Review

Review of OPFRs in animals and humans: Absorption, bioaccumulation, metabolism, and internal exposure research

Rui Hou ^{a, b}, Yiping Xu ^{a, *}, Zijian Wang ^b

^a Key Laboratory of Drinking Water Science and Technology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, PR China

^b State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, PR China

HIGHLIGHTS

- The absorption, bioaccumulation, metabolism and internal exposure of OPFRs are reviewed.
- Inhalation, ingestion and dermal contact are the main OPFRs absorption pathways for humans.
- General *in vivo* and *in vitro* metabolic pathways of three different types of OPFRs are proposed.
- DAPs and MAPs are considered as putative biomarkers for the assessment of internal exposure of OPFRs in humans.

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ABSTRACT

Due to their widespread use, organophosphate flame retardants (OPFRs) are commonly detected in various environmental matrices and have been identified as emerging contaminants. Considering the adverse effects of OPFRs, many researchers have paid their attention on the absorption, bioaccumulation, metabolism and internal exposure processes of OPFRs in animals and humans. In this article, we first review the diverse absorption routes of OPFRs by animals and humans (e.g., inhalation, ingestion, dermal absorption and gill absorption). Bioaccumulation and biomagnification potentials of OPFRs in different types of organisms and food webs are also summarized, based on quite limited available data and results. For metabolism, we review the Phase-I and Phase-II metabolic processes for each type of OPFRs (chlorinated OPFRs, alkyl-OPFRs and aryl-OPFRs) in the animals and humans, as well as toxicokinetic information and putative exposure biomarkers on OPFRs. Finally, we highlight gaps in our knowledge and critical directions for future internal exposure studies of OPFRs in animals and humans.

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* Corresponding author.

E-mail address: ypxu@rcees.ac.cn (Y. Xu).

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1. Introduction

Organophosphorus flame retardants (OPFRs), which extensively used in furniture, textiles, mattresses, electronics and other processing chemicals, constitute one of the most frequently used flame retardants (EPA, 2005). OPFRs are being increasingly consumed since the restriction published on the use of penta- and octa-polybrominated diphenyl ethers (PBDEs) (Marklund et al., 2005b; Van der Veen and de Boer, 2012). According to statistical data, the consumption of OPFRs in Europe had reached to 85,000 tonnes in 2005 (EFRA, 2007). China produced more than 70,000 tons of OPFRs in 2007 (Wang et al., 2010). The global consumption of OPFRs was 500,000 tonnes in 2011 and was expected to 680,000 tonnes in 2015 (Van der Veen and de Boer, 2012; Wang et al., 2015a).

OPFRs basically exist in end-products by physical addition, which is responsible for their leakage from products during service time through volatilization, abrasion and leaching (Sundkvist et al., 2010). After emission, there are several ways in which OPFRs can reach remote areas, such as deposition, washout, infiltration, etc. (Andresen et al., 2004; Bacaloni et al., 2008; Schreder and La Guardia, 2014; Takimoto et al., 1999). Atmospheric washout by precipitation and industrial discharge from factories and wastewater treatment plants (WWTPs) have been identified as the most significant entry modes of OPFRs into aquatic and terrestrial systems (Cristale et al., 2013; Meyer and Bester, 2004; Wei et al., 2015). As a result, OPFRs are globally distributed and ubiquitously present in various environmental mediums, such as dust, indoor air, atmosphere, surface water, sediment and soil (Cequier et al., 2014b; Chung and Ding, 2009; Gao et al., 2014; Kim et al., 2011; Staaf and Ostman, 2005). A number of studies have found that indoor environments have significantly higher levels of OPFRs than brominated flame retardants (BFRs) (Ali et al., 2012; Brommer et al., 2012; He et al., 2015). Over the past decade, almost all of the OPFRs produced have been detected in marine and fresh water animal, avian, insect and human samples (SI Table S1).

OPFRs are synthetic phosphoric acid derivatives, whose structures vary depending on different ester linkages and can be roughly divided into three types: chlorinated OPFRs, alkyl-OPFRs and aryl-OPFRs (Table 1, where the abbreviations of the OPFRs in this review are provided). OPFRs have a wide range of physiological properties in the environment. For example, their solubility, log K_{OW} values, persistence, vapor pressure, bioconcentration factors (BCFs) are quite different (Table 1). These properties are important factors in assessing the behavior of OPFRs in the environment and for assessing their influence on organisms (Van der Veen and de Boer, 2012). Volatile OPFRs with higher vapor pressures, such as TBP, TEP and TCEP, tend to be more likely to emit into air and settled onto dust than heavier OPFRs (Wei et al., 2015). Aryl and alkyl-OPFRs with higher molecular mass are more hydrophobic, have similar BCFs and have an affinity for sediment and soil (Van der Veen and de Boer, 2012; Wei et al., 2015). Chlorinated OPFRs have been

shown to be more water soluble and are considered to be persistent threats to aquatic animals (Reemtsma et al., 2008; Van der Veen and de Boer, 2012).

Considering the occurrence of OPFRs in organisms and in organisms' surroundings, increasing attention has been devoted to their adverse effects to organisms. Toxicological studies have shown that OPFRs have the potential to cause adverse reproductive, endocrine and systemic effects in animals as a result of long term exposure to animals (Dishaw et al., 2011; Liu et al., 2012; Porter et al., 2014; Van der Veen and de Boer, 2012). Aryl-OPFRs have been shown to contribute to heart toxicity by disturbing the expression of transcriptional regulators in zebrafish (Du et al., 2015). Chlorinated OPFRs, such as TCEP, TCIPP and TDCIPP, have been proven to be neurotoxic and carcinogenic (Ni et al., 2007; WHO, 1998, 2000). TDCIPP can easily enter the blood stream and induce tumors in the liver, kidney and testis (OEHHA, 2011). TDCIPP levels in house dust were found to be correlated with reduced concentrations of thyroid hormones (THs) levels and increased prolactin levels in males (Meeker and Stapleton, 2010). TDCIPP and TCEP are prohibited in Washington State, USA, according to the "Toxic Free Kids Act" (Washington Toxics Coalition, 2011). EU Directive 2014/81/EU also introduced specific limits (5 mg kg⁻¹) for TCEP, TCIPP and TDCIPP in certain toys (European Union Commission Directive, 2014).

Studies of the bioaccumulation, metabolism and toxicokinetics of OPFRs date back to the 1970s (Gold et al., 1978; MacFarland and Punte, 1966; Muir et al., 1980; St. John et al., 1976). Fewer studies had been conducted until concerns were raised regarding the reemergence of OPFRs due to their increasing usage and high environmental concentrations (Carlsson et al., 2000). To date, the behaviors of OPFRs in animals and humans have become hot-button issues. However, these long-term studies still have not been fully summarized. Studies of the absorption, bioaccumulation and metabolic processes will facilitate comprehension of the fates and the mechanisms of toxicity of OPFRs. Thus, a review of the available studies in a systematic framework is urgently needed to better understand the behaviors and fates of OPFRs in organisms. In this article, we organized recent studies on the absorption, bioaccumulation, biomagnification and the metabolic processes of OPFRs in animals and humans (Fig. 1), rather than simply listing the occurrences of OPFRs in biota, which has often been conducted in previous reviews (Alves et al., 2014; Reemtsma et al., 2008; Van der Veen and de Boer, 2012; Wei et al., 2015). In addition, we also propose a perspective on research into the internal exposure of OPFRs to animals and humans.

