Factors influencing risk assessments of brominated flame-retardants; evidence based on seafood from the North East Atlantic Ocean

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ABSTRACT

Brominated flame-retardants (BFRs) such as polybrominated diphenyl ethers (PBDE) and hexabromocyclododecane (HBCD) are considered hazardous to human health. Due to their persistence, they are still present in the environment and in biota and seafood is major contributor of BFRs to human exposure. Here, we used data from > 9700 samples of wild and farmed fish, fish feed and fish feed ingredients collected from the North Atlantic between 2006 and 2016 aiming to investigate factors influencing the risk assessments of BFRs.

Due to most representative number of analyses, PBDEs were the main focus of investigation. Mean ΣPBDE in fillet samples ranged from below quantification in Atlantic cod fillet to 2.0 μg kg^{-1} in Atlantic halibut. The main congener contributing to the ΣPBDE in all species was BDE 47. Factors affecting the level of BFR in seafood were multifaceted, and the levels were within species mainly determined by fish age, geographical origin and time of sampling. BDE 47, 99, 153 and HBCD were selected for margin of exposure (MOE) evaluation. When other sources of BFR than seafood were excluded, our risk assessment showed low risk at the current dietary intake of seafood. However, the dietary intake of BDE 99 may be of concern for toddlers when all sources are considered. The choice of fish species, dietary studies, choice of statistics, as well as exposure from other sources than seafood, were all factors that influenced the final MOE of BFRs. We propose the use of regression on order statistics as a tool for risk assessment, to illustrate means and spreads in large surveillance datasets to avoid the issue of measurements below the limit of quantification. A harmonized, updated evaluation of the risk associated with exposure to BFRs from diet, air and dust is warranted, where the fish species most commonly consumed also is taken into consideration.

1. Introduction

Brominated flame retardants (BFRs) constitute a diverse group of compounds used in several commercial commodities to prevent or restrain fire. For instance, the legacy BFRs such as polybrominated diphenyl ethers (PBDE), hexabromocyclododecanes (HBCD) and tetrabromobisphenol A (TBBPA) have been used in electrical components, furniture and insulation-foam. The European Union (EU) has taken precautionary measures regarding these specific BFRs, and has issued bans or restrictions on their production and use (EC, 2003; EC, 2008; EFSA, 2012; Koch et al., 2015). However, due to their persistence they are still present in the environment and consequently in biota (Danon-Schaffer et al., 2013), particularly in aquatic organisms. Hence, the legacy BFRs, are still relevant BFR classes for monitoring (EC, 2014; EFSA, 2006).

