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Research Article

Brominated flame retardants in fish and shellfish – levels and contribution of fish consumption to dietary exposure of Dutch citizens to HBCD

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In order to determine the contamination with brominated flame retardants (BFR) in fish regularly consumed by Dutch citizens, 44 samples of freshwater fish, marine fish, and shellfish were analyzed for polybrominated diphenyl ethers (PBDE), tetrabromobisphenol-A (TBBP-A) and its methylated derivative (me-TBBP-A), and hexabromocyclododecane (HBCD), including its α-, β- and γ-diastereomers. The highest BFR concentrations were found in pike-perch and eel from the highly industrialized and urbanized rivers Rhine and Meuse. The sum concentrations of BDE 28, 47, 99, 100, 153, 154, 183, 209, and brominated biphenyl (BB) 153 and HBCD (selection based on The European Food Safety Authority monitoring recommendation) ranged from below quantification limits to 17 ng/g wet weight (ww) in marine fish and in freshwater fish from 0.6 ng/g ww in pike-perch to 380 ng/g ww in eel. The BDE congener profile in all fish and shellfish samples is dominated by BDE 47, followed by BDE 99, except for eel in which BDE 100 is higher than BDE 99. BDE 209 was detected in two mussel samples, most likely due to BDE 209 contaminated particulate matter in their intestines. Total-HBCD (as determined by GC/electron capture negative ion (ECNI)-MS) was detected in 22 out of the 44 samples in concentrations between 0.20 ng/g in marine fish and 230 ng/g ww in eel. Three HBCD diastereomers were determined by HPLC/ESI-MS/MS. α-HBCD was the prevalent congener in most fish samples, followed by γ-HBCD. β-HBCD, TBBP-A and me-TBBP-A were only detected in a few samples and at low concentrations. A considerable difference was found between HBCD results obtained from GC/ECNI-MS and HPLC/ESI-MS/MS: the GC/ECNI-MS results were 4.4 times higher, according to regression analysis. There is hardly any data on human dietary exposure to HBCD available. We have estimated the fish-related dietary exposure of HBCD for the average Dutch population. The medium bound intake was estimated at 8.3 ng/day for a 70-kg person (0.12 ng/kg bodyweight/day). For this estimation, we relied mostly on HPLC/ESI-MS/MS data as we argue that these results are more accurate than those obtained by GC/ECNI-MS.

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1 Introduction

Flame retardants constitute a diverse group of compounds that are added to materials in order to reduce, delay or even prevent them from catching fire. A substantial part of flame retardants consists of brominated compounds. The most frequently used brominated flame retardants (BFR) are tetrabromobisphenol-A (TBBP-A), hexabromocyclododecane

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Abbreviations: **BDE**, brominated diphenyl ether; **BFR**, brominated flame retardant; **bw**, bodyweight; **ECNI**, electron capture negative ionisation; **HBCD**, hexabromocyclododecane; **Me-TBBP-A**, dimethyl derivative of tetrabromobisphenol-A; **PBDE**, polybrominated diphenyl ether; **TBBP-A**, tetrabromobisphenol-A; **ww**, wet weight

(HBCD) and polybrominated diphenylethers (PBDE). The BFR are used at relatively high concentrations in various materials and polymers, such as polyurethane and polystyrene foams, in a wide range of products, such as printed circuit boards, television sets and computers and other electronic household equipment, cars and construction materials [1]. Information on BFR usage figures (from 2001) can be found elsewhere [2]. BFR can be released into the environment through production, use, and especially from disposal of the flame retarded products. Various BFR are present in biota due to their lipophilicity and persistence. Several of the PBDE and HBCD have shown potential for biomagnification in the food chain. Extensive information on the environmental concentrations of PBDE and HBCD can be found in recent comprehensive reviews by Law *et al*. [2] and Covaci *et al*. [3].

BFR have been found in several human samples [4 – 6] showing that also humans are exposed to these chemicals. An important exposure route of the European general human population to PBDE is through the diet. This is confirmed in an UK study showing estimates of median PBDE exposures (sum of 9 PBDE) for UK adults of 98.7, 0.9 and 0.4% for food, air, and house dust, respectively [7]. The exposure route for BDE 209 is presumably different as this is in most cases the predominant congener in dust (up to ca 90%) [8 – 10]. Within the food group as such, local consumption habits determine if, *e.g*. fish [11] or dairy products (Bakker *et al*., this issue, [12]) predominate the exposure to PBDE. HBCD has been detected in breast milk and human blood [3] in the range of 0.08 – 7.0 ng/g lipid weight. The human exposure to HBCD remains to be quantified, as virtually no data is available on the relevance of different exposure routes such as dietary exposure, dust ingestion, air inhalation, and other routes. A study by Lind *et al*. [13] showed a median dietary exposure of 141 ng/day, being dominated by fish. In addition, TBBP-A was found in human blood samples [14].

