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Chapter

Occurrence, Bioaccumulation and Effects of Legacy and Emerging Brominated Retardants in Earthworms

Jean-Philippe Bedell, Claudia Coelho, Olivier Roques, Anais Venisseau, Philippe Marchand and Yves Perrodin

Abstract

The presence of former brominated flame retardants and “emerging” brominated flame retardants (BFRs and e-BFRs) in soils is well documented, but the presence, metabolism and uptake of them in earthworm species are much less. Polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDDs) are the most abundant “legacy” BFRs in soils. Earthworms are a good bioindicator, presenting an integrated view of soil chemical pollution. They bioaccumulate BFRs passively by dermal absorption, and actively through soil ingestion. However, such information is only available for a limited number of species, mostly for *Eisenia fetida*, which shows high bioaccumulation factors (>2). Most of the ecotoxicity studies on earthworms have been done using PBDEs or HBCDDs. PBDEs were reported to effect changes in enzyme activities, which induced oxidative stress and caused metabolic perturbations in some earthworm species. In *E. fetida*, contaminant bioaccumulation is influenced by the lipid and protein contents of tissues, but several different processes (uptake, depuration, metabolism and isomerization) also contribute to the observed tissue levels. To evaluate and manage the risks posed by these chemicals to terrestrial ecosystems, it is important to better understand the transfer processes of emerging brominated flame retardants in earthworms, as well as the potential trophic biomagnification.

Keywords: bioaccumulation, brominated flame retardants, earthworms, *Eisenia fetida*, emerging brominated flame retardants, PBDEs

1. Introduction

Brominated flame retardants (BFRs) are in demand on the market because of their relatively low cost and efficient fire-resistance performance [1], notably because the BFRs' incorporation generates halogen atoms of the parent compound that can delay or “retard” the development of a fire [2]. Thus, BFRs are used in different product

categories, such as textiles, plastics, building materials and very often in electronic equipment [3–5].

Some BFRs have been classified as “traditional” or “legacy” BFRs in contrast to the “novel” or “emerging” BFRs (e-BFRs) [6, 7]. Legacy BFRs mainly refer to the chemicals that are no longer used or commercialized, such as polybrominated biphenyls (PBBs), polybrominated diphenyl ethers (PBDEs), hexabromocyclododecanes (HBCDDs) and tetrabromobisphenol A (TBBPA). Among the e-BFRs, we can list decabromodiphenyl ethane (DBDPE), bis(2,4,6-tribromophenoxy) ethane (BTBPE), bis(2-ethylhexyl) tetrabromophthalate (BEH-TEBP), 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB), hexabromobenzene (HBBz), pentabromobenzene (PBBz), pentabromotoluene (PBT) and pentabromoethylbenzene (PBEB). These e-BFRs remain poorly studied and documented [8], but they present similar physicochemical properties as other persistent organic pollutants (POPs), notably polychlorobiphenyls (PCBs), and may therefore pose a potentially hazardous risk to ecosystems. Thus, BFRs are characterized by medium to low vapor pressures as well as low water solubility and high volatility, with high (> 6) octanol–water partition coefficients (K_{ow}) [5, 6]. Such properties indicate that they are lipophilic. The physicochemical and biochemical properties of BFRs combined with their resistance to chemical and biological degradation make these molecules persistent and easily incorporated in every compartment of the ecosystem [9, 10]. In Ref. [11], it underlined their persistence and bioaccumulative characteristics as well as their potential toxicity to living organisms. In addition, exposure to BFRs results in negative health effects for animals and humans [9, 10, 12]. PBBs, PBDEs and HBCDDs were the most widely produced and used BFRs. Due to volatilization, weathering and adsorption processes, notably on dusts, BFRs can migrate to environmental compartments and can be released into the air [13]. In addition, at the end of a product life, the incorporated BFRs may be released during waste disposal. In Ref. [14], they classified Σ PBDEs by land use in many different soils, with values ranging between 0.05 and 800 $\text{ng}\cdot\text{g}^{-1}$ for urban soils and between 3 and 40,000 $\text{ng}\cdot\text{g}^{-1}$ for electronic and electrical waste contaminated soils. The variability observed between several measurements demonstrates the ubiquitous dispersion of these compounds across the world and the great difficulty in comparing different soil samples. This difficulty is linked to the types and characteristics of soils (such as organic matter (OM) or clay content), which may be factors influencing the retention/adsorption/fixing of the BFRs, as well as the different potential BFR sources (industrial, waste disposal and urban) and their atmospheric transport and dispersion. In the literature, if PBDEs are well documented, there is a lack of studies and data regarding the environmental fate of PBBs and HBCDDs, notably in soils. Despite its extensive use, information on TBBPA concentrations in soils [15, 16] is also scarce compared to data available for PBDEs, for example, see in [14–18]. Legacy BFRs are still present in the environment and are obviously well dispersed in a lot of different soils.

Electronic waste (E-waste) dismantling areas seem to be a major source of e-BFRs in the environment, which are dispersed by atmospheric transport followed by deposition on land surfaces. The hydrophobic nature of e-BFRs is confirmed by the observed $\log K_{ow}$ values that are greater than 3, which is similar to the legacy BFRs. These compounds are therefore highly lipophilic, which can explain their potential to bioaccumulate in organisms. This bioaccumulation has already been reported in the literature, mainly for aquatic animals, but similar data on e-BFRs in soils, earthworms and plants are scarce. Of the e-BFRs, DBDPE was the dominant compound detected in the Arctic soil (average; 9.1 $\text{pg}\cdot\text{g}^{-1}$ dw; [19]) or in the rhizosphere or non-rhizosphere

of an e-waste soil in South China [20]. Other studies also reported that the DBPDE content was higher than BDE 209 in the Chinese background forest soil [21]. In addition, HBBz also generally occurred at high concentrations in soils, ranging from close to, or above $1 \text{ ng.g}^{-1} \text{ dw}$ to a maximum of $\sim 10 \text{ ng.g}^{-1} \text{ dw}$ depending on the type of soil [14, 17, 21–24]. The other compounds, such as PPBz, PBT and PBEB, showed slightly lower values on average, except at some contaminated sites which are generally where most measurements have been made (e-waste or disposal sites) [23, 25, 26]. The physicochemical characteristics, patterns of environmental contamination and toxicity effects of e-BFRs are close to those of PBDEs. These analogies suggest that availability and bioaccumulation processes might be similar to those of PBDEs, as well as to those of PCBs or polychlorinated dibenzo-p-dioxins/polychlorinated dibenzofurans (PCDD/Fs).

