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Research Article

Probabilistic intake assessment of polybrominated diphenyl ethers and omega-3 fatty acids through fish consumption

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Food intake is one of the principal exposure routes of polybrominated diphenyl ethers (PBDEs) in humans. This study focuses on fish consumption as a PBDE exposure route. A probabilistic intake assessment of PBDEs and healthy long chain omega-3 PUFAs (LC n-3 PUFAs) was conducted for Belgian fish consumers in order to study the balance of the intake of LC n-3 PUFAs and PBDEs. Based on the observed fish consumption level in the sample, the mean intake of brominated diphenyl ether (BDE)-28, 47, 99, 100, 153, and 154 via fish was 0.85 ng/kg body weight (bw)/day and the intake of LC n-3 PUFAs was 3.45 mg/kg bw/day, being low compared to the recommendations. Scenario analyses showed that consuming 150 g salmon twice a week is advisable to achieve the recommended LC n-3 PUFA intake with a rather low PBDE intake. When replacing 150 g salmon by herring, the PBDE intake is higher without an increase in LC n-3 PUFAs. In contrast, the combination of cod and salmon leads to a similar PBDE intake compared to twice a week salmon, but to a lower LC n-3 PUFA intake. In conclusion, the methodology presented in the paper allows balancing benefits and risks related to fish consumption.

Keywords: Dietary intake / Fish consumption / Omega-3 fatty acids / Polybrominated diphenyl ethers / Probabilistic

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1 Introduction

Since the seventies, several industries started to add flame retardants (FRs) to an increasing number of consumer products. Polybrominated diphenyl ethers (PBDEs) belong to the group of halogenated organic FRs. There are 209 possible PBDE isomers divided in ten congener groups differing on the level of bromination (mono- to deca-brominated). PBDEs are commercially produced as three products: penta-, octa-, and deca-products, serving as FRs in a wide variety of durable industrial and consumer goods, such as computers, curtains, or paints [1–5].

Despite their benefit of being fire resistant, PBDEs have important negative characteristics: they are highly lipophilic, persistent in the environment, and bioaccumulating in the human food chain, increasing in concentration at each successively higher trophic level; with less brominated congeners demonstrating higher affinity for lipids and being more bioaccumulative in animal and human adipose tissue [4–6]. Since August 2004, the EU and Norway have banned the marketing of consumer products containing more than 0.1% penta- or octa-brominated diphenyl ether (BDE)-products (Council Directive 76/769/EEC; [4, 7, 8]), because these products are related to liver and neurodevelopmental toxicity and have an impact on thyroid hormone

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Abbreviations: BDE, brominated diphenyl ether; **bw,** body weight; **DHA,** docosahexaenoic acid; **EPA,** eicosapentaenoic acid; **FR(s),** flame retardant(s); **LC n-3 PUFAs,** long-chain omega-3 PUFAs; **LOAEL,** lowest observed adverse effect level; **PBDE(s),** polybrominated diphenyl ether(s); **Σ6BDE**, sum of BDE-28, 47, 99, 100, 153, and 154 levels [7–9]. Schecter *et al.* reported that also in the United States, the penta-BDE and octa-BDE-products are no longer produced or sold [10]. In contrast to the lower brominated congeners, current toxicological evidence indicates that human exposure to deca-BDE is not expected to lead to negative health effects [11].

Although they are currently banned, exposure to penta- and octa-BDE present in products sold prior to August 2004 will continue during the coming decades [12, 13]. Moreover, since the worldwide use of PBDEs, these contaminants are now ubiquitous in the natural environment and are of concern because of their persistence. The FR industry argues that the benefits accrued through saving lives by fire prevention outweigh the costs incurred by any medical consequences. However, Siddiqi *et al.* indicate that over time this cost/benefit ratio is likely to shift [4].

Foods are one of the principal exposure routes of PBDEs in adults, especially food with high fat content like fatty fish [6, 10, 14–16], apart from exposure in the indoor environment, *e.g.* inhalation of indoor air. This study focuses on fish as PBDE dietary exposure route because of the wide distribution of PBDEs in these food items and because fish has been the most frequent food group to be studied for PBDE contamination [17].

