Occurrence and Fate of Alternative Flame Retardants in the Environment

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4.3: Tracing organophosphorus and brominated flame retardants and plasticizers in an estuarine food web

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Abstract

Nine organophosphorus flame retardants (PFRs) were detected in a pelagic and benthic food web of the Western Scheldt estuary, The Netherlands. Concentrations of several PFRs were an order of magnitude higher than those of the brominated flame retardants (BFRs). However, the detection frequency of the PFRs (6-56%) was lower than the BFRs (50-97%). Tris(2-butoxyethyl) phosphate (TBOEP), tris(isobutyl) phosphate (TIBP) and tris(2-chloroisopropyl) phosphate (TCIPP) were the dominant PFRs in sediment with median concentrations of 7.0, 8.1 and 1.8 ng/g dry weight (dw), respectively. PFR levels in the suspended particular matter (SPM) were 2-12 times higher than in sediment.

TBOEP, TCIPP, TIBP, tris(2-chloroethyl) phosphate (TCEP) and tris(phenyl) phosphate (TPHP) were found in organisms higher in the estuarine food web. The highest PFR concentrations in the benthic food web were found in sculpin, goby and lugworm with median concentrations of 17, 7.4, 4.6 and 2.0 ng/g wet weight (ww) for TBOEP, TIBP, TCIPP and TPHP, respectively. Comparable levels were observed in the pelagic food web, BDE209 was the predominant PBDE in sediment and SPM with median concentrations up to 9.7 and 385 ng/g dw, respectively. BDE47 was predominant in the biotic compartment of the food web with highest median levels observed in sculpin and common tern eggs of 79 ng/g lipid weight (lw) (2.5 ng/g ww) and 80 ng/g lw (11 ng/g ww), respectively.

Trophic magnification was observed for all PBDEs with the exception of BDE209. Indications of trophic magnification of PFRs was observed in the benthic food web for TBOEP, TCIPP and TCEP with tentative trophic magnification factors of 3.5, 2.2 and 2.6, respectively ($p< 0.05$). Most of the other PFRs showed trophic dilution in both food webs. The relative high PFR levels in several fish species suggest high emissions and substantial exposure of organisms to PFRs in the Western Scheldt.

Introduction

During the last decades many studies have reported the presence of brominated flame retardants (BFRs) like polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) in the environment (De Wit, 2002; Law et al., 2006; De Boer et al., 1998). Some of these BFRs are considered to be toxic, persistent and accumulate in the food chain. New legislation led to a ban of the commercial BFR mixtures, Penta-BDE and Octa-BDE and restrictions on Deca-BDE. Modern fire safety standards still require the presence of FRs in many products. The ban and restriction on the commercial BDE mixtures have led to an increase in the production and use of alternative flame retardants (FRs). Many of these alternative FRs contain bromine, e.g. decabromodiphenylethane (DBDPE) and 1,2-bis-(2,4,6-tribromophenoxy)ethane (BTBPE) as alternatives for Deca-BDE and Octa-BDE, respectively. Also, Firemaster 550 which contains a mixture of bis (2-ethylhexyl) 2,3,4,5-tetrabromophthalate (BEH-TEBP) and 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (EH-TBB) is used as alternative for the replacement of Penta-BDE in polyurethane foam (PUF) (Stapleton et al., 2009). Another group of alternative FRs is the organophosphorus flame retardants (PFRs) (Van der Veen and

De Boer, 2012). In 2012 the global consumption of FRs was 1.8 million tonnes, which is expected to grow 5% each year for the coming 5 years with the highest growth for the PFRs (BCC research, 2013). The total consumption of FRs in Europe in 2006 was 565,000 tonnes, of which 20% were PFRs and 10% BFRs (Van der Veen and De Boer, 2012). Organophosphorus compounds that are used as FRs, are also used as plasticizers, and anti-foaming agents in various products such as upholstered furniture, plastics, textile, electronics, lacquers, floor finishing products, construction and isolation materials (Marklund et al., 2003; Van der Veen and De Boer, 2012). PFRs are ubiquitously detected in indoor air and house dust with concentrations in the μg/g ranges (Brandsma et al., 2014). This indicates that PFRs leach from various products into the environment. PFRs have been detected in surface-water, influents, effluents, sewage sludge, sediment and soil (Reemstra et al., 2008; Fries et al., 2001; Mihajlovíc et al., 2011; Martínez-Carballo et al., 2007; Meyer and Bester 2004; Marklund et al., 2005). The structural differences among PFRs lead to a variety of chemical and physical properties within this class. The log K_{ow} ranges from 1.44 for tris(2-chloroethyl) phosphate (TCEP) to 9.49 for Tris(2-ethylhexyl) phosphate (TEHP) (Reemstma et al., 2008). Some PFRs are volatile and dominate in the air phase, while others are water soluble or sorb strongly to particulate matter (Reemstma et al., 2008). The structural differences also influence the persistence of the various PFRs in the environment. Chlorinated PFRs are more resistant to biodegradation than the alkyl and aryl phosphates (WHO, 1998, 2000, 2001; Reemstma et al., 2008). The chlorinated PFRs TCEP, tris(2-chloroisopropyl) phosphate (TCIPP) and tris(1,3-dichloroisopropyl) phosphate (TDCIPP) are particularly poorly removed by waste water treatment plants (WWTPs) (Martínez-Carballo et al., 2007; Meyer and Bester, 2004; Marklund et al., 2005).

Toxicity data on PFRs is still limited. The chlorinated PFRs like TCEP and TDCIPP were proven to be neurotoxic and carcinogenic (Van der Veen and De Boer, 2012). TCIPP is suspected to be carcinogenic (Van der Veen and De Boer, 2012; WHO 1998, 2000). The non-chlorinated Tris(2-butoxyethyl) phosphate (TBOEP) is possibly carcinogenic and ortho-tris(methylphenyl) phosphate (TMPP) (or ortho-TCP) is neurotoxic (WHO, 2000; Van der Veen and De Boer 2012). Tris(phenyl) Phosphate (TPHP) is acutely toxic to water organisms and tris(butyl) phosphate (TNBP) was associated with the sick-building-syndrome (Van der Veen and De Boer 2012, Kanazawa et al. 2012). Recently, effects on the neurodevelopment in PC12 cell studies were observed for TCEP, TCIPP and TDCIPP (Dishaw et al., 2011). In *in vitro* tests using human nuclear receptors, Kojima et al. (2013) observed that several PFRs may have potential endocrine disrupting effects. Consequently, the EU Directive (2014/81/EU) introduced specific limit values (5mg/kg) for TCEP, TCIPP and TDCIPP in certain toys, which shall be applied from 21 December 2015 (Directive 2014/81/EU).

Very limited information is available on the occurrence of PFRs in biota. Total PFR concentrations of >1000 ng/g lipid weight (lw) have been observed in marine and freshwater biota from Swedish lakes and in different fish species from Manila Bay (Philippines) (Sundkvist et al., 2010; Kim et al., 2011). Lower amounts of PFRs were detected in herring gull eggs from the Great Lakes (Chen et al., 2012), an order of magnitude lower than in fish from the Swedish lakes and from the Philippines.

The objective of the present study was to investigate the trophic magnification of a number of PFRs and BFRs (PBDEs and HBCDs) in a pelagic and a benthic food web in the Western Scheldt estuary in

the Netherlands. To facilitate this study, a fast analytical method was developed to analyze ten PFRs with LC-MS/MS in biota and sediment samples.

Materials and methods

Materials

The solvents and chemicals used were all pro-analysis quality or HPLC grade, unless otherwise stated. Hexane, isooctane, HPLC water, acetone, dichloromethane (DCM), methanol and acetonitrile (ACN) used for the extraction and cleanup were from Promochem (Wesel, Germany). Formic acid (98%) was obtained from Merck (Darmstadt, Germany). TCEP, TDCIPP, TPHP, TMPP, TNBP, EHDPP, TEHP and TBOEP were supplied by Sigma-Aldrich Chemie B.V. (Zwijndrecht, the Netherlands). TIBP was supplied by Merck (Darmstadt, Germany) and TCIPP from Ehrenstorfer (Augsburg, Germany). The internal standards TPHP-$_{d15}$ and TNBP-$_{d27}$ were supplied by Sigma-Aldrich Chemie B.V. (Zwijndrecht, the Netherlands) and Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Native and mass labeled ($^{13}C_{12}$) α-,β-, and γ-HBCD, ($^{13}C_{12}$) decabromodiphenyl ether (^{13}C-BDE209) and the PDBE congeners were purchased as a mixture (BDE-MXE) from Wellington Laboratories (Guelph, Ontario, Canada).

Sample collection

The Western Scheldt estuary, situated in the south of the Netherlands was selected as the sampling location (Figure S1). The Western Scheldt is well-studied in terms of national water monitoring programs and surveys (Holierhoek et al., 2008) and many industries (e.g. textiles, BFR manufacturing) are located in this estuary as well as further upstream on the Scheldt River in Belgium. The Western Scheldt is also an important shipping lane to Antwerp Harbor in Belgium. While many different chemicals including BFRs have been reported in different compartments of the Western Scheldt ecosystem (De Boer et al., 2003; Covaci et al., 2005; Lopez et al., 2011), PFRs have not yet been studied in this area. Samples were collected in September 2008 in cooperation with colleagues from Deltares (Delft, the Netherlands), Grontmij (the Netherlands) and local fishermen. Sample information including lipid and total organic carbon (TOC) contents, dry weights and trophic levels is given in the supported information Table S1. Samples were collected both from the pelagic and benthic food webs, ensuring that the food chains are as long as possible (Table S1). The samples collected were the main species present in this area, which has been studied for many years. More detailed information on the food chains and relationships between the organisms can be found in Veltman et al. (2005). For the benthic aquatic food web macro invertebrates (lug worms, rag worms) were collected from the mudflat. With nets various invertebrate species were collected: cockles, shore crabs, and common shrimp (also called brown or sand shrimp). Benthic fish species included sculpin, plaice, goby, sole. Mysis (zooplankton) were sampled and these organisms are a key link between the benthic and pelagic food chains. For the pelagic food web suspended matter/algae, jellyfish, and planktivorous fish

(European sprat, herring, sandeel) were collected. In addition, a terrestrial piscivorous bird species (common tern), mainly feeding on herring and sprat, was sampled to provide information on the bioaccumulation from the aquatic to the terrestrial environment. The link with the terrestrial food chain is provided by the tern eggs from the colony feeding in the study area (n=5). Samples were combined on board, directly frozen with dry ice and transported to the laboratory and finally stored at -20ºC. Samples were pooled on size and length. For some fish species different length classes were made. The organisms were not depurated because in trophic magnification studies it is a general practice not to depurate as the aim is to investigate the transfer of contaminates along the food chain (Law et al., 2006b; Wu et al., 2009; Yu et al., 2009; Losada et al., 2009; Van Ael et al., 2012; Ma et al., 2013). In that case the intake is the whole organism including sediment particles, which are also a source of contaminants. The use of whole organisms without depuration could lead to elevated levels. However, we investigated the levels of PBDEs in lugworm which were depurated and not depuration (data not shown). These results showed similar levels of PBDEs between the two groups, except for BDE209, which was 3 times higher in the lugworm that was not depurated.

