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# Occurrence and Distribution of Persistent Organic Pollutants (POPs) in Amphibian Species: Implications from Biomagnification Factors Based on Quantitative Fatty Acid Signature Analysis

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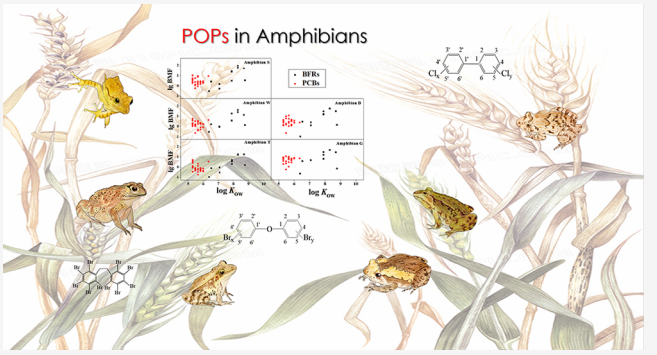
**ABSTRACT:** Contaminants pose a great threat to amphibian populations, but the bioaccumulation and distribution of contaminants in amphibians are still unclear. Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) had median concentrations of 468−3560 ng/g lipid weight (lw) and 206−2720 ng/g lw in the muscle of amphibians, respectively. BDE 209 was the predominant PBDE congener, while CBs 118, 138, 153, and 180 were the main PCB congeners. The diet compositions of amphibians were estimated by quantitative fatty acid signature analysis (QFASA). Dragonfly contributed the most to the diet of amphibians. Biomagnification factors (BMFs) based on quantitative amphibian/insect relationships showed more credible results than BMFs based on amphibian/each insect or amphibian/combined prey relationships. BMFs derived from QFASA declined with log $K_{OW}$ from 5 to 6.5 and then showed a parabolic relationship with log $K_{OW}$ greater than 6.5. BMFs of PCBs were significantly influenced by the elimination capacity of PCBs in amphibians. Less-hydrophobic PCBs preferentially accumulated in the skin than in muscle, which was probably due to the dermal exposure of less-hydrophobic PCBs for amphibians. The biomagnification and distribution of contaminants may be affected by multiple exposure pathways and the toxicokinetics of contaminants in various life stages of amphibians.




**KEYWORDS:** *amphibian, insect, persistent organic pollutants, fatty acid, biomagnification, tissue distribution, maternal transfer*

## 1. INTRODUCTION

Decreases in amphibian populations have raised great concern in the last three decades.[1,2] Many amphibian species have been listed as endangered or threatened species under environmental stress, such as global warming,[3] habitat destruction,[4] spreading of invasive species,[5] human hunting, and exposure to contaminants.[6] Contaminants act as a vital factor in the global decline of amphibian populations.[7−9] Amphibians are sensitive to certain environmental chemicals, which result in morphological malformations and hormone disruption in amphibians.[10] Persistent organic pollutants (POPs) are persistent and toxic chemicals for organisms. Adverse effects such as disruption of thyroid homeostasis induced by polybrominated diphenyl ethers (PBDEs) in amphibian metamorphosis were previously reported.[11−13] Hind limbs, reduced body weight and length, inhibition of tail resorption, delayed metamorphosis, and skin pigmentation impacts were caused by BDEs 47 and 99.[11] Amphibians are considered important environmental stress bioindicators.[14,15] However, an understanding of the bioaccumulation of contaminants in amphibians is incomplete to date.

Amphibians have unique characteristics compared with other vertebrates because they inhabit aquatic and terrestrial environments in different life stages. In addition, amphibians' highly permeable skin facilitates breathing via the skin but may lead to dermal exposure to contaminants.[16] Nevertheless, the occurrence and composition of POPs in amphibians have been reported in limited studies.[17−19] Compositions of PBDEs in a frog species (*Rana limnocharis*) were considered an intermediate between PBDE patterns in terrestrial and aquatic wildlife.[19] Amphibians have diverse terrestrial and aquatic diet items contaminated by distinct profiles of POPs.[20] Biomagnification factors (BMFs) describe the transfer of contaminants from prey to predators. However, most studies reported discrepant BMFs of POPs, mainly because of the uncertainties in predator/prey relationships in field observations.[20−22] BMFs are normally calculated based on the median or mean concentrations of POPs in all available prey samples or

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concentrations of POPs in each prey species, which results in fluctuating BMFs for one chemical.[20−22] Estimation of quantitative diet composition for predators is an important premise for credible BMFs. Quantitative fatty acid signature analysis (QFASA) is a promising method to investigate the diet compositions of wildlife.[23,24] Fatty acids are recalcitrant during digestion and distribution in vertebrates and can be conservatively transferred to higher trophic levels,[24,25] potentially reflecting the diet sources of vertebrates. QFASAR (using R for QFASA) has been increasingly applied in marine organisms such as marine mammals[26,27] and fish[28,29] to achieve quantitative diet compositions but has not yet been reported in terrestrial and riparian food chains.

Embryos are sensitive to contaminants, and maternal transfer of POPs is essential in the toxicological risk assessment for embryos. In the very limited studies on the maternal transfer of POPs in amphibians, the concentrations of POPs in the liver were used to represent the maternal burden of POPs in *R. limnocharis*,[19] and the maternal tissue was not noted in another study.[17] Muscle, liver, fat, blood, and whole body were analyzed in studies on the maternal transfer of POPs in birds, suggesting that selection of maternal tissue led to different maternal transfer results (MTRs).[30−32] The choice of maternal tissue is still a controversial issue in maternal transfer studies of POPs. For amphibians, the distribution and transport of POPs in adult tissues and consequent transfer to eggs are scarcely reported.[17−19]

In the present study, six amphibian species and eight insect species were collected from South China. Biomagnification factors of POPs from insects to amphibians were evaluated based on quantitative results of diet compositions, which were achieved by the QFASAR method. Eggs and several amphibian tissues, including liver, muscle, fat, and skin, were also analyzed for POPs. The aims of the present study were to assess credible BMFs of POPs in different amphibian species and the influences on BMFs and to fill the knowledge gap in the distribution and transfer of POPs in tissues and eggs of amphibians.

## 2. MATERIALS AND METHODS

**2.1. Sample Information.** Six amphibian species, including spot-legged treefrogs (Amphibian S, *Polypedates megacephalus*, $n$ = 4), black-spectacled toads (Amphibian B, *Duttaphrynus melanostictus*, $n$ = 13), wrinkled frogs (Amphibian W, *Hoplobatrachus rugulosus*, $n$ = 13), piebald digging frogs (Amphibian P, *Kaloula pulchra*, $n$ = 2), terrestrial frogs (Amphibian T, *Fejervarya multistriata*, $n$ = 8), and guenther's frogs (Amphibian G, *Boulengerana guentheri*, $n$ = 2), were collected during 2019−2021. The sampling site was an abandoned e-waste recycling site in South China (N 23°36′ E 113°04′). Muscle ($n$ = 42) and skin ($n$ = 42) samples from all amphibian individuals were analyzed. To acquire enough sample weight for analysis of POPs, liver ($n$ = 18) and fat ($n$ = 12) samples were only available for Amphibians B and W. Eggs ($n$ = 10) of Amphibians B and T were also collected (Table S1, Supporting Information). Insect species, including dragonfly (*Dragonfly*), moth (*Lepidoptera*), locust (*Locustoidea*), cricket (*Gryllotalpa spp.*), water scavenger beetle (WSB, *Hydrophilidae*), mantis (*Mantodea*), chafer (*Scarabaeoidea*), and stinkbug (*Hemiptera*), were collected. Approximately, 30−50 individuals of insects were mixed as one composite sample to meet the limits of quantification (LOQs) of POPs. More details are provided in the Supporting Information. Samples were lyophilized and stored in a −20 °C freezer before further analysis.