An intake assessment of PBDEs was executed for Belgian fish consumers focussing on fish as exposure route, being the richest natural dietary source of long-chain omega-3 PUFAs (LC n-3 PUFAs), in particular eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3). These omega-3 fatty acids are related to several beneficial health aspects, which is among the main reasons for which the American Heart Association recommended eating seafood (particularly fatty fish) at least two times a week [18]. The Belgian Health Council formulated the advice to eat fish once to twice a week [19]. The intake of EPA plus DHA was assessed simultaneously and the balance between the intake of LC n-3 PUFAs and PBDEs was calculated, compared, and evaluated for different consumption scenarios. A probabilistic approach was applied for the intake assessment, which essentially represents the complexity of real situations by taking into account the variability of the consumption, body weight (bw), and concentration data. To the authors' knowledge, this is the first paper documenting a simultaneous intake assessment of PBDEs and omega-3 fatty acids from fish.

2 Materials and methods

2.1 Simulation model and probabilistic methodology

The intake was calculated using the following model, combining fish consumption data with contaminant and nutrient concentrations (Eq. 1):

$$Y_i = \frac{\sum_v \sum_t (X_{v,i,t} \cdot C_v)}{T \cdot bw_i} \qquad (1)$$

where Y_i = average daily intake of subject i; $X_{v,i,t}$ = amount (g) of fish v consumed by subject i (with bw_i), at day t ($t = 1,\ldots,T$); and C_v = contaminant or nutrient concentration in fish species v. The variability of the intakes was estimated using a probabilistic approach, with a one-dimensional Monte Carlo simulation (implemented in a software module called ProbIntake[UG], applicable in the software program R® [20]), taking into account the variability of food consumption, bw, and nutrient and contaminant concentration data. The simulation procedure for each individual works as follows: each single consumption data point is multiplied with a concentration data point. And this combination is conducted for all consumed fish species and all different compounds. Next, the assessed intakes per compound were enumerated and this sum was divided by the number of days and the individual's bw. Finally, this procedure was repeated for all individuals. As an extensive consumption database was at our disposal, the inter-individual variability of the consumption data was taken into account in a non-parametric way, *i. e.* using all the individual data as such and without assuming any underlying probability model. As for some species the number of available concentration data for a certain contaminant or nutrient was limited, the inter-species and spatial variability of the nutrient and contaminant concentrations in fish were taken into account in a parametric way, *i. e.* by applying and assuming probability distributions. The latter procedure was also applied for the third parameter, *i. e.* the bw.

2.2 Consumption and bw data

Four different consumption scenarios were evaluated. In the first scenario, the current fish consumption pattern of Belgian fish consumers was used. These consumption data were collected between November and December 2004 by means of a food frequency questionnaire, as part of the pan-European SEAFOODplus consumer survey [21, 22]. The data were collected from a sample of 852 Belgian fish consumers aged between 19 and 83 years, representative for the Belgian population with respect to age and region. In total, 821 of them (202 men and 619 women) provided all information needed for the intake assessment (consumption data, age, and gender). Despite the requirement that the participants of the survey had to be fish consumers, 52 of the respondents answered not to consume any fish. The mean calculated fish consumption was 215.5 ± 203.5 g/week. Table 1 shows the average amount consumed for each species and the relative importance of the different species. In the second, third, and fourth scenario, it was assumed that the whole adult population consumed fish twice a week. In

Table 1. The different seafood species in the Belgian fish consumers consumption dataset: mean consumption and number of available species- and congener-specific data points