Sample extraction and cleanup

PFRs

Whole organisms were used and all samples were freeze-dried and homogenized before extraction. The sample intake for biota was adjusted according to the lipid content of the sample, as a maximum of 200 mg of lipids could be removed during cleanup. Before extraction with DCM/acetone (1:1, v/v), by pressurized liquid extraction (PLE) on an ASE350 (Dionex, Sunnyvale, CA) at 70 °C, 1500 psi with three extraction cycles, TNBP-$_{d27}$ (50 ng) and TPHP-$_{d15}$ (50 ng) were added as internal standards (IS). The extract was evaporated to 0.5 mL in isooctane. Cleanup was performed with SPE-NH_2 cartridges (500 mg 6 mL Discovery® DSC-NH2). The SPE-NH2 cartridge was washed and conditioned with 10 mL of DCM followed by 6 mL of hexane. After addition of the sample, the matrix was removed by eluting with 6 mL of hexane. The PFRs were eluted from the SPE cartridge with 4 mL 20% DCM in hexane (v/v) followed by 15 mL DCM. This fraction was evaporated to 0.5 mL isooctane and the solvent was changed to 0.5 mL methanol (MeOH). For the blank, sediment, and suspended particulate matter (SPM) an extra fraction of 10 mL acetone was collected. The biota extracts were centrifuged for 10 min at 3000 rpm after which 200 µL was transferred to a glass vial with insert for analysis.

PBDEs and HBCDs

After freeze-drying and homogenizing the samples were extracted by PLE on an ASE350 (Dionex, Sunnyvale, CA) at 70 °C, 1500 psi with three extraction cycles using hexane: acetone (3:1, v/v) as extraction solvent. Before extraction the internal standards, BDE58 (50 ng), ^{13}C-BDE209 (50 ng) and ^{13}C α, β and γ- HBCDs (100 ng) were added. Cleanup was performed with sulfuric acid-treated silica

(40:60, w/w) with 100 mL of 30% DCM in hexane (v/v). After cleanup the samples were fractionated with 1.5 g silica gel (1.8% (w/w) H_2O) column using 12 mL of hexane followed by 25 mL of 15% diethyl ether in hexane (v/v) (second fraction). The total fraction was evaporated under nitrogen to 0.5 mL in isooctane and transferred to a glass vial for PBDE analysis with GC-MS. After the GC-MS analysis the solvent was changed to 0.5 mL MeOH for analyzing the isomers of HBCD with LC-MS/MS.

Sample analysis

PFRs

Analysis of the PFRs was performed on a 1260 infinity high performance liquid chromatograph (HPLC) (Agilent Technologies, Amstelveen, The Netherlands). With a 150 mm x 3 mm Luna C18 (2) 3 µm column (Phenomenex, Utrecht, The Netherlands). The HPLC was coupled to a 6410 triple quadrupole MS (Agilent Technologies, Amstelveen, The Netherlands) using positive electro-spray ionization mode (ESI). The MS was run in the MS-MS mode using multiple-reaction monitoring of the parent and daughter ions. More detailed information can be found in Brandsma et al., 2014.

PBDEs and HBCD

Analysis of PBDEs was performed on a HP6890 GC (Agilent Technologies, Amstelveen, The Netherlands) with a CP-Sil-8CB fused silica capillary column (50 m x 0.25 mm ID, 0.25 µm film thickness, Varian, Middelburg, the Netherlands) coupled to a HP5973 quadrupole MS (Agilent Technologies, Amstelveen, the Netherlands). For more details see SI.

The analysis of α-, β- and γ-HBCDs was performed on a 1260 HPLC (Agilent Technologies, Amstelveen, The Netherlands) with a 30 x 2.1 mm 1.8 µm ZORBAX eclipse column (Agilent Technologies). The injection volume was 20 µL and the flow 300 µL/min with a run time of 8 min using 85% of eluent A (ACN/MeOH (75/25 v/v)) and 15% of eluent B (2 mM ammonium acetate). The HPLC was coupled to a 6410 triple quadropole MS (Agilent Technologies) with ESI measuring in the negative mode. Capillary voltage was set to 4000 V with a source temperature of 160 °C, nebulizer gas of 50 psi with a flow of 8 L/min. The MS was run in the MS-MS mode using multiple-reaction monitoring of the parent and the daughter ions, m/z 640.7 to 81 and 79 for HBCDs and 652.7 to 79 and 81 for ^{13}C HBCDs internal standards.

Lipids and total organic carbon

The lipid contents of the benthic and pelagic food web samples were analyzed using a modified Bligh and Dyer method (De Boer, 1988). TOC was determined in the sediment and SPM using the NEN-ISO method (NEN-ISO 10694, 2008).

Quality control

PFRs

The PFR concentrations were calculated using two internal standards TNBP-$_{d27}$ and TPHP-$_{d15}$. TBP-$_{d27}$ was used as internal standard for TCEP, TCIPP, TDCIPP, TIBP, TNBP, TBOEP and TEHP, while TPP-$_{d15}$ was used for TPHP, TMPP and EHDPP. Because high PFR concentrations in house dust had been reported (Brandsma et al., 2014), all glassware was pre-cleaned by rinsing with hexane and acetone. The limit of detection (LOD) was calculated as three times the standard deviation of the mean of the blanks. For compounds that were not present in the blanks, the LOD was calculated by the signal-to-noise ratio (S/N=3). The mean procedural blank value was subtracted from the samples. The PFR levels in the mean of five blanks and standard deviations are given in Table 1. Due to the high blank level for TNBP and the high variation we decided not to report any data about TNBP in this paper.

Table 1. PFRs measured in five procedural blanks showing means and standard deviations and the limit of detection calculated from the standard deviation.

	TCEP	TCIPP	TDCIPP	TPHP	TIBP	TNBP	TBOEP	TMPP	EHDPP	TEHP
Mean blank (n=5) (ng)	0	3.6	0	1.7	1.7	36	7.8	0	0.8	0
Stdev (ng)	0	2.4	0	0.8	1.0	48	6.9	0	0.6	0
LOD (ng/g w/w)*	0.2	1.4	0.2	0.5	0.6	29	4.2	0.2	0.3	0.2

* LOD is calculated based on a sample intake of 5 gram wet weight (w/w)

The extracts were also analyzed by GC-MS (data not shown). However, due to matrix interferences LC-MS/MS was selected as the most robust tool for the analysis of PFRs in biota and sediment. Recovery experiments were performed by spiking 2.5 g of freeze-dried sediment and 250 mg lipids of sole (*Solea solea*) with a PFR analytical standard (50 ng/ml). Recoveries were calculated by subtracting the measured concentrations in the spiked sample (sediment or sole) from those in the non-spiked sample (sediment or sole) (Table 2).

The recoveries given in Table 2 were all corrected for the internal standards TNBP-$_{d27}$ or TPHP-$_{d15}$. Recoveries of both internal standards (TNBP-$_{d27}$ and TPHP-$_{d15}$) ranged from 74 to 128% for both matrices, which were considered acceptable recoveries. Cleanup of the sediment extracts was efficient. The final sole extract still contained some matrix which could not be removed during the cleanup, resulting in lower recoveries for TMPP, EHDPP and TEHP, probably due to ion suppression.

Table 2. Recoveries of PFRs (percentage of spiked amount measured) in a spike experiment of ten PFRs in sediment (in triplicate) and sole lipids (in six-fold).

	Sediment n=3		Sole n=6	
	Recovery (%)	STD (%)	Recovery (%)	STD (%)
TCEP	71	8	73	12
TCIPP	128	9	95	9
TDCIPP	78	5	65	12
TPHP	117	9	106	3
TIBP	104	2	108	4
TNBP	107	3	105	5
TBOEP	130	10	134	6
TMPP	88	2	51	7
EHDPP	80	9	62	7
TEHP	115	7	43	19

PBDEs and HBCDs

The PBDEs and HBCD isomers were measured in our laboratory under certified quality assurance conditions according to accredited protocols. Food web samples were analyzed in series of 15 samples, each containing one internal reference material (IRM) and one blank sample. PBDEs, deca-BDE and α-, β-, and γ-HBCD were calculated by using the following internal standards: BDE58, ^{13}C-labeled deca-BDE and ^{13}C-labeled α-, β-, and γ-HBCD, respectively. The limit of detection (LOD) was set at three times the standard deviation of the mean of the blanks; for compounds not present in the blanks, LOD was calculated by the signal to noise ratio (S/N=3). The mean procedural blank value was subtracted from the concentrations measured in the samples.

Characterization of food web using stable isotope analysis

Trophic magnification factors (TMFs) were calculated for the PFRs and PBDEs in the food web as the slope of the curve of trophic level versus the logarithm of the body residue. Trophic levels were determined using the stable nitrogen isotopes. The nitrogen (δ^{15}N) and carbon (δ^{13}C) isotopic composition of tissues are integrated measures of diet that can be used to distinguish the relative food chain (food web) positions (Fisk et al., 2001) and help interpret trophic transfer of chemicals that bioaccumulate. The (logarithms of) the concentrations in biota are then plotted against relative trophic level. The TMF is calculated by 10^x were x is the slope of the curve of trophic level versus the logarithm of the body residue. Stable isotopes ratios (^{15}N/^{14}N, ^{13}C/^{12}C) were determined in biological samples using an elemental analyzer (NC2500, ThermoQuest Italia, Rodana, Italy) coupled with an Isotope Ratio Mass Spectrometer (Delta Plus, Thermo-Quest Finnigan, Bremen, Germany). Stable isotope values were expressed as a ratio (R) of the heavy to the light isotope (^{13}C/^{12}C or ^{15}N/^{14}N) standardized with respect to internationally recognized reference materials (atmospheric air for nitrogen and VPDB for carbon) as follows:

$$\delta\ (‰) = [(R_{sample}/R_{reference}) - 1] \times 1000.$$

The trophic level (TL) of each organism ($TL_{organism}$) was estimated by using the following formula:

$$TL_{organism} = 1 + (\delta^{15}N_{organism} - \delta^{15}N_{primary\ producer})/\ 3.4$$

As baseline organism ($\delta^{15}N_{primary\ producer}$) the average of the $\delta^{15}N$ of the primary producers, and an enrichment factor of 3.4‰ as discussed by Jardine et al. 2006 were used.

Statistical treatment of the data

IMB SPSS Statics 21 was used for the calculations of the TMF and a Pearson correlation was performed to investigate if the TMFs were significant and for the correlation between the PFR and BFR concentration and the lipid content. ½ LOD was used for the non-detects.