**2.2. Sample Preparation.** After spiking with internal standards (100 ng of CBs 24, 82, and 198; 20 ng of BDEs 118 and 128, and 50 ng of $^{13}$C-BDE 209), the sample was ultrasonically extracted with 4 mL of dichloromethane three times. The extract was purified with 5 mL of concentrated sulfuric acid to remove lipids and then centrifuged for 5 min at 3000 rpm. The supernatant was transferred to a new tube and concentrated to 1 mL under gentle nitrogen flow. The extract was purified by 4 g of acidic silica, and POPs were eluted by 12 mL of dichloromethane. The eluate was reconstituted in 100 $\mu$L of isooctane after spiking with recovery standards (100 ng of CBs 30, 65, and 204; 20 ng of BDEs 77, 181, and 205). Approximately, 0.2 g of the sample was ultrasonically extracted in the same manner as mentioned in the above method, and the extract was used for gravimetric determination of the lipid content.

**2.3. Instrumental Analysis.** The instrumental analysis methods of the target compounds were the same as those described in a previous study.[33] Briefly, PBDEs and 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) were analyzed by a 7890 Agilent gas chromatograph (GC) coupled with a 5975 mass spectrometer (MS) operated in an electron capture negative ionization mode. Tri- to hepta-BDE congeners (BDEs 47, 99, 100, 153, 154, and 183) were separated on a DB-XLB (30 m × 0.25 mm × 0.25 $\mu$m, J&W Scientific) capillary column. Octa- to deca-BDEs (BDEs 196, 197, 202, 203, 206, 207, 208, and 209), BTBPE, and decabromodiphenyl ethane (DBDPE) were separated on a DB-5HT (15 m × 0.25 mm × 0.10 $\mu$m, J&W Scientific) capillary column. The concentrations of polychlorinated biphenyls (PCBs) were determined by a 7890 Agilent GC coupled with a 5975 MS in an electron ionization mode, and PCBs were separated by a DB-5MS (60 m × 0.25 mm i.d., 0.25 $\mu$m film thickness) capillary column. Target PCBs included CBs 40, 41, 52, 60, 66, 70, 74, 87, 99, 101, 110, 118, 128, 138, 141, 146, 149, 153, 170, 172, 177, 180, 183, 187, 194, 199, 203, and 209. All concentrations of POPs were expressed as ng/g lipid weight (lw) in the present study.

**2.4. Quality Control (QC) and Statistical Analysis.** Quality control was carried out by analyzing blanks and spiked matrices. Spiked mixtures consisted of 10 ng of PCBs and 3 ng of PBDEs. A mixture of POPs was spiked in virgin matrices (frog muscle and cricket) and analyzed in replicate. Recoveries of spiked chemicals ranged from 77.8 to 130% with relative standard deviations less than 15%. Recoveries of surrogate standards were 81.4, 95.9, 91.4, 95.4, 107, and 103% for CB 30, CB 65, CB 204, BDE 77, BDE 181, and BDE 205, respectively. Two blank samples were analyzed in the same manner as other samples in each batch, and the concentrations of POPs in the blank samples were subtracted from those in the study samples. The limits of quantification (LOQs) were set as three times the standard deviations of detected values of POPs in blanks or responses at a signal/noise ratio of 10 when POPs were not detected in blanks. The LOQs ranged from 0.22 to 7.30 ng/g lw (Table S2, Supporting Information).

All statistical analyses were performed using SPSS 22.0. The concentrations of individual chemicals were not normally distributed before and after log transformation. The Mann−Whitney test was employed to test the significance of differences between concentrations of PCBs, PBDEs, and DBDPE in different groups of samples. DBDPE concentrations


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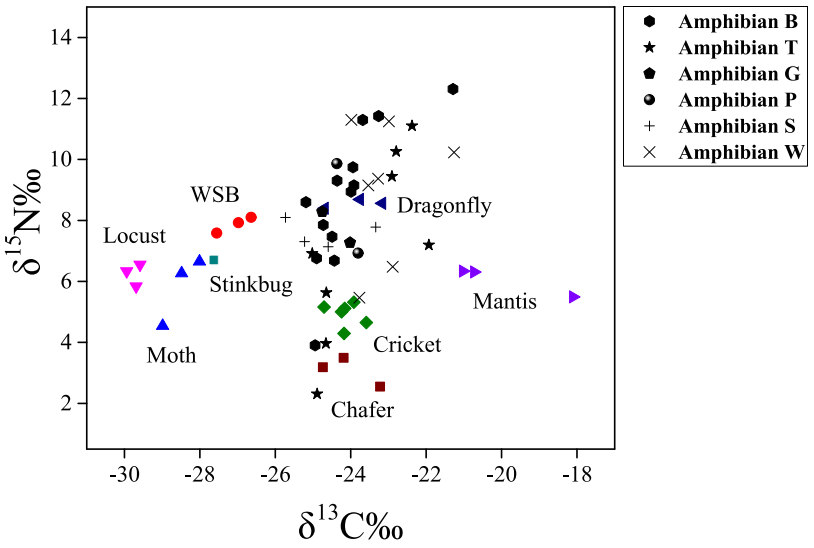


**Figure 1.** Stable isotope results of amphibians and insects.

below the LOQs were set as half of the LOQs before statistical analysis. One-way analysis of variance was used to assess differences between lg-transformed BMFs of PCB congeners in different metabolic groups. Correlations between lg-transformed BMFs and elimination rates of PCB congeners were evaluated by Pearson correlation analysis. QFASAR was conducted using RStudio 1.4.1717. Significance was set as $p < 0.05$.

**2.5. Diet Composition Analysis of Amphibians.** The measurement method of $\delta^{13}C$ and $\delta^{15}N$ was the same as that described in a previous study.[34] Approximately, 0.5 mg of an amphibian muscle or insect sample was put in a tin capsule and analyzed using a Flash EA 112 series elemental analyzer coupled with a Finnigan MAT ConFlo III isotope ratio mass spectrometer. Stable isotope abundances were calculated using the following equation

$$\delta X = (R_{sample}/R_{standard} - 1) \times 1000 \quad (1)$$

where $X$ is $^{13}C$ or $^{15}N$ and $R_{sample}/R_{standard}$ is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio of the sample. The precision values are ± 0.2 and ± 0.5% for $\delta^{13}C$ and $\delta^{15}N$, respectively.

Sample preparation and quantification of fatty acids were the same as mentioned in a previous study.[34] Muscle samples of Amphibians S, B, W, T, and G were used for the analysis of fatty acids. The dietary compositions of amphibians based on FA signatures were estimated based on the QFASAR method proposed in Bromaghin.[23] Details are described in the Supporting Information.

**2.6. Estimations of BMF and Other Ratios.** BMFs were calculated by different methods as follows. The BMF of individual POPs between a predator and each prey was calculated as

$$BMF_{each} = C_{predator}/C_{each\ prey} \quad (2)$$

where $C_{predator}$ and $C_{each\ prey}$ are the median concentrations of POPs (ng/g lw) in an amphibian species and an insect species, respectively.

The BMF of individual POPs between a predator and combined prey was calculated as

$$BMF_{combined} = C_{predator}/C_{combined\ prey} \quad (3)$$

where $C_{predator}$ and $C_{combined\ prey}$ are the median concentrations of POPs (ng/g lw) in an amphibian species and all insect species, respectively.

The BMF of individual POPs with QFASAR results was calculated as

$$BMF_{QFASA} = C_{predator}/\sum p_i C_i \quad (4)$$

where $p_i$ and $C_i$ are the proportion of diet $i$ in the total diet and the concentration (ng/g lw) of a POP in diet $i$, respectively, and $C_{predator}$ is the concentration (ng/g lw) of a POP in an amphibian species.

