



Dietary Uptake of Highly Hydrophobic Chemicals by Rainbow Trout (*Oncorhynchus Mykiss*)

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Abstract

Rainbow trout (*Oncorhynchus mykiss*) was exposed through the diet to a mixture of non-ionic organic chemicals for 28 d, followed by a depuration phase, in accordance with OECD method 305. The mixture included hexachlorobenzene (HCB), 2,2',5,5'-tetrachlorobiphenyl (PCB-52), 2,2',5,5'-hexachlorobiphenyl (PCB-153), decachlorobiphenyl (PCB-209), decabromodiphenyl ether (BDE209), decabromodiphenyl ethane (DBDPE), bis-(2-ethylhexyl)-3,4,5,6-tetrabromophthalate (TBPH), perchloro-p-terphenyl (p-TCP), perchloro-m-terphenyl (m-TCP), and perchloro-p-quaterphenyl (p-QTCP), the latter six of which are considered highly hydrophobic based on n-octanol/water partition coefficients (K_{OW}) greater than 10^8 . All chemicals had first-order uptake and elimination kinetics except p-QTCP, whose kinetics could not be verified due to limitations of analytical detection in the elimination phase. For HCB and PCBs, the growth-corrected elimination rates (k_{2g}), assimilation efficiencies (α), and biomagnification factors (BMF_L) corrected for lipid content compared well with literature values. For the highly hydrophobic chemicals, elimination rates were faster than the rates for HCB and PCBs, and α 's and BMF_L s were much lower than those of HCB and PCBs, i.e., ranging from 0.019 to 2.8%, and from 0.000051 to 0.023 (g-lipid/g-lipid), respectively. As a result, the highly hydrophobic organic chemicals were found to be much less bioavailable and bioaccumulative than HCB and PCBs. Based on the current laboratory dietary exposures, none of the highly hydrophobic substances would be expected to biomagnify, but Trophic Magnification Factors (TMFs) > 1 have been reported from field studies for TBPH and DBDPE. Additional research is needed to understand and reconcile the apparent inconsistencies in these two lines of evidence for bioaccumulation assessment.

The bioaccumulation of highly hydrophobic non-ionic organic chemicals (defined here as chemicals with n-octanol/water partition coefficients [K_{OW}] greater than 10^8) by fish has not been extensively studied in the laboratory (Arnot and Quinn 2015). The lack of data is due, in part, to the difficulties in working with chemicals having very low aqueous solubilities and, in some cases, relatively high laboratory background levels (e.g., brominated flame retardants). The

Organization for Economic Co-operation and Development (OECD) method 305 for measuring bioaccumulation by fish in the laboratory (OECD 2012) includes an option for dietary exposures, which avoids the difficulties in working with chemicals having very low aqueous solubilities. More specifically, OECD 305 recommends dietary feeding studies for chemicals with K_{OW} greater than 10^5 and aqueous solubilities below ~0.01–0.1 mg/L (OECD 2012). With dietary exposure, the elimination rate (k_2), assimilation efficiency (α), and biomagnification factor (BMF) of the chemical are measured, and from these data, a bioconcentration factor (BCF) can be estimated by using the approach of Gobas and Lo (2016), based on reference chemicals included in the dietary exposure.

Arnot and Quinn (2015) assembled a database of dietary bioaccumulation data from the literature, containing 3032 measurements across 477 discrete organic chemicals, and providing 964 half-lives, 1199 α 's, and 869 BMFs across 19 species (primarily trout and carp). Many of these data were not generated according to standardized methodology,

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resulting in more than half of the measurements having identifiable sources of uncertainty. Common uncertainties included incomplete reporting of experimental results, incomplete descriptions of experimental designs, not measuring elimination, and not accounting for growth of the fish. Based on their review, Arnot and Quinn recommended the use of reference chemicals (chemicals with similar K_{OW} s to the test chemicals) and positive controls (e.g., hexachlorobenzene) to improve data quality and comparability. They also suggested further evaluations of the guidance to include higher hydrophobic chemicals, i.e., $\log K_{OW} > 8$.

The objective of this study was to apply the OECD 305 guidance to measure chemical uptake and elimination by rainbow trout (RBT; *Oncorhynchus mykiss*) exposed to six highly hydrophobic chemicals. Three of the chemicals studied are brominated flame retardants: decabromodiphenyl ether (BDE209), decabromodiphenyl ethane (DBDPE), and bis-(2-ethylhexyl)-3,4,5,6-tetrabromophthalate (TBPH). Because these three chemicals are thought to be subject to biotransformation (Barr et al. 2010; Munschy et al. 2011; Stapleton et al. 2004; Tomy et al. 2004; Wang et al. 2019; Zheng et al. 2018), we also included three chemicals thought to be less susceptible to biotransformation, perchloro-p-terphenyl (p-TCP), perchloro-m-terphenyl (m-TCP), and perchloro-p-quaterphenyl (p-QTCP) (see Table 1 for estimated biotransformation half-lives). Although not widely studied, these terphenyls are structurally similar to PCBs and have estimated K_{OW} s in a similar range to the brominated compounds. Of the six highly hydrophobic chemicals, BDE209 and TBPH have measured $\log K_{OW}$ s of 8.37 (SD = 0.12, $n = 2$) and 9.21 (0.19, 2), respectively (Hanson et al. 2019), and the remaining four chemicals have only estimated values ranging from 7 to 18 (Table 1). As a basis for comparison with other studies, several positive control and reference chemicals were also included, specifically hexachlorobenzene (HCB), 2,2',5,5'-tetrachlorobiphenyl (PCB-52), 2,2',5,5'-hexachlorobiphenyl (PCB-153), and decachlorobiphenyl (PCB-209), representing a range in $\log K_{OW}$ from 5.5 to 8.22 (Table 1). Given the structural similarities of PCBs and TCPs, this work can help expand the range of reference chemicals available for applying the OECD 305 dietary methodology to highly hydrophobic chemicals.

Methods & Materials

Reagents and Chemicals

Chemicals used for spiking the trout chow were as follows: PCB-52 (2,2',5,5'-tetrachlorobiphenyl), PCB-153 (2,2',4,4',5,5'-hexachlorobiphenyl), and PCB-209 (deca-chlorobiphenyl) obtained from Supelco (St. Louis, MO) as neat analytical standard grade; decabromodiphenyl ether

(BDE209) from Aldrich (St. Louis, MO) at 98% purity; hexachlorobenzene from Aldrich (St. Louis, MO) at > 97% purity; decabromodiphenyl ethane (DBDPE) obtained from TCI America (Portland, OR) at 96% purity; bis-(2-ethylhexyl)-3,4,5,6-tetrabromophthalate (TBPH) at 95% purity from ARK Pharm, Inc. (Arlington Heights, IL). Perchloro-m-terphenyl (m-TCP), perchloro-p-terphenyl (p-TCP) and perchloro-p-quaterphenyl (p-QTCP) were synthesized in-house using published methods (Mochiike et al. 1983), using m-terphenyl, p-terphenyl and p-quaterphenyl starting material with 95% + purity as determined by GC/MS analysis. Analytical standard solutions for instrument calibration included HCB, PCB-52, PCB-153, PCB-209, tetradecachloro-o-terphenyl (o-TCP), m-TCP, p-TCP, BDE209, TBPH, and decabromobiphenyl obtained from Accustandard (New Haven, CT). Isotopically labeled $^{13}\text{C}_6$ -HCB, $^{13}\text{C}_{12}$ -PCB-52, $^{13}\text{C}_{12}$ -PCB-153, $^{13}\text{C}_{12}$ -PCB-155, $^{13}\text{C}_{12}$ -PCB-209, $^{13}\text{C}_{12}$ -BDE209, and DBDPE were obtained from Cambridge Isotope Laboratories (Tewksbury, MA); $^{13}\text{C}_{14}$ -DBDPE and mass labeled TBPH [TBPH-L; bis(2-Ethylhexyl-d17)-tetrabromo[$^{13}\text{C}_6$] phthalate]) were obtained from Wellington Laboratories (Guelph, Ontario, Canada). The surrogate solution for sample preparation contained $^{13}\text{C}_6$ -HCB, $^{13}\text{C}_{12}$ -PCB-52, $^{13}\text{C}_{12}$ -PCB-153, $^{13}\text{C}_{12}$ -PCB-209, $^{13}\text{C}_{12}$ -BDE209, o-TCP, $^{13}\text{C}_{14}$ -DBDPE, TBPH-L and TBMEHP-L at 100 ng/mL, and o-TCP was used as the surrogate for m-TCP, p-TCP, and p-QTCP. Internal standard solution contained $^{13}\text{C}_{12}$ -PCB-155 and decabromobiphenyl.