Food of animal origin, particularly fat-rich seafood, is traditionally
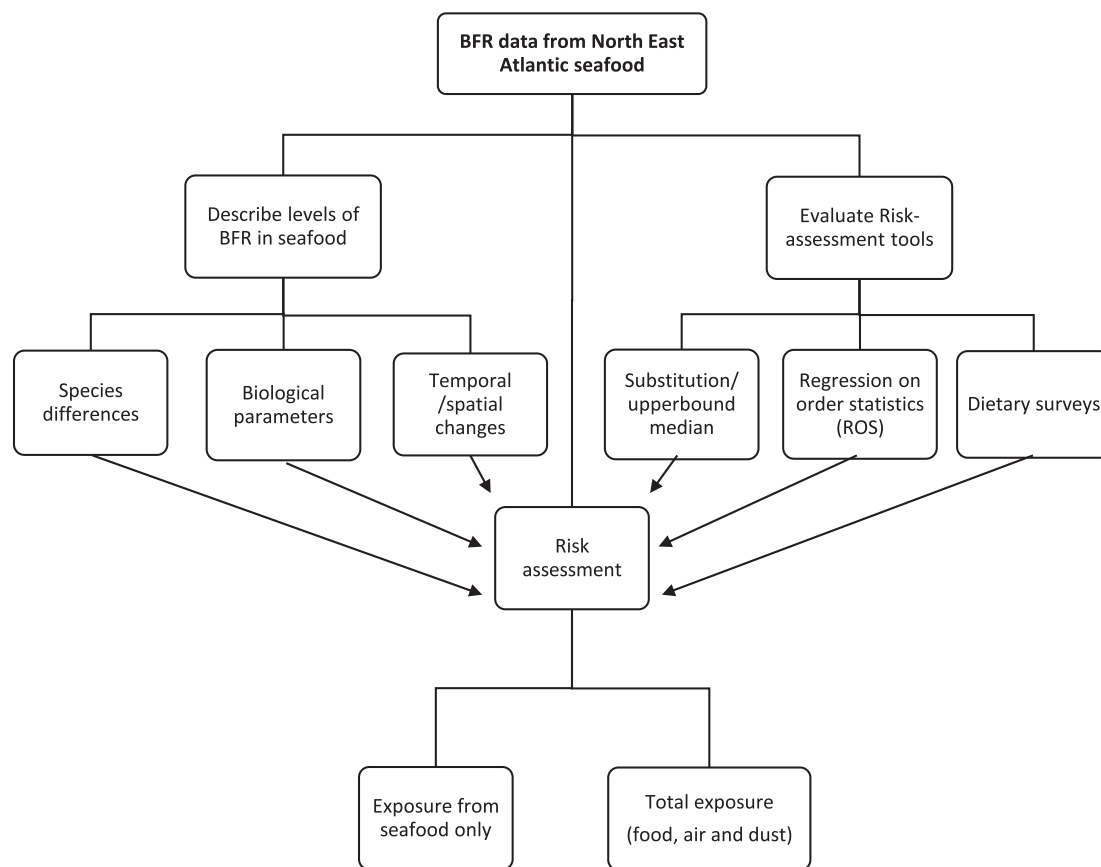

Fig. 1. Flowchart describing the objective and aim of the study. This study has evaluated both levels of BFR in seafood, and the factors affecting these, and different risk assessment tools. These two aspects have been assessed according to their effect on risk assessment from both multiple sources of exposure and from seafood exposure alone.

regarded as a major contributor of BFRs to human exposure (Cruz et al., 2015; EFSA, 2011a; EFSA, 2011b; Koch et al., 2015), although the impact of non-food sources should also be considered (Koch et al., 2015; Martellini et al., 2016). The contribution of Norwegian seafood for human BFR exposure is of interest not only for the Norwegian population who traditionally have a high seafood intake, but also for the population of countries which import seafood from Norwegian waters. Norway is the world's second largest exporter of fish and fishery products including both farmed and wild fish (FAO, 2016). Whereas food exposure assessments generally use "fish" or "seafood" as general food intake categories, commercial Norwegian seafood consist of several different species with a large variation in fat content, age, position in the marine food chain, harvest location and season of capture, which all affect BFR levels and congener composition and thus cause different exposure. In this paper, we highlight factors that may cause variation in risk assessments of BFRs in seafood. We assessed the impact of exposure from other sources than seafood, and how choice of statistics related to reporting limit of quantification (LOQ) in surveillance data, affects risk assessment (Fig. 1). Based on an extensive dataset, we highlight species-specific risk of seafood consumption in terms of the legacy BFRs. The levels of PBDEs, HBCD and TBBPA in the main commercial fish species harvested in and near Norwegian waters are also described. Further, we evaluate factors affecting the level of the different BFRs in seafood species, such as age, fat content, geographical origin, time of sampling and feed.

2. Materials and methods

2.1. Sample material

The data presented in the current study comprise results from

analyses of 9764 marine samples including both wild and farmed fish, fish feed and fish feed ingredients collected between 2006 and 2016. A total of 9211 samples were analyzed for ΣPBDE, here defined as sum of BDE 28, 47, 99, 100, 153, 154 and 183, 1453 for HBCD and 352 for TBBPA (Table 1). Additionally 383 samples of fish feed or fish feed ingredients were analyzed; 383 were analyzed for ΣPBDE, 275 for HBCD, and 69 for TBBPA. An overview of all analyses and results are given in [dataset] Appendix data. Sampling was done primarily on commercial fish species used as food, with the exceptions of certain forage fish (capelin and polar cod). Sampling locations for wild fish represent Norwegian fishing grounds including areas beyond the Norwegian territorial boundaries (Fig. 1). Fish were mainly sampled in seasons when commercial fishing occurs for the different species. Farmed Atlantic salmon (*Salmo salar*), were collected from all regions along the Norwegian coast with aquaculture activity. Twelve of the wild Atlantic salmon caught at sea were found to originate from fish farms, using methodology described elsewhere (Fiske et al., 2005; Lund et al., 1991), and are treated as a separate group hereafter called escapees. Fish feed and fish feed ingredients were sampled from Norwegian feed producers or at fish farms, representative of fish-feed production in Norway.

