Tissue-specific Bioaccumulation and Potential Factors of Organophosphorus Flame Retardants in Crucian Carp

Gyojin Choo[1], Hyeon-Seo Cho[2], Jae-Woo Lee[3], Kyunghwa Park[3], Pilje Kim[3] and Jeong-Eun Oh[1,*]

[1]Department of Civil and Environmental Engineering, Pusan National University, Busan, 46241, South Korea
[2]College of Fisheries and Ocean Sciences, Chonnam National University, Yeosu, 550-749, South Korea
[3]National Institute of Environmental Research, Incheon, 22689, South Korea
E-mail contact: gyojin0218@naver.com

Introduction

Organophosphorus flame retardants (OPFRs) are high production volume chemicals and widely used in industries and consumer products as most suitable alternatives of brominated flame retardants (BFRs)[1]. Recently, OPFRs have received attention because they are easily released from various products in industries, offices and homes and are known to have carcinogenic, dermatitis, and neurotoxic potential[2]. Thus, several studies on OPFRs have been conducted in various environments, especially in indoor environments, to identify their fates, risk and human exposure assessment[3]. However, relatively few studies of OPFRs have conducted in biota, particularly on the exposure experiments in fish, even though numerous OPFRs have been detected in environmental media including water, sediment, and air[4]. Therefore, it is needful to be priory screened OPFRs in biota to understand their fates and bioaccumulation characteristics in field study. In this study, the concentrations and tissue-specific distribution patterns of OPFRs were identified in muscle, liver, gonad, and whole blood of 20 crucian carp which are the most representative species of Nakdong river in South Korea. Moreover, several factors were considered such as total length, body weight, sex, maternal transfer, and partition coefficients of the fish to assess the bioaccumulation potential of OPFRs. To our knowledge, this is the first study investigated bioaccumulation characteristics of OPFRs in whole parts of fish.

Materials and methods

Target compounds

Target compounds were 9 OPFRs including triethyl phosphate (TEP), tributyl phosphate (TBP), tris(2-chloroethyl) phosphate (TCEP), tris(1-chloro-2-prpyl) phosphate (TCPP), tris(2-ethylhexyl) phosphate (TDCPP), tricresyl phosphate (TCP), triphenyl phosphate (TPP), tris(2-butoxyethyl) phosphate (TBEP), and tris(2-ethylhexyl) phosphate (TEHP). Deuterated OPFRs including TCEP-d_{12}, TCPP-d_{18}, TDCPP-d_{15}, and TPP-d_{15} were used as internal standards and phenanthrene-d_{10} for recovery standard to analyze OPFRs.

Sample collection

Totally twenty crucian carps including seven males and thirteen females were collected from Nakdong river in South Korea from September to November in 2015 by using drift gill net and fishing. Two sampling sites including up- (Andong, AD1-10) and mid- (Waegwan, WG1-10) streams of river were investigated to assess the bioaccumulation according to the different environmental conditions. AD sites were located near root of the river, and there are many kinds of industrial factories including chemicals, display, textile, and electronic companies near WG sites. The collected crucian carps were directly dissected by the major tissues including muscle, liver, and gonad, and whole blood were extracted from each fishes by needle (total sample n=80). Each tissues were weighed and stored in amber glass bottles at -20 °C until analysis. The biological appearances such as total length (21-29 cm), body weight (177-493 g), and sex were determined. Whole blood samples were directly collected during sampling, and muscle, liver, and gonad of crucian carps were separated before sample treatment. All samples were stored frozen at -20 ℃.

Experimental Procedures and analysis

The tissue samples (0.5 g of muscle and gonad, 0.1 g of liver) were homogenized with 5 g of anhydrous sodium sulfate (Na_2SO_4) after spiking 10 ng of internal standards to obtain recovery correction, and the contents were extracted with 5 mL of the mixture of DCM and hexane (1:1, v/v) by using ultra-sonication extraction for 30 min twice. The extracts were separated from sample by centrifugation and were concentrated under N_2 flow to approximately 1 mL. After extraction steps, the samples were passed through with Oasis® HLB cartridge (200 mg, 6 cm^3; Waters, Massachusetts, USA) conditioned by DCM for cleaning-up. The cartridge was eluted with 8 mL of DCM, and fitted to 100 μL of DCM for analysis. A 0.25 mL of whole blood samples which were spiked with 10 ng of internal standards were mixed with 2 mL of formic acid and 3 mL of water, then homogenized by ultra-sonication for 10 min, respectively. Sample extraction and clean-up were conducted by solid phase extraction by using Oasis® HLB cartridge (200 mg, 6 cm^3; Waters, Massachusetts, USA) conditioned with 5 mL of DCM, methanol. After sample loading, the cartridge was washed with 3 mL of formic acid and was dried for 30 min. Finally, eluted by 6 mL of DCM, and fitted to 100 μL of DCM. The final eluents were transferred to an amber vial with injection of recovery standard prior to analysis.