Species	Mean consumption (g/week;%)	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154
Cod[a)]	46.6 (21.6)	11	15	15	15	10	8
Salmon	40.5 (18.8)	28	43	43	43	41	41
Tuna	29.4 (13.6)	4	5	4	5	4	4
Saithe[a)]	24.9 (11.5)	11	15	15	15	10	8
Sole	22.0 (10.2)	9	9	9	9	7	7
European plaice	12.9 (6.0)	9	9	9	9	7	7
Herring	12.2 (5.6)	9	9	9	9	5	5
Trout	11.8 (5.5)	7	8	8	8	8	8
Mackerel	10.8 (5.0)	6	7	7	7	4	4
Eel	4.4 (2.0)	7	8	8	7	5	5
Total	215.5 (100.0)						

a) The BDE congener data were pooled in one group ‚lean fish' together with available congener data of whiting assuming that the contamination of these species is similar

the second scenario, consumers were assumed to eat once 150 g cod, which is a lean fish, and once 150 g salmon, which is a fatty fish. These species were the most frequently eaten lean and fatty fish (Table 1). In the third scenario, consumers were assumed to eat twice a week 150 g of the same fatty fish, salmon. In the fourth scenario, they were assumed to consume 150 g salmon and 150 g herring, both fatty fish.

For the purpose of optimising integration of the variability in contaminant and nutrient concentrations in the probabilistic intake assessment simulations, the number of recording days per individual was extended. This was done by extending the fish consumers' consumption data known per week to ten weeks per adult (by repeating the data ten times), assuming that they had every week the same fish consumption pattern. As a result, a good convergence of the population intake estimates was reached. A larger extension would increase the simulation processing time, without significantly improving the intake estimations.

Information about the bw of the Belgian fish consumers was needed in order to be able to express the contaminant intake per kg bw. Therefore, normal bw distributions were applied per age interval and for the two sexes, based on available data of the Belgian population (B.I.R.N.H study [23, 24]). The mean and SD of these normal distributions are given in Table 2. No correlation between the bw and the fish consumption was taken into account, as this was not found in a consumption database of Belgian adolescent for which the individual bw was known (Pearson correlation between bw and seafood consumption for the consumers-only: $r = 0.037$).

Table 2. Mean and SD of the applied bw distributions

Age interval (years)	*Men*		*Women*	
	Mean (kg)	S.D.	Mean (kg)	S.D.
<30	75.5	10.7	60.1	9.6
30–39	77.2	11.2	62.7	10.9
40–49	78.9	11.5	66.7	11.7
50–59	77.4	11.4	69.5	11.2
60–69	75.3	12.3	69.5	11.9
≥70	73.1	10.6	66.1	11.0

2.3 Concentration data and distributions

For both the contaminants and the nutrients, the intake assessment is not based on own analytical data in fish but on previously published concentration data, which is in accordance with the strategy recently proposed by Brüders *et al.* [25], stating that existing data should be used in the most effective and resource efficient way. The PBDE database was built on the basis of publicly available contamination data and data received from personal communication from scientists executing PBDE analyses in fish. An Excel®-database has been constituted with regard to the PBDE concentrations in fish. In the database, all relevant information was included: commercial name, scientific name, period of capture, fat content of the fish, and (mean) contaminant content, if available with extra statistical data (standard deviation, minimum, and maximum). Only concentration data of studies executed after 2000 were included. The concentration data were completed per species and per individual PBDE congener. If concentrations were below the LOQ, they were replaced by half of the LOQ. The construction of this database was based on a previously developed method [26]. In the end, the database contained relevant concentration data from 14 different international sources [10, 27–38] (de Boer, van Leeuwen, personal communication). The collected data were filtered to select those species relevant for the Belgian market (Table 1). For these species, sufficient data were available on BDE-28, 47, 99, 100, 153, and 154 to perform a probabilistic intake assessment. The intake assessment has conse-