2. Absorption

The migration of OPFRs in the environment leads to their exposure to organisms (Abdallah and Covaci, 2014; Alves et al., 2014). There are diverse pathways for organisms to uptake or

Table 1

The names, abbreviations, structures and properties of the studied OPFRs.

Name	Abbr.	Structure	Solubility in water (mg·L ⁻¹) at 25 °C	Vapor pressure (mmHG) at 25 °C	Henry's law constant (atm m ³ ·mol ⁻¹) at 25 °C	log K _{OW}	BCFs
Tricresyl phosphate	TCP		0.36	1.80×10^{-7}	9.2×10^{-7}	5.48	8564.11
Tri- <i>m</i> -cresyl phosphate	TMCP,	$R_1=R_2=R_3=$
Tri- <i>o</i> -cresyl phosphate	<i>m</i> -TCP						
Tri- <i>p</i> -cresyl phosphate	TOCP, <i>o</i> -TCP						
	TPCP, <i>p</i> -TCP						
Tributyl phosphate	TBP,	$R_1=R_2=R_3=$	280	1.13×10^{-3}	1.663×10^{-4}	4.00	39.81
	TnBP						
Tri-iso-butyl phosphate	TiBP	$R_1=R_2=R_3=$	475.57	0.0129	3.19×10^{-6}	3.60	19.51
Triethyl phosphate	TEP	$R_1=R_2=R_3=$	1115	0.393	3.547×10^{-6}	0.87	3.162
Tris(2-ethylhexyl) phosphate	TEHP	$R_1=R_2=R_3=$	0.6	8.25×10^{-8}	2.376×10^{-2}	9.49	1×10^6
Triphenyl phosphate	TPHP	$R_1=R_2=R_3=$	1.9	1.12×10^{-5}	1.960×10^{-7}	4.70	113.3
Tris(2-chloroethyl) phosphate	TCEP	$R_1=R_2=R_3=$	7000	0.0613	1.673×10^{-7}	1.63	0.4254
Tris(chloroisopropyl) phosphate	TCIPP	$R_1=R_2=R_3=$	51.85	5.64×10^{-5}	4.688×10^{-7}	2.89	3.268
Tris(1,3-dichloro-2-propyl) phosphate	TDCIPP	$R_1=R_2=R_3=$	7	2.98×10^{-7}	1.080×10^{-7}	3.65	21.4
Tris(2-butoxyethyl) phosphate	TBOEP, TBEP	$R_1=R_2=R_3=$	1100	1.23×10^{-6}	3.285×10^{-7}	3.00	25.56
Trixylenyl phosphate	TXP	$R_1=R_2=R_3=$	0.89	8.76×10^{-8}	2.779×10^{-5}	7.98	480.1
2-ethylhexyldiphenyl phosphate	EHDPPH	$R_1=$ $R_2=R_3=$	1.9	5×10^{-5}	2.392×10^{-4}	6.30	855.3
Tetrekis(2-chlorethyl) dichloroisopentyl- diphosphate	V6		2.1	1.2×10^{14}	6.5×10^6	1.9	17.07
Resorcinol Bis(diphenylphosphate) RDP			1.11×10^{-4}	2.1×10^{-8}	7.41	5.82	2.05×10^4

The data are compiled from databases (Chemspider, 2015) and Van der Veen and de Boer (2012).

absorb OPFRs through ingestion, gill absorption, skin absorption and inhalation. Several reports over the past decade have provided insight into these routes of absorption in animals and humans.

2.1. Absorption routes in animals

Gill absorption may be one of the most common ways in which aquatic animals take up OPFRs dissolved in ambient water. In an investigation of the distribution of OPFRs in fish, researchers found that OPFRs were primarily absorbed from water through the gills and epithelial tissue rather than through food web biomagnification (Malarvannan et al., 2015; Sundkvist et al., 2010). Kim et al. (2011) also indicated that the gill contributed greatly uptake of OPFRs from water for fish. However, very few studies have thoroughly examined the uptake of OPFRs in aquatic

organisms through gill membranes.

Ingestion is assumed to be another critical pathway for OPFRs to enter the bodies of animals (Sundkvist et al., 2010). Because OPFRs are present in biota, it is possible for predators at the top of the food chain to consume more OPFRs (Eulaers et al., 2014). Studies have shown that TBOEP, TCIPP and TCEP can spread in the benthic and pelagic food web and be detected in predators (e.g., sculpin, plaice, goby, sole and tern) (Brandtsma et al., 2015). For terrestrial birds and mammals, OPFRs can also be absorbed by ingestion of food and water. However, few studies have focused on this route of absorption, except for a study in White-tailed Eagles, in which diet was identified to be the primary means of OPFR absorption (Eulaers et al., 2014).

However, there is insufficient knowledge regarding the different routes by which variant OPFRs can enter organisms under different

Fig. 1. A schematic representation of the fate of OPFRs in the body of animals and humans.

environmental conditions. Additionally, exposure assessments of the biological effects of OPFRs in wildlife have not been studied. Therefore, further research assessing the exposure thresholds of OPFRs for organisms is recommended.

2.2. Absorption routes in humans

Several studies have found that inhalation of indoor air and dust is one of the most important OPFR absorption pathways for people living in indoor environments because building materials are considered to be a significant source of OPFRs (Marklund et al., 2005b; Tajima et al., 2014; Yang et al., 2014). TPHP, TBP, TBOEP, TCEP and TCIPP are the most frequently identified and most abundant compounds among the detected OPFRs in indoor air and dust (Abdallah and Covaci, 2014; Cequier et al., 2014b; Tajima et al., 2014). Moreover, OPFRs exposure through inhalation may be more threatening for people that spend time in offices than PBDEs exposure as more OPFRs are used as alternatives for PBDEs in indoor environments (Tajima et al., 2014; Van der Veen and de Boer, 2012; Wei et al., 2015). OPFRs have also been detected in atmosphere, but their contributions to inhalation are often neglected due to their relatively lower levels in these environments (13–20,300 pg m³) (Wei et al., 2015).

Moreover, dietary exposure of OPFRs via food and drinking water is a major concern for the general population (Li et al., 2014; WHO, 1990, 1991, 1997, 1998, 2000). Fishes were investigated their contribution to OPFRs exposure to human in lakes and coastal areas. In many environmental surveys of fish worldwide, OPFRs concentrations have been reported to be as high as 15,000 ng g⁻¹ (SI Table S1). Ingestion of OPFRs through drinking water cannot be neglected, as the occurrence of OPFRs in drinking water has been established in published literature (Benotti et al., 2009; Li et al., 2014; Stackelberg et al., 2007). Moreover, foodstuffs consumed by humans can be contaminated with OPFRs due to the use of OPFR-

containing packaging plastics (Campone et al., 2010).