The European Food Safety Authority (EFSA) has recognized the concern for the contamination of food and feed with BFR [15]. In 2006, EFSA has adopted an opinion in which the monitoring of the BDE 28, 47, 99, 100, 153, 154, 183, 209, and brominated biphenyl (BB) 153 and HBCD is recommended. This baseline study, conducted in 2003, aimed at the determination of 16 BDE, HBCD, TBBP-A and me-TBBP-A in a broad selection of edible fish and shellfish in order to investigate the importance of fish consumption for human exposure to BFR. More specifically, the human exposure to HBCD in relation to fish consumption was studied. In a separate contribution to this issue, the dietary exposure to PBDE is reported, combining the fish contamination data with BDE data in other food commodities [12].

2 Materials and methods

2.1 Sampling and sample preparation

The choice of samples was based on (i) origin from Dutch marine and freshwaters or (ii) regular consumption by the Dutch population. The analyzed samples and their origins are mentioned in Table 1. Wide varieties of marine species

Table 1. Sample overview

Species		Location	Number of samples
Shrimps	*Crangon crangon*	Rijnmond, Wadden Sea	2
Herring	*Clupea Harengus*	CNS[a)], SNS[b)], Shetlands, The English Channel	4
Cod	*Gadus morhua*	CNS, SNS	2
Coalfish	*Pollachius virens*	CNZ, NNS[c)]	2
Mackerel	*Scomber scombrus*	North Sea, South-west of Ireland, Shetlands	3
Blue Mussels	*Mytilus edulis*	Eastern Scheldt, Western Wadden Sea, Eastern Wadden Sea	3
Eel (farmed)	*Anguilla anguilla*	Wholesale trade	2
Eel (wild)	*Anguilla anguilla*	Nieuwe Merwede, Haringvliet-West and East, Rhine, Lobith, IJssel Lake, Medemblik, Hollands Diep, IJssel, Deventer, Ketel lake, Meuse, Eijsden, Meuse, Keizersveer, Roer, Vlodrop. Noordhollands kanaal, Akersloot, Pr. Margrietkanaal, Suawoude, Waal, Tiel.	14
Flounder	*Platichthys flesus*	Western Scheldt	2
Haddock	*Melanogrammus aeglefinus*	CNS, NNS	2
Plaice	*Pleuronectes platessa*	CNS, SNS	2
Pike-perch	*Sander lucioperca*	Hollands Diep, IJssel Lake	2
Sole	*Solea solea*	CNS, SNS	2
Salmon (farmed)	*Salmo salar*	Wholesale trade	2
		Total number of samples	44

a) Central North Sea.
b) Southern North Sea.
c) Northern North Sea.

were included, as these are important fish in The Netherlands from a consumption perspective. Two popular farmed fish species, eel and salmon, have been included for the same reason. Furthermore, emphasis was placed on eel by sampling a large number of freshwater locations in order to assess the contamination of Dutch fresh waters. Eel has proven to be a valuable indicator of the contamination of Dutch freshwaters with persistent organic pollutants (POP) like polychlorinated biphenyls (PCB) and chlorinated dioxins and furans [16, 17]. Finally, two samples of flounder were obtained from the Western Scheldt to monitor (i) the production of BFR (by a chemical industry) in Terneuzen, The Netherlands, and (ii) the effects of the utilization of BFR in the textile industry in Antwerp and further upstream the river Scheldt in Belgium.

The sample number in Table 1 concerns the number of pooled samples analyzed. Each pooled sample consisted of 16–25 individual fishes (except farmed salmon, which samples contained 7–9 individuals). The majority of locations were sampled between September and December 2003. Sea fish was mostly sampled during surveys of the research vessel Tridens. Remaining samples were obtained directly from anglers, from the auction, or from wholesale traders (farmed fish). Eel was caught between May and June 2003 by electric fishery. After transportation to the laboratory, lengths and weights of the individual fishes were measured. All fishes were within market size. Subsequently, fishes were filleted and equal amounts of filet per fish were pooled. The mussel sample was obtained by taking the whole organism out of the shells after cooking the mussels for 5 min in tap water at 100°C. After rinsing the cooked mussels with water, approx. 100 g mussel meat was pooled. For shrimps, a pooled sample was prepared from approx. 500 g unpeeled and uncooked whole organisms. The pooled samples were homogenized in a Waring Blender and stored at −20°C until analysis. Sampling data, including date of sampling, sampling coordinates, number of individuals per fish, sizes and weights, are given in the Supporting Information.