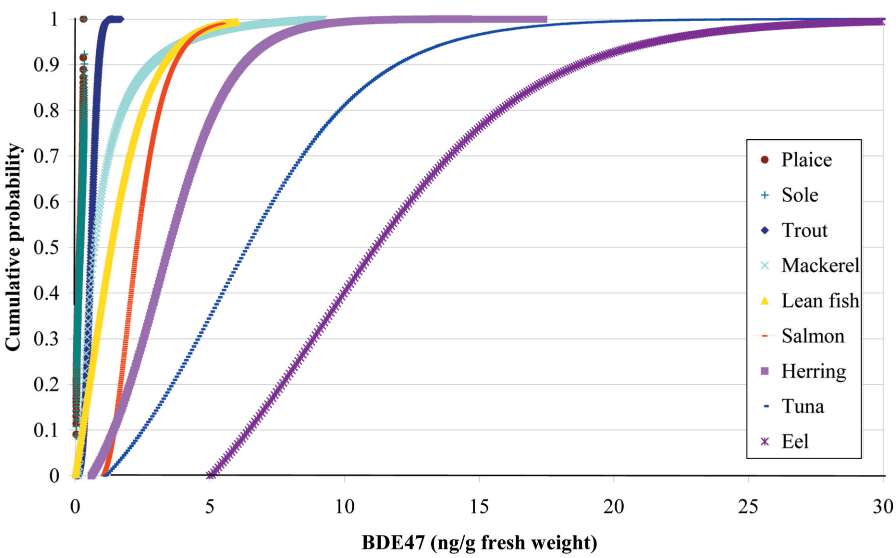

Figure 1. Cumulative probability distributions of BDE47 in ten different fish species

quently focussed on these six PBDE congeners only. Finally, the sum of the intake of these congeners was calculated by summing the intake of the individual congeners per person (further referred to as Σ6BDE). Only for cod and saithe, the available PBDE concentration data were very limited and, therefore, pooled together with whiting in a group called ‚lean fish', assuming that the contamination level of these species is similar. The number of data points per species and per congener is given in Table 1. In the probabilistic approach, the variability of the contaminant and nutrient concentrations in the fish species was taken into account by using appropriate probability distributions. For this, species and congener specific distributions were fitted to the concentration data, using @Risk, Pallisade Corporation (Newfield, NY, version 4.5). The construction of the EPA plus DHA database and distributions has been described by Sioen *et al.* [39].

2.4 Statistical analyses

An independent samples *t*-test was used to compare the intake levels for PBDEs and EPA plus DHA for male versus female fish consumers (p-value < 0.01). Statistical analyses were done with the SPSS software version 12.0 (SPSS, Chicago, IL, USA).

3 Results

Figure 1 shows the probability distributions of the concentration of one of the congeners, *i.e.* BDE-47, in the different fish species of interest. This congener was selected as an example because it occurred at the highest levels in fish as compared to other congeners [6, 36, 40, 41]. The distributions indicate that large inter- and intraspecies differences exist, which can partly be explained by the species' different fat percentage, age, and environmental conditions. The highest BDE-47 concentrations were found in eel, tuna, and herring. No systematic and significant correlations were found between the different BDE-congeners within the species, being supported by the statement in the review of Law *et al.* that the ratio of different congeners was location- and species-dependent, possibly related to differences in metabolism [42]. Therefore, the intakes of the different congeners were assessed without taking into account any correlation between the concentrations of the different congeners.

The results of the probabilistic intake assessment for the different BDE-congeners as well as for EPA plus DHA are summarised in Table 3. No significant differences are found between the PBDE and EPA plus DHA intake of male and female fish consumers in the first scenario (p-values varying between 0.011 for BDE-99 to 0.980 for EPA plus DHA). The mean intake of Σ6BDE is lowest for scenario 1, using the fish consumption data as measured in the SEAFOODplus consumer survey. However, for a small group of heavy fish consumers, the Σ6BDE intake from scenario 1 exceeds the intakes resulting from scenario 2, 3, and 4. The variation in the intakes within scenario 1 is due to variation in the fish consumption pattern (*i.e.* consumption frequency and species variation between individuals) and bw, as well as to variation in the nutrient and contaminant concentrations in different fish species. In contrast, in the other three scenarios the variation in the intakes is only due to variation in the concentration levels and in the bws. The difference in Σ6BDE intake depending on whether one consumes weekly, a portion of cod and a portion of salmon (scenario 2), compared to consuming salmon twice a week (scenario 3) is small. In contrast, weekly consumption of 150 g herring plus 150 g salmon (scenario 4) clearly leads to considerably higher Σ6BDE intakes. On the other hand, the mean EPA plus DHA intake is highest in scenario 3 (twice a week salmon) and lowest in the current fish consumption sce-