Skin also contributes to the total uptake of OPFRs. Touching contaminated water or direct contact with OPFR-containing products may result in the entry of OPFRs into the body through dermal uptake (St John et al., 1976). It has been verified that TDCIPP can be absorbed by human skin (Gold et al., 1978; Nomeir et al., 1981). Accidental exposure of TEHP, TBOEP, TBP and TCP by the dermal route during the manufacturing and use of some products has attracted much attention (WHO, 1990, 1991, 2000). In addition, Hoffman et al. (2015) found the strong relationship between the levels of TDCIPP and TPHP on hand wipes and the levels of their metabolites in urine and suggested that hand-to-mouth contact or dermal absorption may be important absorption pathways of OPFRs.

For the contribution of different absorption pathways, Sundkvist et al. (2010) suggested that humans exposure OPFRs through eating fish was less important than indoor dust inhalation in lakes and coastal areas in Sweden. Cequier et al. (2014a) found that the relative contribution of dermal contact with TDCIPP to total OPFR uptake (i.e. 45% to the intake for children and 58% for women) was as important as inhalation for children and mothers (i.e. 54% and 41%, respectively) in Oslo, Norway. Cequier et al. (2015) subsequently concluded the household inhalation was a more important absorption pathway of OPFRs than through the diet for mother-child pairs in Norway. But more biomonitoring studies are needed to further discuss the contribution of various absorption pathways of OPFRs for human.

2.3. Human exposure assessment

Numerous recent studies have focused on estimating the impact of OPFRs on human health, but information is only available about exposure assessments through inhalation and ingestion.

Most assessments of human exposure to OPFRs via inhalation worldwide are based on the concentrations of OPFRs that are

Table 2
Statistical summary of human exposure assessment to OPFRs via inhalation.

Country	Category	Human exposure to OPFRs (ng·(kg d) ⁻¹)										References
		TCIPP	TCEP	TDCIPP	TBOEP	TBP	EHDPP	TEHP	TPHP	TCP	ΣOPFRs	
Sweden	Adult	—	—	—	—	—	—	—	—	—	5800	Marklund et al. (2005a)
	Children	—	—	—	—	—	—	—	—	—	57,000	
Belgium	Adult	0.5	0.1	—	0.7	1.1	—	—	0.2	0.1	2.7	Van den Eede et al. (2011)
	Toddler	5.6	1.0	—	8.2	12.7	—	—	2.0	1.0	30.5	
Japan	Children	1.41	0.71	0.27	27.25	0.44	—	—	1.99	—	—	Tajima et al. (2014)
	Toddler	9.96	5.02	1.92	193.12	2.1	—	—	13.44	—	—	
Kuwait	Adult	—	—	—	—	—	—	—	—	—	2.55	Ali et al. (2013)
	Toddler	—	—	—	—	—	—	—	—	—	37.1	
Pakistan	Adult	—	—	—	—	—	—	—	—	—	0.18	Ali et al. (2013)
	Toddler	—	—	—	—	—	—	—	—	—	2.7	
Norway	Adult (woman)	4.1	0.27	0.01	0.07	0.4	—	—	0.03	0	—	Cequier et al. (2014b)
	Children	10	0.91	0.02	1.6	1.57	—	—	0.06	0	—	
South China	Adult	1.51	0.29	0.13	0.08	0.09	0.18	—	1.36	34.5	7.02	He et al. (2015)
	Toddler	17.3	3.3.6	1.49	0.87	1	2.1	—	15.5	3.02	80.2	
Egypt	Adult	1.2	0.8	1.6	2	1	0.8	—	1.9	—	5.2	Abdallah and Covaci (2014)
	Toddler	3.0	1.9	4.0	5	2.6	2.1	—	4.8	—	13	
Philippines	Adult	—	0.43	—	—	0.24	1.4	1.8	1.1	0.23	—	Kim et al. (2012)
	Toddler	—	1.5	—	—	0.82	4.7	6	3.8	0.78	—	
UK	Adult	0.92	0.03	0.07	—	0.01	0.09	—	0.13	—	1.3	Brommer and Harrad (2015)
	Children	43	1.7	4	—	0.08	14	—	7	—	70	
	RfD	8000	2200	1500	1500	2400	—	—	7000	—	22,600	Van den Eede et al. (2011)

detected in indoor environments (Table 2). The OPFR intake rates via inhalation in Sweden ($5.8 \mu\text{g} \cdot (\text{kg d})^{-1}$ for adults and $57 \mu\text{g} \cdot (\text{kg d})^{-1}$ for children) were larger than those in other countries (Marklund et al., 2005a) but was lower than the reference dose (RfD) of $22.6 \mu\text{g} \cdot (\text{kg d})^{-1}$ calculated by Van den Eede et al. (2011). These regional differences in exposure are likely driven by differences in the use of consumer products, the frequency of vacuum-cleaning and the regulation of indoor materials among these countries (Dishaw et al., 2014; Wei et al., 2015). In these exposure assessments, children and toddlers were considered to be vulnerable groups due to their more frequent hand-to-mouth activities, lower body weights and more time spent at home than adults (Ali et al., 2012; Tajima et al., 2014). Therefore, the intake rates of OPFRs via inhalation for children and toddlers that were reported in these studies were several orders of magnitude higher than the rates reported for adults.

Absorption of OPFRs via diet has also been assessed recently regarding the risk to human health, but data about this route of absorption are sparse. Sundkvist et al. (2010) estimated that the average ingestion of OPFRs for an adult would be $20 \text{ ng} \cdot (\text{kg d})^{-1}$, based on the recommended amount of fish consumption of 375 g week^{-1} by the National Food Administration. The ingestion rate of OPFRs was $0.67 \mu\text{g} \cdot (\text{kg d})^{-1}$ for a breastfeeding baby who consumes 1 L of mother's milk per day (Sundkvist et al., 2010). Another assessment of OPFR intake through fish consumption was estimated among the population living along Manila Bay, in which the total dietary intake was $5.9 \mu\text{g} \cdot (\text{kg d})^{-1}$ for an adult, based on an annual fish consumption of 30 kg year^{-1} (Kim et al., 2011). However, the total exposure of humans to OPFRs, including all routes of absorption, remains unknown. Hence, for further exposure assessments it is important to consider OPFR concentrations in all pollution sources and the personal habits of the study participants for the population under study.

3. Bioaccumulation

3.1. Bioaccumulation

Measurements of OPFR biodegradability and bioaccumulation in the environment date back to the 1970s, when OPFRs were recognized to have moderate potential to accumulate in aquatic

organisms (Saeger et al., 1979). OPFRs have greater variation in the log Kow range than PBDEs (i.e., log Kow of PBDEs ranges from 4.3 to 9.9 (Van Ael et al., 2013)) and show less bioaccumulation than PBDEs, with bioconcentration factors (BCFs) ranging from 128 to 44,430 (Mansouri et al., 2012). Chlorinated OPFRs generally have relatively lower estimated BCFs than aryl-OPFRs, and BCF increases with molecular mass in non-chlorinated OPFRs (Van der Veen and de Boer, 2012). The OPFRs indicated in the article have a large range of BCFs, from 0.425 for TCEP to 1.00×10^6 for TEHP (Table 1). According to the REACH criterion ($\text{BCF} \geq 500$; European Union (2008)), aryl-OPFRs, such as TCP, TEHP, EHDPP, DCP, RDP and TXP can be classified as bioaccumulative compounds (Table 1).