2.2 Analytical methods and QA/QC

The concentrations of the following BFR were determined: BDE 28, 47, 49, 66, 71, 75, 77, 85, 99, 100, 119, 138, 154 (+BB 153), 183, 190, and 209; HBCD, TBBP-A, and me-TBBP-A. The method for the extraction, clean-up and GC analysis of BDE, HBCD, TBBP-A, and me-TBBP-A is described in detail elsewhere [18]. Briefly, the samples were Soxhlet extracted with hexane/acetone (3:1). The crude extract was treated with acidified water to protonate TBBP-A and thereby force it into the organic extraction solvent. After removal of the aqueous layer, the co-extracted fat and other contaminants were removed by gel-permeation chromatography (GPC). The BFR were separated from other contaminants by silica column chromatography (Merck, Darmstadt, Germany) and the target fraction was treated with concentrated sulfuric acid (Merck) prior to GC/electron capture negative ion (ECNI)-MS analysis (HP-6890 GC and HP5973 MSD, Agilent, USA), monitoring the $[Br]^-$ ions at *m/z* 79 and 81 [18]. BDE 154 is reported as the sum of this BDE and BB 153 as these BFR are not separated on the GC-column used (CP-Sil-8, 50 m, 0.25 mm id, 0.25-μm film thickness; Chrompack, Middelburg, Netherlands). However, given the low production and application volumes of PBB compared to the PBDE [19, 20], it is unlikely that BB 153 will be found at significant concentrations in the analyzed fish samples and will therefore presumably not add significantly to the BDE 154 signal. Deca-BDE (BDE 209) was determined using a shorter column (DB-5, 15 m, 0.25 mm id, 0.25-μm film thickness; J&W Scientific, USA) in order to reduce possible thermal degradation of BDE 209 due to long residence times in the heated GC column [21]. The internal standard (IS) covering the complete method was BDE 116. Furthermore, $^{13}C_{12}$-BDE 209 was added as IS for BDE 209. HBCD was analyzed by both GC/ECNI-MS and LC/ESI (ESI interface)-MS/MS method to enable a method comparison as both methods have their strengths and weaknesses. The GC/ECNI-MS method is very sensitive but provides the HBCD concentration only as a sum, whereas the individual diastereomers (a-, β- and γ-HBCD) can be separated and determined individually by LC/ESI-MS/MS but at the cost of higher LOQ.

The LC separation of the compounds was performed on a Zorbax column (XDB-C18 150 mm × 2.1 mm id, 3.5 μm, Agilent), kept at 20°C, using an ACN (A)-0.01 mM ammoniumchloride (B) gradient. The gradient was programmed as follows: 0–4 min kept 70% A, 4–4.1 min quick ramping to 90% A, 4.1–8 min kept at 90% A and then returning to the initial solvent composition. An LCQ-advantage mass-spectrometer with an ESI (Thermo-Finnigan, USA) was used for detection of the HBCD diastereomers and TBBP-A. The optimized settings were as follows: sheath gas: 46 arbitrary units; capillary spray voltage −4.5 kV; capillary temperature 160°C; capillary voltage −4 V; radio frequency voltage: 500 Vpp and helium was used as collision gas (40% energy for TBBP-A and 20% for HBCD). Initial experiments showed that the $[M\text{-}H]^-$ ion response was too variable for quantitative analysis. A small signal was observed at *m/z* 676.7 being most likely the chlorine adducts of HBCD. By addition of ammonium chloride to the LC-eluens, we forced the formation of the chlorine adducts of the HBCD diastereomers, leading to increased response (and sensitivity). The adduct ions of *m/z* 676.7 $[M+Cl]^- \pm 5$ Da were isolated and fragmented to $[M\text{-}H]^-$ of *m/z* 640.7 (and *m/z* 688.7 and 652.7, respectively for $^{13}C_{12}$-HBCD). For TBBP-A the settings were *m/z* 555 (parent ion) and *m/z* 543 (product ion). LC methods for determination of the individual HBCD diastereomers were recently reviewed by Morris *et al*. [22].

After analysis of the fish extracts by GC/ECNI-MS, the solvent (hexane) was blown down almost to dryness and the

residue was redissolved in methanol for LC analysis. At that stage, ^{13}C-HBCD diastereomers (α-, β- and γ-HBCD) were added to the extract. They could only be added after GC/ECNI-MS analysis of the extracts as otherwise during GC analysis, the $[Br]^-$ originating from the $^{13}C_{12}$-HBCD diastereomers would add to the signal originating from the native HBCD in the sample, making quantification impossible. The $^{13}C_{12}$-HBCD diastereomers were used to correct for the LC-ESI-MS/MS analysis performance only. Possible losses during extraction, clean-up and GC injection and needle wash were corrected using an empirically determined correction factor (multiplying by 1.25). The native standards were obtained from Cambridge Isotope Laboratories (Andover, Canada). The $^{13}C_{12}$-labeled HBCD diastereomers were obtained from Wellington Laboratories (Guelph, Ontario, Canada).

The PBDE, TBBP-A, me-TBBPA and HBCD analyses were performed at the Netherlands Institute for Fisheries Research, accredited under ISO17025 lab no. L097 (see www.RvA.nl). The quality was assured by the analysis of laboratory reference materials, regular duplicate analyze, high numbers of blanks, recovery tests, the use of internal standards, and by an annual participation in interlaboratory studies organized by QUASIMEME [21] with satisfactory results (*i. e.* most results showing z-scores of $<|2|$).