Table 3. Summary of assessed intakes of PBDEs and EPA and DHA through fish consumption for four different consumption scenarios

	BDE28	BDE47	BDE99	BDE100 ng/kg bw/day	BDE153	BDE154	Σ6BDE[a)]	EPA&DHA mg/kg bw/day
Scenario 1: observed consumption in Belgian fish consumer sample								
Mean	0.02	0.56	0.09	0.12	0.02	0.02	0.85	3.54
P50	0.02	0.39	0.06	0.08	0.01	0.01	0.59	2.57
P75	0.03	0.74	0.12	0.16	0.03	0.03	1.12	4.86
P95	0.07	1.76	0.29	0.39	0.07	0.07	2.62	10.05
P99	0.12	2.87	0.48	0.66	0.13	0.12	4.26	18.88
Scenario 2: 150 g cod plus 150 g salmon								
Mean	0.04	0.86	0.13	0.17	0.03	0.03	1.27	6.87
P50	0.04	0.85	0.13	0.17	0.03	0.03	1.26	6.85
P75	0.05	0.95	0.15	0.19	0.03	0.03	1.36	7.39
P95	0.06	1.10	0.17	0.23	0.04	0.04	1.53	8.17
P99	0.07	1.24	0.19	0.26	0.04	0.04	1.66	8.73
Scenario 3: twice 150 g salmon								
Mean	0.06	0.80	0.19	0.16	0.03	0.05	1.28	11.86
P50	0.05	0.79	0.18	0.16	0.03	0.05	1.27	11.82
P75	0.06	0.87	0.20	0.18	0.03	0.06	1.36	12.56
P95	0.07	1.01	0.23	0.21	0.03	0.06	1.50	13.67
P99	0.08	1.14	0.25	0.24	0.04	0.07	1.63	14.52
Scenario 4: 150 g herring and 150 g salmon								
Mean	0.07	1.46	0.39	0.35	0.04	0.07	2.39	9.61
P50	0.07	1.45	0.39	0.34	0.04	0.07	2.38	9.57
P75	0.08	1.59	0.43	0.40	0.05	0.08	2.53	10.15
P95	0.09	1.82	0.50	0.49	0.05	0.09	2.80	11.01
P99	0.10	1.98	0.56	0.56	0.06	0.09	2.99	11.62

a) Σ6BDE This sum was obtained by summating the intake of the individual congeners per person

nario (scenario 1) based on claimed consumption of Belgian fish consumers.

Figure 2 shows the ratio of the intake of EPA plus DHA over the intake of the different PBDE-congeners for all scenarios. This figure clearly indicates that the highest ratio is reached when 150 g of salmon is consumed twice a week. The intake of EPA plus DHA is almost as high for scenario 4 (herring plus salmon) as for scenario 3 (twice salmon). But as herring is more contaminated with PBDEs in comparison to salmon, scenario 4 leads to a less beneficial ratio.

4 Discussion

The results showed that the PBDE concentration within and between species vary a lot (Fig. 1). An attempt was made to compare the EPA plus DHA and the PBDE intake for different fish consumption patterns, in order to reach the recommended EPA plus DHA intake, which is currently too low for the Belgian population [43, 44], with a PBDE intake as low as possible (Fig. 2). To evaluate the EPA plus DHA intake, an "*ad hoc*" reference value was calculated for the population under study based on actual population data starting from the dietary reference intakes formulated by the Belgian Health Council [45]. For EPA plus DHA, the dietry reference intake is 0.3% of the total energy intake per day. Applying a mean bw of 70 kg and a mean energy intake of 2046 kcal, based on the data of the most recent Belgian Food Consumption Survey [46], this leads to a reference value for EPA plus DHA of 681 mg/day or 9.7 mg/kg bw/day. Dividing the reference value for EPA plus DHA by the bw was relevant in this study in order to express the intake of nutrients and contaminants on the same scale. Table 3 shows that the current fish consumption is too low to achieve the recommendation, when considering seafood as the only dietary source of EPA and DHA. Consumption of a portion of cod and salmon once a week led to an equivalent PBDE intake compared to consumption of two portions of salmon a week, but the latter led to a much higher EPA plus DHA intake. The consumption of one portion of cod and one portion of salmon a week was not sufficient to achieve an intake of 9.7 mg/kg bw/day. In contrast, consuming fatty fish twice a week (in this study, salmon and/or herring) led to a sufficient EPA plus DHA intake (Table 3). Nevertheless, replacing one portion of salmon by herring simultaneously led to a higher PBDE intake.