The present work focuses on brominated flame retardants (BFRs) in particular, because certain “new or emerging” BFRs (e-BFRs) have replaced a number of legacy brominated and chlorinated POPs. Moreover, two interesting reviews of BFRs in soil [17] and in wildlife [27] highlighted the requirement for more research, notably on e-BFRs, in order to obtain a better understanding of their environmental fate and dispersion, notably in different environmental compartments. In contrast to many invertebrate organisms that are only indirectly exposed to contaminants through the food chain, invertebrates in the soil are good “sentinel organisms” for the chemical contamination of soil [28] because they are in direct contact with soils, soil pore water and their food sources which generally consist of organic matter, algae, fungi and small microorganisms including bacteria. Thus, one of them, earthworms represent the largest biomass fraction of soils and tend to migrate over very short distances, illustrating again the fact that they are a good candidate for integrating soil chemical pollution [29]. Thus, this work gives concentrations and elements on bioaccumulation of legacy and also with many values on e-BFRs in earthworms which are less developed in bibliography. Moreover, we also address an aspect that is less emphasized in the review articles: toxicological effects of these compounds on some species of earthworms, as well as the “metabolic effect” (physiological or biochemical mechanisms) of these pollutants on earthworms.

2. Potential bioaccumulation and effects of BFRs on earthworms

When soils are contaminated by BFRs, they automatically become a source for the transfer of these pollutants to biotic compartments, and notably to an important key organism of the soil ecosystem: earthworms.

2.1 Earthworms as a bioindicator

Earthworms are also called “ecosystem engineers” due to their role: (i) in soil formation, (ii) in organic matter breakdown processes, (iii) in soil aeration as well and (iv) in nutrient cycling [29]. Consequently, earthworms are good indicators of land-use impact and soil fertility, and affect the physical, chemical and microbiological properties of the soil by their roles in the pedogenesis of the soil profile [30]. In addition, earthworms also increase the mineralization and humification of soil organic matter (OM) by consumption, respiration and passage through their gut [31]. Their castings stimulate an increase in OM breakdown activity and microbial mass, while also improving the mobilization of nutrients [32]. Furthermore, their burrowing

activities significantly promote the increase in water infiltration capacity and aeration of soils [33]. Unfortunately, these activities also result in the direct exposure of earthworms to both organic and inorganic contaminants that are adsorbed in the soil OM particles that constitute a major feed source for these terrestrial invertebrates [34–36].

Due to their interactions with soil, earthworms can be considered as valuable bioindicators, as they are very sensitive to the presence of contaminants in the soil and are significantly affected by them [37]. Earthworms are exposed to soil contaminants through different routes of exposure. They have direct contact with the soil pore water and through that, with the dissolved contaminants present in this water. Earthworm skin is renowned for being very permeable to water [38] and this contact with the skin represents a main route for contaminant uptake [39, 40]. Moreover, soil ingested by earthworms results in continuous exposure to contaminants adsorbed in the solid particles passing through their digestive tract [41].

A species of earthworm, the *Eisenia fetida* (*E. fetida*), is probably the most used standard test organism in terrestrial ecotoxicology evaluations, due to its rapid life cycle, and easily controlled breeding and reproduction [42]. Most of the works on BFR bioaccumulation, effects or metabolism evaluations have been carried out using *E. fetida*, with a small amount being carried out on other earthworms (Tables 1 and 2). Most of these studies have involved legacy BFRs, notably PBDEs (Table 1), and few results are available for HBCDDs or e-BFRs (Tables 2 and 3).

2.2 Bioaccumulation of BFRs in earthworms

Earthworms have the capacity to accumulate organic (and inorganic) contaminants present in soils [57]. Specifically, studies have demonstrated that several earthworm species are able to accumulate POPs, such as PCBs, BFRs, pharmaceuticals, detergent metabolites, polycyclic aromatic hydrocarbons (PAHs) and pesticides [58–60]. Earthworms can accumulate POPs passively by dermal absorption, and actively through soil ingestion. If the relative importance of dermal and dietary exposure depends on the individual contaminant, some results have highlighted that the importance of the dietary pathway for accumulation increases as a function of the hydrophobicity of the contaminant [39, 61]. In Ref. [40], they evaluated the importance of different pathways for metal uptake in *Lumbricus rubellus*. They concluded that the main route to metal accumulation was dermal absorption because ingestion via pore water uptake represented only a small contribution [40]. In a study with four different species (*Eisenia andrei*, *Eisenia fetida*, *Eisenia hortensis* and *Lumbricus terrestris*) [62], they observed the accumulation of PCBs in all earthworm tissues. In the same way [63], they investigated the capacity of *E. fetida* to accumulate doxin-like polychlorinated biphenyls (DL-PCBs). Using contaminated soils from Sweden [64], they showed the occurrence of PCDDs and PCDFs in *E. fetida* tissues resulting from exposure, both *in situ* and in laboratory conditions.