Results and discussion

PFRs in sediment and SPM

PFRs were ubiquitously detected in Western Scheldt sediment and SPM. The results are given in Table 3. TBOEP and TIBP were the dominant compounds in sediment, followed by TCIPP with median concentrations of 7.0, 8.1 and 1.8 ng/g dw, respectively. The PFR levels in the SPM were 3 - 32 times higher than the PFR levels in the sediment, with the exception of TIBP which was 8 times lower. TBOEP was also the dominating PFR in SPM followed by TCIPP with median concentrations of respectively 33 and 16 ng/g dw.

There is limited data on PFR levels in sediment and no data for SPM. Comparable concentrations were found by Cao et al. (2012) in 28 sediment samples from Taihu Lake (China), which were dominated by TBOEP (1.0 - 5.0 ng g dw), TCEP (0.6 - 3.2 ng/g dw) and TCIPP (LOD - 2.3 ng/g) and by Sheldon and Hites, (1978) for TNBP, TBOEP and TPHP (0.1 -3 ng/g dw) in sediment of the Delaware river (US). Higher concentrations were observed for TCEP (13-28 ng/g dw) and TDCIPP (9 - 17 ng/g dw) by Ishakawa et al. (1985) in sediment from a river near Kitakyushu City (Japan) and by Stachel et al. (2005) in 37 sediment samples from the river Elbe (Germany) for TCIPP (5.9 - 311 ng/g dw), TBOEP (<1 - 93 ng/g dw), TCEP (<1 - 41 ng/g dw), TNBP (<1 - 23 ng/g dw) and TDCIPP (<1 - 13 ng/g dw). The highest PFR levels were found by Martínez-Carballo et al. (2007) in sediment from three rivers near Vienna with concentrations up to 130 and 1300 ng/g dw for TBOEP and TCIPP, respectively, and by Kawagoshi et al. (1999) in sediment from a sea-based solid waste disposal sited at Osaka North port (Japan) with concentrations up to 7395, 7122, 2558, 1969 and 1181 ng/g dw for TCEP, TEHP, TMPP,

TBOEP and TCIPP, respectively. TBOEP and TCIPP were also prevalent in the Western Scheldt sediment. Some of the dominating PFRs in the sediment have relatively high water solubility: 7,000 mg/L for TCEP, 1,200 mg/L for TCIPP and 1,100 mg/L for TBOEP. This may indicate that the levels in the river water would be even higher (Cao et al., 2012).

Table 3. Median, mean, maximum and minimum PFR and BFR concentrations in ng/g dw in sediment and SPM from the Western Scheldt.

	Sediment (n=3)					SPM (n=3)				
FRs	**Median ng/g dw**	**Mean ± Std ng/g dw**	**Max ng/g dw**	**Min ng/g dw**	**#**	**Median ng/g dw**	**Mean ± Std ng/g dw**	**Max ng/g dw**	**Min ng/g dw**	**#**
TCEP	0.4	0.5 ± 0.4	1.0	<0.1	*2/3*	1.1	1.2 ± 0.9	2.2	<0.3	*2/3*
TCIPP	1.8	3.6 ± 3.2	7.3	1.7	*3/3*	16	28 ± 34	67	<2.4	*2/3*
TDCIPP	0.3	0.6 ± 0.7	1.4	<0.1	*2/3*	1.0	1.4 ± 1.3	2.8	<0.3	*2/3*
TPHP	0.5	0.6 ± 0.4	1.0	<0.2	*2/3*	1.6	2.0 ± 0.9	3.0	1.4	*3/3*
TIBP	8.1	6.9 ± 2.3	8.3	4.2	*3/3*	1.1	1.5 ± 0.8	2.5	<1.1	*1/3*
TBOEP	7.0	9.5 ± 9.2	19.6	<1.8	*2/3*	33	33 ± 10	43	23	*3/3*
TMPP	0.1	0.4 ± 0.5	0.9	<0.1	*1/3*	2.9	3.5 ± 1.9	5.6	2.0	*3/3*
EHDPP	0.2	0.3 ± 0.3	0.7	<0.1	*1/3*	2.9	3.9 ± 4.0	8.4	<0.6	*2/3*
TEHP	0.8	1.5 ± 1.5	3.3	0.4	*3/3*	2.7	4.1 ± 3.4	8.0	1.6	*3/3*
BDE28	0.02	0.02 ± 0.005	0.03	0.02	*3/3*	<0.03	<0.04 ± <0.01	0.1	<0.03	*0/3*
BDE47	0.04	0.06 ± 0.04	0.1	0.04	*3/3*	0.3	0.4 ± 0.3	0.8	0.2	*3/3*
BDE100	0.02	0.02 ± 0.009	0.03	0.01	*3/3*	0.1	0.1 ± 0.09	0.2	0.04	*2/3*
BDE99	0.04	0.06 ± 0.03	0.1	0.04	*3/3*	0.2	0.4 ± 0.2	0.6	0.2	*3/3*
BDE153	0.04	0.05 ± 0.04	0.1	0.03	*3/3*	0.2	0.2 ± 0.1	0.3	0.1	*3/3*
BDE209	9.7	43 ± 59	111	8.5	*3/3*	385	375 ± 251	622	119	*3/3*
α-HBCD	0.03	0.08 ± 0.10	0.20	<0.02	*2/3*	0.7	1.2 ± 1.2	2.6	<0.5	*2/3*
β-HBCD	0.03	0.05 ±0.05	0.1	0.01	*3/3*	0.4	0.7 ± 0.7	1.6	0.3	*3/3*
γ-HBCD	0.3	1.4 ± 1.9	3.6	0.2	*3/3*	11	36 ± 44	86	9.8	*3/3*

 # frequency number of detected/number of samples analysed

BFRs in sediment and SPM

The BFR levels in the same sediment and SPM samples are also shown in Table 3. Apart from BDE209 and HBCDs, the PBDE levels in both the sediment and SPM were one order of magnitude lower than the PFR levels observed in the same samples. As for the PFRs, the BFR levels in the SPM were 4 – 40-fold higher than the levels in the sediment. BDE209 was the dominating BFR in sediment and SPM, followed by γ-HBCD. This is worldwide a common pattern in sediment, and is related to the high production volumes of the commercial deca-BDE and HBCD formulations, their persistency and their strong hydrophobic character (De Wit, 2002; Covaci et al., 2006; Law et al., 2006, 2014). De Boer et al. (2003) observed similar PBDE patterns in SPM and sediment from the Western Scheldt from 1999, however, with concentrations one order of magnitude higher for each PBDE: e.g. up to 4600 ng/g dw in SPM and up to 510 ng/g dw in sediment for BDE209. The relatively high BDE209 and γ-HBCD concentrations in the Western Scheldt were related to the use of these BFRs by the textile industry in Antwerp (De Boer et al., 2003; Morris et al., 2004). Apparently, both measures taken by industry to

reduce spillage of BFRs and a change in production due to the ban of some PBDEs have most likely led to these lower concentrations. Median HBCD levels (0.3 ng/g dw for γ-HBCD, 0.03 ng/g dw for β-HBCD and 0.03 ng/g dw for α-HBCD) in sediment were lower than those observed by Morris et al., (2004) in Western Scheldt sediment from 2000, which was dominated by γ-HBCD with concentrations of 7.7 - 96 ng/g dw, while α-HBCD concentrations were <0.2 - 2.6 ng/g dw. The HBCD diastereomeric pattern in the sediment samples was similar to that of the commercial formulation (Morris et al., 2004).

PFRs in the food webs

Nine PFRs were detected in the estuarine food web of the Western Scheldt (Fig. 1, Table S2). None of those show a clear correlation with lipid contents. Therefore, in contrast to PBDEs, it seems that PFRs have limited affinity for lipids. The same was reported by Sundkvist et al. (2010), Kim et al. (2011) and Chen et al. (2012). Therefore, all PFR concentrations in the food web study are given in ng/g ww (Fig. 1, Table S2 and S3).

TBOEP and TCIPP were the most frequently detected PFRs in the 34 food web samples, followed by TCEP and TPHP, with a detection frequency of 56, 50, 32 and 32%, respectively (Table 4). TMPP was the least frequently observed PFR and only detected in two of the 34 samples. TBOEP, TPHP, TCIPP and TCEP were more frequently detected in the benthic food web (64%) than in the pelagic food web (26%) (Table 4). PFRs that were found higher up in the pelagic and benthic food webs are TBOEP, TCIPP, TIBP, TCEP and TPHP. In the benthic food web TBOEP, TCIPP and TCEP bioacumulated in some fish (plaice, goby and sculpin) higher in the food web, whereas, TPHP and TIBP levels decreased higher up in the food web. The highest PFR concentrations in the benthic food web were found in sculpin, goby and lugworm with median concentrations for TBOEP, TIBP, TCIPP and TPHP up to 17, 7.4, 4.6 and 2.0 ng/g ww, respectively (Fig. 1, Tables S2 and S3). Comparable levels were observed in the pelagic food web. However, the levels appeared to decrease higher up in the pelagic food web. Highest PFR concentrations in the pelagic food web were found in phytoplankton, pouting and herring, with median concentrations for TBOEP, TIBP, TCIPP and TPHP up to 9.9, 2.2, 4.0 and 0.85 ng/g ww, respectively (Fig. 1, Tables S2 and S3). The median PFR levels were comparable to the mean levels except for herring (Table S2 and S3). For herring the median levels of TBOEP and TCIPP (6.6 and 2.2 ng/g ww) were lower than the mean levels (71 and 8.2 ng/g ww), which is mainly due to the fact that these compounds were detected in 2 of the 5 pooled herring samples only.