Some limitations of this study have to be discussed. First, the approach of combining data from different sources and

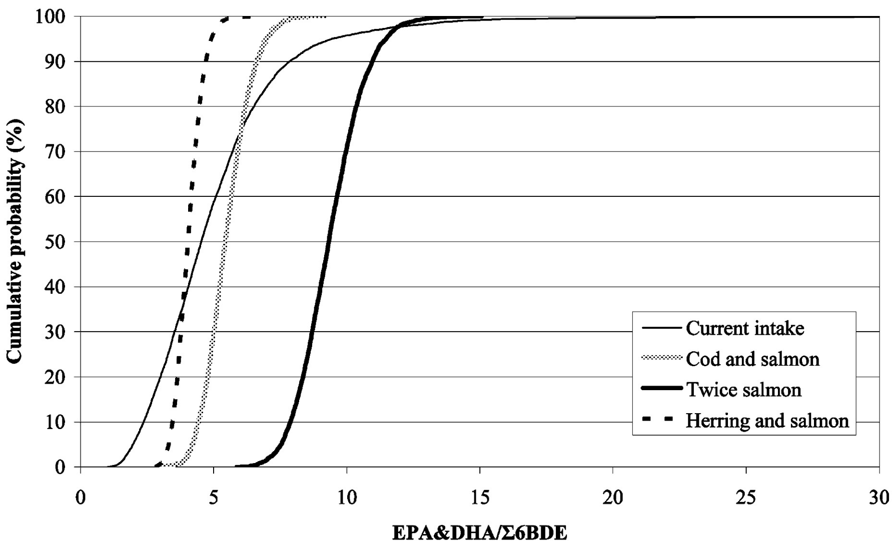

Figure 2. Cumulative probability of the EPA&DHA/Σ6BDE ratio for the four different scenarios

subsequently using them for an intake assessment is for some researchers still equivocal. Nevertheless, it allows to optimally use and benefit from the results available in international literature and to increase the number of data with which to calculate. Moreover, when one should elaborate a sampling plan to gather representative PBDE concentrations in fish on the Belgian market, a very extensive campaign should be set up since fish on the Belgian market is very distinct in species, fishing ground, country of import, and production method. However, some problems were encountered when bringing together data of different sources. These were previously described in a paper about the set up of a large database related to mercury, PCBs, and dioxin-like compounds in seafood [26]. In particular to PBDE concentrations, we encountered the problem that different congeners were analysed over the different studies, and further on used to calculate the overall sum of the PBDE concentrations. This hampered the comparability of the ΣBDE from different studies. Some research recently expressed the need for a common series of BDE congeners to be analysed in all different investigations [16, 42].

Second, the consumption data used in the first scenario were not based on a random sample of the Belgian population, but merely on a random sample of Belgian fish consumers. Therefore, the intake assessment results obtained for scenario 1 are valid for the subpopulation that regularly consumes fish, but not for the overall Belgian population. For reason of comparison, the results of the most recent Belgian Food Consumption Survey [46] indicated a mean consumption of fish and shellfish equal to 168 g/week, while the mean consumption in our sample of fish consumers was 215.5 g/week. Furthermore, the sample contained more women than men. However, a statistical test showed that there was no significant difference in intake levels between men and women, so the inequal gender distribution in the sample is not a major source of bias with respect to the final result. Finally, it is important to stress once more that in this study, only fish was considered while other food groups that potentially contribute to either PBDEs or EPA plus DHA intake were not taken into account.

