on the metabolic capability of the fish species, represented by k_{depl} or $CL_{in\ vitro}$, but also are affected by bioavailability of the compound denoted by f_U, as demonstrated in Eqs. (4)–(6). Higher protein contents lower the bioavailability of hydrophobic BFRs, resulting in decreased k_{MET} when other conditions are identical. Thus, the k_{MET} values in Table 4 do not coincide with the $CL_{in\ vitro}$ values in the same table. In contrast, the $CL_{in\ vitro}$ for BTBPE was greatest under *in vitro* conditions, and k_{MET} was calculated to be smaller than those of the other BFRs. This is because BTBPE has the highest log K_{OW} value (9.15) of the alternative BFRs tested, leading to the lowest unbound fraction in the liver (f_U) and lowest k_{MET} values.

3.4. Evaluation of bioaccumulation potential using a one-compartment fish model

The calculated BCF values based on the total concentration in water (BCF_{TOT}), freely dissolved concentration (BCF_{FD}), and assuming no metabolism ($BCF_{k_{MET}=0}$) are summarized in Table 5. The range of $BCF_{k_{MET}=0}$ values ranged between 1800 (BTBPE in *E. septemfasciatus*) and 34,000 (HBB in *K. punctatus*) and values in *K. punctatus* were greater than those of the other four fish species. The BCF_{FD} values ranged from 710 (TBCT in *M. cephalus*) to 570,000 (BTBPE in *K. punctatus*). Except

Table 4
Calculated whole-body metabolism rate constant (k_{MET}; d^{-1}) for the BFR alternatives.

Species	Abbreviation of BFRs					
	BTBPE	HBB	PBEB	PBT	TBB	TBCT
E. septemfasciatus	0.0012 ± 0.0004	0.0061 ± 0.0019	0.0027 ± 0.0008	0.0048 ± 0.0015	0.0026 ± 0.0008	0.0032 ± 0.0010
K. punctatus	0.00037 ± 0.00012	0.0013 ± 0.0004	0.00040 ± 0.00013	0.00054 ± 0.00017	0.00067 ± 0.00022	0.00045 ± 0.00020
L. japonicas	0.0011 ± 0.0006	0.0045 ± 0.0027	0.0040 ± 0.0022	0.0024 ± 0.0013	0.0022 ± 0.0012	0.0025 ± 0.0014
M. cephalus	0.0075 ± 0.0044	0.041 ± 0.024	0.046 ± 0.027	0.047 ± 0.027	0.024 ± 0.014	0.12 ± 0.07
S. schlegelii	0.0021 ± 0.0011	0.0053 ± 0.0028	0.0048 ± 0.0024	0.0062 ± 0.0031	0.0033 ± 0.0018	0.0044 ± 0.0027

Values of k_{depl} at initial concentration of 1 μM were used to obtain k_{MET} values. Errors were calculated using error propagation of standard deviations of experimental values used for calculation of k_{MET}.

Table 5
Calculated BCF_{TOT}, BCF_{FD}, and $BCF_{KMET=0}$ of the BFR alternatives.

Species	Model results	Abbreviation of BFRs					
		BTBPE	HBB	PBEB	PBT	TBB	TBCT
BCF_{TOT} (L kg_{fish}^{-1})	*E. septemfasciatus*	110 ± 40	5700 ± 1900	1400 ± 500	2300 ± 800	130 ± 40	4300 ± 1400
	K. punctatus	990 ± 370	27,000 ± 10,000	7100 ± 2700	13,000 ± 500	2000 ± 800	1900 ± 9000
	L. japonicas	110 ± 70	5000 ± 3500	1000 ± 670	3100 ± 2000	140 ± 90	4300 ± 2700
	M. cephalus	20 ± 14	1300 ± 900	140 ± 97	360 ± 250	16 ± 11	220 ± 160
	S. schlegelii	97 ± 52	6600 ± 3700	1400 ± 700	2700 ± 1400	160 ± 90	4800 ± 3000
BCF_{FD} (L kg_{fish}^{-1})	*E. septemfasciatus*	64,000 ± 22,000	8500 ± 2900	19,000 ± 6000	11,000 ± 4,00	29,000 ± 1000	14,000 ± 5,00
	K. punctatus	570,000 ± 210,000	40,000 ± 15,000	94,000 ± 36,000	65,000 ± 24,000	470,000 ± 180,000	60,000 ± 29,000
	L. japonicas	66,000 ± 44,000	7400 ± 5100	14,000 ± 9000	15,000 ± 10,000	33,000 ± 32,000	14,000 ± 9000
	M. cephalus	12,000 ± 8000	1900 ± 1300	1800 ± 1300	1800 ± 1300	3700 ± 2600	710 ± 510
	S. schlegelii	56,000 ± 30,000	9700 ± 5400	18,000 ± 10,00	14,000 ± 7500	36,000 ± 21,000	15,000 ± 9000
$BCF_{KMET=0}$ (L kg_{fish}^{-1})	*E. septemfasciatus*	1800 ± 300	16,000 ± 2000	3700 ± 500	6900 ± 1000	1900 ± 300	9500 ± 1400
	K. punctatus	4000 ± 800	34,000 ± 7000	8100 ± 1600	15,000 ± 3000	4100 ± 800	21,000 ± 4000
	L. japonicas	1700 ± 600	8900 ± 3100	3400 ± 1200	6000 ± 2100	1800 ± 600	7700 ± 2700
	M. cephalus	1900 ± 800	10,000 ± 4000	3700 ± 1500	6500 ± 2600	1900 ± 800	8400 ± 3300
	S. schlegelii	2300 ± 400	11,000 ± 2000	4600 ± 800	7900 ± 1400	2400 ± 400	10,000 ± 1700