Most of the works on PBDEs (Table 1) have focused on the genus *Eisenia* and more specifically the specie *fetida*. Only two other genera have been recorded, namely *Lumbriculus* (*variegatus* and *rubellus*) and *Allolobophora caliginosa trapezoides* (Table 1). In a study investigating PCBs and PBDEs in the soil-earthworm-hedgehog food chain, the accumulation of these compounds in *Lumbricus rubellus* tissues was noted [50]. In East China, *E. fetida* and *Allolobophora caliginosa trapezoides* collected from an e-waste dismantling area showed the accumulation of PCBs, PCDDs, PCDFs and PBDEs in their tissues [49]. The results showed measurable and sometimes high

Species	Units	Σ PBDEs	BDE 28	BDE 47	BDE 99	BDE 100	BDE 153	BDE 183	BDE 209	Reference
<i>Eisenia andrei</i>	ng.g ⁻¹ DW	7.52–76.3 (21 congeners)	0.01–0.13	0.14–4.91	0.05–5.42	0.04–1.53	0.02–0.66	0.02–0.41	6.85–59.1	[43]
	BSAF	0.8–4.74	1.32–18.4	0.84–26.9	0.33–14.4	1.04–20.3	0.21–3.57	0.1–1.3	1.08–4.81	
<i>Eisenia fetida</i>	μ g.g ⁻¹ lipid								1.04–865.6 (adult)	[44]
	BSAF								0.074–0.123 (adult)	
<i>Eisenia fetida</i>	μ g.g ⁻¹ DW (max)			16	32			12	5	[45]
	BSAF* OECD spiked			5–12	4–10		0–4	1–3	0–1	
	BSAF*			3–9	3–6		1–3	ND	ND	
<i>Eisenia fetida</i>	BSAF*			5–12	4–9		2–4	0–3	0–1.5	[46]
<i>Eisenia fetida</i>	μ g.kg ⁻¹ lipid	1000–65 000 (6 congeners)								[47]
	BSAF*			5–20	3–13	4–17				
<i>Lumbriculus variegatus</i>	ng.g ⁻¹ lipid (values obtained)		314	5685	2649	813	693	315	8980	[48]
	BSAF*		2.18	4.09	0.905	1.63	0.611	0.320	0.103	
<i>Eisenia fetida</i>	ng.g ⁻¹ DW	0.2–4.44 (14 congeners)								[49]
<i>Allolobophora caliginosa trapezoides</i>	ng.g ⁻¹ DW	0.56–2.13 (14 congeners)								

Species	Units	ΣPBDEs	BDE 28	BDE 47	BDE 99	BDE 100	BDE 153	BDE 183	BDE 209	Reference
<i>Lumbricus rubellus</i>	ng.g ⁻¹ DW	0.08–0.2								[50]
	BSAF*	1.99–5.67								
<i>Eisenia fetida</i>	ng.g ⁻¹ DW	0.537–67.56 (8 congeners)	0.003– 0.024	0.095– 0.854	0.041– 1.145	0.011– 0.659	0.005– 0.203	0.003– 0.095	0.239– 65.161	[51]
	μg.kg ⁻¹ lip	5.03–63.3 (adult)								
		13.7–160 (juvenile)								
	BSAF*	0.01–0.3 (adult)								
		0.02–7.92 (juvenile)								

BSAF, [earthworms DW]/ [soil DW]; BSAF*, [earthworm lipid]/ [soil OC]; OECD, artificial soil used; DW, dry weight; OC, organic content; PBDE, polybrominated diphenyl ethers; BDE, brominated diphenyl ethers + IUPAC Nb.

Table 1.

Polybrominated diphenyl ethers (PBDEs) content and factor of bioaccumulation (biota-to-soil accumulation factor (BSAF)) in several earthworm species. All values gave the min-max observed, except when indicated.

Species	Units	HBBz	TBBPA	HBCDDs			ΣPBBs (=52 + 101 + 153)	Reference
				α	β	γ		
<i>Eisenia fetida</i>	ng.g ⁻¹ DW (max)			2400	400	580		[52]
	BSAF			1.28–11.2	0.42–1.71	0.38–1.95		
<i>Eisenia fetida</i>	ng.g ⁻¹ DW			1500–3000	200–580	200–610		[53]
	BSAF			11–21	1.1–3.2	1.1–4.1		
<i>Metaphire guillelmi</i>	ng.g ⁻¹ DW			500–800	200–480	130–220		
	BSAF			2.5–5.5	1.1–2.7	0.9–1.6		
<i>Eisenia fetida</i>	BSAF*		14					[54]
<i>Metaphire guillelmi</i>	BSAF*		11					
<i>Eisenia fetida</i>	BSAF (TBBPA + Cd)		1.1–4.5					[55]
<i>Metaphire guillelmi</i>	BSAF (TBBPA + Cd)		6.6–7.5					
<i>Eisenia fetida</i>	BSAF			21.8	2.28	3.77		[56]
	BSAF*			2.58	0.27	0.44		
<i>Metaphire guillelmi</i>	BSAF			6.21	2.81	1.16		
	BSAF*			1.10	0.50	0.20		
<i>Eisenia fetida</i>	μg.g ⁻¹ DW (max)	10						[45]
	BSAF* OECD spiked	2–6						
	BSAF*	2.6–8.9						
<i>Eisenia fetida</i>	ng.g ⁻¹ DW	0.21–0.44		0.027–4.68	0.067–0.095	0.045–1.970	0.009–0.207	[51]
	ng.kg ⁻¹ lip						0.12 and 0.17 all other <LOD (adult) all <LOD (juvenile)	
	BSAF*						0–0.26 (adult) 0 (juvenile)	

BSAF, [earthworms DW]/[soil DW]; BSAF*, [earthworm lipid]/[soil OC]; OECD, artificial OECD soil; DW, dry weight; OC, organic content; [PBB, polybrominated biphenyls; HBBz, hexabromobenzene; TBBPA, tetrabromobisphenol A; TBECH, 2-dibromo-4-(1,2-dibromoethyl)cyclohexane; HBCDDs, hexabromocyclododecane]. Limit of detection (LOD) (PBBs), 0.034.