However, the real accumulative potential of OPFRs is variable among different individuals and species (Table 3). Sundkvist et al. (2010) found that total OPFRs levels (e.g., TCIPP, TPHP, TBOEP, TBP, EHDPP, TCP, TDCIPP) in large perch were higher than in small perch. This finding suggested that larger fishes could accumulate more OPFRs. In different studies of TPHP bioaccumulative potential in fishes, BCFs based on most experimental data were reported to range from 0.06 (in *Phoxinus phoxinus*) to 2590 (*Pimephales promelas*) (reviewed by Waaijers et al. (2013)). Wang et al. (2015b) observed that concentrations of TDCIPP in female zebrafish were higher than those in males after OPFRs exposure, indicating gender-dependent bioaccumulation of OPFRs. Differences in feeding behavior and metabolic efficiency between different individuals or species may greatly impact the accumulative potential of OPFRs (Bestvater, 2014; Wang et al., 2015a), but the mechanisms underlying this difference remain unknown. However, what is clear is that many OPFRs (e.g., TCIPP, TPHP, TBOEP, TBP, EHDPP, TCP, TDCIPP and TEHP) have limited affinity for lipid content in fishes, suggesting that the accumulation of OPFRs is not basically associated with lipids (Brandsma et al., 2015; Chen et al., 2012; Kim et al., 2011; Malavannan et al., 2015; Sundkvist et al., 2010).

In addition, some prevalent OPFRs in aquatic environments have been detected in most organisms despite their lower BCFs. TCIPP (with a BCF of 8.51) was found at relatively high levels in rainbow trout from Lake Ontario (Bestvater, 2014) and mussels from lakes and coastal areas in Sweden (Sundkvist et al., 2010). In an investigation of the distribution of OPFRs in fishes from Manila Bay, TEP and TBP (with BCFs of 3.16 and 39.81, respectively), in addition to TEHP and EHDPP (with BCFs of 1.00×10^6 and 855.3,

Table 3
BCFs (bioconcentration factors) of OPFRs in recent reports.

Species	BCFs for OPFRs						References
	TCIPP	TCEP	TDCIPP	TBP	TEHP	TPHP	
Goldfish	—	0.7–0.9	3–5	7–11	—	110–150	Sasaki et al. (1981)
Killifish	—	2.2	33–113	30–35	—	250–300	Sasaki et al. (1981)
Cyprinoid fish	0.8–4.6	0.6–5.1	0.3–22	—	—	—	Green et al. (2007); Evenset et al. (2009); Leonards et al. (2010)
Fish	—	1.1–2.2	31–107	—	250	—	WHO (1998)

respectively), were found to have a high body burden due to their greater environmental levels (Kim et al., 2011). Hence, OPFRs with relatively high background levels should be given more attention for the potential threat that they pose to organisms.

3.2. Biomagnification

Each organic pollutants transferred differently in the food web as the variation of physico-chemical properties and bioavailability and metabolic transformation for organisms. For OPFRs, the biomagnification factors (BMFs) have only been calculated recently in aquatic food webs. Brandsma et al. (2015) calculated BMFs > 1 for TBOEP, TCIPP and TCEP (with BMFs of 3.5, 2.2 and 2.6, respectively) in the benthic food web. There was no observed magnification for TDCIPP, TBP, TMPP, EHDPHP, TCP, TBOEP and TEHP. Brandsma et al. (2015) ascribed the trophic magnification in the benthic food web of TBOEP, TCIPP and TCEP to their high levels of emission and their substantial exposure (Brandsma et al., 2015). Kim et al. (2011) confirmed that there was a significant biomagnification for only TPHP in the demersal fishes of Manila Bay, although they had not calculated its BMF. Nevertheless, results of this type are too few to obtain a clear and thorough understanding of the biomagnification of OPFRs in ecosystems, and more investigations are sorely needed.

OPFRs more likely magnify through the benthic food web rather than through the pelagic food web in aquatic environments. Brandsma et al. (2015) observed that OPFRs (TCEP, TCIPP, TDCIPP, TPHP, TBP, TBOEP, EHDPHP and TEHP) demonstrated trophic dilution in the pelagic food web. This was probably because OPFRs were generally adsorbed to particles, and thus were likely to be more abundant in the sediment than in the water column (Brandsma et al., 2015; Kim et al., 2011). Many OPFRs had low levels of trophic biomagnification based on the limited data likely due to metabolism. However, compared with aquatic species, studies on the bioaccumulation of OPFRs in terrestrial food chains are generally scarce, especially in terrestrial avians and mammals, which are also extensively exposed to OPFRs.

For mechanism of OPFRs biomagnification, the studies are still limit to get clear and holistic comprehension. Unlike PBDEs, OPFRs have limited lipid affinity. Beside the studied passive biotransport (e.g., logK_{OW} and bioconcentration factors of OPFRs, lipid and protein content of animal), the direct interactions of specific biomolecules (i.e., various transport proteins) may give new insights into the intrinsic mechanisms. Assessment of the biotransport ability of OPFRs (i.e., BMFs) have tentatively done, however, more works are required to clarify their species-specific risks, which should take into account the specie, size, gender of the test animals and test conditions.

4. Metabolism

Metabolism is considered to be a major determinant of the bioaccumulation and clearance of xenobiotics, as well as an indirect determinant of the toxicological effects of these compounds (Meyer and Bester, 2004). The study of metabolic processes is an important

component of ecotoxicology and has greatly facilitated our understanding of the fate and mechanisms of toxicity of OPFRs.

4.1. Metabolic processes

In metabolism studies, the recently emerged *in vivo* and *in vitro* approaches are the most robust and widely used approaches (Van de Waterbeemd and Testa, 2015). *In vivo* and *in vitro* OPFR metabolism has been examined in laboratory animals and in humans. Previous studies have demonstrated that OPFRs can be rapidly metabolized through Phase-I and Phase-II biotransformation to metabolites (Table 4), which are more hydrophilic and more readily eliminated (Cooper et al., 2011; Van den Eede et al., 2013a). Although information is still limited, a qualitative summary of the possible metabolic pathways for each type of OPFRs (chlorinated OPFRs, alkyl-OPFRs and aryl-OPFRs) can be proposed (Fig. 2-A–C).