Third, in contrast to the EPA plus DHA intake, the risks related to the assessed PBDE intake could not be evaluated as no data on the tolerable intake have been reported. Nevertheless, PBDEs have potential toxic effects in humans [6, 9]. Generally, the penta-BDEs seem to cause adverse effects at the comparably lowest dose, whereas much higher doses were needed for effects of the deca-BDEs [4, 9]. The critical effects of penta-BDEs are those on neurobehavioural development (from 0.6 mg/kg bw = lowest observed adverse effect level (LOAEL)) and, at somewhat higher dose, thyroid hormone levels in rats and mice. The critical effects of octa-BDEs are on fetal toxicity/teratogenicity in rats and rabbits (from 2 mg/kg bw = LOAEL), and of deca-BDEs on thyroid, liver and kidney morphology in adult animals (from 80 mg/kg bw = LOAEL). Carcinogenicity studies, only performed for deca-BDEs, show some effects at very high levels [9]. Since these LOAELs are expressed per BDE-mixture it was not possible to use them to calculate a margin of exposure based on the intake data that was calculated per individual congener.

The scenarios presented in this study to reduce the intake of PBDEs are only fictive solutions given the current contamination of the environment and in particular the marine food chain. However, a more global approach is needed to reduce the exposure of humans to PBDEs. Fulfilling the regulations related to the ban on marketing of articles or consumer products containing more than 0.1% penta- or octa-BDE-products (Council Directive 76/769/EEC) is therefore crucial to reduce the contamination of the environment with PBDEs.

Related to this study, it is also important to note that for the intake of PUFAs and FRs long term exposure is relevant and that recently remarks have been made about using probabilistic modelling when assessing intakes on a long term basis. Indeed, the variability of nutrient and contaminant concentrations returns to a mean value when calculating the intake over a long term period. Despite this, it remains of crucial importance to characterise the variability in order to be able to make a good and representative assessment of the mean intake (taking into account seasonal variability, temporal variability, and geographical variability). Therefore, it was decided in this study to take the variability into account and to perform the analyses in a probabilistic way.

For comparison, Voorspoels *et al.* [16] executed PBDE analyses on Belgian fish samples and used the results to elaborate a rough intake assessment starting from theoretical estimates on the average daily food consumption and not from the results of a food consumption survey. The estimated intake ranged between 23 and 48 ng/day of total PBDEs (*i.e.* BDEs 28, 47, 99, 100, 153, 154, and 183). They found a contribution of fish of around 40% (9.2–19.2 ng/day), and using a mean bw of 70 kg, the resulting intake was 0.13–0.27 ng/kg bw/day. This value is low when compared to the mean intake assessed in this study using the current fish consumption data of Belgian fish consumers (0.85 ng/kg bw/day). Nevertheless, Voorspoels *et al.* used an estimated daily consumption of fish of 30 g, which matches the mean fish consumption of the population group used in scenario 1 (215 g/week). In contrast, the concentrations measured in fish by Voorspoels *et al.* were lower than the concentrations used in this study [16]. In Finland, total dietary intake of PBDEs by adults was estimated to be 0.57 ng/kg bw/day [47], in Sweden 0.69 ng/kg bw.day [48], in Spain 1.39 ng/kg bw/day [15], and 1.72 ng/kg bw/day in the Netherlands [49]. Domingo *et al.* executed a deterministic intake assessment of PBDEs via seafood only and assessed an intake equal to 0.30 ng/kg bw/day [50], being lower than the mean PBDE intake assessed for Belgian fish consumers based on their current fish consumption.

In conclusion, the simulation results showed that the recommendation to consume fish twice a week would help the population to achieve the recommendation for EPA and DHA. Moreover, consuming regularly fatty fish will not lead to extremely high intakes of PBDEs.

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