3 Results and discussion

3.1 Concentrations of BDE and HBCD in eel

Table 2 shows the results of the GC/ECNI-MS analysis of eight BFR in the eel samples. This is the selection of BFR recently recommended by EFSA for monitoring [15] and includes the BDE 28, 47, 99, 100, 154 (+BB 153), 183, 209 and total-HBCD (by GC). The complete dataset (all BFR analyzed in this study) can be found in the Supporting Information. The results clearly show that eel samples from various freshwater locations contain high BFR concentrations (up to approx 379 ng/g ww) compared to the other samples. In addition to the main rivers Meuse and Rhine, various other freshwater locations are also contaminated with BFR. In these eel samples, HBCD, BDE 47 and BDE 100 predominate, accounting for on average 95% of the sum of the EFSA selection, while the other PBDE were only found at very low concentrations. HBCD concentrations are higher than any of the BDE concentrations in the eel samples. The absence of BDE 99 in nearly all eel samples (contrary to most other fish samples) suggests a specific elimination/metabolism in eel for this congener. The two analyzed pikeperch samples from the same locations could not confirm this specific phenomenon. In another eel study, PCB 77 and 126 were found to be metabolized by eel [23]. It should be noted that the molecular structure of these PCB are different from BDE 99. Therefore, the exact cause of the low BDE 99 accumulation remains to be determined. The BDE 47 and 99 concentrations in eel are comparable to those reported earlier in eel from the same locations [24]. Levels in large mouth bass and Detroit River two US rivers were lower (2.2 – 18 ng/g ww for the sum of 8 BDE) [25]. Levels in Swiss lake whitefish varied from 1.6 – 7.4 ng/g ww for the sum of 7 BDE [26]. Janak *et al*. determined HBCD diastereomers in 2 eel samples from the Western Scheldt estuary and found α-HBCD to be the dominating diastereomer (1.8 – 7.0 ng/g ww) [27]. Levels of γ-HBCD were 0.5 – 0.8 ng/g ww). These data are in the same range as our observations, although eel from some major Dutch river systems showed considerably higher levels (see Table 2 for total HBCD information and Fig. 1 for diastereomer specific information) [28].

3.2 Concentrations of BDE and HBCD in other fish species

The ΣBFR (EFSA selection as specified earlier) concentrations in the fish species other than eel ranged from <LOQ for all congeners to 16.5 ng/g ww. The six samples with <LOQ values were shrimps from the Wadden Sea, haddock from the northern North Sea, plaice and sole from the central North Sea and sole from the southern North Sea. All these species feed on organisms that are relatively low in the food chain. In addition, they have low fat contents (≤2.1%). Low ΣBFR concentrations were found in other lean marine fish (up to 1.7 ng/g ww for Rijnmond shrimps). These concentrations are lower than those observed in the Belgian part of the North Sea [29]. ΣBFR concentrations in flounder from the Western Scheldt (7.3, 16.5 ng/g ww) were somewhat higher than in various fish fillets from the Western Scheldt by Voorspoels *et al*. [29]. Herring and mackerel showed ΣBFR concentrations of 0.7 – 13.6 ng/g ww. Paepke and Herrmann [30] determined several BDE in herring from the North Sea and North East Atlantic and found concentrations ranging from 6.7 – 14 ng/g lipid weight (lw) for the sum of 11 BDE, which is lower than our findings (when expressed on a wet weight basis). Farmed salmon from Norway did not contain detectable BFR concentrations whereas the Scottish salmon sample showed ΣBFR concentrations of approx. 4 ng/g ww. This is consistent with findings in a study by Hites *et al*. [31]. They found approx. 0.1 to 4 ng/g ww for the sum of 43 BDE [31] in farmed salmon from North-West Europe, with concentrations of salmon from Scotland being among the highest. HBCD concentrations in our herring samples were much lower than those reported by Remberger *et al*. [32] in Swedish fish (21 – 180 ng/g lw). Janak *et al*. [27] determined HBCD diastereomers in various biota samples (shrimp whole body and muscle tissue of bib, plaice, sole and whiting) from the Western Scheldt estuary and found 0.2 – 0.3 ng/g ww for the sum of α- and γ-HBCD (except for sole which contained higher levels of 1.2 – 11 ng/g ww).

BDE 209 was not at all detected in any of the samples except for two mussel samples. This is most likely caused

Table 2. BDE and HBCD concentrations in fish and shellfish samples consumed by Dutch citizens (by GC/ECNI-MS, ng/g ww)