4.1.1. Chlorinated OPFRs

Chlorinated OPFRs are the most extensively studied compounds. As early as the 1980s, rodent exposure experiments had been conducted to explore metabolites of TDCIPP and TCEP. Lynn et al. (1981) found that TDCIPP transformed to bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), 1,3-dichloro-2-propyl phosphate (MDCIPP) and 1,3-dichloro-2-propanol (1,3-DCP). Nomeir et al. (1981) identified a glutathione-conjugated TDCIPP as a Phase-II metabolite in rat livers *in vitro*. TCEP can also metabolize to bis (2-chloroethyl) hydrogen phosphate (BCEP), bis (2-chloroethyl) carboxymethyl phosphate and glucuronide of bis (2-chloroethyl) 2-hydroxyethyl phosphate in rats (Burka et al., 1991; Sasaki et al., 1984).

The metabolism of chlorinated OPFRs (TDCIPP, TCEP and TCIPP) in human liver preparations was also examined recently (Van den Eede et al., 2013a). Hydroxylated TDCIPP (OH-TDCIPP), carboxylated TDCIPP (COOH-TDCIPP), BDCIPP and hydroxylated BDCIPP (OH-BDCIPP) were all validated as major Phase-I metabolites for TDCIPP. The identified TCEP metabolites included BCEP and hydroxyethyl 2-chloroethyl hydrogen phosphate (OH-TCEP). Similarly, TCIPP metabolized to hydroxylated TCIPP (OH-TCIPP), bis(1-chloropropyl) phosphate (BCIPP), hydroxylated BCIPP (OH-BCIPP) and carboxylated TCIPP (COOH-TCIPP). For the Phase-II metabolites, glutathione-conjugated TDCIPP and TCEP were identified in all chlorinated OPFRs, except for TDCIPP (Nomeir et al., 1981; Van den Eede et al., 2013a).

The overall metabolic pathway of chlorinated OPFRs is shown in (Fig. 2-A). The Phase-I metabolic pathway of chlorinated OPFRs involves cleavage of the ether bond (O-dealkylation) and oxidative dehalogenation of the terminal carbon atom. These reactions produce the formation of diesters (DAPs) and hydroxylated metabolites and ultimately form carboxylic acids. In addition, the formation of Phase-II metabolites (glutathione conjugate) can occur through direct substitution of Cl atoms in chlorinated OPFRs, which are electrophilic or substitutive in molecules.

Table 4

Summary of major metabolites of OPFRs from recent OPFRs metabolism studies.

Compounds	Species/assays	Methods	Major metabolites	References
TDCIPP	Rat	<i>In vivo</i>	BDCIPP, MDCIPP, 1,3-DCP, 3-MCPD	Lynn et al. (1981)
	Rat liver homogenate	<i>In vitro</i>	BDCIPP, 1,3-DCP, 3-MCPD, glutathione-conjugated TDCIPP	Nomeir et al. (1981); Sasaki et al. (1984)
	Human liver microsome (HLM)	<i>In vitro</i>	BDCIPP, hydroxylated TDCIPP (OH-TDCIPP), hydroxylated BDCIPP (OH-BDCIPP), hydrocarboxylated TDCIPP (COOH-TDCIPP)	Van den Eede et al. (2013a)
	Human liver S9 fraction	<i>In vitro</i>	BDCIPP, hydroxylated TDCIPP (OH-TDCIPP), hydroxylated BDCIPP (OH-BDCIPP), hydrocarboxylated TDCIPP (COOH-TDCIPP), glutathione-conjugated TDCPP	Van den Eede et al. (2013a)
TCEP	Rat	<i>In vivo</i>	BCEP, hydrocarboxylated TCEP (OH-TCEP), glucuronide-conjugated BCEP	Burka et al. (1991)
	Human liver microsome (HLM)	<i>In vitro</i>	BCEP	Chapman et al. (1991)
	Human liver microsome (HLM)	<i>In vitro</i>	BCEP, hydroxylated TCEP (OH-TCEP)	Van den Eede et al. (2013a)
	Human liver S9 fraction	<i>In vitro</i>	BCEP, hydroxylated TCEP (OH-TCEP), glutathione-conjugateed TCEP	Van den Eede et al. (2013a)
TCIPP	Human liver microsome (HLM), human liver S9 fraction	<i>In vitro</i>	BCIPP, hydroxylated TCIPP (OH-TCIPP), hydrocarboxylated TCIPP (COOH-TCIPP), hydroxylated BCIPP (OH-BCIPP)	Van den Eede et al. (2013a)
TnBP	Rat	<i>In vivo</i>	DnBP, MnBP, dihydroxylated TnBP (di-OH- TnBP)	Suzuki et al. (1984)
TBOEP	Rat liver microsome	<i>In vitro</i>	DnBP, hydroxylated TnBP (OH-TnBP), dihydroxylated TnBP (di-OH- TnBP)	Sasaki et al. (1984)
	Human liver microsome (HLM)	<i>In vitro</i>	BBOEP, bis(2-butoxyethyl) hydroxyethyl phosphate (BBOEHP), hydroxylated TBOEP (1-HO-TBOEP, 2-HO-TBOEP and 3-HO-TBOEP), hydrocarboxylated TBOEP (COOH-TBOEP)	Van den Eede et al. (2013a)
	Human liver S9 fraction	<i>In vitro</i>	BBOEP, hydroxylated TBOEP (OH-TBOEP), hydrocarboxylated TBOEP (COOH-TBOEP), glucuronide-conjugate of hydroxylated TBOEP	Van den Eede et al. (2013a)
TPHP	Chicken embryonic hepatocyte	<i>In vitro</i>	DPHP, hydroxylated TPHP (OH-TPHP), dihydroxylated TPHP (di-OH-TPHP)	Su et al. (2014)
	Chicken embryonic hepatocyte	<i>In vitro</i>	DPHP, hydroxylated TPHP (<i>p</i> - and <i>m</i> -OH-TPHP), glucuronide-conjugate of <i>p</i> - OH-TPHP	Su et al. (2015)
	Human liver microsome (HLM)	<i>In vitro</i>	DPHP, MPHP, hydroxylated TPHP (OH-TPHP), dihydroxylated TPHP (di-OH-TPHP), hydroxylated DPHP (OH-DPHP)	Van den Eede et al. (2013a)
	Human liver S9 fraction	<i>In vitro</i>	glucuronide-conjugate and sulfate-conjugate of OH-TPHP, glucuronide conjugate of di-OH-TPHP	Van den Eede et al. (2013a)
TPCP	Rat	<i>In vivo</i>	DPCP, hydroxylated TCP (OH-TPCP), hydrocarboxylated TCP (COOH-TPCP), dihydrocarboxylated TCP (di-COOH-TPCP)	Kurebayashi et al. (1985)
RDP	Rats, mice and monkeys	<i>In vivo</i>	resorcinol, diphenyl phosphate (DPHP), hydroxylated DPHP (OH-DPHP), hydroxylated RDP isomers (OH-RDP, di-OH-RDP)	Freudenthal et al. (2000)
	Human liver microsome (HLM)	<i>In vitro</i>	DPHP, hydroxy-RDP (OH-RDP) and dihydroxy-RDP (di-OH-RDP), glucuronide and sulfate conjugates of OH-RDP and di-OH-RDP	Ballesteros-Gómez et al. (2015)
EHDPHP	Rat	<i>In vivo</i>	DPHP, MPHP, hydroxylated DPHP (OH-DPHP), phenol	Nishimaki-Mogami et al. (1988)
	Human liver microsome (HLM)	<i>In vitro</i>	DPHP, hydroxylated EHDPHP, dihydroxylated EHDPHP,	Ballesteros-Gómez et al. (2015)
	Human liver S9 fraction	<i>In vitro</i>	DPHP, hydroxylated EHDPHP, dihydroxylated EHDPHP, glucuronide conjugate of hydroxylated EHDPHP	Ballesteros-Gómez et al. (2015)