The tissue distribution ratio (TDR) of individual POPs was calculated as

$$TDR = C_{tissue}/C_{muscle} \quad (5)$$

where $C_{tissue}$ is the concentration (ng/g lw) of a POP in the liver, fat, or skin and $C_{muscle}$ is the concentration (ng/g lw) of a POP in muscle.

The maternal transfer ratio (MTR) of individual POPs for amphibians was calculated as

$$MTR = C_{egg}/C_{maternal\ tissue} \quad (6)$$

where $C_{maternal\ tissue}$ is the concentration (ng/g lw) of a POP in muscle, liver, fat, or skin and $C_{egg}$ is the concentration (ng/g lw) of a POP in the egg.

## 3. RESULTS

**3.1. Stable Isotope and Fatty Acid Profiles.** The $\delta^{15}N$ and $\delta^{13}C$ values are shown in Figure 1 and Table S1, Supporting Information. Figure 1 shows the enrichment of $\delta^{15}N$, not $\delta^{13}C$, from insects to amphibians. Insects had a wide range of $\delta^{13}C$ from −18 to −30‰. Amphibian species had similar $\delta^{13}C$ values from −21 to −25‰ but highly variable $\delta^{15}N$ values from 2.3 to 12‰. The compositions of fatty acids in amphibians and insects are shown in Figures S1 and S2, Supporting Information, respectively. C16:0, C18:0, C18:1n9, and C18:2n6 were the main chemicals in total FAs for all amphibians and insects. C23:0 also accounted for a significant fraction of FAs in amphibians, while 18:3n3 was an important chemical in FAs for most insects.



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Table 1. Median and Range of Pollutant Concentrations (ng/g lw) in Amphibian and Insect Samples[a]

| | N | lipid % | PBDEs | PCBs | BTBPE | DBDPE |
|---|---|---|---|---|---|---|
| S-muscle | 4 | 0.86 | 458 (71.0-908) | 1280 (1150−3570) | nd[b] | 71.8 (10.0−108) |
| B-muscle | 13 | 1.50 | 398 (37.2−1340) | 2260 (329−4430) | nd-9.60 | 31.8 (nd-232) |
| W-muscle | 13 | 0.59 | 262 (75.1−1440) | 1320 (278−9000) | nd-49.2 | 40.6 (nd-191) |
| P-muscle | 2 | 0.71 | 2720 (2580−2860) | 3560 (2120−5000) | nd | 245 (25.4−464) |
| T-muscle | 8 | 1.73 | 206 (86.6−716) | 468 (244−1648) | nd-23.2 | 47.4 (36.4−73.1) |
| G-muscle | 2 | 1.51 | 354 (217−491) | 2271 (602−3940) | nd | 12.8 (nd-25.6) |
| B-eggs | 6 | 13.0 | 256 (133−416) | 3880 (1500−7600) | 5.24 (3.00−28.8) | nd-95.0 |
| T-eggs | 4 | 7.84 | 304 (190−746) | 2300 (540−10700) | nd-6.15 | nd-13.4 |
| B-fat | 6 | 57.9 | 488 (70.4−552) | 8250 (704−25100) | 14.9 (3.39−18.0) | nd |
| W-fat | 6 | 76.3 | 184 (27.8−236) | 2380 (444−9460) | nd-4.31 | nd |
| B-liver | 6 | 7.67 | 150 (96.8−569) | 1710 (960−4730) | nd-4.39 | 9.62 (nd-32.4) |
| W-liver | 12 | 6.41 | 220 (4.84−2340) | 1930 (368−9470) | nd-10.9 | 15.3 (nd-133) |
| S-skin | 4 | 1.66 | 314 (54.6−1120) | 2710 (1610−4260) | 226 (16.0−649) | 8.49 (nd-11.7) |
| B-skin | 13 | 1.12 | 331 (102−1670) | 2980 (621−10200) | 4.00 (nd-1540) | 81.5 (14.3−688) |
| W-skin | 13 | 1.46 | 251 (102−2940) | 1890 (275−18900) | 27.4 (nd-202) | 35.6 (nd-155) |
| P-skin | 2 | 1.59 | 284 (248−319) | 1143 (752−1534) | 38.5 (30.6−46.4) | 47.0 (37.8−56.3) |
| T-skin | 8 | 1.78 | 277 (54.0−3990) | 1960 (826−7000) | 24.9 (2.24−76.1) | 154 (9.58−701) |
| G-skin | 2 | 1.46 | 223 (113−333) | 3920 (1140−6700) | 46.6 (32.6−60.6) | 19.6 (nd-39.2) |
| Dragonfly | 4 | 3.36 | 66.5 (58.8−69.1) | 526 (376−555) | nd-3.35 | 28.7 (23.7−38.8) |
| Moth | 6 | 4.16 | 18.8 (9.44−51.7) | 147 (117−165) | nd-3.04 | 8.79 (nd-21.7) |
| Locust | 4 | 3.58 | 5.62 (2.75−7.23) | 43.4 (23.9−53.3) | nd-3.38 | 7.46 (nd-13.2) |
| Cricket | 5 | 4.98 | 9.96 (5.77−10.2) | 206 (179−254) | 1.24 (nd-1.63) | nd |
| WSB | 5 | 3.45 | 6.89 (5.31−18.2) | 173 (160−466) | 35.6 (31.3−43.0) | nd-8.60 |
| Mantis | 3 | 3.00 | 23.4 (17.9−25.4) | 168 (154−204) | nd-4.57 | 37.7 (28.7−73.8) |
| Chafer | 4 | 1.28 | 8.80 (nd-15.1) | 233 (111−248) | 16.6 (4.32−43.4) | 81.9 (28.6−122) |
| Stinkbug | 1[c] | 3.00 | nd | 442 | 108 | nd |

[a]Each insect sample was a pool of 30 to 50 individuals. [b]Not detected. [c]Data available for only one sample.

**3.2. POPs in Amphibians and Insects.** The concentrations of PCBs, PBDEs, BTBPE, and DBDPE in amphibians and insects are shown in Table 1. Most congeners of PCBs and PBDEs and DBDPE were detected in the samples, while BTBPE had low detection frequencies. PCBs were the main POPs, with median concentrations of 1280, 2260, 1320, 3560, 468, and 2271 ng/g lw in muscle samples of spot-legged treefrogs (Amphibian S), black-spectacled toads (Amphibian B), wrinkled frogs (Amphibian W), piebald digging frogs (Amphibian P), terrestrial frogs (Amphibian T), and guenther's frogs (Amphibian G), respectively. The median concentrations of PBDEs were 458, 398, 262, 2720, 206, and 354 ng/g lw in the muscle of Amphibians S, B, W, P, T, and G, respectively. The median concentrations of DBDPE in muscle ranged from 12.8 to 71.8 ng/g lw. Amphibian P had significantly higher concentrations of PBDEs in muscle than those of Amphibians B, W, and T ($p < 0.05$). Amphibian T had significantly lower concentrations of PCBs in muscle than those of Amphibians S, B, W, and P ($p < 0.05$). A significant difference in DBDPE concentrations in muscle was only found between Amphibians T and G ($p < 0.05$). BDE 209 was the predominant congener in PBDEs and contributed to 36−65% of the total PBDEs in muscle (Figure S3, Supporting Information). Other PBDE congeners contributed to less than 10% of PBDEs in muscle in most cases. Amphibians B, W, and G had lower fractions of BDE 209 and slightly higher fractions of BDEs 153 and 183 in muscle than other amphibian species. The compositions of PCBs in muscle were relatively consistent in six amphibian species. CBs 118, 138, 153, and 180 were the main PCB congeners (Figure S4, Supporting Information).