Species	Source area	Lipids (%)	BDE 28	BDE 47	BDE 99	BDE 100	BDE 154 + BB 153	BDE 183	BDE 209	Total HBCD	Sum (EFSA)[a]
Marine											
Mussels	Eastern Scheldt	2.2	<0.1	0.2	<0.1	<0.1	<0.1	<0.1	0.1	0.9	1.2
Mussels	Western Wadden Sea	2.1	<0.1	0.1	0.1	<0.1	<0.1	<0.1	0.8	0.2	1.2
Mussels	Eastern Wadden Sea	2.3	<0.1	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	< 0.1	0.1
Shrimps	Wadden Sea	2.1	<1.0	<0.1	<0.1	<0.1	<0.1	<0.1	<0.7	<0.1	<2.3
Shrimps	Rijnmond	2.2	<0.1	0.7	0.8	<0.1	0.2	<0.1	<0.3	<0.1	1.7
Cod	Central North Sea	0.7	0.2	<0.1	<0.1	0.4	<0.1	<0.1	<0.5	<0.1	0.6
Cod	Southern North Sea	0.9	<0.1	0.4	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.4
Haddock	Central North Sea	0.7	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Haddock	Northern North Sea	0.8	<0.2	<0.1	<0.1	<0.1	<0.1	<0.1	<0.2	<0.1	<1.0
Coalfish	Central North Sea	1	<0.1	0.2	<0.1	0.1	<0.1	<0.1	<0.1	0.2	0.5
Coalfish	Northern North Sea	1.1	<0.1	0.2	<0.1	<0.1	<0.1	<0.1	<0.2	<0.1	0.2
Plaice	Central North Sea	0.9	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	< 0.1	<0.8
Plaice	Southern North Sea	1.3	<0.1	0.2	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.2
Sole	Central North Sea	1.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	< 0.8
Sole	Southern North Sea	1.2	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.8
Herring	Central North Sea	20	0.2	2.8	0.9	0.8	0.2	<0.1	<0.2	2.7	7.6
Herring	Southern North Sea	18	<0.1	3.2	0.9	1.2	<0.1	<0.1	<0.5	<0.1	5.3
Herring	Shetlands	16	0.1	1.8	<0.5	0.5	<0.2	<0.1	<0.7	<1.2	2.4
Herring	The English Channel	14	0.2	3.8	0.9	1.2	0.2	<0.1	<0.5	7.3	13.6
Mackerel	North Sea	17	<0.1	<0.8	0.7	0.1	0.1	<0.1	<0.3	2.1	3.0
Mackerel	South-west of Ireland	13	<0.1	1	0.7	0.2	0.2	<0.1	<1	2	4.1
Mackerel	Shetlands	3.3	<0.1	0.6	<0.3	0.1	<0.1	<0.1	<0.3	<0.5	0.7
Flounder	Western Scheldt (Terneuzen)	1.1	0.2	4.4	0.3	1.1	0.4	<0.1	<0.1	0.9	7.3
Flounder	Western Scheldt	1.7	0.3	11	1.0	2.1	0.7	<0.1	<0.1	1.3	16.4
Dutch freshwater											
Eel	Haringvliet-East	17	0.6	20	1.4	8.1	2.1	0.2	<0.7	70	102.4
Eel	Hollands-Diep	16	0.7	36	<1.3	24	2.8	<0.1	<0.4	150	213.5
Eel	Maas, Eijsden	5.3	<1.9	<4.5	<0.2	2	<0.2	<0.1	<0.5	15	17.0
Eel	Roer, Vlodrop	14	0.3	26	<1.7	11	<0.8	<0.1	<0.5	130	167.3
Eel	Noord-Hollands kanaal, Akersloot	4.1	<0.1	0.4	0.2	0.1	<0.1	<0.1	<0.5	0.7	1.4
Eel	Pr. Margrietkanaal, Suawoude	16	<0.1	<7.4	<7.3	<1.6	0.1	0.1	<4.5	2.5	2.7
Eel	Waal, Tiel	16	0.4	43	<2.6	22	2.3	<0.1	<0.5	210	277.7
Eel	IJssel, Deventer	8.7	<1.9	17	<1.0	7.3	<0.9	<0.1	<0.6	94	118.3
Eel	Ketel lake	21	0.2	<15	<0.7	4.8	1.1	<0.1	<0.5	30	36.1
Eel	Nieuwe Merwede	22	1.7	81	<3.2	61	5.7	<0.1	<0.4	230	379.4
Eel	Maas, Keizersveer	24	0.3	16	<0.6	8.5	2.4	0.1	<0.6	<0.1	27.3
Eel	Haringvliet-West	12	0.1	5.8	<0.6	3.7	0.7	<0.1	<1.0	21	31.3
Eel	Rijn, Lobith	9.8	0.2	21	<1.7	7.6	<0.9	<0.1	<0.7	97	125.8
Eel	IJssel Lake, Medemblik	23	0.4	6.8	0.9	1.7	0.7	<0.1	<0.6	<3.4	10.5
Pike-perch	Hollands Diep	0.9	<0.1	0.5	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.6
Pike-perch	IJssel Lake	1	<0.1	0.3	0.2	0.1	0.1	<0.1	<0.1	<0.1	0.7
Farmed											
Salmon	Fishtrade Norway	12	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.8
Salmon	Fishtrade Schotland	12	0.1	1.6	0.7	0.3	0.1	<0.1	<0.2	1.3	4.1
Eel	Italian Fish Farm	22	<0.1	1.1	0.2	0.2	0.1	<0.1	<0.5	<0.4	1.6
Eel	Dutch Fish farm	36	<0.1	1.3	0.3	0.2	0.2	0.2	<0.6	<0.5	2.2

a) Sum based on selection from EFSA recommendation [14]. Results < LOQ are considered zero and have not been added to the sum.

by the fact that the mussels were analyzed as harvested, without depuration. Sediment particles (known to contain high concentrations of BDE 209, *e.g.* in the Western Scheldt [33]) may have remained in the mussel stomachs, thereby contaminating the meat. Typically, BDE 209 is, if detected at all, only observed at low concentrations in European fish samples [2]. Furthermore, it should be noted that various pitfalls might influence the BDE 209 result [21]. Although we took great care in reducing potential error sources, the BDE 209 results should be treated with care. The BFR patterns vary with type and origin of the samples. In the Western Scheldt flounder, BDE 47 predominates, whereas HBCD is more prominent in some of the North Sea fish samples.