4.1.2. Alkyl-OPFRs

Published information on the metabolism of alkyl-OPFRs exists only for TnBP in laboratory animals and TBOEP in human liver microsomes. Suzuki et al. (1984) studied the metabolism of TnBP in male rats *in vivo*, in which the major metabolites in urine were dibutyl hydrogen phosphate (DnBP), butyl dihydrogen phosphate (MnBP), butyl bis(3-hydroxybutyl) phosphate (di-OH-TnBP) and other metabolites of hydroxylated derivatives of the butyl moieties. In a study of the metabolism in rat liver microsomes *in vitro*, TnBP could easily be oxidized to hydroxylated TnBP and further transformed to dihydroxylated TnBP or DnBP (Sasaki et al., 1984). No Phase-II metabolites of TBP have been reported.

In a human liver microsome study, bis(2-butoxyethyl) phosphate (BBOEP), bis(2-butoxyethyl) hydroxyethyl phosphate (BBOEHP), four isomers of mono-hydroxylated TBOEP (di-OH-TBOEP) and some ketone isomers were reported to be metabolites of TBOEP (Van den Eede et al., 2013a). For Phase-II metabolites, a glucuronide conjugate with BBOEHP was identified in human liver S9 fraction incubation (Van den Eede et al., 2013a). In another study using human liver microsomes, the mono-hydroxylated metabolites of TBOEP were confirmed to be 3-HO-TBOEP (bis(2-butoxyethyl) 3-hydroxyl-2-butoxyethyl phosphate), 1-HO-TBOEP (bis(2-butoxyethyl) 1-hydroxyl-2-butoxyethyl phosphate) and 2-HO-TBOEP (bis(2-butoxyethyl) 1-hydroxyl-2-butoxyethyl

phosphate) (Van den Eede et al., 2015a).

Alkyl-OPFRs, as with the chlorinated compounds, were also thought to be easily dealkylated to their respective diesters via hydrolysis. Hydroxylation of unsubstituted carbon atoms has been shown to occur in alkyl-OPFR, instead of oxidative dehalogenation in chlorinated OPFR metabolism. Hydroxylation was more likely to occur on the terminal carbon atoms for alkyl-OPFRs, which would be more stable than hydroxylation on other carbon atoms (Van den Eede et al., 2015a). Moreover, further hydroxylation, hydrocarboxylation or oxidative dealkylation also partially occurred in the former metabolites of these compounds (Fig. 2-B). Overall, the formation of Phase-II metabolites occurred on hydroxyls formed in Phase-I metabolism, and the formation of these metabolites was distinct from that of chlorinated OPFRs.

4.1.3. Aryl-OPFRs

As for aryl-OPFRs, metabolites of TCP, TPHP and resorcinol bis-diphenylphosphate (RDP) were investigated in laboratory animals. Kurebayashi et al. (1985) studied the metabolism of TCP in rats. In this study, the major metabolites were *p*-hydroxybenzoic acid, di-*p*-cresyl phosphate (DCP), *p*-cresyl *p*-carboxyphenyl phosphate (COOH-TCP) and *p*-cresyl di-*p*-carboxyphenyl phosphate (di-COOH-TCP). Su et al. (2014) studied the metabolism of TPHP in chicken embryonic hepatocytes *in vitro*. The identified metabolites

Fig. 2. General metabolic pathways (Phase-I and Phase-II metabolites) of three types of OPFRs (A: Chlorinated OPFRs; B: Alkyl-OPFRs; C: Aryl-OPFRs) in organisms from both *in vivo* and *in vitro* metabolism studies. Reactions are numbered according to the following annotation (①: O-dealkylation; ②: Hydroxylation; ③: Oxidative dechlorination; ④: Oxidation; ⑤: Conjugation).

were DPHP, hydroxylated TPHP (OH-TPHP) and dihydroxylated TPHP isomers (di-OH-TPHP). Su et al. (2015) then identified the hydroxylated TPHP as *p*- and *m*-OH-TPHP and found that the conjugate with glucuronic was primarily on *p*-OH-TPHP. RDP, which is a structure analogous to TPHP, has been studied only in rats, mice and monkeys *in vivo* (Freudenthal et al., 2000). The major excreted metabolites were resorcinol, diphenyl phosphate (DPPH), hydroxylated DPPH (OH-DPPH), and hydroxylated RDP isomers (OH-RDP, di-OH-RDP). No differences were observed between species.

To date, the metabolism of TPHP and RDP has also been studied in human liver microsomes *in vitro*. In addition to the metabolites in the aforementioned chicken hepatocytes, mono-ester (MPPH) and hydroxylated DPPH (OH-DPPH) have also been identified in the metabolism of TPHP (Van den Eede et al., 2013a). Glucuronide conjugates and sulfate conjugates of TPHP were observed in the human liver S9 fractions in the *in vitro* study of Phase-II metabolites (Van den Eede et al., 2013a). Ballesteros-Gómez et al. (2015) reported the *in vitro* metabolism of RDP using human liver microsomes, which was consistent with the *in vivo* metabolism study. RDP was similarly metabolized to DPPH, hydroxy-RDP (OH-RDP) and dihydroxy-RDP (di-OH-RDP) and to glucuronide and sulfate conjugates.

A general metabolic pathway of aryl-OPFRs was same as alkyl-OPFRs (Fig. 2-C). The Phase-I metabolic processes included hydroxylation, dihydroxylation and carboxylation on the phenyl. Glucuronide and sulfate can only react on hydroxylated or dihydroxylated metabolites in Phase-II reaction. What worth mentioning is EHDPPH, who owns both alkyl and aryl. Nishimaki-Mogami et al. (1988) investigated the *in vivo* metabolism of EHDPPH on rats by oral exposure. DPPH, phenol and a small quantity of *p*-hydroxyphenyl phenyl phosphate (*p*-OH-DPPH) and monophenyl phosphate (MPPH) were identified to be major metabolites. While in a EHDPPH study in human liver microsomes *in vitro*, hydroxylation occurred both in 2-butoxyethyl and phenyl, which formed hydroxylated and dihydroxylated metabolites (OH-TPHP, di-OH-TPHP). Moreover, DPPH and glucuronidated conjugates of Phase-I metabolites were also detected as the major Phase-I and Phase-II metabolites (Ballesteros-Gómez et al., 2015).