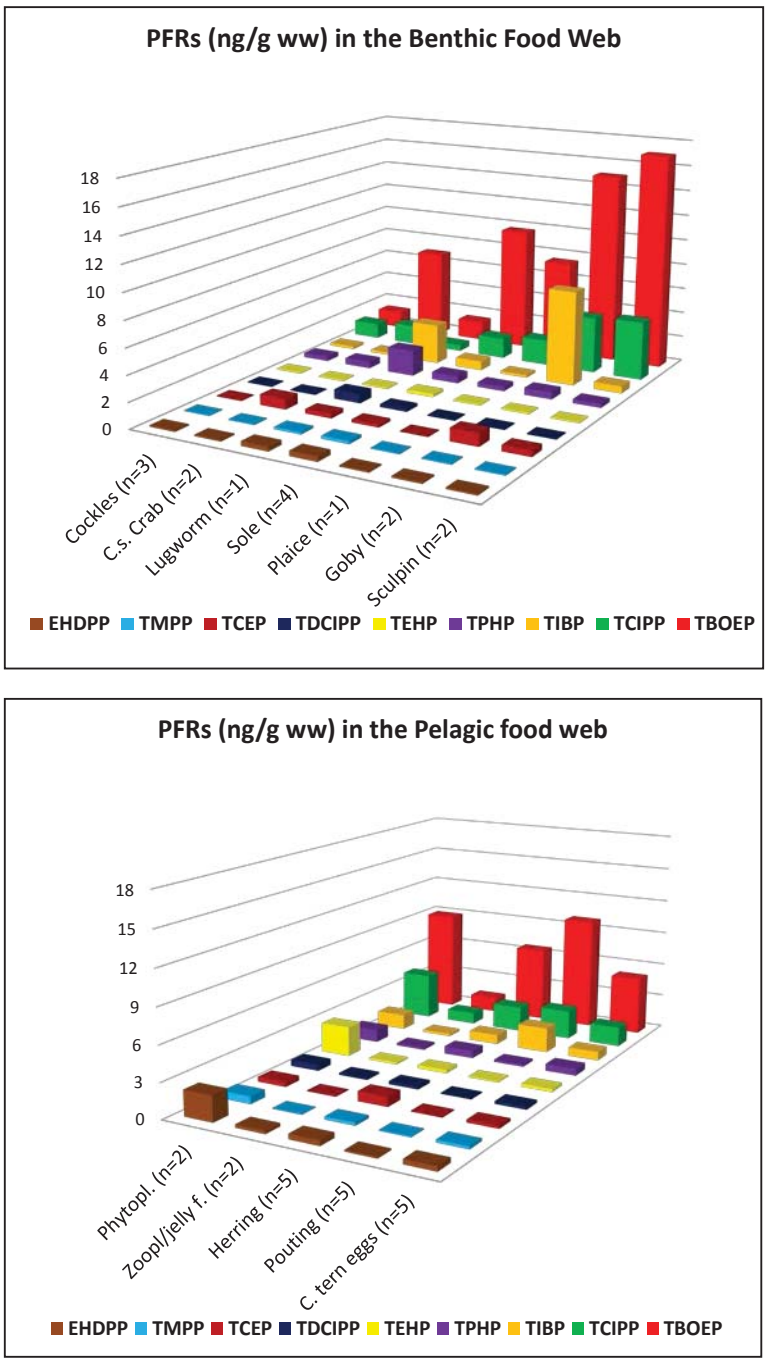


Figure 1. Median PFR levels in ng/g ww in benthic and pelagic organisms from the Western Scheldt.

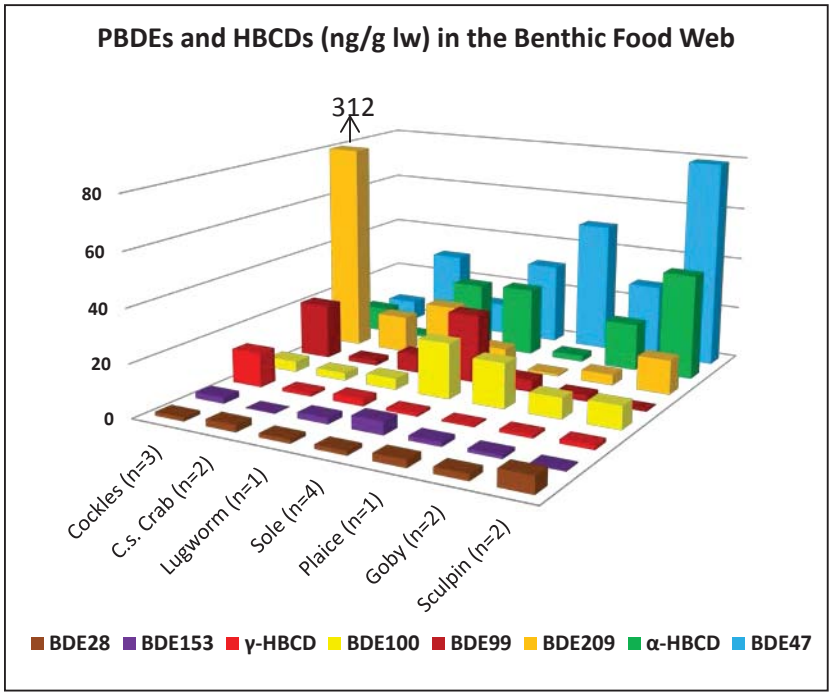
In one of the scarce studies on PFRs in biota, Sundkvist et al. (2010) measured eleven PFRs in different marine and freshwater species. In general, TCIPP and TPHP dominated with levels ranging from 0.4 to 16 ng/g ww for TCIPP and 0.04 to 2.3 ng/g ww for TPHP. Comparable results were found in our study for TPHP and TCIPP with median levels ranging from <0.19 - 2.0 ng/g ww and <0.49 – 4.6 ng/g ww, respectively. However, the dominant PFR in fish from the Western Scheldt was TBOEP (with median levels up to 17 ng/g ww). Sundkvist et al. (2010) observed relatively high TBOEP levels 0.86 - 4.2 ng/g

ww in perch and carp. The perch and carp were collected downstream of WWTPs, in the vicinity of an airport. Sundkvist et al. (2010) also detected high levels of TBOEP (3.1 - 30 μg/L) in effluent from a WWTP and in snow close to an airport and therefore concluded that WWTPs and the airport could be sources of TBOEP. Meyer and Bester, (2004) observed low removal efficiency in WWTPs for the chlorinated PFRs and high removal efficiency of the non-chlorinated PFRs such as TBOEP (84%). The high concentrations observed in the effluents (μg/L range) and detectable concentrations in fish would imply that effluents may be contributing to the TBOEP exposure. WWTP are therefore suspected to be a source of both chlorinated and non-chlorinated PFR emissions.

Kim et al. (2011) detected nine non-halogenated PFRs in twenty fish species from Manila Bay, Philippines. TEHP and EHDPP were the dominating PFRs observed in Manila Bay fish, with highest mean levels of 6.0 and 3.5 ng/g ww in indian-mackerel and common-ponyfish, respectively. In our study TEHP and EHDPP were the less frequently detected PFRs (15%) with the highest mean levels observed in pouting (0.18 ng/g ww) and zooplankton/jelly fish (0.13 ng/g ww), respectively. In addition, TBOEP was the dominating PFRs observed in fish from the Western Scheldt (mean levels ranging from <1.3 -71 ng/g ww), whereas in Manila Bay fish TBOEP was the less frequently detected PFR with mean levels in the low ng/g ww region. However, PFR accumulation and elimination between fish species may vary, the different PFR patterns may also reflect the different PFR usage per regions. TBOEP, TCIPP and TCEP were also detected in herring gull eggs from the Channel-Shelter Island in the Great Lakes of North America collected in 2010 with concentrations ranging from <LOQ - 2.2 ng/g ww, <LOQ - 4.1 ng/g ww, to <LOQ - 0.6 ng/g ww respectively (Chen et al., 2012). In common tern eggs from the Western Scheldt PFR concentrations were all below the LOD, while all PBDEs were detected in all eggs. The lack of PFR accumulation in terns may be due to a number of factors, such as a high metabolisation rate, efficient elimination, or inefficient transfer from mother to egg which is less likely as Chen et al. (2012) found PFRs in eggs.

BFRs in the food webs

The PBDE and HBCD levels measured in ng/g lw in the benthic and pelagic food webs of the Western Scheldt are shown in Figure 2 and Table S3. In general the detection frequencies for the BFRs (between 50-97%) are higher compared to those of the PFRs (6-56%). A significant correlation between the BFR levels and the lipid content was observed for all BFRs ($p < 0.05$) with the exception of BDE209. In the lower part of the benthic and pelagic food webs BDE209 and γ-HBCD dominated with median levels of respectively 1319 and 100 ng/g lw in phytoplankton and 312 and 13 ng/g lw in cockles. Higher in the benthic and pelagic food webs these pattern changes: BDE28, BDE47, BDE99, BDE100, BDE153 and α-HBCD were more concentrated in organisms at higher trophic levels, whereas the levels of BDE209 and γ-HBCD in organisms at higher trophic levels decrease (Figure 2).



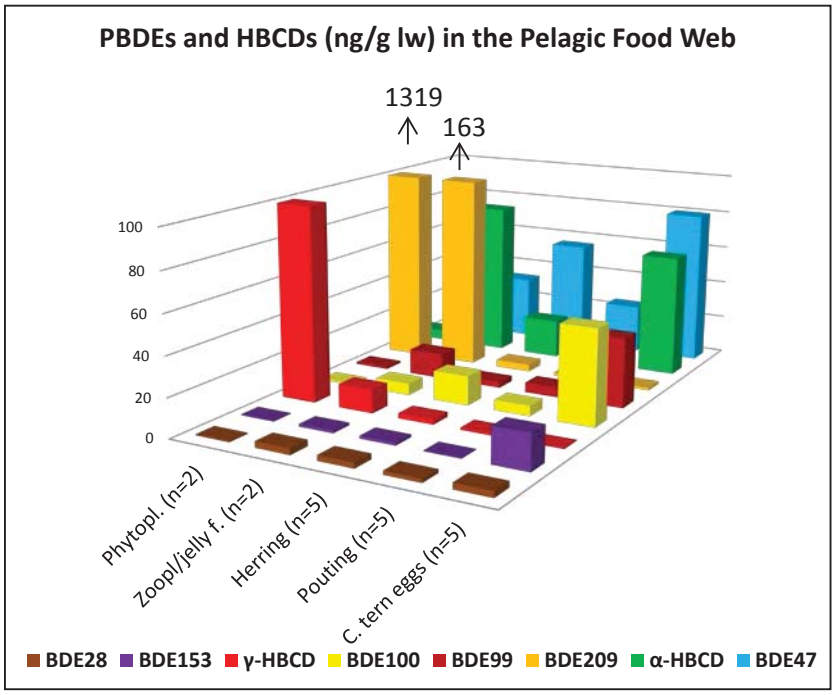


Figure 2.Median PBDE and α-,γ-HBCD levels in ng/g lw in benthic and pelagic organisms in the Western Scheldt.

The reviews of De Wit et al. (2002), Covaci et al. (2006), Law et al. (2006, 2014) extensively describe the detection of PBDEs and HBCDs in the environment all over the world. In this study we will only compare our result with the PBDEs and HBCD levels observed in the Western Scheldt.

PBDEs

The Western Scheldt has been studied for many years for the presence of BFRs by De Boer et al. (2003), Voorspoels et al. (2003), Morris et al. (2004), Janak et al. (2005), Van Leeuwen and De Boer (2008), Van Ael et al. (2012), (2013). In all these studied BDE47 was the predominant PBDE observed in biota followed by BDE100 and BDE99. In our study BDE47 was also dominant with median levels of 80 ng/g lw (11 ng/g ww) in common tern eggs and 79 ng/g lw (2.5 ng/g ww) in sculpin. Van Ael et al. (2012) detected BDE47 is worms, crab and sole from the Western Scheldt with median concentrations of 0.52 (0.33-0.85), 0.11 (0.05-0.75) and 0.17 (0.05-0.59) ng/g ww, respectively. The median levels observed in our study are roughly in the same range: lungworm 0.13 ng/g ww, crab 0.95 ng/g ww and sole 0.73 ng/g ww. However, in another study of Van Ael et al., (2013) the median BDE47 concentrations of 0.04, 0.15, 0.06, 0.05 and 0.13 ng/g ww in lugworm, crab, sole, pouting and sculpin from the Western Scheldt, respectively, were 3, 6, 12, 15 and 19 times lower that the levels observed in our study. The use of whole organisms in our study may possibly partly explain the difference, whereas Van Ael et al. (2012 and 2013) analyzed the PBDEs in fish muscles. The differences are, however, substantial.