All samples were analyzed at the Institute of Marine Research (IMR) or Eurofins Gfa GmbH (Hamburg, Germany). The farmed fish, feed ingredients and fish feed were sampled by the Norwegian Food Safety Authority, while the wild fish were sampled by the IMR. The current study includes data on the legacy brominated flame-retardants PBDEs (28, 47, 66, 99, 100, 119, 138, 153, 154 and 183), HBCD and TBBPA.

2.2. Sample preparation

Fish length, weight and sex were recorded for each fish sampled

Table 1
Fish species analyzed; matrix, origin, time of sampling and sample size.

Species	Latin	Farmed or wild	Matrix sampled	Years of sampling	Samples	PBDE N	HBCD N	TBBPA N	References
Atlantic cod	*Gadus morhua*	Farmed and wild	Muscle/liver	2006–2007, 2009–2011, 2013–2014	2101	2099	58	23	(Julshamn et al., 2013)
Atlantic halibut	*Hippoglossus hippoglossus*	Wild	Fat muscle/lean muscle	2013–2015	688	688	0	0	(Nilsen et al., 2017)
Atlantic mackerel	*Scomber scombrus*	Wild	Muscle	2007–2009	812	812	23	0	(Frantzen, 2010)
Atlantic salmon	*Salmo salar*	Farmed and wild	Muscle (NQC for farmed fish)	2008, 2010–2015	708	546	286	277	(Lundebye et al., 2017; Nostbakken et al., 2015)
Blue Whiting	*Micromesistius poutassou*	Wild	Muscle	2013	25	25	0	0	
Capelin	*Mallotus villosus*	Wild	Whole fish	2007–2010, 2012–2014	22	22	6	8	
Common ling	*Molva molva*	Wild	Muscle	2008	52	51	0	15	
European eel	*Anguilla anguilla*	Wild	Muscle	2006, 2008, 2010	167	167	38	0	
European hake	*Merluccius merluccius*	Wild	Muscle	2014	18	18	0	0	
European plaice	*Pleuronectes platessa*	Wild	Muscle	2007	25	25	25	0	
Greenland Halibut	*Reinhardtius hippoglossoides*	Wild	Muscle	2006–2008	1030	1030	59	0	(Nilsen et al., 2010)
Herring	*Clupea harengus*	Wild	Muscle	2006–2007, 2009–2011, 2014	1887	1887	840	0	(Frantzen et al., 2011)
Northern shrimp	*Pandalus borealis*	Wild	Whole	2008–2014	31	31	3	4	
Polar cod	*Boreogadus saida*	Wild	Whole fish	2006, 2008–2014	22	22	3	3	
Rainbow trout (farmed)	*Oncorhynchus mykiss*	Farmed	Muscle	2007, 2010, 2012–2015	17	12	6	7	
Rose fish	*Sebastes norvegicus*	Wild	Muscle	2007	44	44	44	0	
Saithe	*Pollachius virens*	Wild	Muscle/liver	2006, 2010–2013	1664	1664	63	0	(Nilsen et al., 2013b; Nilsen et al., 2013a)
Tusk	*Brosme brosme*	Wild	Muscle	2008	68	68	0	15	
Total				2006–2015	9381	9211	1454	352	