Solvents used for food preparation, sample extraction and cleanup, and GC/MS analytical standards preparation were hexane (GC Resolv™ grade), dichloromethane (DCM) (Optima™ grade), chloroform (CHCl₃) (HPLC grade), methanol (MeOH) (HPLC grade), corn oil (food grade), toluene (Optima™ grade), glacial acetic acid (certified ACS grade), acetonitrile (ACN) (HPLC grade), and acetone (Optima™ grade) all from Fisher Scientific (Pittsburgh, PA); tetrahydrofuran (HPLC grade ≥ 99.9%) was obtained from Sigma (St. Louis, MO). Phenomenex Novum 3 mL SLE Cartridges (Phenomenex; Torrance, CA) and sodium sulfate (ACS grade; 80–200 mesh) were used in sample processing.

Skretting (St. Andrews, NB, Canada) (PN:4602053) commercial trout chow 3 mm extended sinking pellets were used for all fish diets, with a minimum of 40% crude protein, 12% oil, and 1% phosphorus, and a maximum of 3% crude fiber.

Food Preparation

Chemical-spiked food was prepared in 5 batches; for each batch, test chemicals were dissolved in 100 mL of toluene containing 0.50 g of corn oil at nominal concentrations of 100 mg/L (DBDPE @ 50 mg/L due to limited solubility). The chemical mixture was combined with 100 g trout

Table 1 Properties of the test chemicals

Chemical**	CAS #	Molecular Weight	Measured n-Butanol/ Water Partition Coef- ficient (K_{BW}) ^a	Measured n-Octanol/ Water Partition Coef- ficient (K_{OW})	Estimated n-Octanol/Water Partition Coefficient (K_{OW})				Biotransformation Half-Life in Fish (hours) ^f		
					Hanson et al. ^a	OPERA ^c	ACD ^d	EPISuite ^b		ALOGP 2.1 ^e	ppLFER ^f
HCB	118-74-1	284.77	4.19	5.53 (0.25; 17) ^a	5.93	4.1 ^e	4.56	4.72	5.70	5.53 (0.22 i) ^h	296 (30.8 i) ^h
PCB-52	35693-99-3	291.98	–	6.17 ^b	–	6.18 i	5.94	6.34	6.24	6.20 (0.22 i)	2950 (9.3 i)
PCB-153	33979-03-2	360.86	–	7.23 (7.23; 4) ^a	–	7.28 i	6.73	7.62	7.27	7.32 (0.72 i)	10,500 (9.3 i)
PCB-209	2051-24-3	498.63	5.20 (0.04; 5)*	8.22 (8.22; 10) ^a	7.72	8.38 i	8.30	10.2	8.59	9.13 (1.55 o)	13,800 (9.3 i)
BDE209	1163-19-5	959.17	5.47 (0.04; 4)	8.37 (0.21; 2) ^a	8.21	7.74 o	10.4	12.1	7.68	11.5 (0.74 o)	312 (31.9 o)
TBPH	26040-51-7	706.15	6.21 (0.19; 2)	9.21 (0.19; 2) ^a	9.52	7.55 o	9.48	11.9	8.36	12.3 (0.71 i)	410 (30.8 i)
DBDPE	84852-53-9	971.23	5.60 (0.14; 2)	–	8.43	7.52 o	10.6	13.6	7.86	12.3 (1.08 o)	1030 (31.9 o)
m-TCP	42429-89-0	712.5	5.78 (0.06; 4)	–	8.76	8.46 o	11.8	14.5	9.79	12.7 (1.74 o)	24,000 (30.8 o)
p-TCP	No CAS	712.5	5.82 (0.08; 4)	–	8.82	NA	NA	14.5	9.80	12.7 (1.74 o)	24,000 (30.8 o)
p-QTCP	No CAS	926.36	6.47 (0.13; 3)	–	9.98	6.15 o	15.0	18.9	10.38	16.3 (1.75 o)	24,000 (30.8 o)

Sources ^aHanson et al. (2019), ^bUS-EPA 2012, ^cUS-EPA via <https://comptox.epa.gov/dashboard> (accessed September 5, 2023), ^dTetko et al. 2005, ^eEAS-E-Suite 2023

*Average (standard deviation, count) ^fFor OPERA predictions: *i* inside Global Applicability Domain and *o* outside Global Applicability Domain. ^hFor ppLFER and biotransformation predictions: Value (error and applicability domain (AD)) where *i* inside AD and estimates are reliable and *o* outside AD and estimates are unreliable (accessed September 5, 2023)

**Abbreviations: HCB = Hexachlorobenzene, PCB-52 2,2',5,5'-Tetrachlorobiphenyl, PCB-153 2,2',4,4',5,5'-Hexachlorobiphenyl, PCB-209 Decachlorobiphenyl, BDE209 decabromodiphenyl ether, DBDPE decabromodiphenyl ethane, TBPH bis(2-ethylhexyl)-3,4,5,6-tetrabromophthalate, m-TCP perchloro-m-terphenyl, p-TCP perchloro-p-terphenyl and p-QTCP perchloro-p-quaterphenyl. CAS # Chemical Abstracts Service number, ppLFER Poly-parameter Linear Free Energy Relationship

chow, then placed in a 4-L jar on a slowly rolling bed with vacuum applied until solvent. Each batch had greater than 97% weight recovery after evaporation. All 5 batches were pooled, mixed well, and stored at 4 °C. Spiked food samples were collected on days 1, 15 and 28 of the uptake exposures for chemical analysis.

Blank spike (control) food was prepared similarly except there were 6 batches of 100 g of trout chow and 100 mL aliquots of only toluene containing 0.50 g corn oil. After vacuum evaporation, all 6 batches were pooled, mixed well, and then, stored at 4 °C throughout the exposure. Samples of the blank spike food were collected on day 1 of the experiment for chemical analysis.

Experimental Design

The dietary exposure followed OECD 305 dietary methodology (OECD 2012). Fish were housed in 40-gallon fiberglass tanks (Dura-Tech Industrial and Marine) supplied with 1440 L/day/tank of filtered and UV-treated Lake Superior water at a nominal temperature of 11 °C and a 12h light:12h dark photoperiod. A total of 7 tanks were used including 3 control tanks and 4 exposure tanks, and each tank started with 19 rainbow trout (RBT). Rainbow trout (*Oncorhynchus mykiss*, Erwin strain) was obtained as eggs from the US Geological Survey Upper Midwest Environmental Sciences Center in LaCrosse, Wisconsin, and reared to desired size (~ 10 g) in our in-house culture facility.