BDE209

BDE209 is predominant in sediment and due to the large size of the molecule and possible fast metabolism it was initially expected not to accumulate in organisms. However, in an *in vivo* study with rainbow trout Kierkegaard et al., (1999) demonstrated that BDE209 can be taken up by fish. This led not much later to the first detection of BDE209 in wildlife samples such as freshwater and marine fish and birds (peregrine falcon) (Akutsu et al., 2001; Lepom et al., 2002; Lindberg et al., 2004). In our study we also observed BDE209 in both the pelagic and the benthic food webs of the Western Scheldt with a detection frequency of 85% (Figure 2, Table 4). The highest BDE209 levels were found in cockles and phytoplankton with median concentrations of 312 ng/g lw (2.0 ng/g ww) and 1319 ng/g lw (24 ng/g ww), respectively. At higher trophic levels in the food web of the Western Scheldt the BDE209 levels decrease although still detectable BDE209 levels were found in goby (median of 4.0 ng/g lw, 0.14 ng/g ww) and sculpin (median of 13 ng/g lw, 0.39 ng/g ww) of the benthic food web and in pouting (median of 1.4 ng/g lw, 0.04 ng/g ww) and common tern egg (median of 1.2 ng/g lw, 0.17 ng/g ww) of the pelagic food web. Data on BDE209 in organisms from the Western Scheldt is scarce. Voorspoels et al. (2003) detected BDE209 in only 8 (bib, sole and whiting) liver samples from the Western Scheldt with levels ranging from 3.4 – 37 ng/g ww. These levels were higher than the median BDE209 levels we observed in sole (0.16 ng/g ww) or in the other fish species like goby (0.14 ng/g ww), sculpin (0.39 ng/g ww), herring (0.05 ng/g ww) and pouting (0.04 ng/g ww). BDE209 levels in benthic fish (sole, goby and sculpin) are slightly higher than those observed in the pelagic fish (herring and pouting), which is probably associated with the living and feeding area of the benthic fish, on or near the BDE209 enriched sediment. Also the lower metabolic capability of benthic organisms compared to pelagic organisms may play an imported role (Wilson et al., 2013) Elevation of BDE209

levels due to BDE209 bound to the outside of the skin of the organism or that BDE209 is incorporated into the gastrointestinal tract could not be excluded in this study.

HBCDs

HBCDs have been detected in organisms from all over the world (Covaci et al. 2006; Law et al. 2014). α- and γ- HBCD have been detected in both the benthic and pelagic food webs of the Western Scheldt (Figure 2 and 3). β-HBCDs was only detected in 29% of the samples with levels close to the LOD, and is therefore not included in Figure 2. The diastereoisomeric patterns of HBCDs in sediment and biota are different. In sediment the γ-diastereoisomer is dominant and in biota the α-diastereoisomer (Morris et al., 2004; Covaci et al., 2006). This was also observed in the present study. Figure 3 shows that γ-HBCD concentrations decrease and α-HBCD concentrations increase with an increase of trophic level. The pelagic food web shows that at the lower trophic levels (cockles and common shore crabs) γ-HBCD is still present (up to 30%) (Fig. 3). This change in diastereomeric pattern through the food web could be explained by the differences in water solubility, bio-isomerization and metabolism between the isomers of HBCD (Zegers et al., 2005; Law et al., 2006; Covaci et al., 2006).

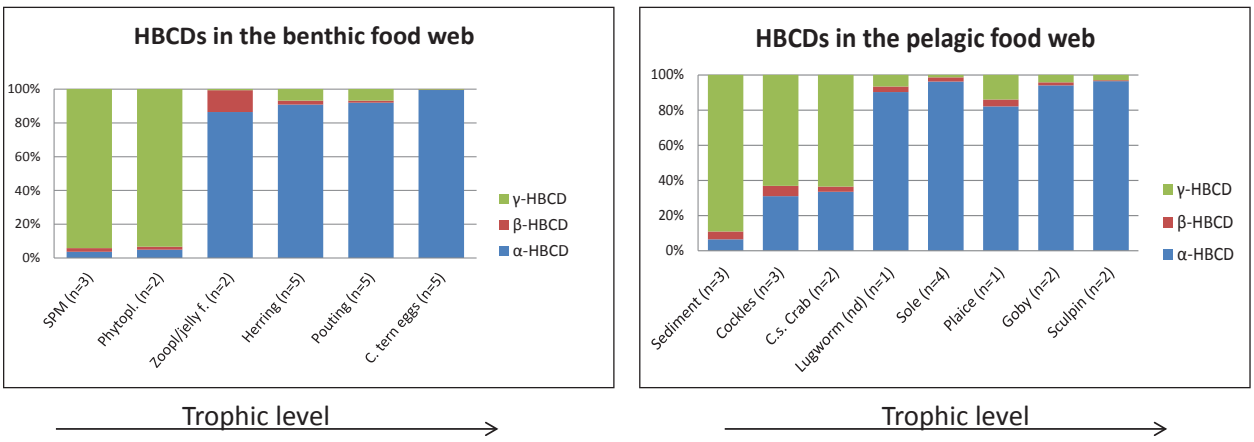


Figure 3. The diastereoisomer pattern of HBCDs trough the benthic and pelagic food web of the Western Scheldt.

Morris et al. (2004) observed total-HBCDs levels of 300 - 7100 ng/g lw in common tern eggs (n=10) from the Western Scheldt from 2001. Janak et al., (2005) analyzed HBCD in sediment and biota from the Western Scheldt from 2001 and detected in sole tissue (n=4) α-HBCD and γ-HBCD at levels of 110 - 1100 ng/g lw and 6 - 11 ng/g lw, respectively. In plaice tissue (n=1) the levels were lower, with α-HBCD and γ-HBCD levels of 38 ng/g lw and <2 ng/g lw, respectively. The β-HBCD levels in sole and plaice were all below the LOQ. We found lower levels for both α-HBCD and γ-HBCD in the common tern eggs, sole and plaice with levels respectively, of 22 - 205 ng/g lw and <0.3 - 0.37 ng/g lw in

common tern eggs (n=5), 22 - 37 ng/g lw and 0.2 - 1.8 ng/g lw in the sole (n=4) and 2.3 and 0.5 ng/g lw in plaice (n=1). The HBCD levels in the western Scheldt have clearly decreased from 2001 to 2008. Decreasing HBCD levels after 2000 in Europe were also observed by Esslinger et al. (2011) in herring gull eggs. Sellström et al. (2003) observed that HBCD levels in guillemot eggs from the Baltic Sea leveled off between 1995 and 2001.

Trophic magnification of PFRs and BFRs

PFRs

TMFs were calculated for PFRs in the two food webs and in the total food web (benthic + pelagic) (Table 4). TMF were only calculated if the detection frequency was above 40%.The TMFs of the PFRs were calculated on a wet weight basis. In general a number of samples between 30 and 60 is recommended to achieve statistical reliable TMFs (Borgå et al., 2012). The sample size of the total food web (benthic and pelagic) in our study is 34. However, for the individual food webs the number of samples is smaller: pelagic (n=15) and benthic (n=19). Therefore the TMFs calculated for the individual food webs are assigned as *tentative* because of the higher uncertainty due the lower sample size, notwithstanding some TMFs were statistically significant. PFRs demonstrated trophic dilution in the pelagic and in the total food web, probably due to metabolism (Table 4). However, in the benthic food web trophic magnification (i.e. TMF>1) was observed for TCEP, TCIPP and TBOEP with tentative TMFs of 2.6, 2.2 and 3.5, respectively ($p < 0.05$). An example of the relation between the log concentration and the trophic level is given for TCIPP and TBOEP in figure 4.
In the only other study on trophic magnification of PFR published to date (Kim et al., 2011), no accumulation was observed for nine PFRs, with exception of TPHP in demersal species. Kim et al., (2011) suggested that exposure to bottom sediments enriched in TPHP has caused the accumulation of TPHP in bottom dwelling species. This may also explain the trophic magnification we observed in the benthic food web for TCEP, TCIPP and TBOEP (Table 3).

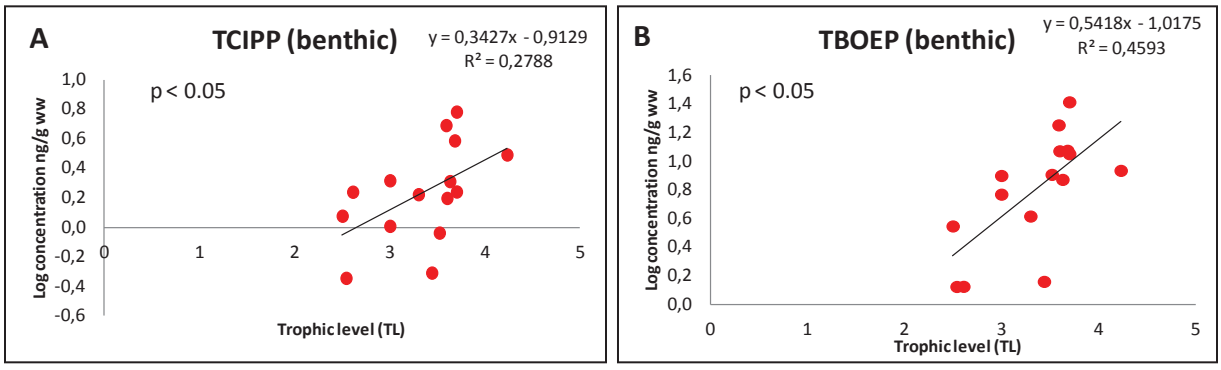


Figure 4. Relation between the log PFR concentration (ng/g ww) and the trophic level in the benthic food web for A) TCIPP and B) TBOEP.

