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Tissue-specific distribution and bioaccumulation potential of organophosphate flame retardants in crucian carp☆

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ABSTRACT

The concentrations, distributions, and bioaccumulation of nine organophosphate flame retardants (OPFRs) were investigated in both abiotic and biotic media, comprising river water, sediment, and crucian carp. The highest concentrations were observed in liver (6.22–18.1 ng/g ww), and the levels in muscle (4.23–7.75 ng/g ww) and gonad (3.08–7.70 ng/g ww) were similar. In whole blood, tris(2-butoxyethyl) phosphate (TBOEP; 31.1–256 ng/mL) accounted for 90% of the total OPFR concentration. Distributions of OPFRs differed between biotic and abiotic media, as tris(2-chloroethyl) phosphate (TCEP), tris(1-chloro-2-propyl) phosphate (TCIPP), and TBOEP were dominant in abiotic media, whereas triethyl phosphate (TEP), tri-n-butyl phosphate (TNBP), TCEP, and TBOEP dominated in crucian carp. The TNBP had remarkable accumulation potential among nine OPFRs, which the TNBP concentrations in muscle increased with increased total length and body weight. The higher perfusion rate of TNBP to female eggs were observed rather than to male gonads as the concentrations were higher in males than in females, while the opposite results were observed in gonad. Moreover, the concentration of TNBP in female muscle began to decrease near maximum growth as a sexually dimorphic difference in crucian carp. This is the first study to simultaneously investigate the fate of OPFRs in biotic and abiotic media and to show sex differences.

1. Introduction

Recently, organophosphate flame retardants (OPFRs) have begun to attract great interest, as worldwide OPFR demand has substantially increased owing to the designation of polybrominated diphenyl ethers (PBDEs) as persistent organic pollutants (POPs) by the Stockholm Convention (Lee et al., 2016). The production of chlorinated OPFRs, including tris(2-chloroethyl) phosphate (TCEP), tris(1-chloro-2-propyl) phosphate (TCIPP), and tris(1,3-dichloro-2-propyl) phosphate (TDCIPP), increased from 14,000 tons per year in the 1980s to 38,000 tons per year in 2012 in the US (Greaves and Letcher, 2016; Schreder et al., 2016). Due to their high production volume and usage, OPFRs have been frequently detected in various environmental media and were known to cause diverse toxicity in biota by lab-exposure experiment. TCEP, TDCIPP, and tri-n-butyl phosphate (TNBP) in particular are known carcinogens, and TNBP, triphenyl phosphate (TPHP), and tricresyl phosphate (TCP) are reported to have neurotoxic effects in animals (Kim et al., 2011; Van den Eede et al., 2013).

Despite the ubiquitous presence of OFPRs in biota (Kim et al., 2011; Brandsma et al., 2015), relatively few field-based OPFR monitoring studies have investigated aquatic wildlife, focusing on fish species. Most of these studies investigated the edible parts (muscle) of fish to evaluate the presence and risk assessment of OPFRs (Kim et al., 2011; Malarvannan et al., 2015), and only one study identified different accumulation characteristics of OPFRs in several organs of freshwater fishes, observing the highest concentrations in liver, followed by kidney, muscle, intestine, and ovarian tissues (Hou et al., 2017). Recently, two studies were conducted to assess the effects of various factors such as trophic levels and fish size on the bioaccumulation of OPFRs in diverse fish species (Kim et al., 2011; Brandsma et al., 2015). They found no correlations

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between the concentrations of OPFRs and trophic levels through the food web or lipid contents, and no growth-dependent accumulation of OPFRs in any fish species. However, despite these studies, growth parameters (length and weight) should be considered together with sex, because sexually dimorphic growth patterns are related to the maternal transfer of resources to eggs (Nyholm et al., 2008; Sühring et al., 2015; Zheng et al., 2015).

Like these, research on OPFR accumulation in fish species is still limited, despite the importance of evaluating tissue-specific distribution and potentially diverse factors such as growth, sex, and lipid contents (because trace pollutants assimilated from food first accumulate in the blood and are transported to diverse tissues based on the characteristics of each tissue compartment) (Kojadinovic et al., 2007). Moreover, their presence, fate, and distribution are not yet fully understood, because no field-based studies have investigated OPFRs in both biotic and abiotic environments simultaneously to better understand accumulation characteristics of each OPFRs in biota from surrounding environments.

Therefore, the first objective of this study was to determine the concentrations and distributions of nine OPFRs in aquatic environments, including river water, sediment, and multiple tissues, including muscle, liver, gonad, and whole blood, of crucian carp (*Carassius auratus*), which are the most representative predator species in the Nakdong River of South Korea. We also evaluated the growth-dependent accumulation and sexually dimorphic growth differences related to maternal transfer as potential bioaccumulation factors of OPFRs. Finally, the field-based bioconcentration factor (BCF) and biota-sediment accumulation factor (BSAF) were compared with other POPs as indicators of bioaccumulation potential. To the best of our knowledge, this is the first study to simultaneously investigate both biotic and abiotic environments, as well as the maternal transfer of OPFRs.