Fish were assigned arbitrarily to tanks seven days before chemical dosing began for an acclimation period (days -7–0), followed by a 28-day uptake phase with dietary exposure (days 0–28), and then 28 days of depuration (days 28–56). All tanks were fed blank spike food during the acclimation phase, blank spike or chemical-spiked food as applicable during the uptake phase, and unmanipulated trout chow during the depuration phase, all at a target rate of 1.25% of wet body weight per day. One hour after each feeding, any uneaten pellets at the bottom of the tank were removed using a pipette and counted. During the exposure phase, all tanks consumed all ration offered.

Fish for chemical analysis were sampled prior to daily feeding on days 0, 3, 7, 14, 21, 28, 29, 31, 35, 42, 49, and 56. Fish were sampled by netting out one or two fish per tank (Table S4) and euthanizing them with an overdose of pH-buffered ethyl-3-aminobenzoate methanesulfonate (Finquel; Argent Laboratories). Each fish was weighed, then the abdominal cavity was opened by incision and all tissue in the abdominal cavity was removed to avoid inclusion of the gastrointestinal tract. The fish was then re-weighed, wrapped in muffled aluminum foil and stored at -20 °C until further processing.

Homogenates of each fish were prepared by cutting the frozen fish into ≤ 5 mm pieces which were frozen in liquid nitrogen then transferred into a pre-chilled 50 mL stainless steel ball-mill cell with 25 mm stainless steel ball. Two balanced samples were processed on a Retsch MM-301 ball mill for 3.00 min at 30 Hz. The resulting homogenates were stored at -20 °C in glass jars with PTFE lined caps for analysis. The homogenates were later subsampled separately for lipid content, for TBPH analysis, and for all other chemicals.

Analytical

For all analytes except TBPH, tissue and food samples were spiked with surrogate chemicals and extracted by sonicating with CHCl_3 :MeOH (1:1 v/v) for 2 min (Branson Sonifer 450 with MicroAdapter tip, power level 3 and 60% duty cycle; Branson Ultrasonics), followed by centrifugation at 3000 RPM for 15 min and decanting of the solvent. This process was repeated two more times. Subsequently, the combined extracts were passed through an acid silica column (US-EPA 2008). After concentrating under nitrogen, the final extract was spiked with internal standards for analysis.

For TBPH, tissue and food samples were spiked with mass labeled surrogate and extracted via ultrasonic probe three times with CHCl_3 :MeOH (1:1 v/v) and then once with an ACN:DCM (1:1 v/v):glacial acetic acid (20:1 v/v) solvent system following Bradley et al. (2013a,b). The CHCl_3 :MeOH extracts were combined and concentrated to 2.0 mL. Subsequently, the ACN:DCM and CHCl_3 :MeOH extracts were combined, evaporated to dryness, and reconstituted in 40 μL mixture of acetonitrile:acetone (3:1 v/v). After the addition of 360 μL of aqueous 1% formic acid, the extract was transferred to a 3-mL SLE cartridge and eluted with hexane.

For TBPH, BDE209, DBDPE, p-TCP, m-TCP, and p-QTCP, samples were analyzed using selected-ion monitoring with Thermo Scientific Orbitrap GC/MS using MS resolution of 60,000. For HCB, PCB-52, PCB-153, and PCB-209, an Agilent GC/MS (6890N/5975C) selected ion monitoring was used (see Table S25 for detailed parameters). Isotope dilution with internal standards was used for quantification for all analytes. For analytes not detected, not quantified (not meeting ion abundance ratio criteria of $\pm 15\%$), and below reporting limit (RL), concentrations were reported as the RL. Reporting limits (Table S1) were determined from the lowest amount detectable with the Orbitrap and Agilent MS systems (Instrument Detection Limit; Table S1).

Lipid contents of all RBT samples and foods were measured using a modified version of the Bligh and Dyer method (Bligh and Dyer 1959). The method was modified to exclude use of a partitioning phase between aqueous and chloroform

due to excessive emulsions using whole-body rainbow trout tissues. The modified method was evaluated by analyzing NIST Lake Superior Fish Tissue SRM 1946, which averaged $12.17 \pm 0.33\%$ (SD; $n = 5$) extractable fat by our method. As might be expected, this falls between the NIST reported value of $10.17 \pm 0.48\%$ and a value of $13.5 \pm 0.4\%$ from Dodds et al. (2004), whose method is inclusive of more polar lipids.

Data Analysis

Results of the dietary bioaccumulation test were interpreted using OECD 305 methodology (OECD 2012). Elimination rates (k_2) were determined using the equation:

$$\ln C_{f,i} = \ln C_{0,d} - k_2 t \quad (1)$$

where $C_{0,d}$ is derived concentration in fish at time zero of the depuration phase (ug/kg-ww), $C_{f,i}$ is the concentration in fish on day i , and t is days of depuration.

The growth rate of the fish (k_g) was determined using the equation:

$$\ln W_{(f,t)} = \ln W_{(f,0)} + k_g t \text{ or } W_{(f,t)} = W_{(f,0)} e^{(k_g t)} \quad (2)$$

where $W_{f,t}$ is the fish weight at day t , $W_{f,0}$ is fish weight at the start of the exposure, and t is day of exposure.

The growth-corrected elimination rates (k_{2g}) were determined using the equation:

$$k_{2g} = k_2 - k_g \quad (3)$$

The growth-corrected half-life ($t_{1/2g}$) was calculated using the equation:

$$t_{1/2g} = \ln(2)/k_{2g} \quad (4)$$

The assimilation efficiencies (α) were determined using the equation:

$$\alpha = \frac{C_{0,d} \times k_2}{I \times C_{\text{food}}} \times \frac{1}{(1 - e^{-k_2 t})} \quad (5)$$

where I is the food ingestion rate constant (g food-dw/ g fish-ww/day) and C_{food} is the measured concentration of chemical in the food fed to the fish ($\mu\text{g}/\text{kg-dw}$).

Biomagnification factors (BMF) were determined using the equation:

$$\text{BMF} = \frac{I \alpha}{k_2} \quad (6)$$

and lipid-corrected biomagnification factors (BMF_L) were determined using the equation:

$$\text{BMF}_L = \text{BMF}_L \frac{L_{\text{food}}}{L_{\text{fish}}} \quad (7)$$

where L_{fish} and L_{food} are the mean lipid fractions in fish and food, respectively. Growth-corrected BMFs were not computed based upon the recommendation by Gobas and Lee (2019) because correction for growth violates mass balance constraints in the calculations.

The computation of the k_2 , k_g , α , $C_{0,d}$, and BMF were performed with the *bcmfR* version 0.4–18 R statistics package (OECD 2019) and the log-linear regression statistics were used in this report. For samples with quantifications less than the RL, $1/2$ of the RL was used in the calculations. Values for the first-order uptake rate constant (k_u) were determined using nonlinear regression with the equation:

$$C_{u,i} = (k_u/k_2) \times C_{\text{food}} \times (1 - e^{(-k_2 t)}) \quad (8)$$

where $C_{u,i}$ is the concentration in the RBT on day i in the uptake exposure. The nonlinear regressions were performed using the *nlrmt nls* algorithm (R Core Team 2013) in RStudio (RStudio Team 2020) with the measured C_{food} and k_2 determined from the elimination data. For p-QTCP where k_2 could not be determined from the elimination data, k_u and k_2 were determined using the *nlrmt nls* algorithm using the uptake data and C_{food} , with Eq. 8.