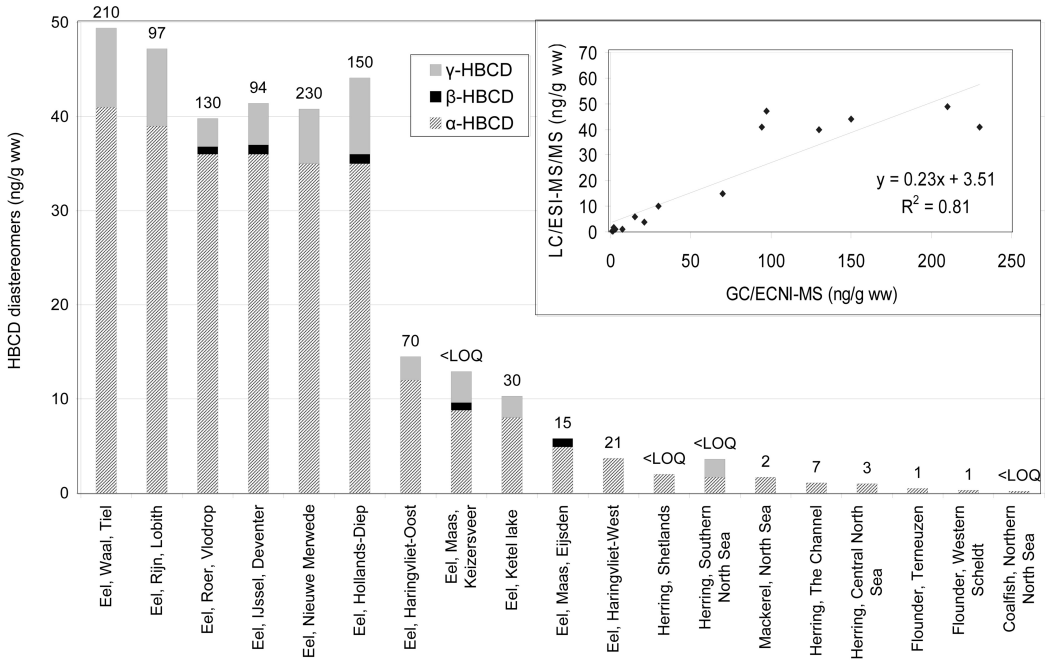

Figure 1. HBCD diastereomers (α, β and γ) profile as determined by LC/ESI-MS/MS. For comparison, the value determined by GC/ECNI-MS is plotted at the top of each bar. Only the fish samples with (at least 1) diastereomer concentrations above LOQ are plotted. The regression plot (inlay) shows the correlation between the GC and LC data.

3.3 TBBP-A and me-TBBP-A

TBBP-A and me-TBBP-A have been analyzed in all samples. In nearly all samples TBBP-A was below the LOQ. Me-TBBP-A was detected in some marine fish, shellfish and farmed fish samples but at concentrations close to the LOQ. Me-TBBP-A concentrations in eel and pikeperch samples range from <0.1 to 1.4 ng/g ww, which is lower than the BDE and HBCD concentrations. The low concentrations of TBBP-A can be explained by its relatively low Log Kow value of 4.5–5.3 [34]. Furthermore, TBBP-A is often applied as flame retardant in printed circuit boards. In this application, TBBP-A is covalently bound to the epoxy resin [34] and, therefore, leaching into the environment during the life-time cycle of the product is less likely. Detailed concentration data are presented in the Supporting Information.

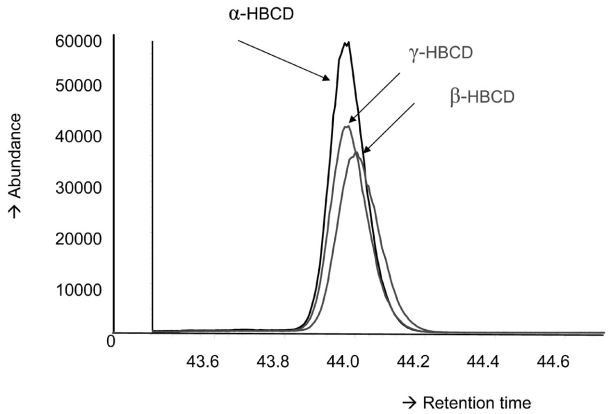

Figure 2. GC-ECNI-MS response of α-, β- and γ-HBCD diastereomers. Injected amounts were 0.53 ng (α-HBCD), 0.54 ng (β-HBCD) and 0.55 ng (γ-HBCD). Relative responses are 100, 71 and 73% for α-, β- and γ-HBCD.