Table 2.

Content and bioaccumulation factors of some brominated flame retardants (BFRs) in several earthworm species. All values gave the min-max observed except when indicated or when just one value was available.

		PCDD/Fs			PCBs		Legacy BFRs			e-BFRs						
		Σ PCDDs	Σ PCDFs	Σ DL-PCBs-no	Σ DL-PCBs-mdio	Σ_6 NDL-PCBs	Σ_8 PBDEs	Σ HBCCD	Σ_3 PBBs	PBEB	PBT	TBCT	HBBz	PBBz	pTBX	OBIND
Adult	Min	94.30	60.7	2390	24 0420	1 988 320	4.393	<0.31	<0.034	< 0.029	0.058	< 0.032	0.289	0.058	< 0.037	<0.178
	Max	11 310.9	2 377.9	36 930	2 539 830	20 087 510	63.29	4.96	0.173	0.116	4.913	< 0.032	4.393	1.098	< 0.037	<0.178
Juvenile	Min	109.02	107.7	960	52 260	380 670	13.71	<0.31	<0.034	< 0.029	0.337	< 0.032	0.421	0.084	< 0.037	<0.178
	Max	7 653.3	1 219	11 330	843 960	6 077 920	159.33	4.85	<0.034	0.337	4.972	< 0.032	8.12	1.798	< 0.037	<0.178

[PCDDs, polychlorinated dibenzo-p-dioxins; PCDFs, polychlorinated dibenzofurans; DL-PCBs-no, dioxin-like none ortho polychlorinated biphenyls; DL-PCBs-mdio, dioxin-like mono + di-ortho polychlorinated biphenyls; NDL-PCBs, non-dioxin-like polychlorinated biphenyls; PBDE, polybrominated diphenyl ethers; PBB, polybrominated biphenyls; PBEB, pentabromoethylbenzene; PBT, pentabromotoluene; TBCT, tetrabromo-o-chlorotoluene; HBBz, hexabromobenzene; PBBz, pentabromobenzene; pTBX, 2,3,5,6-Tetrabromo-p-xylene; OBIND, octabromotrimethylphenylindane]. Values indicated in italic with < symbol are the limit of quantitation (LOQ) for the compound.

Table 3.

Concentrations of several organic contaminants measured in *Eisenia fetida* adults and juveniles exposed during 4 weeks on eight different soils (all results in ng.kg^{-1} lipid dw) [51].

concentrations (of the order of $\mu\text{g}\cdot\text{kg}^{-1}$ lw), demonstrating the transfer of PBDEs to earthworms (**Table 1**). In addition, it was noted that some biota-to-soil accumulation factors (BSAFs) for PBDEs are very high, and not only for the most abundant congener in soils (BDE 209). These factors are just as important for other congeners such as BDE 47 (**Table 1**). The predominance of BDE 47 and BDE 99 is typical for biota [65, 66]. BDE 209 displayed a strong sorption tendency to soils/sediments and biological samples due to its extremely hydrophobic character (the $\log K_{ow}$ for BDE 209 is 9.97) [67]. Generally, PBDE profiles in environmental samples were dominated by BDE 209 [18]. High concentrations of PBDEs (average $\Sigma_{10}\text{PBDEs}$ 2665 $\text{ng}\cdot\text{g}^{-1}$ dw) were detected in residential soils from an electronic waste polluted region in Guiyu, China, where BDE 209 accounted for 73% of the total $\Sigma_{10}\text{PBDEs}$ [68]. *E. andrei* showed concentrations ranging over a factor of 10 between the minimum and maximum levels, from 7.5 to 75 $\text{ng}\cdot\text{g}^{-1}$ dw, with a predominance of BDE 209 in the tissues. On the other hand, comparing BSAFs, BDE 47 expressed the highest global factor (26.9), which reduced to 4.74 for ΣPBDEs (**Table 1**). In an experiment where earthworms were exposed to a low spiked artificial soil, *E. fetida* showed ΣPBDE concentrations of up to 65,000 $\mu\text{g}\cdot\text{kg}^{-1}$ lw [47]. Other studies showed ranges of *E. fetida* concentrations between 5 and 160 $\mu\text{g}\cdot\text{kg}^{-1}$ lw and 1 and 865.6 $\mu\text{g}\cdot\text{g}^{-1}$ lw (**Table 1**). The maximum BSAF values were 20 for BDE 47, 17 for BDE 100 and up to 7.92 for ΣPBDEs (**Table 1**). Tissue burdens decreased in the following order: BDE 47 > 99 > 100 > 85 > 154 > 153 [47]. In the present study, adult *E. fetida* showed a BSAF <1 for BDE 209, as observed in [44], and the highest values of up to 7.92 were seen in the tissues of juveniles [51] (**Table 1**). The *Lumbriculus* and *lumbricus* genera showed concentrations of up to 8900 $\text{ng}\cdot\text{g}^{-1}$ lw for BDE 209 and some BSAF values were showed up to 4.09 for BDE 47 and up to 5.67 for ΣPBDEs (**Table 1**). In a study on Canadian (British Columbia) soils [69], they reported BSAF (normalized to lipid in biota and organic content (OC) in soil) values of 4.74 for BDE 47, 33 for BDE 85, 2.56 for BDE 99 and 2.47 for BDE 100, for the “group” *lumbricidae*. Some field studies investigated the accumulation of PBDEs in earthworms, and expressed the relationship between BSAFs and the octanol–water partition coefficient ($\log K_{ow}$), to assess bioavailability to earthworms, and did not address the direct relationship between soil and earthworm concentrations [70, 71].