individually. Age was determined by reading of otoliths by the IMR. All fillet samples, except for farmed Atlantic salmon and wild Atlantic halibut (*Hippoglossus hippoglossus*), were collected by excising the whole fillet of the fish from one or both sides, with subsequent removal of the skin prior to homogenization. Fillets from farmed Atlantic salmon were sampled as described by Nostbakken et al. (2015) and fillets from Atlantic halibut were divided according to fat content (lean B-cut and fatty I-cut) as described by Nortvedt and Tuene (1998). Whole fish livers from farmed and wild Atlantic cod (*Gadus morhua*) and Atlantic saithe (*Pollachius virens*) were extracted and homogenized. Capelin (*Mallotus villosus*), polar cod (*Boreogadus saida*) are both forage species and also used in fish feed, they are normally used whole, and the whole fish were used for analyses. Northern shrimp (*Pandalus borealis*) were also taken whole for analyses, since these are normally cooked whole for human consumption. Fish feed, and fish feed ingredients were analyzed for BFRs as described by Sissener et al. (2013).

Farmed Atlantic salmon were analyzed in pools of five individuals while northern shrimp, blue whiting (*Micromesistius poutassou*), capelin, and polar cod were analyzed in pools of 25 individuals.

2.3. Analyses

Fat content of seafood matrices was determined gravimetrically using ethyl acetate extraction (Norwegian standard NS9402).

2.3.1. PBDE

From 2006, PBDE 28, 47, 99, 100, 153, 154, 183 were determined by an accredited in-house method, as described by Bethune et al. (2005). Since 2010, PBDEs were also analyzed together with PCBs and dioxins/furans, in a multicomponent method, described by Julshamn et al. (2013). This method is an adaption of the EPA standard methods 1613 and 1668 (US-EPA, 1994; US-EPA, 2010a). The PBDEs were analyzed in a relevant solvent fraction from the EPA clean-up procedure (Pirard et al., 2003). Throughout the entire period, the quantification of PBDE congeners in both methods were carried out by GC/MS operating in the negative ion chemical ionization mode, monitoring a bromine ion mass fragment. Both methods were accredited according to the ISO 17025 standard. The accuracy of both methods was maintained by regularly participation in laboratory proficiency tests (PTs) (Quasimeme and Norwegian Institute of Public Health). To ensure comparability between the two methods, a joint in-house QC reference sample (Atlantic salmon) was included in each analytical series. The in-house reference sample was prepared in large batches, lasting for 2–4 years of continuous use. When a new in-house QC reference sample was prepared, the old and the new QC samples were analyzed together over a time period to ensure continuity. Also sample material from previously successfully participated PTs was analyzed in both methods to ensure accuracy.

2.3.2. HBCD

From 2006 ΣHBCD (sum of α-HBCD, β-HBCD and γ-HBCD) were determined by an in-house method together with PBDEs as described above. The quantification of ΣHBCD was performed by GC/MS operating in a negative ion chemical ionization mode, by monitoring a bromine ion mass fragment. The method was accredited according to the ISO 17025 standard. From 2013 to 2016, HBCD was analyzed by Eurofins Gfa GmbH (Hamburg, Germany). For the latter method, internal standard, $^{13}C_{12}$-labeled HBCD, was added to a homogenized freeze dried sample. The sample was extracted by a Soxhlet apparatus, using a mixture of acetone and hexane, for at least 16 h. The extract was reduced under a stream of nitrogen, before hexane was added. Sulfuric acid was used for purification. The sample was further cleaned up by an alumina column. The extract was subsequently dried under a stream of nitrogen, and dissolved in a mix of methanol and acetonitrile. The HBCD isomers were separated by a Nucleodur C18 Isis column and quantified by LC-MS/MS using Electrospray Ionization (ESI). The

method was accredited according to the ISO 17025 standard.