Based on the former studies, a general metabolic pathway of OPFRs includes O-dealkylation, hydroxylation, carboxylation and oxidative dehalogenation (only for chlorinated OPFRs), resulting in a wide array of dialkyl phosphates (DAPs) and hydroxylated and carboxylated metabolite isomers (Table 4). The dehalogenation in all chlorinated OPFRs was driven by oxidation (a replacement of Cl

with OH), which was different from PBDEs, where reductive dehalogenation pathways were more common (Hakk and Letcher, 2003). Further hydrolysis and oxidation of the alkyl- and aryl-moieties were also possible (Kurebayashi et al., 1985). However, the common occurrence of methoxylation in PBDEs has never been reported to occur in OPFR metabolism (Van den Eede et al., 2013a). More likely, these metabolites can be further metabolized to secondary metabolites by dihydroxylation, oxidation of the hydroxylated carbon atom and further O-dealkylation (producing more hydrophilic metabolites). Alternatively, these metabolites can be conjugated with sulfate, glutathione or glucuronic acid (producing Phase-II metabolites), which renders these metabolites much more soluble for elimination (Van den Eede et al., 2013a). Generally, there have been some inconsistencies in the metabolites identified between study approaches (*in vivo* and *in vitro*) and between species (Table 4). Nomeir et al. (1981) hypothesized that the *in vitro* metabolites identified in studies may be further metabolized before being excreted, thereby making them undetectable in some *in vivo* assays. Different species (rodent, human), matrices (urine, liquid supernatant) and analytical techniques (low or high resolution mass spectrometry) may also be responsible for the inconsistent results (Ballesteros-Gómez et al., 2015).

4.2. Kinetics of metabolism

OPFRs can be metabolized through Phase-I and Phase-II biotransformation to metabolites that are more hydrophilic and more readily eliminated (Van den Eede et al., 2013a). OPFRs possess much lower $t_{1/2}$ (Table 5) than PBDEs (25d–115d in rat) (Hakk and Letcher, 2003). In general, the OPFRs with the lowest bioaccumulation potential ($\log K_{OW} < 3$) have a higher speed of elimination and shorter half-life ($t_{1/2}$) in organisms (Green et al., 2007). Sasaki et al. (1981) reported that chlorinated OPFRs (TDCIPP and TCEP) have rapid elimination rates compared with TBP and TPHP in fish. However, among chlorinated OPFRs, Minegishi et al. (1988) considered di-chlorinated OPFRs (TDCIPP) to be the more slowly absorbed, distributed and excreted than the mono-chlorinated compounds (TCEP and TCIPP).

In many studies of OPFR metabolism conducted in exposed laboratory rodents, urine, feces or expired gas has been assumed to be the main carrier of the excreted metabolites (Table 5). After administration of 14C-TDCIPP in rats, Lynn et al. (1981) found that 92% of 14C had been excreted in the urine (54%), feces (16%), and expired air (22% as 14 CO₂), where 63% of TDCIPP was recovered as BDCIPP. This high recovery rate may be due to the fact that BDCIPP has a slow metabolic rate in the body and can be directly eliminated by excretion before being metabolized. In another similar study, after 168 h of oral exposure to 14C-TDCPP, recovery of 43.2% of 14C in urine, 39.2% in feces and 16.2% in expired air (Minegishi et al.,

1988). Burka et al. (1991) studied TCEP exposure in rats and mice. They found that more than 70% of oral-dosed 14C-labelled metabolites were excreted in urine within 8 h, and BCEP was found to be a major metabolite.

Su et al. (2015) most recently studied TPHP metabolism in chicken embryonic hepatocytes *in vitro*. In this study, the detected *p*-OH-TPHP (after β -glucuronidase treatment) accounted for 60% of the initial TPHP dose at 36 h, which was 3.5-fold higher than that detected for DPHP and 12-fold higher than that detected for *m*-OH-TPHP. They suggested that the glucuronic acid conjugate of *p*-OH-TPHP may serve as a more critical metabolite than the formerly accepted DPHP. However, because of the large time span and the limited magnitude of the reviewed studies, we cannot determine clear differences of OPFRs metabolites rates among individuals, sexes and species.

Crump et al. (2012) have mentioned that some metabolites of OPFRs are comparatively more stable than their parent compounds in the body; hence, some of the adverse effects of OPFRs may, in fact, be the result of their metabolites. Meeker et al. (2013a) reported a significant or suggestive relationship between two urinary OPFR metabolites (BDCIPP and DPHP) and serum hormone levels in men. 1,3-DCP, the metabolite of TDCIPP, has been verified to be a powerful direct-acting mutagen (Gold et al., 1978). DPHP has been reported to alter transcripts associated with the lipid/cholesterol metabolism in chicken embryonic hepatocytes (Su et al., 2014). However, although some kinetic data on absorption and excretion are available, available toxicokinetic studies of OPFRs are still scarce. Toxicokinetics models, which can establish relationships between dosage, bioaccumulation, metabolism and clearance utilizing dose-response measurements (Mošat' et al., 2013), may be a promising direction for future research. Therefore, studies that fully characterize the toxicokinetics of OPFR metabolism and measure circulating and excreted metabolites are sorely needed for the study of OPFR toxicokinetics.

4.3. Enzymes in metabolism

The overall evidence from aforementioned studies suggests that multiple metabolic systems may manage the metabolism of OPFRs. Enzyme catalysis is acknowledged to be largely responsible for the metabolism of drugs. The same is likely to be the case for OPFRs, as their metabolism primarily occurs in the liver, kidney, and adipose tissue (Sasaki et al., 1984).

Cytochromes P450 (CYP) was thought to play a critical role in several steps of Phase-I metabolism. For OPFRs metabolic processes, the major CYPs that catalyzed Phase-I reactions were hydroxylation of unsubstituted carbon atoms, O-dealkylation/Phase-II reactions, and possible mono- and di-hydroxylated Phase-I metabolites (Ballesteros-Gómez et al., 2015). Although CYPs appear to

Table 5
Toxicokinetic studies of OPFRs in laboratory animals.

Compounds	Species	Exposure approaches	Major disposition tissues	$t_{1/2}$	Excretion pathway and proportion	References
TDCIPP	Killifish	Water exposure	—	31 h	—	Sasaki et al. (1981)
	Goldfish	Water exposure	—	42 h	—	Sasaki et al. (1981)
TCEP	Killifish	Water exposure	—	0.7 h	—	Sasaki et al. (1981)
	Mice	Oral	—	—	Urine (70%)	Burka et al. (1991)
	Rat	Oral	Liver, kidney, adipose tissue, spleen, lung	n/a	Urine (96%), feces (6%), expired air (2%)	Minegishi et al. (1988)
TBP	Killifish	Water exposure	—	58 h	—	Sasaki et al. (1981)
	Goldfish	Water exposure	—	>100 h	—	Sasaki et al. (1981)
TEHP	Rat	Inhalation	Lungs, stomach contents, brain and liver	n/a	Urine (low), feces (high)	WHO (2000)
TPHP	Rainbow trout	—	—	79 h	—	Green et al. (2007)
	Chironomus	—	—	30–79 h	—	Green et al. (2007)
	Killifish	Water exposure	—	5 h	—	Sasaki et al. (1981)
	Goldfish	Water exposure	—	>100 h	—	(Sasaki et al. (1981))

be the main family of enzymes responsible for the formation of Phase-I metabolites, some hydrolysed metabolites may be catalyzed by NADPH independent liver microsomal phosphatase enzymes, such as, for example, paraoxonases and aryl esterases (Van den Eede et al., 2013a). However, the role of CYPs is likely to be more significant than that of the hydrolases in these processes, such as O-dealkylation Phase-II reactions via the hydroxylation metabolic pathway (Chapman et al., 1991; Sasaki et al., 1984; Van den Eede et al., 2013a). In Phase-II reactions, the conjugating enzymes consist of many superfamilies of enzymes, including glucuronyl transferases (UGTs), sulfotransferases (SULTs) and glutathione S-transferases (GSTs) (Van den Eede et al., 2013a).