Skin samples were available for all amphibian species and had median concentrations of 223−331 and 1143−3920 ng/g lw for PBDEs and PCBs, respectively (Table 1). The skin had similar concentrations of PBDEs and higher concentrations of PCBs than those of muscle, and a significant difference was observed in the concentrations of PCBs in the skin and muscle of Amphibian T ($p < 0.05$). Significant differences in the concentrations of PCBs and PBDEs were observed between tissues of Amphibian B ($p < 0.05$), but no differences were observed in the concentrations of POPs in tissues of Amphibian W ($p > 0.05$). The compositions of PBDEs in the liver and fat are described in Figure S5, Supporting Information. Fractions of BDE 209 in tissues ranked as muscle > liver > fat, while other BDE congeners ranked as muscle < liver < fat for Amphibians B and W. Higher fractions of BDE 209 were observed in the skin than in muscle in Amphibians S, W, T, and G, while lower fractions of BDE 209 were observed in the skin than in muscle in Amphibian B (Figure S6, Supporting Information). Fractions of BDE 209 were comparable in the skin and muscle in Amphibian P (Figure S6, Supporting Information). The compositions of PCBs were similar in liver and fat. Muscle and skin had higher fractions of tri- and tetra-CBs than those of liver and fat for Amphibians B and W (Figures S7 and S8, Supporting Information).

Dragonfly insects had the highest median concentrations of PBDEs (66.5 ng/g lw) and PCBs (526 ng/g lw) (Table 1). The highest median concentration of DBDPE in insect species was observed in chafer. Insects have different composition patterns of PBDEs and PCBs (Figures S9 and S10, Supporting Information). Dragonfly, moth, and WSB had higher fractions of BDE 47 than other insect species. Cricket had the lowest fractions of BDEs 47 and 99 and the highest fractions of BDEs


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153 and 183 in all insect species. PBDEs were dominated by BDE 209 in insect species other than dragonflies, moths, and WSBs. CBs 118, 128, 153, and 180 were the main PCB congeners in insects, while moths had higher fractions of tri- to penta-CBs than those of other insect species.

## 4. DISCUSSION

**4.1. Comparisons with Previous Studies.** The occurrence of POPs in amphibians has only been reported in a limited number of studies.[17−19] A frog species (*R. limnocharis*) collected at the same site as this study was measured for PBDEs[19] and had similar PBDE concentrations as all amphibians except for Amphibian P in the present study. However, distinct compositions were observed in Wu et al.[19] compared to in this study. BDEs 99 and 153 were the main PBDE congeners, followed by BDEs 47, 100, and 209 among PBDEs in *R. limnocharis*.[19] In contrast, BDE 209 was the predominant PBDE in all amphibian species in this study. BDE 99 was also the predominant PBDE congener (more than 40% of PBDEs) in *R. limnocharis* from another e-waste recycling site in China.[18] Species-specific bioaccumulation of PBDEs in amphibians cannot be ruled out, but the local PBDE source is more likely responsible for the results. Samples were collected in 2006 and 2009 in Wu et al.[19] and Liu et al.,[18] respectively, when technical mixtures of PBDEs were not banned worldwide. Penta- and Octa-BDEs were listed among POPs by the Stockholm Convention in 2009, and Deca-BDE was also added to POPs in 2017.[35] It is reasonable to observe higher fractions of BDE 209 in samples collected in recent years than those collected at earlier times (especially before 2009) in e-waste recycling areas.

**4.2. Biomagnification of POPs.** *4.2.1. Calculation of Biomagnification Factors.* Biomagnification factors were calculated based on the concentrations of individual POPs in muscle samples of amphibians relative to those in insects. Estimation of reliable diet compositions of amphibians was the primary issue before calculations of BMFs. The $\delta^{13}C$ values of amphibians were in the middle of the $\delta^{13}C$ values of insects (Figure 1), indicating that these insect species are potential sources of food for amphibians. Moreover, the similar $\delta^{13}C$ values and highly variable $\delta^{15}N$ values of amphibians suggest that these amphibian species have similar diet sources but different diet compositions. $\delta^{13}C$ values can provide a general view of diet items but cannot provide quantitative results of diet items for a predator.[36] $\delta^{15}N$ values enriched by 3−5‰ from prey to predator and are commonly used to estimate the trophic level of organisms. However, BMFs cannot be normalized by the enrichment of $\delta^{15}N$ in the present study. Amphibian species had average $\delta^{15}N$ values from 7.10 to 9.03, which were higher than the $\delta^{15}N$ values in most insects but comparable to the $\delta^{15}N$ values in dragonflies (mean $\delta^{15}N$: 8.55) and water scavenger beetles (mean $\delta^{15}N$: 7.87) (Table S1, Supporting Information). Thus, the complex diet sources of amphibians cannot be clearly elucidated by the $\delta^{13}C$ and $\delta^{15}N$ values.

The QFASAR method was applied to amphibian and insect samples in this study. A total of 12 FAs (C12:0, C14:0, C15:0, C16:0, C16:1, C17:0, C18:0, C18:1n9, C18:2n6, C18:3n3, C20:2n6, and C20:5n-3) had detection frequencies greater than 50% in all species of amphibians and insects and were used to calculate diet compositions for amphibians. Dragonfly was recognized as the main food for all amphibians and contributed 64.2−99.7% of the total diet (Table S3, Supporting Information). Stinkbug, moth, locust, and cricket were also included in the diets of amphibians and contributed 0−24% of the total diet for different amphibian species. It should also be noted that the investigated insect species are only a proportion of the insect populations within the studied site, which is located in a subtropical area with high biodiversity. Dragonflies migrate from the aquatic environment in the larval stage to the terrestrial environment in the adult stage, which is similar to amphibians. Dragonfly individuals and amphibians may have similar diet sources during different life stages, leading to similar $\delta^{13}C$ and fatty acid signatures. This assumption warrants further study.

BMFs were calculated in different ways for comparison (Table S4, Supporting Information). $BMF_{each}$ was set as a ratio between the median concentrations of POPs in a predator species and each prey species, leading to fluctuating BMF data for a single chemical. $BMF_{combined}$ was set as a ratio between the median concentrations of POPs in a predator species and all insect species, and $BMF_{QFASA}$ was derived from quantitative diet compositions according to QFASAR results. $BMF_{each}$ varied by several orders of magnitude for the same chemical, and sometimes $BMF_{each}$ even ranged from less than 1 to more than 1000 (Table S4, Supporting Information). Most POPs had $BMF_{combined}$ and $BMF_{QFASA}$ greater than 1, and $BMF_{combined}$ was generally several times higher than $BMF_{QFASA}$. The $BMF_{combined}$ values of BDEs 196 and 207 were not available because the two PBDE congeners were not detected in most insect species. The maximum BMFs of POPs were predicted to be less than 100 in various taxa of animals, and the highest predicted BMFs were observed for some carnivorous mammals and birds.[37] For insectivores, the predicted maximum BMFs of POPs were 2.9, 31.1, and 57.8 for wolf spiders (*Lycosa rabida*), great tits (*Parus major*) adults, and bats (*Plecotus auritus*), respectively. The observed maximum BMF of POPs was 31.0 for great tit adults.[37] In the present study, most $BMF_{QFASA}$ were in the range of 1−10, with a few exceptions up to almost 100 for amphibians. Endotherms are considered to have a higher biomagnification potential of POPs than ectotherms due to their relatively longer lifetime and higher metabolic capacity for endotherms.[38] Therefore, the maximum BMFs for amphibians are expected to be higher than those for spiders and lower than those for birds and mammals, which have maximum BMFs of 10 to 100, as predicted in Debruyn and Gobas.[37] The results indicate that the estimated BMFs without quantitative diet compositions are not reliable and may be misleading in the interpretation of data. Surprisingly, $BMF_{QFASA}$ values for Amphibian T were generally lower than 1. For instance, the $BMF_{QFASA}$ of CB 153 was 0.70 for Amphibian T, although CB 153 was considered a persistent chemical in different food webs.[39] The $BMF_{QFASA}$ values for Amphibian T still need further investigation considering the limited sample size ($n$ = 8) of Amphibian T.