As comparisons to measurements from this study, k_{2g} , α , and K_{OW} (K_{OWs} from Hawker and Connell, 1988) data were retrieved from the data set of Arnot and Quinn (2015), supplemented with α , BMF_L and K_{OW} data from Gobas et al. (2020) for chemicals with $\log K_{\text{OWs}}$ greater than 8.0 (Table S2). For the chemicals used in the current study without measured K_{OWs} , i.e., DBDPE, m-TCP, p-TCP, and p-QTCP, estimates derived from *measured* n-butanol-water partition coefficients (K_{BW}) reported by Hanson et al. (2019) with the $K_{\text{OW}}-K_{\text{BW}}$ predictive relationship developed by Hanson et al. (2019) were used (Table 1). Estimates using other predictive methods are provided in Table 1; these estimates vary considerably and are beyond the applicability range of some models for these four chemicals.

Results

The dietary exposure met requirements for exposure conditions and performance as outlined in OECD method 305. Ammonia, conductivity, hardness, and pH were consistent throughout the exposure (Tables S4, S5, and S9). Dissolved oxygen averaged 93.3% of saturation (minimum of 85.2%) and temperature averaged 11.1 °C (range 10.3–11.9 °C), meeting their respective criteria of > 60% saturation and ± 2 °C. Lake Superior water is very stable in composition, and previous measurements of total organic

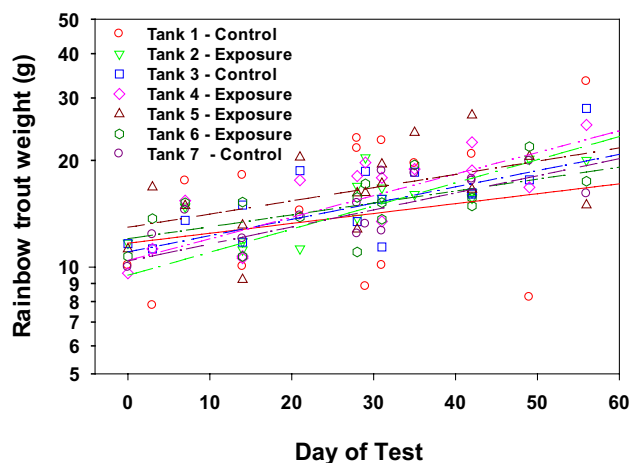


Fig. 1 Rainbow trout weights over time in the test with regression fits using Eq. 2 for each exposure tank

carbon (TOC; 1.60 ± 0.29 mg/L [Saunders et al. 2020]) have shown conformance to the recommendation of < 2 mg/L in the OECD method. Concentrations of the chemicals in the spiked food were stable and were homogeneous, and the control food had non-detectable and/or trace levels of the spiked contaminants (Table S3). Overall mortality rate was 3% (4 of 133 fish), well below the OECD 305 criterion of $\leq 10\%$ (Table S4).

Prior to computing growth rates of the fish, we examined the weight data of the RBT by tank and noticed a small number of anomalously small fish in the samples (Table S10). Using the Bonferroni outlier test in RStudio on each tank, four outliers were detected ($p < 0.10$) and these samples were eliminated from the analyses of growth, lipid content, and uptake and elimination (Table S11). The samples eliminated were from tanks 2, 3, 4, and 7 collected on days 3, 28, 28, and 14, respectively (Table S8).

The RBT grew from an average of 10.72 g (SD 0.84) on day 0 to an average of 22.16 g (SD 6.88) on day 56 of the test (Table S8). The rate of increase in fish weights were similar across all 7 tanks (Fig. 1) and growth was not significantly different among tanks ($p > 0.05$, Tables S12 and S13). Growth rate constants (k_g) were computed using Eq. 2. For the control and exposed RBT, growth rates were 0.00875 (SE 0.00305) and 0.01011 (SE 0.00149), respectively, and when combined, provided an overall growth rate of 0.00952 (SE 0.00156) for the test (Table S9). Based on the fish weights over time, the overall average feeding rate was 0.0118 g-food/g-fish/d, slightly less than the target rate of 0.0125 g food/g fish/day. The average lipid content in the controls and exposed RBT was 6.23% (SE 0.19) and 5.80% (SE 0.091), respectively, and there was no change in lipid content over the entire test ($p > 0.05$) (Figure S1, Table S14). ANOVA revealed the lipid contents of the control and

exposed RBT were significantly different (p value = 0.0193); uptake and depuration computations used the 5.80% lipid content for exposed fish. Measured lipid in the food was 10.97% (SE 0.119, $n = 6$).