5. Internal exposure

OPFRs are emerging contaminants, thus, internal exposure to OPFRs is a critical concern. In contrast to external exposure, which concerns the source or pathway to the body, studies of internal exposure have focused on the total concentration of circulatory OPFRs and their metabolites within the body, which indicates the total burden of exposure (Alves et al., 2014).

OPFRs can be transported in the plasma and be distributed through the body and metabolized in liver. OPFRs have been found in various tissues in rodents, fishes and avians. OPFRs are considered to have a weak correlation with lipid content in tissues, which is different from observations of PBDEs (Brandsma et al., 2015; Chen et al., 2012; Greaves and Letcher, 2014; Kim et al., 2011; Sundkvist et al., 2010). For wild animals exposed to OPFRs at the environment level, the contaminants are highly distributed in metabolically inactive tissues, such as adipose tissue, muscle and feathers. Thus, these OPFRs could be transferred to eggs in ova (Evenset et al., 2009). Greaves and Letcher (2014) found that the effect of hydrophobicity was weak with respect to OPFR accumulation in the body of herring gulls. They concluded that other processes, such as metabolism, may play a critical role in the distribution of OPFRs. In the herring gulls of the Great Lakes, metabolically inactive tissues, such as fat, egg yolk, egg albumin, muscle and red blood cells, contained relatively higher OPFR concentrations, whereas no OPFRs were detected in the liver and plasma (Greaves and Letcher, 2014). In surveys of white-tailed eagle nestlings in Norway, the levels of OPFRs detected in feathers were much higher than those detected in other tissues (Eulaers et al., 2014).

When estimating internal burdens of OPFRs in wild animals, the sampling matrices should be fully considered. Plasma can equilibrate with internal tissues and can be easily and non-destructively sampled. Thus, plasma is commonly used as sampling matrix to represent the internal body burden of OPFRs (Eulaers et al., 2014). In the aforementioned studies, OPFRs tend to accumulate in storage organs or metabolically inactive tissues, such as fat, egg and feather. Thus, these tissues may serve as more valuable alternatives or complementary sampling matrices for the conventional analysis of homogenates and muscle samples of animals (Greaves and Letcher, 2014) (SI Table S1). In human biomonitoring, non-invasive matrices are required. Aside from plasma, urine, saliva and hand wipes are the most frequently used matrices in human biomonitoring (Alves et al., 2014; Hoffman et al., 2015; Meeker et al., 2013b). Hair and nails are less metabolically active tissues that may also be promising samples for internal exposure studies (Alves et al., 2014).

OPFR diesters (DAPs) and monoesters (MAPs) are thought to be the major metabolites of many OPFRs, their distribution and concentrations throughout the body would be highly valuable information for understanding the pharmacokinetics of OPFR interactions in the body (Greaves and Letcher, 2014). Recently, quantified DAPs and MAPs in urine have been identified as

biomarkers to assess human exposure. In total, DAPs of BDCIPP, BCIPP, BCEP, BBOEP, DBP, DPHP, DEHP and some MAPs of MEHP, MBP, MCIPP, MBOEP and MPHP have been quantified in human urine, with median concentrations in the ng ml⁻¹ range (Butt et al., 2014; Cequier et al., 2014b, 2015; Chu et al., 2011; Cooper et al., 2011; Dodson et al., 2014; Fromme et al., 2014; Hoffman et al., 2015; Meeker et al., 2013a,b; Moller et al., 2004; Petropoulou et al., 2016; Reemtsma et al., 2011; Schindler et al., 2009a,b; Van den Eede et al., 2015b; Van den Eede et al., 2013b) (SI Table S2). However, no information is available for DAPs and MAPs or other OPFR metabolites from internal exposure studies of wild animals. Quantitative whole-body autoradiography (QWBA) or whole-body autoradioluminography (WBAL) may provide a good solution for the characterization of OPFR burdens and their metabolites throughout the body.

6. Conclusion

The wide use of OPFRs and their ubiquitous occurrence in environments has increased their exposure to organisms. An overview of OPFR fates in animals and humans is provided in Fig. 1. Generally, ingestion, inhalation and dermal contact are more important absorption routes for humans, whereas ingestion is more important for wildlife exposed to OPFRs. After being absorbed, OPFRs can accumulate in the body, depending on their physicochemical properties and exposure levels. It is further suggested that OPFRs can be transferred through aquatic and terrestrial food chains, but to our knowledge there are no clear conclusion that OPFRs can biomagnify in food webs. Assessment of the bioaccumulation and biomagnification of OPFRs (BCFs and BMFs, respectively) have tentatively done, however, more works are required to clarify their species-specific risks, which should take into account the specie, size, gender of the test animals.

The common metabolic pathways are summarized for all three types of OPFRs (chlorinated OPFRs, alkyl-OPFRs and aryl-OPFRs), including O-dealkylation, hydroxylation and oxidative dehalogenation. Studies on OPFR metabolism have been limited to laboratory rats and *in vitro* human models. There is no information on the *in vivo* metabolic process of OPFRs on other wildlife. Using advanced analysis methods (i.e., UPLC-QTOF-MS with targeted and untargeted screening tools), more *in vivo* studies can be conducted to identify a wide variety of metabolites to provide confirmation that metabolism of OPFRs readily occurs in organisms. For the study of OPFRs metabolism, *in silico* models can be used to identify the metabolites formed to simplify the screening process. Combinations of *in vitro* and *in silico* methods, which have been called the *in combo* approach, may be the most promising strategy for studies of metabolism.

In recent studies, investigators have mainly focused on DAPs and MAPs as major biomarkers in urine for internal studies of human. However, information about the other *in vivo* Phase-I or Phase-II metabolites (i.e., hydroxylated metabolites, glucuronic and sulfate conjugates) was less available in human excreta monitoring reports. For exposure assessments of wild animals, only residual OPFRs were analyzed in the plasma or in different tissues. The major OPFR metabolites formed in *in vitro* studies are valuable candidate markers for monitoring wild animal exposure (Alves et al., 2014). Future research focusing on the quantification of more OPFRs and their metabolites in other metabolically inactive tissues may provide a comprehensive picture of the internal exposure of OPFRs in animals and humans.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.chemosphere.2016.03.003>.

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