*4.2.2. Influences on the Biomagnification Factors of POPs.* The BMFs of POPs may be influenced by the physiochemical properties of POPs and biological processes in amphibians. The BMFs of POPs were plotted as the log $K_{OW}$ of POPs. The data source of log $K_{OW}$ is introduced in the Supporting Information. A parabolic relationship was observed between log $K_{OW}$ and lg-transformed $BMF_{each}$ or lg-transformed $BMF_{combined}$ (Figures S11−S17, Supporting Information). The peak values of $BMF_{each}$ and $BMF_{combined}$ were always observed for POPs with log $K_{OW}$ of approximately 8.5. In contrast, the relationships between lg-transformed $BMF_{QFASA}$


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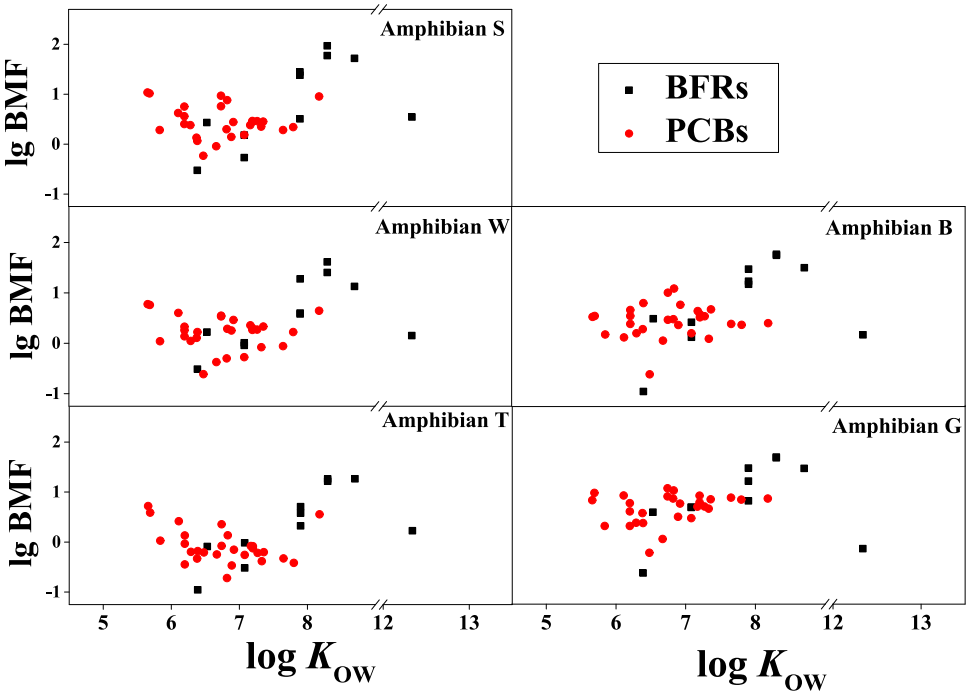


**Figure 2.** Lg-transformed BMF-FAs based on results using R for quantitative fatty acid signature analysis (QFASA). Brominated flame retardants (BFRs) included PBDEs, BTBPE, and DBDPE. The break in the horizontal axis is from 9 to 12.

and log $K_{OW}$ showed a unique pattern. $BMF_{QFASA}$ was negatively related to log $K_{OW}$ between 5 and 6.5, increased with higher log $K_{OW}$ until 8.5, and declined with further increases in log $K_{OW}$ (Figure 2). Wu et al.[19] reported a parabolic relationship between lg-transformed BMF and log $K_{OW}$ of PBDEs in amphibians and insects, which was consistent with the results of PBDEs and DBDPE in this study. The parabolic relationship between lg-transformed BMF and log $K_{OW}$ of PBDEs can be explained by the preferential accumulation of more-hydrophobic POPs with fewer bromine atoms and the influence of steric hindrance derived from the high molecular weight of POPs with more bromine atoms.[19] The relationship between lg-transformed BMF and log $K_{OW}$ of PCBs showed contradictory trends for $BMF_{each}$, $BMF_{combined}$, and $BMF_{QFASA}$. In addition, the correlations between lg-transformed $BMF_{QFASA}$ and log $K_{OW}$ of PCBs were not significant ($p > 0.05$), which can account for the similar log $K_{OW}$ of PCBs with the same chlorine atoms.

The metabolism of PCBs in most vertebrates is dependent on the PCB structure, as suggested in Kannan et al.[39] PCBs with both *meta-para-* and *ortho-meta* vicinal hydrogens were considered susceptible to metabolic attack.[39] The target PCBs in this study were divided into four metabolic groups based on the substitution of chlorine atoms.[39] PCBs in groups 1 and 3 were theoretically more resistant to metabolism than PCBs in groups 2 and 4.[39] To explore the effect of the metabolic efficiency of PCBs on BMFs, lg-transformed $BMF_{QFASA}$ of PCBs was compared between the four groups of PCBs. As shown in Figure S18, Supporting Information, the lg-transformed $BMF_{QFASA}$ values of PCBs in group 2 were lower than those in other groups for all amphibians. Significant differences in the lg-transformed $BMF_{QFASA}$ values of PCBs in groups 2 and 3 were observed for Amphibians S, B, and W ($p < 0.05$). Moreover, the lg-transformed $BMF_{QFASA}$ values of PCBs in group 4 were not significantly different from those in groups 1 and 3 with few exceptions. In addition, amphibians show different elimination rates of PCBs in different life stages, such as tadpole,[40] metamorphosis,[40] and adults.[41] Correlations between the lg-transformed $BMF_{QFASA}$ values of PCBs in this study and the elimination rate constants of PCBs in metamorph[40] and adult[41] amphibians from the literature were assessed. Negative and significant correlations were observed between the lg-transformed $BMF_{QFASA}$ values of PCBs for Amphibians S, B, W, and G and the elimination rates of PCBs in green frog (*Rana clamitans*) metamorphs and leopard frog (*Rana pipiens*) metamorphs[40] (Tables S5 and S6, Supporting Information). The results suggest that BMFs of PCBs are strongly affected by the metabolic efficiency of PCBs, and the metabolism of PCBs during the metamorphosis stage is an important factor mediating BMFs of PCBs, which warrants more toxicokinetic studies to confirm the results.

Protein was suggested as another important binding site for organic contaminants in lipid-poor tissues, and the sorptive capacity of animal protein was estimated as 5% of that of lipids in model predictions.[42] The protein contents in insects and amphibians were collected from the literature and converted to lipid contents by multiplying by 5%. The calculations of $BMF_{QFASA}$ normalized by protein and lipid contents are given in the Supporting Information. The results are shown in Tables S7 and S8, Supporting Information. The $BMF_{QFASA}$ values adjusted by protein and lipid contents were generally 50−100% of $BMF_{QFASA}$ based on lipid-normalized concentrations of POPs, mainly due to the relatively high protein (approximately 20%) and low lipid contents (approximately 1%) in amphibian muscle. It should be noted that the adjusted BMFs are only rough estimations because binding capacity data of proteins with different POPs are still scarce and may differ from lipid sorption with POPs.