BFRs

Trophic magnification (i.e. TMF>1) was observed for all PBDEs with the exception of BDE209 in the two food webs (Table 4). Significant correlations between the BFR levels and the lipid contents were observed for all BFRs ($p < 0.05$) with the exception of BDE209. Therefore, the BFR TMFs were calculated using lipid weigh based concentrations. Trophic magnification of PBDEs has been reported by Law et al. (2006b), Kelly et al. (2008), Tomy et al. (2008), Wan et al. (2008), Yu et al. (2009), Wu et al. (2009), Losada et al. (2009), Mizukawa et al. (2013), Van Ael et al. (2013) and Ma et al. (2013). The present results are consistent with the literature data. Significant positive slopes were found for BDE47 in all studies with the exception of the study of Van Ael et al., (2013), this indicates that BDE47 undergoes trophic magnification (Fig. 5A). However, the variations between the TMF in the different studies were high, e.g. for BDE47 the TMF varies from 1.6 (Kelly et al., 2008) in Canadian arctic marine food web to 7.2 in the marine food web of Bohai Bay, Northern China (Wan et al., 2008). The size of the food web may influence the trophic magnification of the PBDEs. Wan et al. (2008) and Ma et al. (2013) found higher TMFs when they included mammals in their food web. The only PBDE that showed a significant negative slope in our study was BDE209 with a TMF of 0.2 (Fig. 5B). Tomy et al. (2008) reported a TMF of 0.3 in a Canadian Arctic marine food web for BDE209. However, Law et al. (2006b) found a TMF of 3.6 for BDE209 which indicated high trophic magnification of BDE209 in Lake Winnipeg food web of Canada. These differences between the TMFs of BDE209 may be related to the different organisms studied within the food web and the metabolic/debromination capability of the different organisms. For example, Tomy et al. (2008) suggested that relatively high BDE209 levels in Arctic cod and redfish may be influenced by the greater exposure to zooplankton that contained high BDE209 levels and the lower metabolic/debromination capabilities. In the same food web Tomy et al. (2008) observed lower BDE209 levels in the higher trophic organisms like beluga and narwhal and suggested that these organisms may have a higher metabolism/debromination capacity of BDE209. If beluga and narwhal were excluded from the Arctic food web by Tomy et al. (2008) probably higher TMF are found for BDE209. This again highlights that the length of the food web influences the magnitude of the TMF of BDE209.

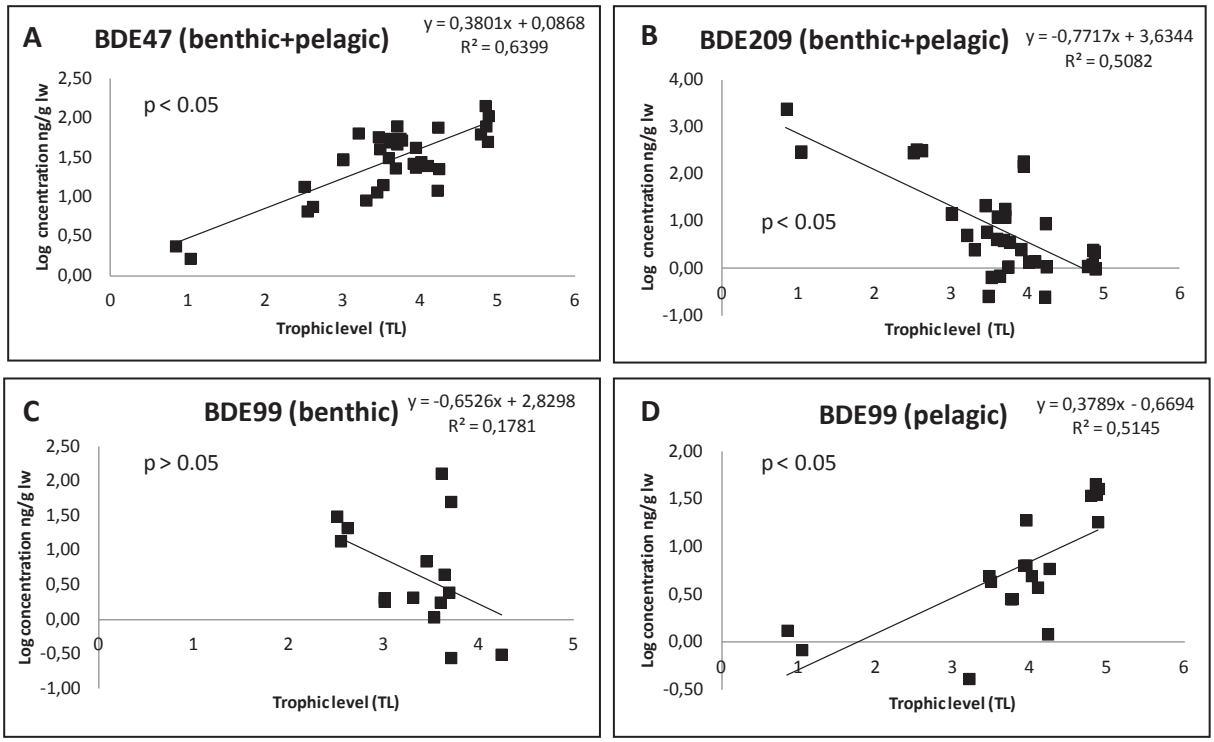


Figure.5. Relation between the log PBDE concentration (ng/g ww) and the trophic level in the total food web for A) BDE47 and B) BDE209 and in the benthic food web for C) BDE99 the pelagic food web for D) BDE99.

By comparing the tentative TMFs in the benthic food web with those in the pelagic food web we observed a different slope for BDE99. In the pelagic food web the slope for BDE99 is 0.35 and in the benthic food web -0.65 (Fig. 5C and D). However, in the benthic food web the correlation is not significant. Therefore, no TMF was calculated. Several studies have indicated that BDE99 is easily metabolized to BDE47 (Voorspoels et al., 2003; Stapleton et al., 2004; Noyes, 2009). Possibly, BDE99 may be metabolized faster in the benthic food web compared to the pelagic food web.

A significant correlation ($p < 0.05$) between the log α- and γ-HBCD concentration (ng/g lw) and the trophic level was observed in both food webs (Table 4). α-HBCD shows a significant positive slope and γ-HBCD a significant negative slope. which indicates that α-HBCD undergoes trophic magnification γ-HBCD trophic dilution (Fig. 3). The TMF of α-HBCD in the total food web is 2.2 and is comparable with TMFs reported for α-HBCD by Tomy et al. (2008), Hauskas et al. (2010), Wu et al. (2010) and Zhang et al. (2013) of 2.1, 2.6, 2.2, 1.7 and 2.6, respectively. For γ-HBCD Tomy et al., (2008) observed a TMF of 0.5 and Hauskas et al. (2010) a TMF of 0.3, which is also comparable with the TMF in our study of 0.3 for γ-HBCD. The TMF of α-HBCD in the benthic food web (3.7) is higher than in the pelagic food web (2.0), which is probably related to the higher exposure of the benthic organisms to the HBCD enriched sediment (Table 3).

Table 4. *TMFs for the PFRs and BFRs calculated as the regression coefficient of the trophic level versus the logarithm of the concentration in organisms from the benthic and pelagic food webs.*

	Total food web n = 34 (benthic + pelagic)					**Benthic food web n = 15**					**Pelagic food web n = 19**				
	p	R	slope	TMF	DF %	p	R	slope	**TMF	DF %	p	R	slope	**TMF	DF %
TCEP	0.50	0.00	0.00	ns	32%	0.04	0.46	0.42	**2.6**	47%	0.30	0.13	-0.07	ns	21%
TCIPP	0.48	0.03	-0.01	ns	50%	0.02	0.53	0.34	**2.2**	60%	0.14	0.26	-0.09	ns	42%
TDCIPP	0.13	0.20	-0.09	ns	12%	0.26	0.18	-0.16	ns	13%	0.08	0.03	-0.11	ns	11%
TPHP	0.37	0.06	-0.02	ns	32%	0.45	0.03	0.03	ns	67%	0.29	0.14	-0.04	ns	5%
TIBP	0.13	0.20	0.10	ns	29%	0.03	0.50	0.53	*	33%	0.44	0.04	0.01	ns	26%
TBOEP	0.28	0.10	0.06	ns	56%	0.00	0.68	0.54	**3.5**	80%	0.43	0.05	-0.03	ns	37%
TMPP	0.20	0.15	-0.58	ns	6%	0.18	0.26	0.13	ns	7%	0.04	0.41	-0.14	*	5%
EHDPP	0.09	0.24	-0.94	ns	15%	0.18	0.26	0.13	ns	7%	0.01	0.58	-0.19	*	21%
TEHP	0.03	0.34	-0.16	*	15%	0.50	0.00	0.00	ns	7%	0.00	0.59	-0.27	*	21%
BDE28	0.00	0.56	0.17	**1.5**	76%	0.01	0.63	0.29	**1.9**	100%	0.00	0.61	0.17	**1.5**	58%
BDE47	0.00	0.80	0.38	**2.4**	97%	0.00	0.73	0.52	**3.3**	100%	0.00	0.84	0.37	**2.3**	95%
BDE100	0.00	0.76	0.43	**2.7**	94%	0.01	0.58	0.40	**2.5**	100%	0.00	0.80	0.45	**2.8**	89%
BDE99	0.03	0.33	0.24	**1.7**	88%	0.06	0.42	-0.65	ns	100%	0.00	0.72	0.38	**2.4**	79%
BDE153	0.00	0.45	0.31	**2.1**	50%	0.41	0.06	0.08	ns	80%	0.01	0.58	0.35	*	26%
BDE209	0.00	0.71	-0.77	**0.2**	85%	0.00	0.71	-1.20	**0.1**	87%	0.00	0.72	-0.69	**0.2**	84%
α-HBCD	0.00	0.57	0.35	**2.2**	97%	0.04	0.46	0.57	**3.7**	93%	0.00	0.66	0.29	**2.0**	100%
γ-HBCD	0.00	0.71	-0.52	**0.3**	76%	0.01	0.63	-0.79	**0.2**	100%	0.00	0.78	-0.51	**0.3**	58%

*Detection frequency was below 40%. therefore no TMF was calculated. ns: not significant.
**Tentative TMF

Conclusions

In conclusion, tentative TMFs >1 have been observed for TBOEP, TCIPP and TCEP in the benthic food web, which may imply that these PFRs undergo trophic magnification. A higher number of samples may be needed for a final confirmation of the tropic magnification. Several PFRs were also found at elevated levels in the food web (fish) with concentrations similar to PBDEs. In the abiotic compartments (sediment, SPM) concentrations of PFRs were often higher than those of PBDEs. It has been shown that most PFRs can easily be metabolized (WHO, 1990, 1991, 1997, 1998, 2000) and this would imply that the emissions and exposure of PFRs in the Western Scheldt area must be high and continuous in order to explain the relatively high levels observed in fish. The increased demand for PFRs following the ban and phase out of the PBDEs may lead to a further increase of environmental levels and a higher exposure of organisms to PFRs.

Acknowledgements

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Appendix. Supplementary data available

Sample information and location of the two food webs including the lipid and total organic carbon (TOC) contents, dry weights and trophic levels is given in Table S1 and Figure S1. PFR levels in ng/g wet weight are given in Table S2 and S3. PBDE levels in ng/g lipid weight in Table S4 and S5.