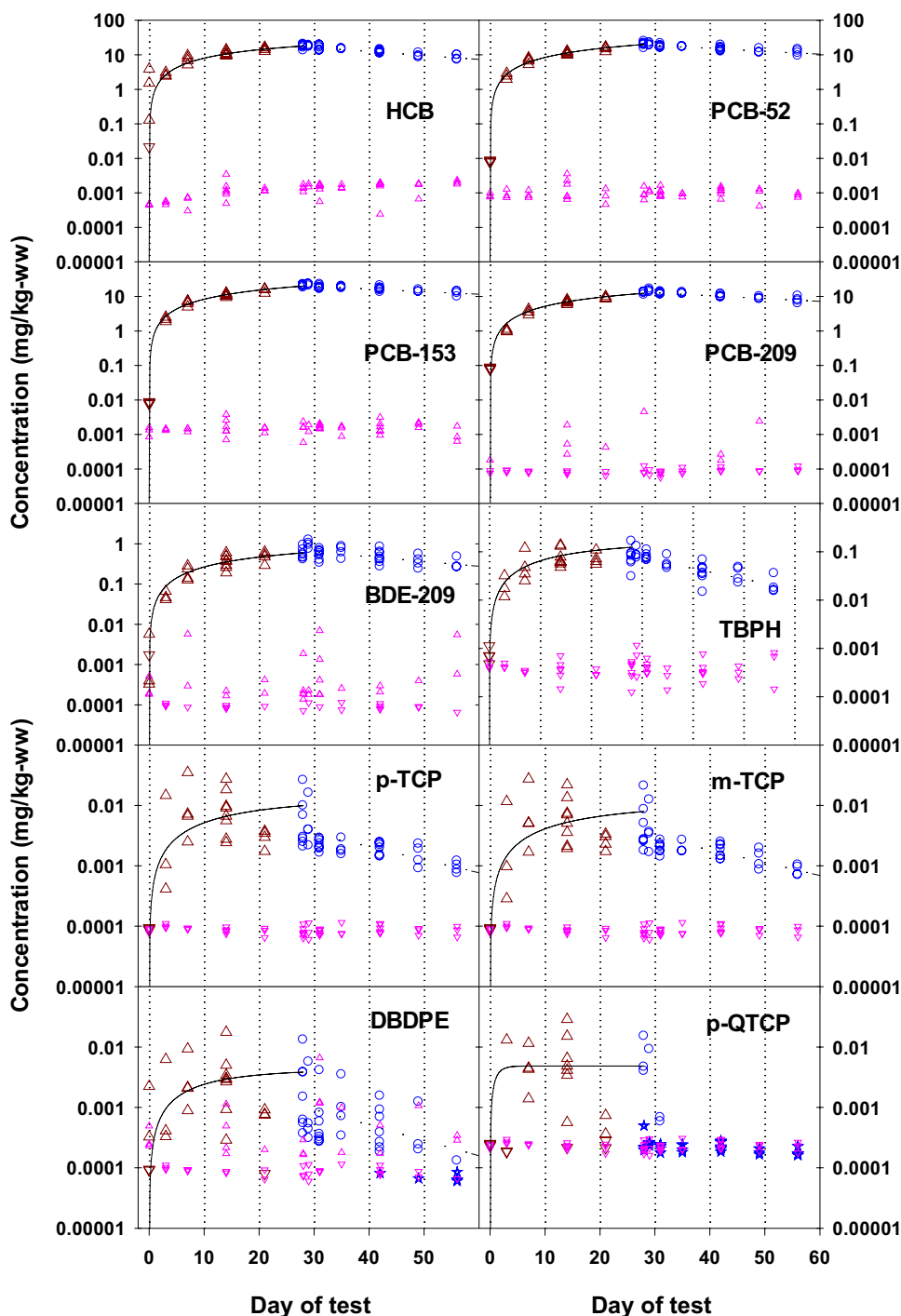
Uptake and Elimination Rates

Figure 2 shows the uptake and elimination data for all chemicals along with the data from the control RBT (see Tables S15–S24 for numerical data). Nine of the ten chemicals had uptake and elimination profiles consistent with first-order kinetics. There was limited uptake of p-QTCP and after 3 days of elimination, concentrations in the RBT were below our method reporting limits. With the non-detects in the elimination phase, we were unable to confirm first-order uptake and elimination kinetics for p-QTCP. In contrast to our study, Bruggeman et al. (1984) reported no accumulation of p-TCP with guppies (*Poecilia reticulata*) in a 10-week dietary exposure using an analytical method with a practical detection limit of 50 $\mu\text{g}/\text{kg}$. As shown in Fig. 2, uptake of p-TCP is limited, and improved reporting limits in this study, i.e., 0.17 $\mu\text{g}/\text{kg}$ -ww, permitted successful measurement of p-TCP residues in the uptake and elimination phases of the dietary exposure.

Coefficients of variation (CV) by sampling day were 12 versus 13% for HCB, 10 versus 12% for PCB52, 9 and 10% for PCB153, and 6 and 9% for PCB209, respectively. For the highly hydrophobic chemicals, coefficients of variation for residues (ww) by sampling day were larger than the PCBs; within and across tanks, coefficients of variation for BDE209 were 23 versus 39%, TBPH 30 versus 40%, p-TCP 41 versus 60%, m-TCP 43 versus 60%, and DBDPE 80 versus 115%, respectively. The higher CVs corresponded with chemicals with low concentrations in tissues, which presumably contributed to the higher variances.

The elimination rate constants (k_2) were computed for all chemicals using Eq. 1 except p-QTCP (Table 2). For p-QTCP, only 6 of 39 samples had detectable amounts of p-QTCP and performing the regression using $\frac{1}{2}$ RL for 33 samples yields a very uncertain estimate of its elimination rate constant. Consequently, no elimination rate for p-QTCP was determined. For DBDPE, 34 of 39 samples had detectable amounts of DBDPE and its elimination rate constant was 0.0840 (0.0181 SE) (1/d) using $\frac{1}{2}$ RL values for the 5 samples with responses below the RL. For comparison purposes, the elimination rates determined with the 5 samples set to their RL values and with 5 samples eliminated from the regression were 0.0690 (0.0172 SE) and 0.0586 (0.0234 SE), respectively. These three elimination rates were not significantly different ($p = 0.05$). For all other chemicals, all quantifications were above their RLs in the elimination phase of the exposure.

Fig. 2 Measured concentrations in rainbow trout. Exposure uptake samples \triangle , exposure uptake samples less than reporting limit with half of RL plotted ∇ , exposure elimination samples \circ , exposure elimination samples less than reporting limit with half of RL plotted \star , control samples \triangle , control samples less than reporting limit with half of RL plotted ∇ . The dashed line is the regression fit of the elimination data using Eq. 1. The solid line is regression fit of uptake data using Eq. 8 with k_{2ww} determined from regression fit of the elimination data



Growth-corrected elimination rate constants (k_{2g} ; Table 2) from the current study are in good agreement with the k_{2g} s for HCB and PCBs from the data compilation of Arnot and Quinn (2015) for RBT tissue samples comparable to those assessed in this study (i.e., whole body without GIT, whole body without GIT and liver; Fig. 3). Additionally, the k_{2g} for BDE209 is consistent with values for lake trout (*Salvelinus namaycush*) from the same source. Chemicals with K_{OW} higher than BDE209 show higher k_{2g} in both the current

study and in literature data. Similar behavior was observed in a dietary study by Cantu and Gobas (2021) with dodecamethylcyclohexasiloxane (D6) ($\log K_{OW} = 9.06$; US-EPA 2012), with an elimination rate larger than those for the reference PCBs. From the available data, it is difficult to parse the degree to which this increase reflects higher intrinsic elimination rates associated with higher K_{OW} versus elimination enhanced by in vivo biotransformation. The two TCPs are thought to be resistant to biotransformation, and DBDPE

Table 2 Elimination rates, assimilation efficiencies and biomagnification factors

Units	HCb**	PCB52	PCB153	PCB209	BDE209	TBPH	p-TCP	m-TCP	DBDPE	p-QTCP
$k_{2\text{ ww}}$	1/d 0.0265 (0.00215)*	0.0196 (0.00217)	0.0178 (0.00179)	0.0191 (0.00175)	0.0273 (0.00577)	0.0504 (0.00626)	0.0495 (0.00959)	0.0487 (0.00908)	0.084 (0.01806)	ND**
k_{2g}	1/d 0.0152 (0.00215)	0.0083 (0.00217)	0.00652 (0.00179)	0.00781 (0.00175)	0.016 (0.00577)	0.0391 (0.00626)	0.0382 (0.00959)	0.0374 (0.00908)	0.0727 (0.01806)	-
$C_{0,avg}$	ug/g-ww 17.5 (0.883)	20.3 (1.03)	20.5 (0.573)	12.8 (0.302)	0.577 (0.0698)	0.0833 (0.0158)	0.007574 (0.003275)	0.006301 (0.002595)	0.00338 (0.00171)	0.003554 (0.00206)
$C_{0,d}$	ug/g-ww 17.3 (0.489)	19.5 (0.558)	20.5 (0.481)	13.3 (0.306)	0.665 (0.0505)	0.0836 (0.00688)	0.003703 (0.000467)	0.003254 (0.000389)	0.00111 (0.000263)	-
k_u	kg-ww/(kg-dw x d) 0.00856 (0.000195)	0.00911 (0.000155)	0.00592 (0.0000961)	0.000296 (0.0000168)	0.0000507 (0.00000459)	0.00000758 (0.00000185)	0.00000723 (0.00000173)	0.00000724 (0.00000193)	0.0000498 (0.000232)	-
C_{food}	mg/kg-dw 83.4 (2.51)	108.2 (4.57)	99.6 (1.66)	96.1 (2.59)	102.6 (3.56)	137.0 (2.13)	86.1 (2.58)	71.5 (2.08)	44.8 (2.31)	90.6 (4.51)
α	% 88.9% (4.5%)	71% (3.69%)	79.1% (3.4%)	54.2% (2.27%)	2.8% (0.38%)	0.34% (0.048%)	0.024% (0.0051%)	0.025% (0.0051%)	0.019% (0.0072%)	-
BMF	kg-dw/kg-ww 0.395 (0.0158)	0.427 (0.0282)	0.524 (0.0329)	0.335 (0.0185)	0.0121 (0.00124)	0.000806 (0.0000468)	0.000057 (0.000005)	0.000061 (0.000005)	0.000027 (0.0000055)	0.0000392 (0.0000601) #
BMF _L	kg-lipid/kg-lipid 0.749 (0.0158)	0.809 (0.0282)	0.991 (0.0329)	0.634 (0.0185)	0.023 (0.00124)	0.00153 (0.0000468)	0.000108 (0.000005)	0.000115 (0.000005)	0.000051 (0.0000055)	0.0000743 (0.0000601) #
$t_{1/2g}$	d 26.1	35.4	38.9	36.3	25.4	13.7	14.0	14.2	9.5	-
k_g	1/d 0.0101 (0.00149)									
L_c	% Lipid _{fgT} / % Lipid _{d,food} 0.5283									
I	g-food-dw/g fish-ww/day 0.0118 (0.00045)									