**4.3. Tissue Distribution and Maternal Transfer of POPs.** As hydrophobic chemicals, POPs are supposed to be distributed in relation to lipid contents in the tissues of organisms. However, significant differences were observed in


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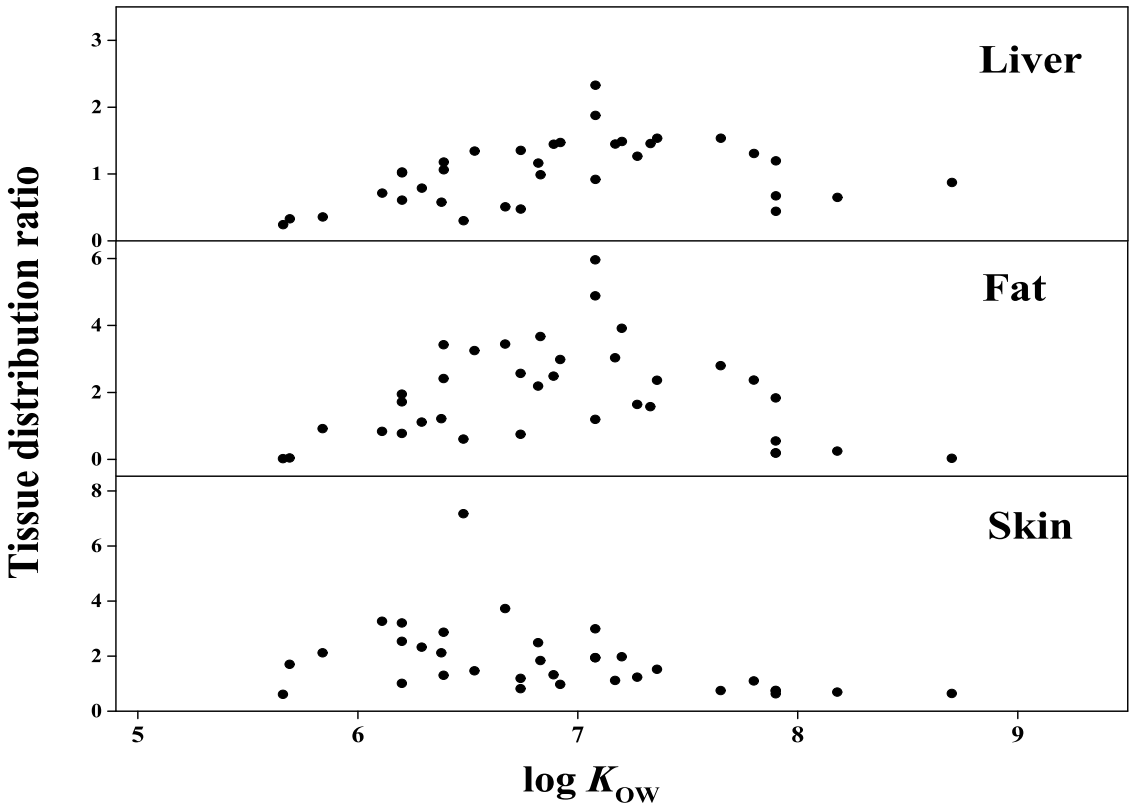


**Figure 3.** Tissue distribution ratios of POPs in Amphibian W.

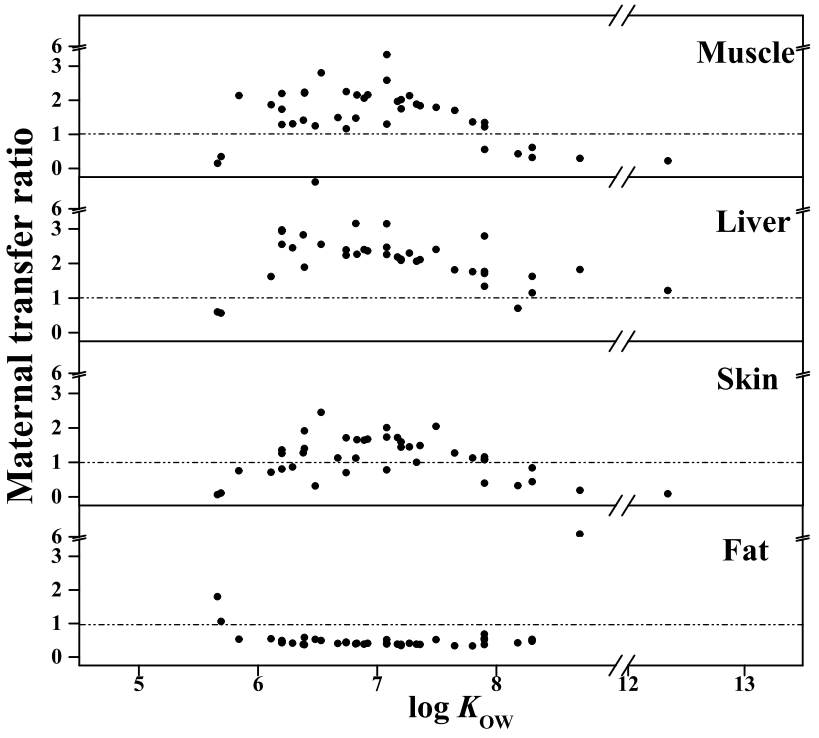


**Figure 4.** Maternal transfer factors of POPs in Amphibian B. The breaks in the horizontal axis and the vertical axis are from 9 to 12 and 3.5 to 6, respectively. The dotted line means a maternal transfer factor of 1.

the lipid-normalized concentrations of PCBs between muscle and skin of Amphibian T ($p < 0.05$) and in the concentrations of PCBs, PBDEs, and DBDPE between different types of tissues of Amphibian B ($p < 0.05$). The compositions of PCBs and PBDEs were also different in tissues. Fat and liver had much lower fractions of BDE 209 and tri- to tetra-CBs and higher fractions of tetra- to hepta-BDEs than muscle and skin for Amphibians B and W. To clearly elucidate the tissue



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distribution characteristics of POPs, the tissue distribution ratios (TDRs) were calculated between the concentrations of POPs in a certain tissue and muscle. The TDRs were plotted with log $K_{OW}$ in Figure 3 for Amphibian W and in Figures S19−S23, Supporting Information, for other amphibian species.

Most POPs had TDRs greater than 1, indicating higher concentrations of POPs in the liver, fat, and skin than in muscle, except for those in the liver of Amphibians B and P. TDRs between the liver and muscle were consistent for PBDEs except BDE 209, which preferentially accumulated in the liver of *R. limnocharis*.[19] The TDRs of POPs between skin and muscle were negatively related to log $K_{OW}$ for all six amphibian species. Amphibian skin is thin and involved in both gas and water exchange. Dermal exposure presents a potentially significant but understudied route for the uptake of POPs, especially less-hydrophobic POPs in amphibians. However, the estimated absorption rates showed no clear relationship with the log $K_{OW}$ of the selected pesticides.[16] It should be noted that Van Meter et al.[16] reported dermal uptake of five pesticides with log $K_{OW}$ of 0.57−5.18, rather than more-hydrophobic PCBs and PBDEs. The dermal uptake efficiency of PCBs and PBDEs is still unclear. Another potential factor affecting the TDR is the binding of POPs with different organic matter in amphibians. Skin and muscle tissues of amphibians had relatively low lipid contents (approximately 1%) in the present study. Another nonlipid organic matter may also contribute to the distribution of POPs in amphibian tissues.[42]

The maternal transfer ratios (MTRs) were calculated between the concentrations of POPs in eggs and a certain tissue (Figures 4, S24 and S25, Supporting Information). The MTRs between skin and eggs were lower than the MTRs between liver/muscle and eggs. A parabolic relationship between the MTRs and log $K_{OW}$ of POPs in the liver, muscle, skin, and eggs was observed. Wu et al.[19] also found a parabolic relationship between the maternal transfer ratios and log $K_{OW}$ of PBDEs. The MTRs between fat and egg samples showed a different feature and were approximately 0.5 for all POPs except for BDE 209 (Figure 4). Previous studies seldom concerned the maternal transfer of contaminants from different tissues to eggs. In the maternal transfer of PCBs from chicken tissues to eggs, fat was suggested to represent the maternal burden of PCBs in chickens because fat has congener compositions and chiral signatures similar to those of eggs.[43] Maternal transfer of PCBs occurs along with the mobilization of fat to form yolk in chickens.[43] In amphibians, the direct transfer of POPs from fat to eggs may provide an explanation for the similar MTRs of most POPs between fat and eggs.