PBDEs analysis

The injector temperature was 275 °C and the samples were injected in the pulsed splitless mode with a pulse pressure of 277 KPa for 1.5 min. Helium was used as carrier gas with a flow of 3.0 mL/min. The oven temperature program was 90°C held for 2 min, followed by an increase of 30 °C/min to 210 °C. The temperature was subsequently increased to 320 °C (5 °C/min) and held for 10 min. The post run temperature was 320 °C, held for 15 min. DecaBDE was analyzed on the same GC-MS as the PBDEs but using a DB-5 capillary column (15 m x 0.25 mm ID, 0.25 µm film thickness, J&W Scientific, Amstelveen, the Netherlands). The injector temperature was 275 °C and the samples were injected in the pulsed splitless mode with a pulse pressure of 277 KPa for 1.5 min. Helium was used as a carrier gas with a flow of 1.0 ml/min. The oven temperature program was 90°C held for 3 min, followed by an increase of 30 °C/min to 210 °C. The temperature was then increased to 315 °C (5 °C/min) and held for 10 min. For all PBDEs, including decaBDE, the MS was run in the electron capture negative chemical ionization (ECNI) mode using methane as reagent gas. The temperatures of the source and quadrupole were 200 and 106 °C, respectively. Selective ion monitoring (SIM) was performed for m/z 79 and 81 for the PBDEs, m/z 484 and 486 for BDE209 and 496 and 498 for the internal standard ^{13}C-BDE209.

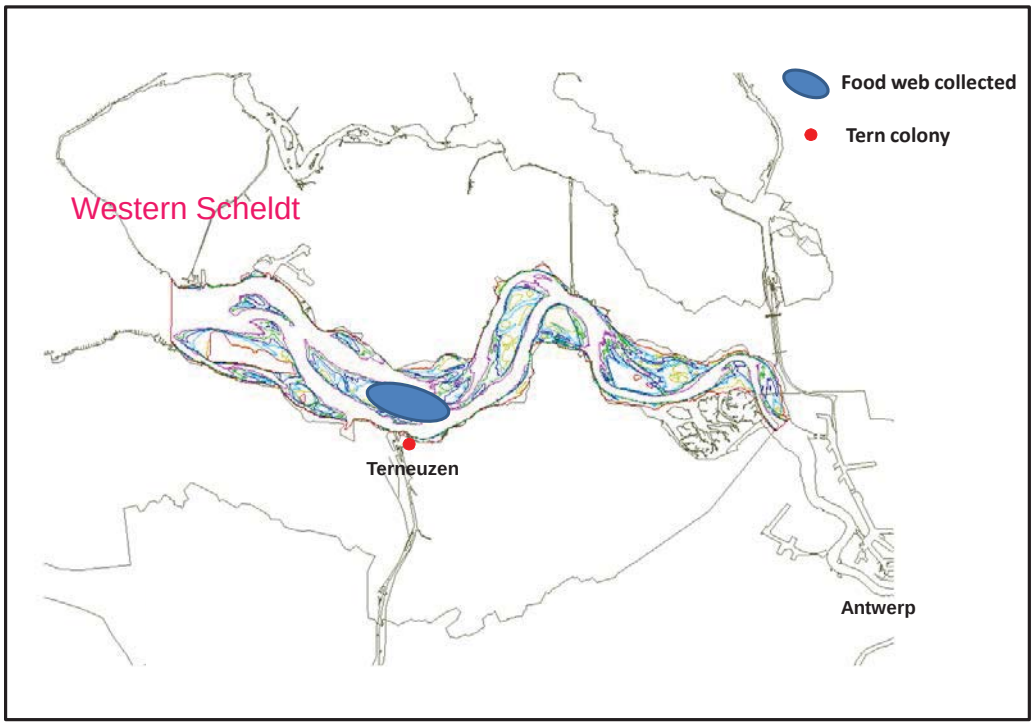

Figure S1. Western Scheldt study site located in the south of the Netherlands.

Table S1. Samples list of the benthic and pelagic food web of the Western Scheldt.

Matrix	Species name	Sample type	TOC**/Lipids* (%)	Trophic level	Dry weight (%)
Pelagic food web					
suspended particule matter 1		Pooled (centrifuged)	2.60	1.02	52
suspended particule matter 2		Pooled (centrifuged)	1.20	0.97	52
suspended particule matter 3		Pooled (centrifuged)	2.74	1.02	29
Phytoplankton 1/SPM	*Algae*	Whole organism (pooled)	0.30	1.03	25
Phytoplankton 2/SPM	*Algae*	Whole organism (pooled)	0.30	0.84	48
Zooplankton/jelly fish 1	*Zooplankton and Pleurobrachia sp.*	Whole organism (pooled)	0.59	3.94	11
Zooplankton/jelly fish 2	*Zooplankton and Pleurobrachia sp.*	Whole organism (pooled)	0.20	3.94	4.8
Pouting-1	*Trisopterus luscus*	Whole organism (pooled)	2.90	4.09	22
Pouting-2	*Trisopterus luscus*	Whole organism (pooled)	2.20	4.01	22
Pouting-3	*Trisopterus luscus*	Whole organism (pooled)	3.70	4.24	24
Pouting-4	*Trisopterus luscus*	Whole organism (pooled)	2.80	3.91	23
Pouting-5	*Trisopterus luscus*	Whole organism (pooled)	4.10	4.22	23
Herring-1	*Clupea harengus*	Whole organism (pooled)	3.90	3.48	24
Herring-2	*Clupea harengus*	Whole organism (pooled)	2.40	3.46	21
Herring-3	*Clupea harengus*	Whole organism (pooled)	1.40	3.76	19
Herring-4	*Clupea harengus*	Whole organism (pooled)	1.40	3.74	21
Herring-5	*Clupea harengus*	Whole organism (pooled)	1.20	3.20	19
Common tern egg-1	*Sterna hirundo*	Egg	18.5	4.88	31
Common tern egg-2	*Sterna hirundo*	Egg	15.5	4.78	26
Common tern egg-3	*Sterna hirundo*	Egg	11.0	4.84	22
Common tern egg-4	*Sterna hirundo*	Egg	13.7	4.85	28
Common tern egg-5	*Sterna hirundo*	Egg	16.3	4.87	27
Bentihic food web					
Sediment-1		Pooled	0.20	1.20	76
Sediment-2		Pooled	0.20	0.33	77
Sediment-3		Pooled	0.86	0.95	56
Lugw orm	*Arenicola marina*	Whole organism (pooled)	1.14	3.44	15
Cockle-1	*Cerastoderma edule*	Whole organism (pooled)	0.63	2.61	13
Cockle-2	*Cerastoderma edule*	Whole organism (pooled)	0.67	2.54	12
Cockle-3	*Cerastoderma edule*	Whole organism (pooled)	0.70	2.50	27
Common shore crab-1	*Carcinus menas*	Whole organism (pooled)	3.28	3.00	22
Common shore crab-2	*Carcinus menas*	Whole organism (pooled)	3.00	3.00	22
Goby-1	*Goby sp.*	Whole organism (pooled)	3.59	3.68	24
Goby-2	*Goby sp.*	Whole organism (pooled)	3.62	3.59	25
Sculpin-1	*Myoxocephalus scorpius*	Whole organism (pooled)	2.29	3.70	22
Sculpin-2	*Myoxocephalus scorpius*	Whole organism (pooled)	4.17	4.23	26
Plaice	*Pleuronectes platessa*	Whole organism (pooled)	2.19	3.63	22
Sole-1	*Solea solea*	Whole organism (pooled)	1.68	3.70	22
Sole-2	*Solea solea*	Whole organism (pooled)	3.80	3.60	25
Sole-3	*Solea solea*	Whole organism (pooled)	4.76	3.30	27
Sole-4	*Solea solea*	Whole organism (pooled)	4.64	3.52	14

** For biota the value represents the percentage lipid of the wet weight of the sample. **For sediment and SPE the value represents the percentage total organic carbon (TOC) of the dry weight of the sample.*

Table S2. Median and mean organophosphorus flame retardant (PFR) levels ± standard deviation in ng/g wet weight (ww) for the benthic food webs of the Western Scheldt.

Benthic food web ng/g ww		TCEP	TCIPP	TDCIPP	TPHP	TIBP	TBOEP	TMPP	EHDPP	TEHP
Sediment (n=3)	Median	0.23	1.4	0.20	0.39	6.2	5.4	0.07	0.12	0.63
	Mean	0.35	2.3	0.35	0.38	5.0	5.9	0.22	0.20	0.92
	Std	0.35	1.6	0.38	0.20	2.2	4.8	0.27	0.15	0.80
	#	*2/3*	*3/3*	*2/3*	*2/3*	*3/3*	*2/3*	*1/3*	*1/3*	*3/3*
Cockle (n=3)	Median	<0.06	1.2	0.06	0.35	<0.20	1.3	<0.06	<0.11	0.06
	Mean	<0.06	1.1	0.51	0.41	<0.20	2.1	<0.06	<0.11	0.11
	Std	0.0001	0.64	0.77	0.29	0.0003	1.3	0.0001	0.0002	0.08
	#	*0/3*	*2/3*	*1/3*	*2/3*	*0/3*	*1/3*	*0/3*	*0/3*	*1/3*
C.s. crab (n=2)	Median	0.82	1.5	<0.06	0.51	<0.19	6.9	<0.06	<0.11	<0.06
	Mean	0.82	1.5	<0.06	0.51	<0.19	6.9	<0.06	<0.11	<0.06
	Std	0.15	0.74	0.003	0.01	0.008	1.5	0.003	0.005	0.003
	#	*2/2*	*2/2*	*0/2*	*2/2*	*0/2*	*2/2*	*0/2*	*0/2*	*0/2*
Lugworm nd (n=1)	Median	0.33	<0.49	0.73	2.0	3.1	<1.5	0.2	0.35	<0.07
	Mean	0.33	<0.49	0.73	2.0	3.1	<1.5	0.2	0.35	<0.07
	Std	–	–	–	–	–	–	–	–	–
	#	*1/1*	*0/1*	*1/1*	*1/1*	*1/1*	*0/1*	*1/1*	*1/1*	*0/1*
Sole (n=4)	Median	<0.23	<1.6	<0.23	0.57	<0.71	9.7	<0.23	<0.39	<0.23
	Mean	<0.21	<1.5	<0.21	0.45	<0.64	8.9	<0.21	<0.35	<0.21
	Std	0.05	0.38	0.05	0.28	0.16	3.5	0.05	0.09	0.05
	#	*0/4*	*0/4*	*0/4*	*1/4*	*0/4*	*4/4*	*0/4*	*0/4*	*0/4*
Plaice (n=1)	Median	<0.06	2.0	<0.06	0.35	<0.19	7.5	<0.06	<0.10	<0.06
	Mean	<0.06	2.0	<0.06	0.35	<0.19	7.5	<0.06	<0.10	<0.06
	Std	–	–	–	–	–	–	–	–	–
	#	*0/1*	*1/1*	*0/1*	*1/1*	*0/1*	*1/1*	*0/1*	*0/1*	*0/1*
Goby (n=2)	Median	1.0	4.4	<0.07	0.56	7.4	15	<0.07	<0.12	<0.07
	Mean	1.0	4.4	<0.07	0.56	7.4	15	<0.07	<0.12	<0.07
	Std	0.33	0.73	0.001	0.22	7.5	4.3	0.001	0.001	0.001
	#	*2/2*	*2/2*	*0/2*	*2/2*	*2/2*	*2/2*	*0/2*	*0/2*	*0/2*
Sculpin (n=2)	Median	0.46	4.6	<0.06	0.32	0.55	17	<0.06	<0.11	<0.06
	Mean	0.46	4.6	<0.06	0.32	0.55	17	<0.06	<0.11	<0.06
	Std	0.08	2.1	0.001	0.24	0.02	12	0.001	0.001	0.001
	#	*2/2*	*2/2*	*0/2*	*1/2*	*2/2*	*2/2*	*0/2*	*0/2*	*0/2*

frequency of detected/number of samples analysed

Table S3. Median and mean organophosphorus flame retardant (PFR) levels ± standard deviation in ng/g wet weight (ww) for the and pelagic food webs of the Western Scheldt.