However, positive relationships between soil and earthworm (*Lumbricus rubellus*) contents were found for PBDEs (sum of two congeners 47 and 99) for the combined data for two parks where hedgehogs foraged [50].

Concerning the other legacy BFRs (**Table 2**), we noted that only two types of earthworms, *Eisenia fetida* and *Metaphire guillelmi*, were used to study soil interactions. Several studies have shown the transfer of HBCDDs to earthworms (**Table 2**). In studies with *E. fetida*, the highest concentrations were observed for α -HBCDD, showing a range from 2400 to 3000 $\text{ng}\cdot\text{g}^{-1}$ dw. Corresponding levels were 10 times lower for β -HBCDD with values between 200 and 400 $\text{ng}\cdot\text{g}^{-1}$ dw, with similar concentrations for γ -HBCDD ranging from 200 to 610 $\text{ng}\cdot\text{g}^{-1}$ dw (**Table 2**). BSAFs reflected the dominance of α -HBCDD with a maximum value of ~ 22 and a range from 1 or 2, up to 21, in comparison to the lower factors for β -HBCDD and γ -HBCDD, ranging from <1 to 4.1 (**Table 2**). Similarly, in studies on *M. guillelmi*, the highest concentrations at 500 and 800 $\text{ng}\cdot\text{g}^{-1}$ dw were noted for α -HBCDD, one third of the maximal observed values for *E. fetida* for the same congener (**Table 2**). However, the concentration ranges for β -HBCDD (200 and 480 $\text{ng}\cdot\text{g}^{-1}$ dw) and γ -HBCDD (130 and 220 $\text{ng}\cdot\text{g}^{-1}$ dw) were similar to the corresponding ranges seen in *E. fetida* (**Table 2**). Such transfer can be explained by the highly hydrophobic character of individual

HBCDDs, with $\log K_{ow}$ values of 5.38 (α -), 5.47 (β -) and 5.80 (γ -), and their association, primarily with soil particles [72]. BSAFs reflect this dominance of α -HBCDD in *M. guillelmi* with a maximum α -HBCDD factor at 5.5 in comparison to β -HBCDD and γ -HBCDD factors, with maximum BSAF values of 2.7 and 1.6, respectively (Table 2). Also, reflecting the relatively lower concentrations, *M. guillelmi* expressed a lower transfer factor than *E. fetida*, with BSAFs at least two to four times lower (Table 2). This observed difference in the bioaccumulation of β -HBCDD between the two species can be due to other processes, such as uptake, depuration, metabolism and isomerization. In earthworms, β - and γ -HBCDDs are strongly bioisomerized to the α -form, but this isomerization occurs to a larger extent in *E. fetida* [56]. The difference in stereostructure of the three HBCDD diastereoisomers leads to substantial differences in their physicochemical, biological and toxicological properties [73, 74]. The lipids in an organism are an important depot for the storage of organic pollutants. In Ref. [52], they report the lipid content of the body wall and gut at higher than 5%, underlining the important potential role that the tissue lipid plays in affecting the accumulation and distribution of HBCDDs in earthworms, compared to protein. In comparison, *Lumbricus rubellus* showed a lipid content of between 6.16 and 6.96% lipid weight (lw)/dry weight (dw) basis [50]. In Ref. [56], they explained the much greater BSAF values of α - and γ -HBCDDs in *Eisenia fetida* relative to *Metaphire guillelmi*, by the higher lipid and protein contents of tissues in *E. fetida*. Some studies have reported that the relative accumulation capacity of animal protein was approximately 5% that of lipid. Therefore, in animals with lipids at <5% of the dry weight content, protein sorption will exert a significant role in the accumulation and distribution of contaminants [75, 76]. The uptake in earthworms of other BFRs such as TBBPA has also been investigated (Table 2). TBBPA was studied in combination with cadmium [55] (Table 2), with results for TBBPA showing a BSAF value between 1.1 and 14 for *E. fetida* and between 6.6 and 14 for *M. guillelmi*. Table 2 also shows studies on HBBz, uptake in *E. fetida*, with concentrations of 0.021–10,000 ng.g⁻¹ dw and BSAF values between 2 and 8.9. In our studies on Σ PBBs, we noted low transfer values from soil to earthworms for the eight samples tested, with only two quantifiable values of 120 and 170 ng.kg⁻¹ lw in adults. All other concentrations were below the limit of detection (LOD), including in all juvenile earthworms [51] (Table 2). The three PBBs (32, 101 and 153) investigated, seemed to indicate little, if any transfer to the earthworms, particularly in juveniles. The lack of other studies on PBB uptake in earthworms limits the possible comparisons and discussion.

If we compare the e-BFR concentrations in *Eisenia fetida* to values obtained for other POPs such as PCBs and PCDD/Fs (Table 3), we also notice the concentration differences between adults and juveniles exposed to eight different soils during 28 days [51].

For adults, we noted concentrations decreasing in the following order: NDL-PCBs (non-dioxin-like polychlorinated biphenyls) >>> mono-di-ortho-PCBs >> non-ortho-PCBs >>> PCDDs >> PCDFs > PBDEs >> HBCCD \approx HBBz \approx PBT > PBBz > PBBs \approx PBEB >> LOQ (limit of quantitation): TBCT (tetrabromo-o-chlorotoluene); pTBX (2,3,5,6-tetrabromo-p-xylene); OBIND (octabromotrimethylphenylindane). For juveniles, we noted small differences in this order: NDL-PCBs >>> mono-di-ortho-PCBs >> non-ortho-PCBs >>> PCDDs >> PCDFs > PBDEs >> HBBz > HBCCD \approx PBT >> PBBz >> PBEB >> LOQ: TBCT; pTBX; OBIND (Table 3). The preferential accumulation of some POPs in earthworms, especially NDL-PCBs and DL-PCBs, was expected in this species, following a study by [63], on the capacity of *E. fetida* to DL-PCBs.