2.3.3. TBBPA

From 2007 to 2012, TBBPA was determined by an in-house validated method. Stable isotope labeled internal standard, $^{13}C_{12}$ TBBPA, was added to the sample and the analyte was extracted using a mix of acetone, cyclohexane and sodium chloride. The extract was concentrated by pressurized evaporation (Turbovap II™ Zymark, USA). The sample was dissolved in hexane, and sulfuric acid was used for purification. The hexane extract was dried under a stream of nitrogen and redissolved in a mix of water and methanol containing ammonium acetate. The sample was analyzed by LC-MS/MS equipped with an electrospray ionization (ESI) source operated in a negative mode. An acquity UPLC BEH C18 column (150 mm × 2.1 mm i.d., 1.7 µm particle size) was used for separation using a 0.5 ml/min flow. The mobile phases used in the assay were 2 mM ammonium acetate in water and 2 mM ammonium acetate in methanol. Chromatography was performed according to a stepwise gradient: 0–5 min, 70% ammonium acetate in methanol; 6–7 min, 100% ammonium acetate in methanol; 7,1–10 min, 70% ammonium acetate in methanol. All gradient steps were linear, and the flowrate was 0.5 ml/min. This method was discontinued in 2012 due to a relative high LOQ of 1.0 ng/g. However, these early data were used to verify relative low levels below the LOQ. From 2013 to 2016, TBBPA was analyzed by Eurofins Gfa GmbH (Hamburg, Germany). Internal standard, $^{13}C_{12}$-labeled TBBPA was added to a homogenized freeze dried sample, before phosphoric acid was added for acidification. Extraction was performed by a Soxhlet apparatus using a mix of acetone and hexane for at least 16 h. The extract was reduced under a stream of nitrogen. The sample was redissolved in hexane, and sulfuric acid was used for purification. Subsequently the sample was dried under a stream of nitrogen and redissolved in *O*-bis (trimethylsilyl)trifluoroacetamide (BSTFA) for derivatization. After 25 min in a drying oven at 50 °C, the solution was dried under a stream of nitrogen and redissolved in hexane. The extract was purified using column chromatography. The extract was reduced under a stream of nitrogen. TBBPA was analyzed by GC–MS using and Electron Ionization (EI). A XLB column was used for separation. The method was accredited according to the ISO 17025 standard.

2.4. Spatial evaluation of BFRs in wild fish

Geographical coordinates of sampling location were registered for 8551 of the 8768 wild fish samples. All fish samples were sorted based on catch site, according to sea, latitude and proximity to the coast. Fish caught within the Norwegian baseline border were categorized as "near shore". Fish caught between the baseline border and six nautical miles outside the baseline border (6 NM border) were categorized as "intermediate", and fish caught outside the 6 NM border were categorized as "open sea". The definitions of the baseline border and the 6 NM border were obtained from the Norwegian Mapping Authority (www. kartverket.no/en). Fish caught near the shore of Svalbard or other islands in open sea were categorized as open sea. Only samples collected in open sea (N = 6436) were used in the spatial and temporal analyses, to avoid statistical noise from local pollution near shore. Wild salmon, which were all caught near the coast, were excluded from spatial assessment as wild salmon spend most of their adult life in the open sea (IMR, 2016). The borders for the seas were derived from The International Hydrographic organization (IHO, 1953), with one exception being the border between the Barents Sea and the Norwegian Sea, which was based on the Eggakanten line (the upper edge of the continental slope between the shallow area to the east and the deep sea to the west). This was considered more ecologically correct based on the distribution of important migratory fish stocks such as Northeast Arctic cod and Norwegian Spring Spawning (NSS) herring (*Clupea harengus*). A line was drawn along the Eggakanten edge at approximately 500 meter depth using Google maps to determine the Eggakanten border. The

Arctic Circle, defined as 66°33′N, was used as the cut-off for classifying samples as Arctic or not.

2.5. Categorization according to fat content

Individual species and matrices were grouped according to mean fat content. Fat content was either analyzed in the samples, or the average fat content of the species was obtained from the IMR seafood database (https://sjomatdata.nifes.no). To assess the impact of fat on the accumulation of BFRs, the species were divided into three groups based on fat content: group 1 = < 10 g total lipid/100 g, group 2 = between 10 and 20 g total lipid/100 g, and group 3 > 20 g total lipid/100 g.

2.6. Evaluation of food safety

In order to evaluate the seafood examined in this study in terms of food safety, we initially evaluated the BFRs in individual fish species without considering other sources of BFR intake. This was done for all consumer ages using different published Norwegian and European dietary surveys. Additionally, we did a worst-case evaluation for the most sensitive group in the population (toddlers), including other sources of BFR exposure including air, dust and food other than fish.