The identification of 9 OPFRs were performed by a gas chromatography (Agilent 7890B) coupled with tandem mass spectroscopy using an Agilent 7000C (Agilent Technologies, Santa Clara, California, USA) with DB-5MS UI (15 m long, 0.25 mm i.d., 0.10 μm film thickness; from 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). The helium was used for carrier gas with constant flow at 1.5 mL/min. Inlet, interface, and source temperature were retained at 300 °C, 280 °C, and 300 °C, respectively. Multiple reaction monitoring (MRM) and positive electron ionization mode were used with 70 eV ionization voltage.

Results and discussion

Concentration and distribution of BFRs in treatment plants and river

The average concentration of OPFRs in each parts of crucian carp were shown in Table 1. The total concentration of OPFRs were 4.2-7.8 ng/g ww in muscle, 6.2-18.1 ng/g ww in liver, 3.1-7.7 ng/g ww in gonad, and 31-256 ng/mL in whole blood. The observed concentration of OPFRs in this study was similar with previous studies in China (Gonad: 0.4-4.9 ng/g ww)[6] and Canada (muscle: N.D.-5.6 ng/g ww)[1]. TEP and TBP which had short chain and low biodegradation rates were highly detected in muscle, liver, and gonad samples, and large amounts of TBEP were observed in whole blood. 2-fold higher concentrations of OPFRs were observed in liver samples compared to muscle and gonad, indicating relatively stronger bioaccumulation potential of OPFRs in liver. Especially, there is a statistically difference (Mann-Whitney U test, $p<0.01$) in liver tissues, particularly two times higher levels of TEP, TCPP, and total OPFRs were observed in WG which have many potential sources compared to AD, implying that liver tissues sensitively bioaccumulated by environmental conditions rather than other tissues.

Table 1. Concentrations of OPFRs from each parts of crucian carps.

Media		Mean	Min	Max
Muscle	AD	5.13	4.59	5.87
	WG.	5.73	4.23	7.75
Liver	AD	7.90	6.22	9.34
	WG.	11.2	8.47	18.1
Gonad	AD	4.66	3.08	5.97
	WG.	4.82	3.16	7.70
Whole blood	AD	73.7	38.3	123
	WG.	98.3	31.1	256

Bioaccumulation potential factors

Growth dependent accumulation

There were moderate correlations between physical appearances and TBP concentrations (total length and TBP: r=0.447, $p<0.05$; body weight and TBP: r=0.451, $p<0.05$), implying that TBP might be accumulated in muscle tissues as crucian carps grew. On the other hand, the concentrations of TBEP were decreased as the body weight were increased (r=-0.448, $p<0.05$), which are regarded as a dilution effect by the growth of biota.

In case of other media including liver, gonad, and whole blood, TEP was strongly associated with the growth of fishes. The positive relationships between body weight and TEP concentrations were observed in liver (r=0.475, $p<0.05$), gonad (r=0.570, $p<0.01$), and whole blood (r=0.530, $p<0.05$). Additionally, the significant correlations between total length and TEP concentrations were found in gonad (r=0.533, $p<0.05$) and whole blood (r=0.494, $p<0.05$). It can be seen that TEP and TBP were highly accumulated by growth dependent.

The detailed discussion on maternal transfer and partition between tissues and whole blood will be presented during the conference.

Acknowledgements

This study was funded by the National Institute of Environmental Research (NIER-SP2016-053) for supporting this research in 2015.

References:

[1] McGoldrick, D.J., Letcher, R.J., Barresi, E., Keir, M.J., Small, J., Clark, M.G., Sverko, E., Backus, S.M., 2014. Organophosphate flame retardants and organosiloxanes in predatory freshwater fish from locations across Canada. Environ. Pollut. 193, 254–261.

[2] Möller, A., Sturm, R., Xie, Z., Cai, M., He, J., Ebinghaus, R., 2012. Organophosphorus Flame Retardants and Plasticizers in Airborne Particles over the Northern Pacific and Indian Ocean toward the Polar Regions: Evidence for Global Occurrence. Environ. Sci. Technol. 46, 3127–3134.

[3] Ali, N., Dirtu, A.C., Eede, N.V. den, Goosey, E., Harrad, S., Neels, H., 't Mannetje, A., Coakley, J., Douwes, J., Covaci, A., 2012. Occurrence of alternative flame retardants in indoor dust from New Zealand: Indoor sources and human exposure assessment. Chemosphere 88, 1276–1282

[4] Greaves, A.K., Letcher, R.J., 2016. A Review of Organophosphate Esters in the Environment from Biological Effects to Distribution and Fate. Bull. Environ. Contam. Toxicol. 1–6.

[5] Kojadinovic, J., Potier, M., Le Corre, M., Cosson, R.P., Bustamante, P., 2007. Bioaccumulation of trace elements in pelagic fish from the Western Indian Ocean. Environ. Pollut. 146, 548–566.

[6] Chen, D., Letcher, R.J., Chu, S., 2012. Determination of non-halogenated, chlorinated and brominated organophosphate flame retardants in herring gull eggs based on liquid chromatography–tandem quadrupole mass spectrometry. J. Chromatogr. A 1220, 169–174.