Pelagic food web ng/g ww		TCEP	TCIPP	TDCIPP	TPHP	TIBP	TBOEP	TMPP	EHDPP	TEHP
Phytoplankton (n=2)	Median	<0.42	4.0	0.55	<1.1	<1.3	<8.9	0.66	2.2	2.6
	Mean	<0.42	4.0	0.55	<1.1	<1.3	<8.9	0.66	2.2	2.6
	Std	0.46	2.0	0.28	1.2	1.4	9.7	0.14	1.3	1.4
	#	*0/2*	*1/2*	*1/2*	*0/2*	*0/2*	*0/2*	*1/2*	*2/2*	*2/2*
SPM (n=3)	Median	0.55	8.2	0.54	0.83	0.28	12	1.5	1.5	1.4
	Mean	0.43	9.4	0.48	0.80	0.42	15	1.4	1.4	1.5
	Std	0.30	9.5	0.37	0.08	0.25	6.9	0.30	1.1	0.75
	#	*2/3*	*2/3*	*2/3*	*3/3*	*1/3*	*3/3*	*3/3*	*2/3*	*1/3*
Herring (n=5)	Median	0.85	2.2	<0.26	<0.66	<0.82	6.6	<0.26	<0.45	<0.26
	Mean	1.6	8.2	<0.27	<0.67	<0.83	71	<0.27	<0.46	<0.27
	Std	1.8	12	0.09	0.23	0.28	119	0.09	0.15	0.09
	#	*3/5*	*2/5*	*0/5*	*0/5*	*0/5*	*2/5*	*0/5*	*0/5*	*0/5*
Zoopl./jelly f. (n=2)	Median	<0.06	0.99	0.16	0.21	<0.20	<1.3	<0.06	0.27	0.13
	Mean	<0.06	0.99	0.16	0.21	<0.20	<1.3	<0.06	0.27	0.13
	Std	0.06	0.30	0.20	0.09	0.20	1.36	0.06	0.27	0.16
	#	*0/2*	*1/2*	*1/2*	*1/2*	*0/2*	*0/2*	*0/2*	*2/2*	*1/2*
Pouting (n=5)	Median	0.08	2.54	<0.08	<0.19	2.15	9.91	<0.08	<0.13	0.08
	Mean	0.36	2.94	<0.09	<0.24	2.37	26.59	<0.09	<0.16	0.18
	Std	0.60	2.19	0.04	0.09	1.16	40.58	0.04	0.06	0.23
	#	*1/5*	*4/5*	*0/5*	*0/5*	*5/5*	*5/5*	*0/5*	*0/5*	*1/5*
C. tern eggs (n=5)	Median	<0.24	<1.7	<0.24	<0.60	<0.75	<5.1	<0.24	<0.41	<0.24
	Mean	<0.26	<1.9	<0.26	<0.65	<0.81	<5.5	<0.26	<0.44	<0.26
	Std	0.05	0.35	0.05	0.12	0.15	1.03	0.05	0.08	0.05
	#	*0/5*	*0/5*	*0/5*	*0/5*	*0/5*	*0/5*	*0/5*	*0/5*	*0/5*

frequency of detected/number of samples analysed

Table S4. Average PBDEs and α-, β- and, γ-HBCD levels ± standard deviation in ng/g lw in the benthic food web of the Western Scheldt. Sediment and SPM (suspended particulate matter) concentrations are given in units of ng/g organic carbon (OC).

Benthic food web ng/g lw		BDE28	BDE47	BDE100	BDE99	BDE153	BDE209	a-HBCD	b-HBCD	g-HBCD
Sediment (n=3)	Median	6.0	13.7	5.0	16	10	3718	10	7.0	120
	Mean	5.2	12.4	4.6	13	10	4730	10	7.3	146
	Std	3.2	4.6	2.4	5.7	3.6	2174	2.6	2.5	77
	#	*3/3*	*3/3*	*3/3*	*3/3*	*3/3*	*3/3*	*2/3*	*3/3*	*3/3*
Cockle (n=3)	Median	1.4	7.6	3.9	21	2.3	312	9.5	1.5	13
	Mean	1.4	9.3	4.6	22	2.4	305	9.5	1.6	21
	Std	0.49	3.8	1.8	8.8	1.7	21	0.52	0.26	14
	#	*3/3*	*3/3*	*3/3*	*3/3*	*2/3*	*3/3*	*3/3*	3/3	3/3
C.s. crab (n=2)	Median	2.6	30	2.8	1.9	<0.12	14	0.62	<0.06	1.2
	Mean	2.6	30	2.8	1.9	<0.12	14	0.62	<0.06	1.2
	Std	0.11	0.52	0.11	0.16	0.02	0.14	0.41	0.04	1.3
	#	*2/2*	*2/2*	*2/2*	*2/2*	*0/2*	*2/2*	*1/2*	*0/2*	*2/2*
Lugworm nd (n=1)	Median	1.5	12	4.1	7.0	2.3	21	25	0.90	2.6
	Mean	1.5	12	4.1	7.0	2.3	21	25	0.90	2.6
	Std	–	–	–	–	–	–	–	–	–
	#	*1/1*	*1/1*	*1/1*	*1/1*	*1/1*	*1/1*	*1/1*	*1/1*	*1/1*
Sole (n=4)	Median	1.6	31	21	26	5.0	7.4	26	0.75	1.1
	Mean	1.7	32	21	46	6.8	6.9	28	0.70	1.1
	Std	0.40	23	15	61	7.6	6.2	6.5	0.54	0.87
	#	*4/4*	*4/4*	*4/4*	*4/4*	*4/4*	*3/4*	*4/4*	*3/4*	*4/4*
Plaice (n=1)	Median	2.8	50	17	4.5	1.7	<0.69	2.3	<0.11	0.5
	Mean	2.8	50	17	4.5	1.7	<0.69	2.3	<0.11	0.5
	Std	–	–	–	–	–	–	–	–	–
	#	1/1	1/1	1/1	1/1	1/1	0/1	1/1	0/1	1/1
Goby (n=2)	Median	2.4	28	7.8	2.1	1.3	4.0	18	0.36	1.1
	Mean	2.4	28	7.8	2.1	1.3	4.0	18	0.36	1.1
	Std	0.05	5.9	1.9	0.49	0.01	0.15	4.0	0.35	0.39
	#	*2/2*	*2/2*	*2/2*	*2/2*	*2/2*	*2/2*	*2/2*	1/2	2/2
Sculpin (n=2)	Median	6.2	79	8.7	0.30	0.46	13	40	<0.25	1.5
	Mean	6.2	79	8.7	0.30	0.46	13	40	<0.25	1.5
	Std	0.80	2.2	0.32	0.02	0.14	6.3	0.73	0.14	0.39
	#	*2/2*	*2/2*	*2/2*	*2/2*	*2/2*	*2/2*	*2/2*	0/2	2/2

frequency of detected/number of samples analysed

Table S5. Average PBDEs and α-, β- and, γ-HBCD levels ± standard deviation in ng/g lw in the pelagic food web of the Western Scheldt. Sediment and SPM (suspended particulate matter) concentrations are given in units of ng/g organic carbon (OC).

Pelagic food web ng/g lw		BDE28	BDE47	BDE100	BDE99	BDE153	BDE209	a-HBCD	b-HBCD	g-HBCD
Phytoplankton (n=2)	Median	<0.56	2.0	<0.56	1.1	<0.42	1319	5.2	1.9	100
	Mean	<0.56	2.0	<0.56	1.1	<0.42	1319	5.2	1.9	100
	Std	0.40	0.52	0.40	0.35	0.20	1453	5.8	1.9	134
	#	*0/2*	*1/2*	*0/2*	*1/2*	*0/2*	*2/2*	*2/2*	*1/2*	*2/2*
SPM (n=3)	Median	<0.77	8.8	2.2	6.6	3.6	6569	20	11	425
	Mean	<1.1	8.2	2.4	6.8	3.6	6476	20	12	520
	Std	0.53	2.2	1.7	2.3	0.55	1265	7	4	354
	#	*0/3*	*3/3*	*2/3*	*3/3*	*3/3*	*3/3*	*3/3*	*3/3*	*3/3*
Herring (n=5)	Median	2.8	56	15	2.9	<1.4	3.6	20	<0.71	2.5
	Mean	2.7	55	15	3.1	<1.7	3.1	24	<0.62	2.4
	Std	1.1	8.8	4.0	1.8	1.2	2.4	9.4	0.4	1.4
	#	*1/5*	*5/5*	*5/5*	*2/5*	*0/5*	*3/5*	*5/5*	*0/5*	*3/5*
Zoopl./jelly f. (n=2)	Median	3.2	33	5.9	13	<1.3	163	80	14	12
	Mean	3.2	33	5.9	13	<1.3	163	80	14	12
	Std	0.91	13	1.6	9.0	0.49	24	96	19	15
	#	*1/2*	*2/2*	*2/2*	*2/2*	*0/2*	*2/2*	*2/2*	*1/2*	*2/2*
Pouting (n=5)	Median	1.9	25	5.2	5.0	<0.37	1.4	15	<0.17	1.0
	Mean	1.7	23	4.7	4.5	<0.41	1.3	14	<0.19	1.2
	Std	0.70	6.4	1.6	2.1	0.08	0.81	3.9	0.07	0.76
	#	*4/5*	*5/5*	*5/5*	*5/5*	*0/5*	*4/5*	*5/5*	*0/5*	*4/5*
C. tern eggs (n=5)	Median	3.1	80	49	36	18	1.2	63	0.09	<0.27
	Mean	2.9	90	52	35	17	1.6	80	0.12	<0.41
	Std	1.1	38	20	10	5.3	0.68	72	0.10	0.37
	#	*5/5*	*5/5*	*5/5*	*5/5*	*5/5*	*5/5*	*5/5*	*1/5*	*0/5*

frequency of detected/number of samples analysed

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