For the risk assessment we used a margin of exposure (MOE, see Eq. (1)) approach as suggested by the European Food Safety Authority (EFSA) (EFSA, 2011a; EFSA, 2011b). The MOE is defined as the ratio of the no-observed-adverse-effect level (NOAEL) or benchmark dose lower confidence limit (BMDL) for the critical effect to the theoretical, predicted, or estimated exposure dose or concentration. EFSA has established $BMDL_{10}$ (Lower confidence limit at 10% incidence) for BDE 47, 99, 153, HBCD and TBBPA of 309, 12, 83, 930 and 16,000 µg kg^{-1} b.w., respectively (EFSA, 2011a; EFSA, 2011b; EFSA, 2011c). These equate to body burden at $BMDL_{10}$ of 232, 9, 62, 790 µg kg^{-1} b.w. for BDE 47, 99, 153 and HBCD, respectively. Body burdens at BMDLs were converted to chronic human dietary intake ($D_{r,h}$) by EFSA, and we used the derived $D_{r,h}$ from EFSA which was further divided by the level of BFR in the seafood in question in our study. The resulting value was compared to the MOE threshold as determined by EFSA (EFSA, 2011a; EFSA, 2011b). Typically, a MOE of 100 is used as threshold level, but for BFRs EFSA derived a threshold of 2.5 which has been rationalized in their risk assessments (EFSA, 2011a; EFSA, 2011b). > 98% of the results for TBBPA were below the LOQ, and it was therefore not feasible to calculate a MOE for this compound. Hence, we assessed the BFRs BDE 47, 99, 153 and HBCD.

$$\frac{D_{r,h}}{[BFR_i]\text{ng/g} \times Seafood\ consumed\ \text{g/bw/day}} = MOE \tag{1}$$

where $D_{r,h}$ is the estimated chronic human consumption corresponding to the body burden at $BMDL_{10}$. BFR_i is the level of BFR in the fish species evaluated, and Seafood consumed is based on consumption data from different food surveys.