In a study with four different species (*Eisenia andrei*, *Eisenia fetida*, *Eisenia hortensis* and *Lumbricus terrestris*), the accumulation of PCBs was observed in all earthworm tissues [62]. In Japan, a study of earthworms in rice fields showed tissue levels of $150 \mu\text{g.kg}^{-1}$ fresh weight of DL-PCBs [77]. In East China, *E. fetida* and *Allolobophora caliginosa trapezoides* species from a typical e-waste dismantling area showed PCB accumulation in tissues at levels of 1.17 up to $78.6 \mu\text{g.kg}^{-1}$ dw [50]. In Ref. [50], they also observed that earthworm PCB concentrations increased proportionately with increasing soil levels of PCBs. However, BSAFs decreased significantly with increasing soil concentrations of dichlorodiphenyltrichloroethane (DDT) ($r = -0.52$, $p = 0.005$), PCBs ($r = -0.89$, $p = 0.001$) and PBDEs ($r = -0.85$, $p = 0.01$) suggesting that steady-state equilibrium of compound distribution was not reached in soils and worms in this field study [50]. In Ref. [64], they also demonstrated, both *in situ* and during exposure in laboratory conditions, the accumulation of PCDDs and PCDFs in *E. fetida* tissues at concentrations of $1.5\text{--}15,000 \mu\text{g.kg}^{-1}$ dw, using contaminated soils from Sweden. The accumulation of PCDDs and PCDFs in the tissues of two other earthworm species, *Allolobophora caliginosa trapezoides* and *Lumbricus rubellus*, was also observed by [78], and in [77] they reported concentrations of $0.9 \mu\text{g.kg}^{-1}$ dw of PCDDs and PCDFs in earthworms taken from rice fields in Japan. In another study [49], PCDD and PCDF accumulation at a range between 0.13 and $0.59 \mu\text{g.kg}^{-1}$ dw was recorded in *E. fetida* and *Allolobophora caliginosa trapezoides* species collected from a typical e-waste dismantling area (in East China). Using radio labeled ^{14}C -TBBPA, they showed an uptake (ks) of this BFR at $0.03 (\pm 0.002) \text{g}_{\text{oc}}.\text{g}^{-1} \text{lw}.\text{day}^{-1}$ for *E. fetida* and $0.08 (\pm 0.03) \text{g}_{\text{oc}}.\text{g}^{-1} \text{lw}.\text{day}^{-1}$ for *M. guillelmi* [54].

Considering the BFRs measured, we noted that while PBDEs were frequently detected, some e-BFRs, such as HBBz and PBBz, were also efficiently bioaccumulated (Tables 2 and 3). Some BSAF values presented (Tables 1–3) clearly show the potential of BFRs to accumulate in *E. fetida* tissues. In our results, adult and juvenile *E. fetida* can be considered as poor bioaccumulators of PCDDs, PCDFs, PBDEs and PBBs (bioaccumulation factor (BAF) < 1) but show strong bioaccumulation of PCBs, HBBz, PBBz, PBT and PBEB (BAF > 2) (Table 3) [51]. Moreover, in our studies on *E. fetida*, bioaccumulation factors (BAFs) for some of the studied BFRs were lower than 10, indicating the potential transfer of these POPs to higher trophic levels along with the potential toxicity effects. The data show that BFRs are present in the soils at much lower levels than PCBs, PCDDs and PCDFs, but despite this, they are also available for incorporation in soil-dwelling organisms and possible biomagnification through upper levels of food chains.

Both brominated and chlorinated compounds undergo the same processes of volatilization in the atmosphere, and of dissolution in water leading to accumulation by aquatic organisms and adsorption to soil and/or sediment particles. This fact supports the hypothesis that some brominated compounds (notably BDE 153, BDE 99, BDE 47 and BDE 28) have a similar environmental behavior to chlorinated compounds such as PCBs, PCDDs and PCDFs.

2.3 Effects of BFRs on earthworms

Earthworms are more and more recognized as an important model test organism for studying the effects of contaminants on soil organisms, notably for establishing standard guidelines for risk evaluation. First, the European Union (EU) and the Organization for Economic Cooperation and Development (OECD) defined acute toxicity tests for earthworms based on mortality and growth rate [79] and also an

avoidance test [78, 80]. Later, OECD proposed chronic toxicity assays based on reproduction rate measurement [81]. Most of these studies were done with legacy BFRs, usually PBDEs. Moreover, in order to normalize the tests, as well as for reasons of reproducibility and “simple” breeding, the most widely used genus is *Eisenia* and particularly *Eisenia fetida*, as a model organism.

2.3.1 Global toxicological effects and distribution in the organism

Most studies on bioaccumulation from soils have been done on PBDEs, notably BDE 209. Thus, avoidance behavior studies show that *E. fetida* was insensitive to BDE 209 exposure for 48 h, at concentrations between 0.1 and 1000 mg.kg⁻¹ [44], or to a 231–3720 mg.kg⁻¹ decabromodiphenyl ethane exposure [82]. The European Commission [83] also observed that no abnormal avoidance behaviors occurred when *E. fetida* was exposed to BDE 209.

No significant effects of BDE 209 on earthworm cocoon production were detected but a statistically significant decrease in the number of juveniles per hatched cocoon was reported when artificial soils were spiked at 1000 mg.kg⁻¹ with BDE 209 [44]. In Ref. [82], they also reported no differences between decabromodiphenyl ethane exposure and the control, in terms of percent mortality, mass and weight gain for the adult earthworms. But they did report adverse effects on reproduction at a high dose (3720 mg.kg⁻¹ dw [82]).