*value (standard error) ** ND Not Determined # BSAF computed using day-28 average residue ($C_{0,avg}$) divided by C_{food}
 **HCB Hexachlorobenzene, PCB-52 2,2',5,5'-Tetrachlorobiphenyl, PCB-153 2,2',4,4',5,5'-Hexachlorobiphenyl, PCB-209 Decachlorobiphenyl, BDE209 decabromodiphenyl ether, DBDPE Decabromodiphenyl Ethane, TBPH bis(2-ethylhexyl)-3,4,5,6-tetrabromophthalate, m-TCP perchloro-m-terphenyl, p-TCP perchloro-p-terphenyl and p-QTCP perchloro-p-quatphenyl

Fig. 3 Growth-corrected elimination rates (k_{2g} (1/d)) for whole body without GIT and viscera. Data shown are for rainbow trout (RBT) for HCB and all PCBs from Arnot and Quinn (2015) (circle); for RBT for chemicals with $\log K_{OWs} > 8.0$ from Gobas et al. (2020) (square); for RBT from this study (diamond); and for lake trout (*Salvelinus namaycush*) from Tomy et al. (2004) (triangles). The dashed line connects the measurements from this study. Labeled chemicals are highly hydrophobic chemicals from the present study

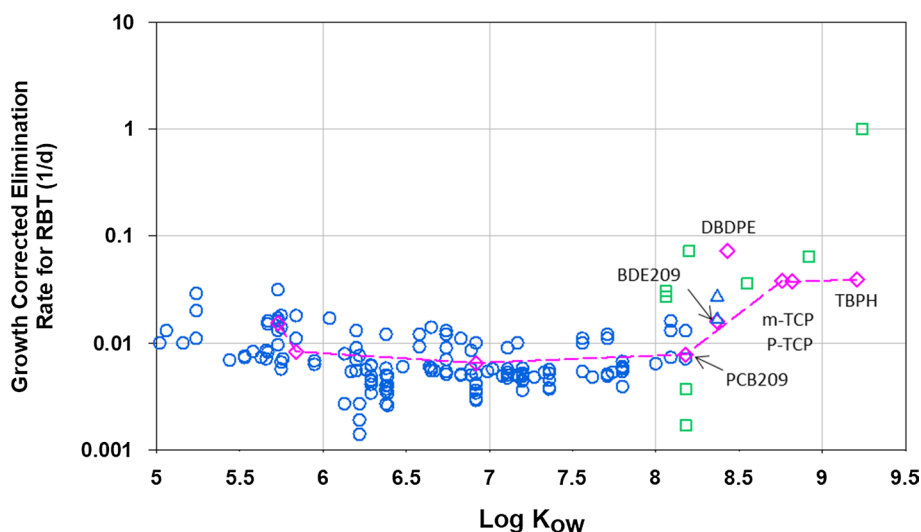
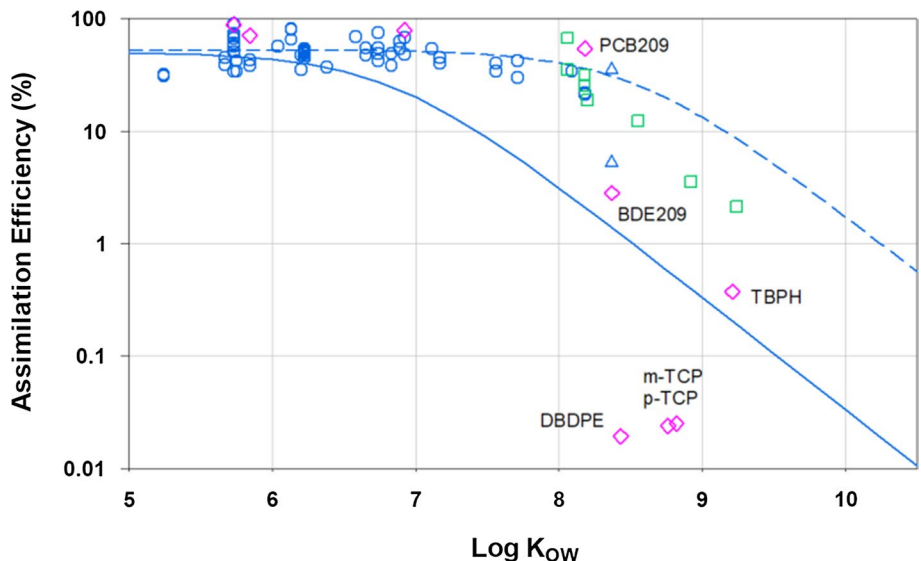


Fig. 4 Assimilation Efficiencies (α) for rainbow trout (RBT) for HCB and all PCBs from Arnot and Quinn 2015 (circle); for RBT for chemicals with $\log K_{OWs} > 8.0$ from Gobas et al. 2020 (square); for RBT from this study (diamond); and for lake trout (*Salvelinus namaycush*) from Tomy et al. (2004) (triangles). Solid line is predictive equation for α from Arnot and Gobas (2004) and dashed line is the predictive equation for α from Gobas et al. (2020). Labeled chemicals are highly hydrophobic chemicals from the present study



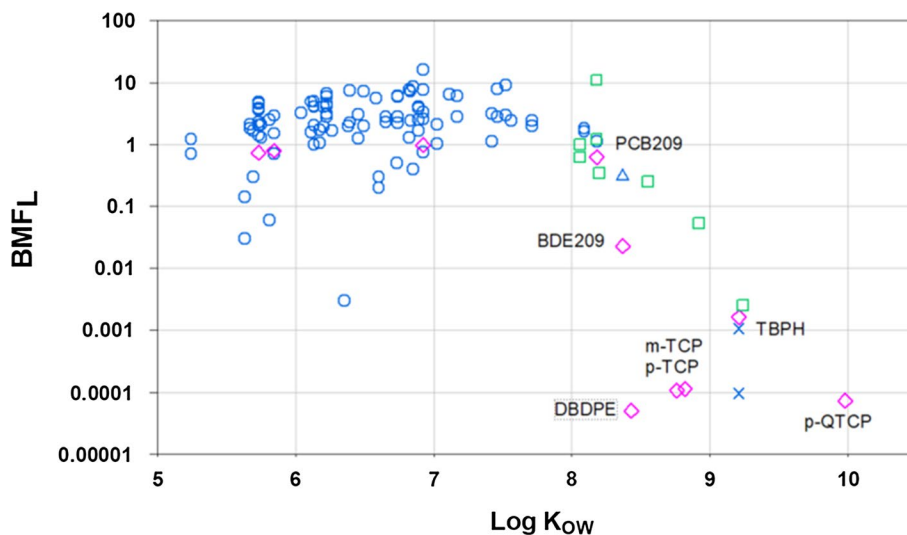
lies above the line connecting PCB209 and the TCPs, which is suggestive of in vivo biotransformation.