Errors of BCF values were obtained by error propagation of standard deviations of input parameters.

for *M. cephalus*, BTBPE and HBB had the highest and lowest BCF_{FD} values, respectively, in the marine fish species. The calculated BCF_{TOT} values of HBB and PBT for four fish species (except for *M. cephalus*) were ≥2,000, the regulatory limit for bioaccumulation (TSCA, 2017). The lowest value was 16 (TBB in *M. cephalus*) and the highest values was 27,000 (HBB in *K. punctatus*) and the BCF_{TOT} values for HBB were highest for all marine fish species. For all BFRs, the lowest BCF_{TOT} values were estimated for *M. cephalus*, mostly because of the highest $CL_{in\ vitro}$ for the individual BFRs. Because metabolic transformation is regarded as the key process determining the bioaccumulation potential (Arnot and Gobas, 2006; Nichols et al., 2013; Papa et al., 2014), the calculated BCF_{TOT} values are inversely proportional to k_{MET}. The lowest k_{MET} values in *K. punctatus* led to the highest BCF_{TOT} of the five species for the tested BFR.

Experimental BCF_{TOT} values for selected BFRs are scarce. To the best of our knowledge, only two peer-reviewed publications reported the BAF of alternative BFRs in this study (Law et al., 2006; Wu et al., 2011). The reported BAF values of BTBPE in Lake Winnipeg ranged between 1.3 and 26 L kg^{-1} (Law et al., 2006), slightly lower than the estimated values in this study (20–990 L kg^{-1}). On the other hand, the reported BAF values of BTBPE, HBB, PBEB, and PBT for mud carp, crucian carp, and northern snakehead in South China were mostly >2000. Those values are close to our estimated BCF_{TOT} values for HBB, PBEB, and PBT. For highly hydrophobic BTBPE, the measured values were higher than the estimated BCF_{TOT}. The differences might be from the method used for determining the concentration in water. Wu et al. (2011) used XAD-2/XAD-4 adsorption, in which DOC and POC phases are partly separated. Thus, the water concentration might be between $C_{W,TOT}$ and $C_{W,FD}$. Other aspects that need to be considered when comparing BCF with field derived BAF include dietary exposure resulting BAFs higher than BCFs and long time to attain steady-state.

The use of *in vitro* clearance experiments coupled with the IVIVE model to determine whole-body k_{MET} and BCF/BAF values has limitations. One issue is whether liver metabolism is dominant for alternative BFRs. In fish, other organs, such as kidney and intestines, also transform xenobiotics *via* multiple pathways (Chambers and Yarbrough, 1976; Lo et al., 2016) When xenobiotics are readily metabolized in other organs at comparable rates to those in the liver, the k_{MET} values obtained may be underestimated because only liver metabolism was considered. The IVIVE model employs physiological parameters (*e.g.*, V_{WBL}, Q_{HFRAC}, and f_U) that were developed for rainbow trout, which adds uncertainties of the model predictions of BFR accumulation potential in the marine fish studied here. Another issue is the assumption of a steady-state in the model. Because the time to reach the steady-state increases with increasing hydrophobicity (*i.e.*, log K_{OW}) or decreasing water solubility, an extremely high value of log K_{OW} for alternative BFRs, such as for BTBPE, inevitably includes great uncertainties. The sensitivity of the model-calculated BCF value to incremental changes in K_{OW} as an input variable was evaluated, and the detailed results are presented in Tables S8 and S9 (Supplementary Material). The calculated BCF values decreased moderately with increasing K_{OW} except for that of HBB, the least hydrophobic BFR tested. Decrease in the bioavailable fraction ($C_{W,FD}/C_{W,TOT}$) with increasing K_{OW} (Eq. (12)) would explain this. For example, the calculated BCF_{TOT} of BTBPE in *E. septemfasciatus* decreased from 110 to 52 when the log K_{OW} value increased by 0.50 log units (from 9.15 to 9.65). On the other hand, BCF_{TOT} of HBB in *E. septemfasciatus* showed a maximum value of 5700 at log K_{OW} of 6.07. The sensitivity indices, defined as the increase in the calculated BCF with respect to the increase in K_{OW} ($dBCF/dK_{OW}$ at the K_{OW} value used), were negative for the more hydrophobic BFRs, whereas they were close to zero for HBB, implying that the greatest BCF_{TOT} value is likely for chemicals with a logK_{OW} of approximately 6. Although there are other factors to be considered such as bioavailability, the very low biotransformation rates of the studied BFRs in the liver S9 fraction warrant further study to evaluate their bioaccumulation potential.

Acknowledgments

We appreciate anonymous reviewers for constructive comments and suggestions. This research was partly supported by the Korea Ministry of Environment (MOE) as "Technology Program for establishing biocide safety management" (2018002490001) and by a Korea University grant.

Appendix A. Supplementary data

Extraction recoveries are shown in Table S1. Chemical- and fish-specific input parameters for the *in vitro* to *in vivo* extrapolation (IVIVE) model and one-compartment BCF model for all fish species are presented in Tables S2–S6. Values of k_{depl} are shown in Table S7. Sensitivity analyses are presented in Tables S8 and S9. Experimental determination of k_{depl} is described in Figs. S1–S5. A plot of the $CL_{in\ vitro}$ values *versus* the EROD activity is presented in Fig. S6. Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.10.432.

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