2. Material and methods

2.1. Chemicals

Of the nine target OPFRs, triethyl phosphate (TEP), TNBP, TCEP, TCIPP, TDCIPP, TCP, and TPHP were obtained from AccuStandard (New Haven, CT, USA), and tris(2-butoxyethyl phosphate (TBOEP) and tris(2-ethylhexyl) phosphate (TEHP) were purchased from Wellington Laboratories (Guelph, Canada). Deuterated OPFRs, including TCEP-d_{12}, TCIPP-d_{18}, TDCIPP-d_{15}, and TPHP-d_{15} (Cambridge Isotope Laboratories, Andover, MA, USA) were used as internal standards, and phenanthrene-d_{10} was purchased from AccuStandard as a recovery standard for analyzing OPFRs. Detailed physicochemical properties of the nine OPFRs are given in Supporting Information, Table S1.

2.2. Sample collection

Twenty crucian carp (*Carassius auratus*), seven males and thirteen females, were collected from upstream (Andong, AD1–10) and midstream (Waegwan, WG1-10) sites of the Nakdong River, South Korea, from September–November 2015. Tissue samples (muscle, liver, and gonad [gonad of males and eggs of females]) were dissected from the collected crucian carp and whole blood was taken directly from each fish by needle after capture. Tissue samples were weighed and stored in amber glass bottles at −20 °C until analysis. The average total length and body weight of the crucian carp were 24.9 ± 2.37 cm and 312 ± 102 g, respectively. Detailed physical descriptions of each fish sample are given in Supporting Information, Table S2. In addition, river water and sediment samples ($n = 4$ each, 8 in total) were collected by grab sampling in the same river to screen the levels of OPFRs in the aquatic environments surrounding the biota.

2.3. Experimental procedures and analysis

Wet based tissue samples (0.5 g muscle and gonad, 0.1 g liver) were grinded and well homogenized by a blender with 5 g of anhydrous sodium sulfate (Na_2SO_4) after spiking 10 ng of each internal standards to obtain recovery correction, and the contents were extracted twice with 5 mL of a mixture of dichloromethane (DCM) and hexane (1:1 v/v) using ultra-sonication extraction for 30 min. The extracts were separated from samples by centrifugation and were concentrated under N_2 flow to approximately 1 mL. After the extraction steps, the samples were passed through an Oasis HLB cartridge (200 mg, 6 cc; Waters Corporation, Milford, MA, USA) conditioned by DCM, methanol, acetonitrile, and water, and the cartridge was eluted with 8 mL of DCM. Additionally, the extract was passed through Supelclean Envi Florisil SPE (6 cc; SUPELCO, USA) to reduce remaining lipid contents and interference. Whole blood samples (0.25 mL) were spiked with 10 ng of internal standards and mixed with 2 mL of formic acid and 3 mL of water to remove lipid contents in blood samples, then homogenized by ultra-sonication for 10 min. Sample extraction and clean-up were conducted by solid phase extraction (SPE) using an Oasis HLB cartridge (Waters Corporation) conditioned with 5 mL of DCM and methanol. After sample loading, the cartridge was washed with 3 mL of formic acid and dried for 30 min. Finally, the cartridge was eluted with 8 mL of DCM and adjusted to 100 μL with DCM. The final eluents were transferred to an amber vial and spiked with recovery standard prior to analysis. Information on experimental procedures using water and sediment samples are given in Supporting Information.

2.4. Instrumental analysis

Identification of the nine OPFRs was performed by gas chromatography (7890B; Agilent Technologies, Santa Clara, CA, USA) coupled with tandem mass spectrometry using an Agilent 7000C with DB-5MS UI (15 m long, 0.25 mm i.d., 0.10 μm film thickness; J&W Scientific, Palo Alto, CA, USA). The oven program was 50 °C (3 min), 15 °C/min to 230 °C, and 15 °C/min to 300 °C (1 min). Helium was used as the carrier gas with constant flow at 1.5 mL/min. Inlet, interface, and source temperatures were retained at 300 °C, 280 °C, and 300 °C, respectively, and splitless mode was used for injector. Multiple reaction monitoring (MRM) and positive electron ionization mode were used with 70 eV ionization voltage as described in Table S3.

2.5. Quality assurance/quality control

As OPFR-containing materials, including plastic tubes, cartridges, and gloves, were widely used in the experimental procedures, all products were pre-cleaned with acetonitrile three times, and all glassware was baked at 450 °C and washed in the same manner as the plastic products to reduce background. Additionally, all used apparatus, including collection vial, SPE cartridge, amber vial, and insert etc., was extracted with a mixture of DCM/hexane (1:1 v/v), and the extracts were analyzed prior to the sample analysis to confirm detection of OPFRs in background as concerned in many previous studies (Cristale and Lacorte, 2013; Liang et al., 2015). Procedural blanks in every batch (5–10 samples) and extracts of apparatus were mostly not detected or lower than the method detection limits (MDLs), which were obtained by a signal to noise ratio of three in each type of samples. Some of target OPFRs in blank samples in several experimental batches were