**4.4. Environmental Implications.** The present study reported BMFs of POPs from insects to amphibians based on quantitative diet compositions of amphibians derived from QFASA. The tissue distribution and the maternal transfer process of POPs in amphibians were also preliminarily discussed. Although the observed BMFs were based on highly variable concentrations of POPs in a limited number of samples in a field study, more reliable BMFs were acquired based on QFASA results than BMFs with unknown diet compositions of predators. QFASA has been increasingly used to study marine food webs in the last two decades.[23−29] The present study suggests that QFASA is a promising tool to trace credible prey/predator relationships in terrestrial environments, which will largely extend knowledge on the trophic transfer of essential nutrients and contaminants in riparian and terrestrial food webs.

BMFs derived from QFASA results are influenced by log $K_{OW}$ and elimination rates of POPs, but the key processes in the exposure pathway and metabolism of POPs remain unclear in amphibians.[44−47] The developmental stage (tadpole, metamorphosis, adult, hibernation), sex, age, species, and environmental conditions such as temperature and habitat are important factors affecting the BMF of contaminants. Further *in vivo* laboratory studies and *in silico* studies are necessary to create a life-cycle model describing the toxicokinetics of POPs in frogs, which can be combined with field observation results.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.1c07416.

Detailed description of sample information, compositions of POPs and fatty acids, BMF data, tissue distribution ratios, and maternal transfer ratios (PDF)

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### Notes

The authors declare no competing financial interest.

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## ■ REFERENCES

(1) Wake, D. B. Declining amphibian populations. *Science* **1991**, *253*, 860−861.
(2) Houlahan, J. E.; Findlay, C. S.; Schmidt, B. R.; Meyer, A. H.; Kuzmin, S. L. Quantitative evidence for global amphibian population declines. *Nature* **2000**, *404*, 752−755.
(3) Pounds, J. A.; Bustamante, M. R.; Coloma, L. A.; Consuegra, J. A.; Fogden, M. P. L.; Foster, P. N.; La Marca, E.; Masters, K. L.; Merino-Viteri, A.; Puschendorf, R.; et al. Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* **2006**, *439*, 161−167.
(4) Fonseca, C. R.; Becker, C. G.; Haddad, C. F. B.; Prado, P. I. Response to comment on "Habitat split and the global decline of amphibians. *Science* **2008**, *320*, No. 874.
(5) Stolzenburg, W. Double agents. *J. Nat. Conserv.* **1999**, *49*, 18−24.
(6) Hayes, T. B.; Case, P.; Chui, S.; Chung, D.; Haeffele, C.; Haston, K.; Lee, M.; Mai, V. P.; Marjuoa, Y.; Parker, J. Pesticide mixtures, endocrine disruption, and amphibian declines: Are we underestimating the impact? *Environ. Health. Perspect.* **2006**, *114*, 40−50.
(7) Collins, J. P.; Storfer, A. Global amphibian declines: Sorting the hypotheses. *Diversity Distrib.* **2003**, *9*, 89−98.
(8) Kiesecker, J. M. Global stressors and the global decline of amphibians: Tipping the stress immunocompetency axis. *Ecol. Res.* **2011**, *26*, 897−908.
(9) Brühl, C. A.; Schmidt, T.; Pieper, S.; Alscher, A. Terrestrial pesticide exposure of amphibians: An underestimated cause of global decline? *Sci. Rep.* **2013**, *3*, No. 1135.
(10) Burkhart, J. G.; Ankley, G.; Bell, H.; Carpenter, H.; Fort, D.; Gardiner, D.; Gardner, H.; Hale, R.; Helgen, J. C.; Jepson, P.; Johnson, D.; Lannoo, M. Strategies for assessing the implications of malformed frogs for environmental health. *Environ. Health Perspect.* **2000**, *108*, 83−90.
(11) Carlsson, G.; Kulkarni, P.; Larsson, P.; Norrgren, L. Distribution of BDE-99 and effects on metamorphosis of BDE-99 and -47 after oral exposure in Xenopus tropicalis. *Aquat. Toxicol.* **2007**, *84*, 71−79.
(12) Balch, G. C.; Velez-Espino, L. A.; Sweet, C.; Alaee, M.; Metcalfe, C. D. Inhibition of metamorphosis in tadpoles of Xenopus laevis exposed to polybrominated diphenyl ethers (PBDEs). *Chemosphere* **2006**, *64*, 328−338.
(13) Schriks, M.; Zvinavashe, E.; Furlow, J. D.; Murk, A. J. Disruption of thyroid hormone-mediated Xenopus laevis tadpole tail tip regression by hexabromocyclododecane (HBCD) and 2,2′,3,3′,4,4′,5,5′,6-nonabrominated diphenyl ether (BDE206). *Chemosphere* **2006**, *65*, 1904−1908.
(14) Du, X.; Yuan, B.; Zhou, Y.; Zheng, Z.; Wu, Y.; Qiu, Y.; Zhao, J.; Yin, G. Tissue-Specific Accumulation, Sexual Difference, and Maternal Transfer of Chlorinated Paraffins in Black-Spotted Frogs. *Environ. Sci. Technol.* **2019**, *53*, 4739−4746.
(15) Cui, Q.; Pan, Y.; Zhang, H.; Sheng, N.; Wang, J.; Guo, Y.; Dai, J. Occurrence and tissue distribution of novel perfluoroether carboxylic and sulfonic acids and legacy per/polyfluoroalkyl substances in black-spotted frog (Pelophylax nigromaculatus). *Environ. Sci. Technol.* **2018**, *52*, 982−990.
(16) Van Meter, R. J.; Glinski, D. A.; Hong, T.; Cyterski, M.; Henderson, W. M.; Purucker, S. T. Estimating terrestrial amphibian pesticide body burden through dermal exposure. *Environ. Pollut.* **2014**, *193*, 262−268.
(17) Kadokami, K.; Takeishi, M.; Kuramoto, M.; Ono, Y. Maternal transfer of organochlorine pesticides, polychlorinated dibenzop-dioxins, dibenzofurans, and coplanar polychlorinated biphenyls in frogs to their eggs. *Chemosphere* **2004**, *57*, 383−389.
(18) Liu, P. Y.; Du, G. D.; Zhao, Y. X.; Mu, Y. S.; Zhang, A. Q.; Qin, Z. F.; Zhang, X. Y.; Yan, S. S.; Li, Y.; Wei, R. G.; Qin, X. F.; Yang, Y. J. Bioaccumulation, maternal transfer and elimination of polybrominated diphenyl ethers in wild frogs. *Chemosphere* **2011**, *84*, 972−978.
(19) Wu, J. P.; Luo, X. J.; Zhang, Y.; Chen, S. J.; Mai, B. X.; Guan, Y. T.; Yang, Z. Y. Residues of polybrominated diphenyl ethers in frogs (*Rana limnocharis*) from a contaminated site, South China: tissue distribution, biomagnification, and maternal transfer. *Environ. Sci. Technol.* **2009**, *43*, 5212−5217.
(20) (a) Liu, Y.; Luo, X. J.; Huang, L. Q.; Yu, L. H.; Mai, B. X. Bioaccumulation of Persistent Halogenated Organic Pollutants in Insects: Common Alterations to the Pollutant Pattern for Different Insects during Metamorphosis. *Environ. Sci. Technol.* **2018**, *52*, 5145−5153. (b) Gobas, F. A. P. C.; de Wolf, W.; Burkhard, L. P.; Verbruggen, E.; Plotzke, K. Revisiting bioaccumulation criteria for POPs and PBT assessments. *Integr. Environ. Assess. Manage.* **2009**, *5*, 624−637.
(21) Weisbrod, A. V.; Woodburn, K. B.; Koelmans, A. A.; Parkerton, T. F.; McElroy, A. E.; Borgå, K. Evaluation of bioaccumulation using in vivo laboratory and field studies. *Integr. Environ. Assess. Manage.* **2009**, *5*, 598−623.
(22) Kelly, B. C.; Ikonomou, M. G.; Blair, J. D.; Morin, A. E.; Gobas, F. A. P. C. Food web−specific biomagnification of persistent organic pollutants. *Science* **2007**, *317*, 236−239.
(23) Bromaghin, J. F. QFASAR: quantitative fatty acid signature analysis with R. *Methods Ecol. Evol.* **2017**, *8*, 1158−1162.
(24) Iverson, S. J.; Field, C.; Don Bowen, W.; Blanchard, W. Quantitative fatty acid signature analysis: a new method of estimating predator diets. *Ecol. Monogr.* **2004**, *74*, 211−235.
(25) Budge, S. M.; Penney, S. N.; Lall, S. P. Estimating diets of Atlantic salmon (Salmo salar) using fatty acid signature analyses; validation with controlled feeding studies. *Can. J. Fish. Aquat. Sci.* **2012**, *69*, 1033−1046.
(26) Ning, X.; Gui, D.; He, X.; Wu, Y. Diet Shifts Explain Temporal Trends of Pollutant Levels in IndoPacific Humpback Dolphins (Sousa chinensis) from the Pearl River Estuary, China. *Environ. Sci. Technol.* **2020**, *54*, 13110−13120.
(27) Bromaghin, J. F.; Lance, M. M.; Elliott, E. W.; Jeffries, S. J.; Acevedo-Gutiérrez, A.; Kennish, J. M. New insights into the diets of harbor seals (Phoca vitulina) in the Salish Sea revealed by analysis of fatty acid signatures. *Fish. Bull.* **2013**, *111*, 13−26.
(28) Guo, F.; Lee, S. Y.; Kainz, M. J.; Brett, M. T. Fatty acids as dietary biomarkers in mangrove ecosystems: Current status and future perspective. *Sci. Total. Environ.* **2020**, *739*, No. 139907.
(29) Happel, A.; Stratton, L.; Kolb, C.; Hays, C.; Rinchard, J.; Czesny, S. Evaluating quantitative fatty acid signature analysis (QFASA) in fish using controlled feeding experiments. *Can. J. Fish. Aquat. Sci.* **2016**, *73*, 1222−1229.
(30) Verreault, J.; Villa, R. A.; Gabrielsen, G. W.; Skaare, J. U.; Letcher, R. J. Maternal transfer of organohalogen contaminants and metabolites to eggs of Arctic-breeding glaucous gulls. *Environ. Pollut.* **2006**, *144*, 1053−1060.