3.4 Evaluation of HBCD concentrations measured by GC and LC

HBCD consists of three diastereomers, α-, β- and γ-HBCD. These diastereomers, were determined by LC/ESI-MS/MS, whereas total-HBCD was measured by GC/ECNI-MS. The LC results show that α-HBCD is the predominant isomer in the samples analyzed, followed by γ- and β-HBCD (Fig. 1). Due to the limited MS sensitivity in combination with the low concentrations, β-HBCD was only detected in a few samples. The GC/ECNI-MS is more sensitive, resulting in less HBCD non-detects. The results in Fig. 1 show a mismatch between total-HBCD determined by GC/ECNI-MS (printed on top of the bars) and the sum of the three diastereomers determined by LC/ESI-MS/MS (indicated by the bars). The GC-based results are on average a factor of 4.4 higher compared to the LC based results (based on the regression curve in Fig. 1). There can be several causes for that phenomenon. On the GC-side, the thermally labile HBCD diastereomers can rearrange above oven temperatures of 160°C [22] and this may considerably affect the

results. Secondly, the ECNI-MS response factors are different for the different diastereomers. We have injected equal amounts of the individual diastereomers and found that the response for α-, β- and γ-HBCD were 100, 71 and 73%, respectively (see Fig. 2). This phenomenon is relevant as the diastereomer profile in our biological samples (where α-HBCD predominates) is different from the profile in our standard solution (equal concentrations of α-, β- and γ-HBCD). As a result, the GC/ECNI-MS results may have been altered by 10 – 20%, causing biased results. It is therefore recommended, when doing GC analysis, to match the diastereomer profile in the sample with the profile in the standard. On the LC-side, the issue of different response factors is not relevant because the diastereomers are separated chromatographically. However, signal suppression may occur in the ESI due to co-eluting matrix constituents and in case of high HBCD concentrations. These effects were found insignificant by Dodder *et al*. [35] although Tomy *et al*. [36] did report on occurring matrix effects in LC/MS. In the present study, compensation for matrix effects was achieved by using a $^{13}C_{12}$-labeled HBCD IS for each diastereomer. In addition, the samples have been submitted to a very thorough clean up, which strongly reduces the chance of matrix effects in the MS. For these reasons, we regard the LC results to be the most accurate ones. Obviously, the additional information from the diastereomer profile information is beneficial and crucial when assessing the fate and behavior of these chemicals. Covaci and coworkers [37] recently presented a comparison of LC and GC techniques used for analyzing HBCD in eel samples from the Scheldt basin. He found a <2-fold difference. This may be due to the fact that the levels in eel were very well detectable (400 – 1400 ng/g w/w). Also, due to the high levels, the molecular ion of HBCD could be used for GC-MS quantification, which enabled the use of $^{13}C_{12}$-labeled α-HBCD for correcting the GC results. Most likely this considerably improved the quantification by GC-ECNI-MS. Still, a 2-fold difference requires further study on the causes of it.

Haug *et al*. [38] reported on a comparison of GC-MS and LC-MS results from an interlaboratory study. The test materials provided to the laboratories were a Baltic Sea herring and a cod liver oil sample. They also observed that GC-MS results were higher than the LC-MS results, although less pronounced. Median HBCD results were 27% higher for the herring sample and 11% higher for the cod liver oil sample. These findings and our study results cannot fully explain the factor 4.4 difference between the LC- and GC-based results. This shows the need for further investigations into the differences between GC- and LC-MS results.

HBCD profiles in fish are different from those observed in sediment and in technical HBCD. In fish, α-HBCD is the major diastereomer as shown above. In sediments, however, β-HBCD can reach comparable concentrations to γ-HBCD and in technical HBCD, γ-HBCD is the predominant diastereomer [3]. The processes causing these differences in isomer composition are not fully understood yet. However, in a laboratory experiment, bio-isomerization was found to occur in juvenile trout (β to α and γ to α) [39]. α-HBCD showed the highest biomagnification factor [39]. These findings may be a reason for the predominance of α-HBCD in fish.

3.5 Estimates of dietary exposure to HBCD from fish consumption

Virtually no information is available on dietary human exposure to HBCD, although studies reporting HBCD in human blood and breast milk [3] show that HBCD can enter the human body. We have therefore made an estimation of the fish-related dietary exposure of the average Dutch citizen. This estimation is based on HBCD data from the present study combined with consumption data from the Dutch National Food Consumption survey (DNFCS) of 1997/1998 [40]. The DNCSF contains files of 6250 people in the age of 1 to 90 years, who recorded the food they consumed during two consecutive days in a food diary. The HBCD exposure calculation is based on the daily fish consumption multiplied by the average lower bound concentrations (per species) is in $DE = \Sigma(Qa \cdot Ca + Qb \cdot Cb + \ldots Qz \cdot Cz)$, in which DE = Daily Exposure, Qa = Quantity of daily consumption of fish species a and Ca = average lower bound concentration of HBCD in fish species a (measured in this study). The HBCD data used originated from the LC/ESI-MS method (sum of α-, β- and γ-HBCD diastereomers), but for four species, coalfish, mackerel, mussels and farmed salmon, we have used the GC/ECNI-MS data available because the LC data of all diastereomers was <LOQ (due to a low sensitivity as discussed earlier). These GC/ECNI-MS data were divided by a factor 4.4 to account for the difference between GC/ECNI-MS and LC/ESI-MS/MS results, as discussed earlier. Concentrations per fish species were averaged. Results of HBCD diastereomers that were <LOQ were assumed to be zero ("lower bound" approach, LB), 0.5 × LOQ ("medium bound", MB) and equal to LOQ ("upper bound", UB). The fish samples included in this study covered 88% of the Dutch daily fish intake, thereby representing very well the fish consumed by Dutch citizens.