Dietary Assimilation Efficiencies

Dietary assimilation efficiencies (α) were computed using Eq. 5 for all chemicals (Table 2) except p-QTCP for which there were insufficient data. The α values for HCB and PCBs from the present study show consistently high α values largely independent of K_{OW} ; these values were within the range derived/assembled by Arnot and Quinn (2015; Fig. 4), though the current values are on the high end of the range. For the high K_{OW} chemicals, assimilation efficiencies from this study were lower than for similarly hydrophobic chemicals from Gobas et al. (2020), though the value for BDE209

is not greatly different than that from Tomy et al. (2004) for lake trout. Assimilation efficiencies for m-TCP, p-TCP, and DBDPE were the lowest of all, and lower than BDE209 and TBPH even though their plotted K_{OW} ranges overlap. It is worth noting that the K_{OWs} for m-TCP, p-TCP, and DBDPE were estimated rather than measured, whereas the values for BDE209 and TBPH were measured. We opted to use the K_{OW} estimates from Hanson et al (2019) for the former three chemicals, because they are based on measured values (K_{BW}) rather than prediction; however, several of the K_{OW} calculators place their estimated K_{OWs} above those for BDE209 and TBPH, raising the possibility that the K_{OWs} used in Figs. 3–5 are underestimates (see Figures S2, S3, and S4 for plots with other estimates for these chemicals). Also shown in Fig. 4 are the default assimilation efficiency equations of Gobas et al. (2020; $\alpha = 1 / (5.6 \times 10^{-9} \times K_{OW} + 1.9)$), and Arnot and

Fig. 5 Lipid-corrected $BMFL_S$ for rainbow trout (RBT) for HCB and all PCBs from Arnot and Quinn (2015) (circle); for RBT for chemicals with $\log K_{OW} > 8.0$ from Gobas et al. (2020) (square); for RBT from this study (diamond); for lake trout (*Salvelinus namaycush*) from Tomy et al. (2004) (triangles); and for fathead minnows (*Pimephales promelas*) from Beare et al. (2010) (×). Labeled chemicals are highly hydrophobic chemicals from the present study



Gobas (2004; $\alpha = 1/(3.0 \times 10^{-7} \times K_{OW} + 2.0)$). The equation from Gobas et al. (2020) appears to significantly overestimate α values at $\log K_{OW} > 8.2$, while the equation from Arnot and Gobas (2004) is close to BDE209 and TBPH, but substantially overestimates efficiencies for other high K_{OW} compounds.

Biomagnification Factors (BMFs)

Lipid-corrected biomagnification factors ($BMFL_S$) computed using Eqs. 6, 7 (Table 2) are shown in Fig. 5 along with other literature data. The $BMFL_S$ from this study are consistent with $BMFL_S$ from the data compilation of Arnot and Quinn (2015) (Fig. 5) for HCB and PCBs, though our values are on the low end of the range. For chemicals with $\log K_{OW}$ greater than 8.2, $BMFL_S$ decreased rapidly with increasing K_{OW} . For TBPH, we derived two $BMFL_S$ from the effects study of Beare et al. (2010) who exposed fathead minnows (*Pimephales promelas*) via the diet for 56 days. The $BMFL_S$ in Fig. 5 are the ratio of day 56 residues in the carcass after the removal of brain, liver, blood, and gonads divided by the concentration in food. These values are slightly lower in compared to the corresponding $BMFL$ measured in this study for TBPH. The Beare et al. (2010) study did find genotoxic effects from exposure, which may have influenced the computed $BMFL_S$. DBDPE, m-TCP, and p-TCP, are outliers to the low side, which could again reflect the selection of K_{OW} values for these chemicals (see discussion under the Dietary Assimilation Efficiencies section). For all six of the highly hydrophobic chemicals, $BMFL_S$ were substantially less than

1, meaning that the lipid-normalized concentration in fish tissue was below that in the diet, and that biomagnification would not be expected based on the current study.

Influence of K_{OW} Estimation

A pervasive uncertainty in the evaluation of relationships shown in Figs. 3–5 is the K_{OW} values chosen. As evidenced in Table 1, estimates of K_{OW} for the more hydrophobic compounds vary widely among the estimation approaches, by as much 9 orders of magnitude. For compounds without measured K_{OW} , we chose to use values estimated from K_{BW} values reported by Hanson et al. (2019) because they were based on actual measurements, and the measured values were within, or very close to, values for other chemicals with measured K_{OW} , and in that way could be argued to be within the applicable domain of the approach. Other estimation approaches, such as poly-parameter linear free energy relationships (ppLFER), have a strong theoretical underpinning, but their applicability domains do not extend to all of the tested chemicals, and diverge strongly for some of the chemicals with measured K_{OW} (e.g., 2.8 log units for TBPH; Table 1). Whether these differences relate to measurement uncertainty or model uncertainty is not clear. However, to qualitatively evaluate the influence of K_{OW} value selection on data interpretation, Figures S2–S4 provide a direct comparison of how patterns in elimination rate, assimilation efficiency, and $BMFL$ vary when different sources K_{OW} estimates are used (Hanson et al. [2019], ALOGP 2.1, ppLFER). The coherence of the three accumulation-related parameters with K_{OW} varies across Figures S2–S4, but not in a way that would make a convincing argument that any one of the three K_{OW} estimations consistently rationalizes the data broadly.

Table 3 Field measured trophic magnification factors (TMFs)

Location	Species	Measured/Estimated/Derived Metric			
		TMFs	Tissues	Reference	Range in TL
<i>BDE209</i>					
Bohai Sea, China	plankton—fish	0.72 (0.65–0.83) DF=49.0% *	Invertebrates – soft tissue, fish—muscle	1	1.9
Longtang Town, China	Dragonfly—watersnake	0.34	Insects and fish—whole body, watersnake—muscle	2	1.5
Lake Ontario	6 fish species	0.34	Whole body	3	1.5
Lake Erie	9 fish species	0.23	Whole body	3	2.59
Lake Winnipeg, Canada	plankton – fish	3.6	Whole-body-muscle	4	2.18
e-waste recycling site, South China	Snail, prawns, fish	0.26	Whole body	5	2.6
<i>TBPH</i>					
Lake Taihu, China	plankton—fish	2.42 DF > 50%	Invertebrates – soft tissue, fish—muscle	6	3.22
South China Sea, China	plankton—fish	1.62 DF = 78.4%	Invertebrates – soft tissue, fish—muscle	7	3.21
<i>DBDPE</i>					
Lake Winnipeg, Canada	plankton – fish	2.7	Invertebrates – soft tissue, fish—muscle	4	2.18
South China Sea, China	plankton—fish	4.22 DF = 82.2%	Invertebrates – soft tissue, fish—muscle	7	3.21
Lake Taihu, China	plankton—fish	0.37 DF > 50%	Invertebrates – soft tissue, fish—muscle	6	3.22
Bohai Sea, China	plankton—fish	0.47 (0.32–0.66) DF = 87.0%	Invertebrates – soft tissue, fish—muscle	1	1.9
Longtang Town, China	Dragonfly—watersnake	0.23	Insects and fish—whole body, watersnake—muscle	2	1.5

1 (Liu et al. 2021), 2 (Liu et al. 2020), 3 (Su et al. 2017), 4 (Law et al. 2006), 5 (Wu et al. 2009), 6 (Zheng et al. 2018), 7 (Hou et al. 2022)

*DF Detection frequency in residue analyses and ½ MDL used in TMF derivation, *BDE209* decabromodiphenyl ether, *TBPH* bis(2-ethylhexyl)-3,4,5,6-tetrabromophthalate, *DBDPE* decabromodiphenyl ethane