Concerning toxicological endpoints, the NOEC (no observed effect concentration) and LOEC (lowest observed effect concentration) for 56-day reproduction were set, respectively, at 1910 and 3720 mg.kg⁻¹ dw for decabromodiphenyl ethane (DBDPE) for *Eisenia fetida* [82]. The lowest observed effect level (LOEL) for the parameters of survival and growth in *E. fetida* was reported at more than 1000 mg.kg⁻¹ of BDE 209 [44].

This is a high level and one hypothesis is that adult earthworms are more tolerant to BDE 209 exposure in soils, unlike juveniles, that seem to be more prone to the potential toxicity [44].

Studies on the HBCDD tissue distribution in *E. fetida* found a gradient order of concentration as: gut>body fluid>body wall [52, 84]. The report on the lower concentration of HBCDDs in earthworm’s casts relative to the paired bulk soil, observed in [52], allows us to conclude that earthworms take up HBCDDs mainly through digestion of soil particles. This was confirmed by the fact that the highest HBCDD concentrations and BAFs were found in the gut [52]. Thus, HBCDD accumulated in the gut was translocated into the body fluid and distributed to the body wall notably by a series of passive diffusion and partition processes. Moreover, more than 5% of lipid content was measured in the body wall and gut, indicating that tissue lipids can play an important role in the HBCDDs’ accumulation and distribution in earthworms compared to protein [52].

2.3.2 Metabolic and physiological effects

The metabolic responses of earthworms to BFRs appear to be more sensitive than growth inhibition and/or the reproduction global test. PBDEs were reported to increase the intracellular reactive oxygen species (ROS) level and then induce oxidative stress with some effects on the activities of enzymes, such as catalase (CAT), glutathione-S-transferase (GST), superoxide dismutase (SOD) or on the level of malondialdehyde (MDA) [85].

In Ref. [86], enzyme activity modifications in earthworms, which were in contact with soils treated with BDE 209, relative to the controls, showed that: (i) superoxide dismutase (SOD) activities were elevated significantly after 21 and 28 days of exposure; (ii) catalase (CAT) activities were much higher in all tests during the entire exposure period; (iii) peroxidase (POD) and glutathione-S-transferase (GST) activities generally decreased and indicated a contrary response trend; and (iv) total antioxidant capacity (T-AOC) after exposure to a low level of BDE 209 (1 mg.kg^{-1}) was induced, whereas it was suppressed at 10 and 100 mg.kg^{-1} .

In Ref. [85], they showed a slight induction of oxidative stress for *Eisenia fetida* caused by lower levels of BDE 209, and severe oxidative stress at higher levels. The electron paramagnetic resonance (EPR) spectra suggested that hydroxyl radicals ($\bullet\text{OH}$) in earthworms were significantly induced by BDE 209, and that the changes in MDA content indicated that reactive oxygen species (ROS) might lead to cellular lipid peroxidation in earthworms [86]. These results demonstrated that BDE 209 toxicity plays an important role in inducing severe oxidative stress in *E. fetida* [86].

Another study on *E. fetida* exposed to sublethal concentrations of BDE 47 and 209 [87] demonstrated that there is an increase in protein degradation to amino acids (with lactate and glutamate accumulation) in earthworms. The increase in the betaine content indicated that *E. fetida* may maintain cell osmotic pressure and protect enzyme activity by metabolic regulation [87]. The exposure interval and concentration of contaminants also showed a significant effect on the lysosomal stability of earthworms [88]. Earlier studies had shown a decrease in the lysosomal stability of *Eisenia fetida* [89, 90]. These reported observations clearly demonstrate that PBDEs induce changes in the enzyme activities, causing oxidative stress as well as disruption to the metabolic activities of *Eisenia fetida*. A recent study carried out using artificial soil fortified with different concentrations of DBDPE also showed biochemical toxicity in *Eisenia fetida* [91]. This e-BFR caused an increase in ROS and MDA levels as well as some modifications in the levels of antioxidant enzymes [91]. The authors showed DNA damage at all tested DBDPE concentrations, additionally suggesting a genotoxic response [91].

2.3.3 Kinetics of absorption/depuration

Polybrominated diphenyl ethers in soil become less bioavailable when their $\log K_{ow}$ increases [43]. The limited bioavailability of some PBDE congeners can be due to their large molecular size ($>0.95 \text{ nm}$), high molecular weight ($959.17 \text{ g.mol}^{-1}$ for BDE 209) and the high hydrophobicity of these compounds [43]. Such properties limit the ability of PBDEs to penetrate cell membranes and decrease the uptake efficiency by earthworms. But in Ref. [88], they demonstrated the effects of earthworms on the removal of BDE 209 in contaminated soils, demonstrating the decrease with kinetic curve plots of the decline in BDE 209 soil content. They also observed that this process followed a normal biphasic pattern *i.e.* a fast-initial decrease during the first few days, followed by a slower phase with a smooth decrease until the end of exposure [88]. A similar two-step elimination was also observed in an earlier study on other terrestrial organisms such as *Enchytraeus albidus* and *Enchytraeus luxuriosus* exposed to lindane and HCB [92]. BFR concentrations in the oligochaete, *Lumbriculus variegatus*, also increased rapidly in the 14 first days before arriving at a “plateau” toward the end of 28 days of exposure [48].

Earthworms take up organic contaminants through two main pathways, passive epidermal uptake and dietary uptake. It has been reported that the contributions of the two routes are different, depending on the properties of the contaminant. Dietary uptake

is the dominant route for hydrophobic compounds that have a $\log K_{ow}$ value > 5 [93, 94]. HBCDDs are highly hydrophobic, with $\log K_{ow}$ values of 5.38–5.80, and are primarily associated with soil particles [72]. As $\log K_{ow}$ values for PBDEs were between 5.9 and 10, dietary uptake is also likely to be the dominant exposure route for these compounds in earthworms. The lipid contents of earthworms are also an important parameter for the accumulation of organic pollutants. In fact, the greater the lipid and protein contents in *E. fetida*, the higher the bioaccumulation potential [56], but several different processes (such as uptake, depuration, metabolism and isomerization) also play a role in the final accumulation of contaminants. For example, *E. fetida* showed 1.73% wt dw and 3.56% wt dw of lipids, respectively, for adults and juvenile earthworms, predicting differences in the uptake potential/capacity between adults and juveniles [51].