The results are shown in Table 3. The average Dutch exposure varied from 4.3 – 12 ng/day from LB to UB (0.06 – 0.17 ng/kg bw/day for a 70-kg individual). Figure 3 shows the contribution of the various fish species to the exposure. This figure shows that herring is responsible for approx. 65% of the exposure (MB), followed by cod, farmed salmon and mackerel. Although high BFR concentrations were found in wild eel, this species hardly contributes to the exposure, as the consumption of eel is low. Furthermore, approx. 95% of the consumed eel consists of farmed eel, and the HBCD concentrations in both farmed eel samples were <LOQ. There is a considerable difference

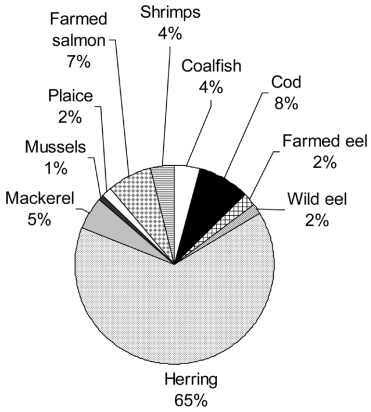

Figure 3. Relative contribution of various fish species to the exposure of Dutch consumers to α-, β- and γ-HBCD (determined by LC/ESI-MS/MS) For coalfish, mackerel, mussels and farmed salmon samples, corrected GC/ECNI-MS data (see text for explanation) was used in case all diastereomers determined by LC were < LOQ. In other cases, diastereomer values < LOQ were considered 0.5 × LOQ.

Table 3. Average dietary exposure of Dutch population to HBCD resulting from fish consumption

Species	Average dietary exposure (ng/day)		
	Lower bound (< LOQ = 0)	Medium bound (< LOQ = 0.5 × LOQ)	Upper bound (< LOQ = LOQ)
Herring	3.5	5.4	7.2
Cod	0	0.69	1.4
Farmed salmon	0.16	0.61	1.1
Mackerel	0.20	0.44	0.59
Shrimps	0	0.33	0.65
Coalfish	0.22	0.35	0.48
Farmed eel	0	0.17	0.27
Wild eel	0.14	0.14	0.14
Mussels	0.04	0.08	0.11
Plaice	0	0.13	0.25
Total	**4.3**	**8.3**	**12.2**

between the LB and UB result (factor 2.8). This is mainly caused by the low levels in some popular fish (*e.g.* cod, coalfish and farmed salmon) resulting in a large number of < LOQ values. By increasing the sensitivity of the analytical method, this situation can be improved. Lind *et al*. [13] found mean dietary exposures of 2.5 ng/kg bw/day for Swedish females (age 17 – 74 years). About 1.8 ng/kg bw/day could be attributed to fish consumption, being considerably higher than our observation. Presumably, this is due to a combination of higher HBCD concentrations in their fish samples (Remberger *et al*. [32] found HBCD levels of 21 – 180 ng/g lw in Swedish herring samples), combined with a higher daily intake of fish (although the authors did not report the underlying data). De Bakker *et al*. [12] determined the dietary exposure for BDE for Dutch citizens, in which they used the BDE dataset of the fish samples in this study. They found a long term median dietary exposure of 0.79 ng/kg bw/day (MB) for the sum of BDE 47, 99, 100, 153 and 154. Fish accounted for 28%, being 0.22 ng/kg bw/day, which is higher than the 0.12 ng/kg bw/day that we found for HBCD (MB in both studies). It should be noted that in this study, we calculated the average exposure based in a 2-day average fish consumption pattern, whereas de Bakker *et al*. used different (statistical) approaches to determine the long term dietary exposure for a wide range of food commodities.

From a 28-days endocrine effects toxicity study with Wistar rats a benchmark dose (based on 10% thyroid weight) of 1.6 mg/kg bw/day was derived for HBCD [41]. Germer *et al*. [42] found significant induction of drug metabolizing enzymes in female Wistar rats in a 28-day oral exposure study at a concentration of 3.0 mg/kg bw and higher (technical HBCD mixture). In both cases, the effect level is approx. 10 000 000 times higher than the intake calculated in this study, suggesting a large margin of safety.

The above estimation is only a first attempt towards human risk characterization due to dietary exposure to HBCD in fish. Apart from fish, other sources will contribute to the human exposure as well, including other food commodities (*e.g.* dairy and meat products), exposure through dust and air [3], and dermal exposure. The increasing environmental HBCD concentrations call for more efforts on this contaminant, preferably by evaluating the individual diastereomers from an exposure and toxicity point of view.

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