Comparison with Field Measurements

For TBPH, BDE209, and DBDPE, field studies have reported trophic magnification factors (TMFs) for each chemical (Table 3). TMFs describe the average biomagnification of a chemical across the entire food web. In contrast, BMFs measured in this study and in field studies represent only one predator–prey interaction and capture only the biomagnification from that single change in trophic level (Borgå et al. 2012; Conder et al. 2012). Measurement and uncertainties associated with TMFs have been discussed in detail by Borgå et al. (2012), Conder et al. (2012), and Burkhard et al. (2013). Some of the important considerations in performing quality TMF measurements include having a minimum range of 2 trophic levels in the organisms collected, having a good baseline species, having even distribution of species across all trophic levels, collecting adequate numbers of samples at all trophic levels, collecting organisms that are in the same food web, measuring lipids accurately, measuring concentrations in

the whole body of the organisms (as opposed to blood, liver or muscle), minimizing non-detects, and using proper statistical techniques. Based on these considerations, the TMFs for BDE209, TBPH and DBDPE in Table 3 are of varying quality. Reasons for varying quality include limited ranges in trophic level, using muscle tissue samples for fish, and using species covering only a part of the entire food web. Additionally, based on their reports, determining detection frequency and how non-detects were addressed was often very difficult to ascertain.

For BDE209, 5 of 6 studies reported TMFs with values less than 1, which indicate trophic dilution, i.e., concentrations in the food web decrease with increasing trophic level of the organisms. The BMF_L for BDE209 of 0.0255 our laboratory study is consistent with the reported TMFs. For the DBDPE, a mixed result is provided where only 3 of 5 studies have TMFs less than 1. The BMF_L from this study of 0.0000501 strongly indicates trophic dilution and predicts concentrations should decline with increasing trophic level. For TBPH, its reported TMFs are greater than 1 and the

BMF_L from this study of 0.00214 strongly indicates concentrations should decline with increasing trophic level. TMFs for DBDPE and TBPH being greater than 1 are difficult to rationalize with the much lower BMF_L s measured in this study. However, our laboratory experiment differs from field studies in the way the chemical is incorporated into food (applied versus naturally incorporated) and in not having commensurate exposure from water and other media which occurs in field studies. Additionally, lipid content, digestibility, and feeding rates of food influence uptake measurements in the laboratory (Gobas et al. 2021) and influential differences from field conditions may exist. Further, field measurements may better approximate steady-state conditions than do laboratory measurements, and treatment of non-detects could influence the measured BMFs and/or TMFs. The consequences of these differences between laboratory and field studies on uptake and trophic magnification are not clear. However, we suspect uptake from water and other media might be the primary cause of the inconsistency of field measured TMFs indicating increasing concentrations with increasing trophic level (TMF greater than 1) while laboratory measured BMF_L s indicating decreasing concentrations with increasing trophic level (BMF_L less than 1).

The BMF_L s for the highly hydrophobic chemicals are much smaller than those for HCB and PCBs, as would be expected from the lower assimilation efficiencies and faster elimination rates measured.

Discussion

Although exposing fish via the diet rather than water avoided difficulties of creating and measuring waterborne exposures at extremely low concentrations, the current study was not without challenges. Dosing the food was difficult because of the low solubility of the six highly hydrophobic chemicals in organic solvents used to dose the food. Levels of accumulation for p-TCP, m-TCP, DBDPE, and p-QTCP were quite low, requiring sensitive analytical techniques, which were inadequate for p-QTCP in the elimination phase. For chemicals with low uptake, high concentrations in the food relative to fish tissues create great potential for bias if there is any contamination of the carcass with contents of the GI tract during its removal. We believe the high variability of measurements for DBDPE and p-QCTP (Fig. 2) are a reflection of these difficulties.

For the five highly hydrophobic chemicals with measurable values, k_{2g} s are larger than those for the control and reference chemicals in this study (HCB and PCBs). If these five chemicals behave like control and reference chemicals where passive diffusion controls their partitioning behavior within the fish, the only explanation for their k_{2g} s being larger than those for the HCB and PCBs is additional elimination via

biotransformation. Biotransformation by fish is well documented for BDE209 (Kierkegaard et al. 1999; Munsch et al. 2011; Stapleton et al. 2004, 2006; Wan et al. 2013; Wang et al. 2019) and DBDPE (Hou et al. 2022; Wang et al. 2019; Zheng et al. 2018). For TBPH, measurements with fish liver microsomes have extremely slow depletion rates (Hou et al. 2022; Zheng et al. 2018) and non-detectable depletion rates (Barr et al. 2010). For the m- and p-TCP, there are no measurements of biotransformation rates in fish, and m-TCP and p-TCP elimination rates are 4.7 and 5.0 times faster than PCB-209, respectively. Given the structural similarities to PCB209 and their extremely large predicted biotransformation half-lives (Table 1), we suspect little, if any, biotransformation by the RBT even though their fast elimination rates suggest otherwise.

With the faster elimination rates for the TBPH, m-TCP, and p-TCP, what mechanism(s) beyond biotransformation might explain this behavior? In examining the model of Gobas et al. (2020) for computations with data from the OECD 305 dietary method, fecal elimination rate is inversely related to α , i.e., $k_{BE} = k_{BG}(1 - \alpha)$ where k_{BG} = rate constant for chemical transfer from the fish body to the gastro-intestinal contents (d^{-1}), and k_{BE} = rate constant for fecal excretion of chemical (d^{-1}) (Eq. 5 in Table 1 of Gobas et al. 2020). For the five chemicals in this study, their α values were very small in comparison to those of the reference PCBs, which, according to the Gobas et al. (2020) model, would suggest fecal elimination rates two to four times faster than the reference PCBs. The measured k_{2g} s for TBPH, p-TCP and m-TCP were about fivefold faster than the measured k_{2g} for PCB209 (Table 2). The model and measurements are consistent, suggesting increased elimination caused by the small α values may be responsible for the faster elimination rates.

In this study, the RBT were fed food contaminated with chemicals at nearly the same concentrations (see Table 1) and as shown in Fig. 2, the six highly hydrophobic chemicals have low bioaccumulation potential in comparisons to the PCBs. Additionally, other well controlled laboratory studies with highly hydrophobic chemicals demonstrate similar behavior (Gobas et al. 2020). Low bioaccumulation potential is driven by their low α values, and existing models to estimate α appear to systematically overestimate the measured α values for chemicals with $\log K_{OW}$ s greater than 8.2. Low α values for the highly hydrophobic chemicals lead to these chemicals not being very bioavailable or bioaccumulative.

Conclusions

This study demonstrates that the OECD 305 dietary method (OECD 2012) can be done successfully with highly hydrophobic organic chemicals. Further, it demonstrates that large highly hydrophobic chemicals can be accumulated by fish

and reaffirms the arguments of Larisch and Goss (2018) that there is “no hydrophobicity/size cut off” in fish for highly hydrophobic organic chemicals. The data from this study provide measured k_{us} , k_{2g} s and α values for highly hydrophobic organic chemicals that can be used to improve models for predicting their uptake and elimination by fish, especially for existing models to estimate assimilation rates, as existing models overestimated many of the measured values. The calculated BMF_L s for the highly hydrophobic chemicals were substantially less than 1, i.e., ranging from 0.000051 to 0.0230 (Table 2), suggesting that the highly hydrophobic chemicals should not biomagnify in aquatic food webs, a finding that is not entirely in line with all field-derived BMF_L values. Further research focused on understanding the mechanisms behind low assimilation efficiencies, and for potential inconsistencies between laboratory- and field-derived BMF_L values are key research needs.

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Data Availability Detailed data are provided in the Supplementary Information.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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