In Ref. [88], they observed that BDE 209 was likely to lose one bromine atom in the biota. The proportions of lower brominated congeners (BDE 153, BDE 99, BDE 47 and BDE 28) in earthworms (34.5–52.5%) were generally higher than in soil (27.9–40.5%), indicating either that the debromination mechanism occurred more easily in earthworms or that BDE 153, BDE 99, BDE 47 and BDE 28 were easily absorbed by earthworms with a low elimination rate [88].

The depuration rates for the three HBCDD diastereomers in two earthworm species, *E. fetida* and *Metaphire guillelmi*, followed the order β - $>$ γ - $>$ α - [56]. β - and γ -HBCDDs were bioisomerized to α -HBCDDs, to a greater extent in *E. fetida* than in *M. guillelmi*. The depuration half-time values ($t_{1/2}$) were greater for α -HBCDDs (30.1–12.8 days) than for γ HBCDD (14.7–6.86) or β -HBCDD (7.88–5.82) [56].

The BFR, TBBPA and cadmium (Cd) are three ubiquitous pollutants in soils and are often found together, notably in electronic waste recycling sites. In an experimental study [55], they investigated the toxicity of these pollutants, as well as the accumulation and subcellular partitioning in two earthworms species, *Eisenia fetida* and *Metaphire guillelmi*, exposed for 14 days to Cd (at 1 mg.kg⁻¹) and TBBPA (at 10, 50, 100 and 500 mg.kg⁻¹) separately and in combination. In general, the Cd-TBBPA co-exposure resulted in synergistic effects in terms of acute toxicity, growth inhibition, histopathological changes in body walls and oxidative stress responses to earthworms [55]. Moreover, *M. guillelmi* showed higher sensitivity to Cd-TBBPA co-exposure and a higher Cd BSAF than *E. fetida*, [55]. By contrast, the increased Cd accumulation in *M. guillelmi* suggested a different uptake route than that of *E. fetida*, specifically gut processes and epidermal absorption, respectively [55]. The study showed that, in addition to specific pathways or lipid contents, other interactions between pollutants can modify the preferential transfer route of POPs in earthworms.

2.4 Conclusions on BFRs in earthworms

Earthworms can be considered as valuable bioindicators of soil pollution as they are very sensitive to contaminants in the soil and are significantly affected by them. Earthworms are exposed to soil contaminants through different routes of exposure such as direct contact with soil pore water or through their food. Their position at the base of the food chain and their abilities to uptake POPs make them an important route for pollutant entry into the terrestrial ecosystem and biomagnification to higher trophic levels. Several studies have already demonstrated the accumulation of POPs, such as PCBs, PCDD/Fs and PBDEs, in earthworm tissues at considerable levels without causing mortality or severe toxic effects. Our study is one of the few that show the transfer and bioaccumulation of e-BFRs in earthworms, and the differences in effects between adult and juvenile *E. fetida*.

Earthworms bioaccumulate BFRs passively through dermal absorption, and actively through ingestion. Effectively, the dietary pathway to bioaccumulation is facilitated by the lipid content of the earthworms' gut and body wall, and is also influenced by the hydrophobicity of the contaminants. But the phenomenon of bioaccumulation is very complex, with a species specificity (for dermal composition, metabolism and/or depuration, behavior) that is controlled by physicochemical properties of both the contaminants and the soil: the concentration and speciation of the contaminants, the type and characteristics of the soil, the temperature, the duration of exposure, the bioaccessibility/mobility of the contaminants and their interactions with the other contaminants present in the soil.

3. General conclusion and future directions

The data on BFR levels and trends in soil need to be extended, notably for e-BFRs, for which little data exist. The levels of these contaminants in soils are currently low but measurable. The time trends of BFRs showed that legacy BFRs, such as the well-studied PBDEs, were increasing in terrestrial environments and accumulating in wildlife, notably in earthworms. A study of the differences in the environmental distributions between legacy BFRs and e-BFRs seems to be, for now at least, hindered by the lack of occurrence data that would have allowed dispersion modeling and/or bioaccumulation studies. This makes us wonder whether in the near future, e-BFRs will overtake the levels and dispersion in ecosystems of legacy BFRs such as PBDEs for example. In the same way as what has been studied for PCBs, we should investigate if the persistence, or maybe the decrease of legacy BFRs levels in soils, could be tracked in relation to the source-limiting effects of political and regulatory decisions to no longer use these chemicals.

The risk evaluation for food chain linked to bioaccumulation remains to be investigated. Bioaccumulation or bioconcentration factors (BAF/BCF), which have been studied for legacy BFRs, should now be performed for e-BFRs. In risk assessments, BAFs are used to estimate and predict the potential trophic transfer of contaminants from soil to wildlife. Therefore, in order to obtain a better understanding of the environmental fate of e-BFRs, their accumulation, dispersion, or in order to model their fluxes, further studies would be useful and should be encouraged. Due to the increasing levels of these organic compounds in terrestrial ecosystems, it is important to study the occurrence, fate and transfer processes of BFRs in earthworms, as well as the potential phenomenon of trophic biomagnification. These studies are essential to evaluate and manage the risks posed by organic pollutants, such as BFRs or e-BFRs, to ecosystems and human health.

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Conflict of interest

The authors declare no conflict of interest.

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