(31) Van den Steen, E.; Jaspers, V. L. B.; Covaci, A.; Neels, H.; Eens, M.; Pinxten, R. Maternal transfer of organochlorines and brominated flame retardants in blue tits (Cyanistes caeruleus). *Environ. Int.* **2009**, *35*, 69−75.
(32) Tanabe, S.; Subramanian, A.; Hidaka, H.; Tatsukawa, R. Transfer rates and pattern of PCB isomers and congeners and p,p'-DDE from mother to egg in Adelie Penguin (Pygoscelis adeliae). *Chemosphere* **1986**, *15*, 343−351.
(33) Zheng, X. B.; Wu, J. P.; Luo, X. J.; Zeng, Y. H.; She, Y. Z.; Mai, B. X. Halogenated flame retardants in home-produced eggs from an electronic waste recycling region in South China: levels, composition profiles, and human dietary exposure assessment. *Environ. Int.* **2012**, *45*, 122−128.
(34) Tao, L.; Zhang, Y.; Wu, J. P.; Wu, S. K.; Liu, Y.; Zeng, Y. H.; Luo, X. J.; Mai, B. X. Biomagnification of PBDEs and alternative brominated flame retardants in a predatory fish: Using fatty acid signature as a primer. *Environ. Int.* **2019**, *127*, 226−232.
(35) UNEP. Stockholm Convention Text and Annexes as Amended in 2009, 2009. http://chm.pops.int/Convention/tabid/54/language/en-US/Default.aspx#convtext.
(36) Cabana, G.; Rasmussen, J. B. Modeling food-chain structure and contaminant bioaccumulation using stable nitrogen isotopes. *Nature* **1994**, *372*, 255−257.
(37) Debruyn, A. M. H.; Gobas, F. A. P. C. A bioenergetic biomagnification model for the animal kingdom. *Environ. Sci. Technol.* **2006**, *40*, 1581−1587.
(38) (a) Romero-Romero, S.; Herrero, L.; Fernandez, M.; Gómara, B.; Acuna, J. L. Biomagnification of persistent organic pollutants in a deep sea, temperate food web. *Sci. Total. Environ.* **2017**, *605*−*606*, 589−597. (b) Walters, D. M.; Jardine, T. D.; Cade, B. S.; Kidd, K. A.; Muir, D. C. G.; Leipzig, S.. P. Trophic magnification of organic chemicals: A global synthesis. *Environ. Sci. Technol.* **2016**, *50*, 4650−4658.
(39) Kannan, N.; Reusch, T. B. H.; Schulz-Bull, D. E.; Petrick, G.; Duinker, J. C. Chlorobiphenyls: Model compounds for metabolism in food chain organisms and their potential use as ecotoxicological stress indicators by application of the metabolic slope concept. *Environ. Sci. Technol.* **1995**, *29*, 1851−1859.
(40) Leney, J. L.; Drouillard, K. G.; Haffner, G. D. Metamorphosis increases biotransformation of polychlorinated biphenyls: a comparative study of polychlorinated biphenyl metabolism in green frogs (Rana clamitans) and leopard frogs (Rana pipiens) at various life stages. *Environ. Toxicol. Chem.* **2006**, *25*, 2971−2980.
(41) Leney, J. L.; Balkwill, K. C.; Drouillard, K. G.; Haffner, G. D. Determination of polychlorinated biphenyl and polycyclic aromatic hydrocarbon elimination rates in adult green and leopard frogs. *Environ. Toxicol. Chem.* **2006**, *25*, 1627−1634.
(42) Debruyn, A. M. H.; Gobas, F. A. P. C. The sorptive capacity of animal protein. *Environ. Toxicol. Chem.* **2007**, *26*, 1803−1808.
(43) Zheng, X. B.; Luo, X. J.; Zeng, Y. H.; Wu, J. P.; Mai, B. X. Chiral Polychlorinated Biphenyls (PCBs) in Bioaccumulation, Maternal Transfer, and Embryo Development of Chicken. *Environ. Sci. Technol.* **2015**, *49*, 785−791.
(44) Angell, R. A.; Haffner, G. D. Polychlorinated biphenyl elimination rates and changes in chemical activity in hibernating amphibians. *Environ. Toxicol. Chem.* **2010**, *29*, 700−707.
(45) Cary, T. L.; Karasov, W. H. Toxicokinetics of polybrominated diphenyl ethers across life stages in the northern leopard frog (Lithobates popiens). *Environ. Toxicol. Chem.* **2013**, *32*, 1631−1640.
(46) Katagi, T.; Ose, K. Bioconcentration and metabolism of pesticides and industrial chemicals in the frog. *J. Pestic. Sci.* **2014**, *39*, 55−68.
(47) Leney, J. L.; Drouillard, K. G.; Haffner, G. D. Does metamorphosis increase the susceptibility of frogs to highly hydrophobic contaminants? *Environ. Sci. Technol.* **2006**, *40*, 1491−1496.





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