For food consumption data, we compared Norwegian consumption to European consumption. This was done for Norwegian consumers since they are exposed to the seafood in question, and since Norway has more than twice the fish supply per capita compared to other nations in Europe (FAO, 2015). We used several published dietary surveys of Norwegian fish consumption (Hansen et al., 2015; Kristiansen and Andersen, 2009; Meltzer et al., 2002; Totland et al., 2012; Øverby et al., 2009), whereas the European fish consumption data was from the EFSA comprehensive database (EFSA, 2015). Consumption data for fish liver were obtained from Meltzer et al. (2002). Food consumption data are summarized in brief in Appendix 1. The evaluation distinguished between consumption data for fish fillet and liver due to large differences between these foods, both in terms of consumption and BFR content. Otherwise, each species of fish were assumed to be representative of the total seafood intake, so the mean BFR content of the fillet of each species were multiplied with the intake data for seafood from the
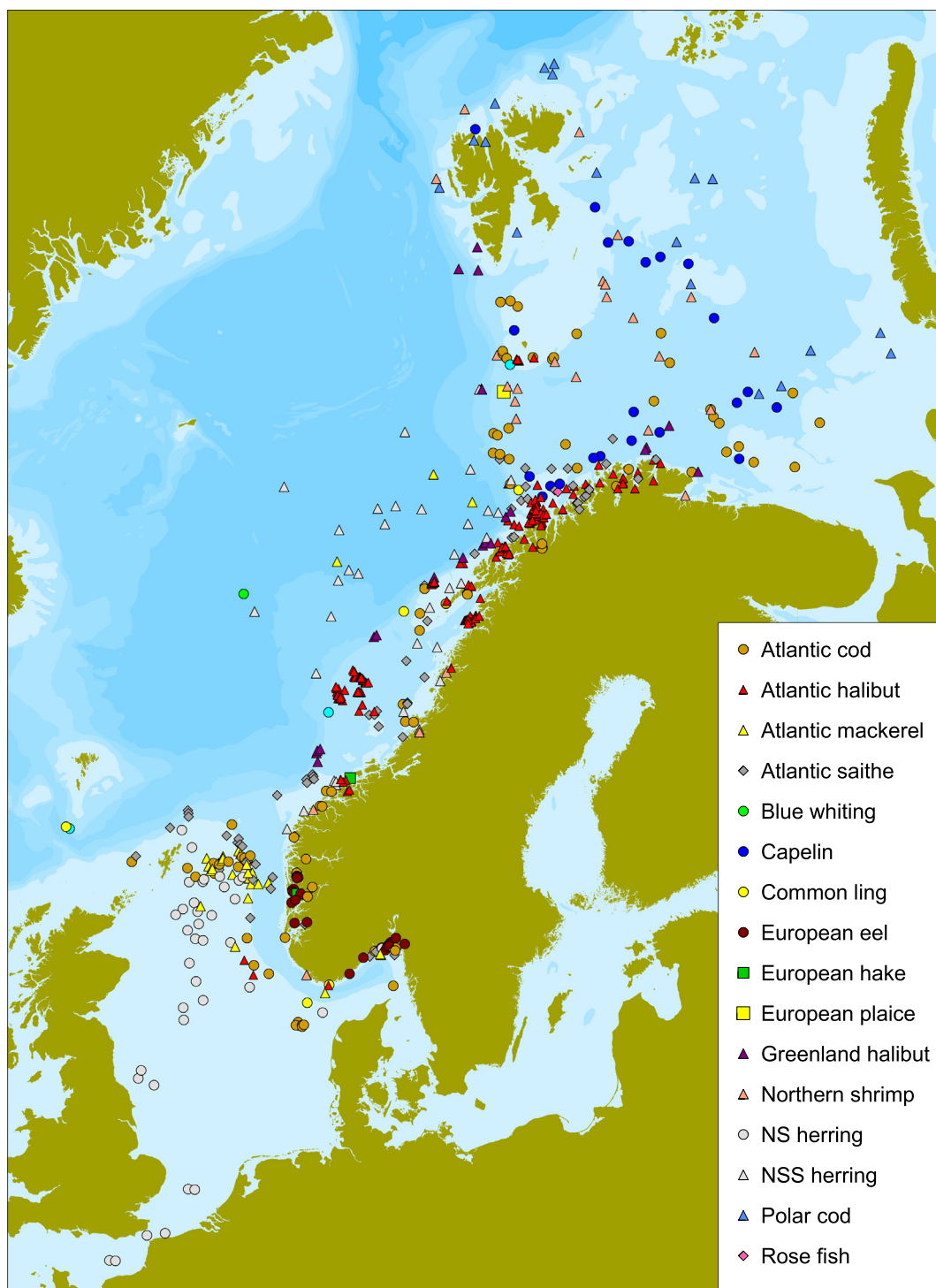
Fig. 2. Sampling area for all species with geographical coordinates. Each species is denoted with a coloured dot. For higher resolution and more information per sample, see: https://drive.google.com/open?id=1wYX8z3UntxnHGNq0_70zyAti-28&usp=sharing

dietary surveys. Although this is an oversimplification, it serves to illustrate the food safety of the specific fish species in question.

The mean seafood intake was used to calculate the mean MOE. Specifically for Norwegian consumption data, the average was calculated from several surveys or groups (such as gender, and age group). In order to evaluate high-risk groups of the populations we included the MOE for the 95th percentile intake data. In this case, the highest consumption displayed within a particular group or survey was used as a basis for our MOE calculation of the 95th percentile.

To calculate the BFR contribution from other sources, BFR intake

from food groups other than fish, was based on the total upper-bound dietary intake reported by EFSA (EFSA, 2011a; EFSA, 2011b). The worst case exposure from air and dust was obtained from data for those European countries described by Fromme et al. (2016). We subsequently replaced the contribution of BFR from fish reported by EFSA with our own BFR data, and used a minimum and maximum dietary intake from fish as reported by EFSA (EFSA, 2011a; EFSA, 2011b; EFSA, 2011a; EFSA, 2011b).

Neither total upper-bound dietary intake nor the relative contribution of HBCD from fish consumption in Europe was calculated by EFSA

for toddlers, hence, values from "other children" were used (EFSA, 2011b). Although this can potentially overestimate the exposure, a high MOE would indicate even lower risk for the actual toddler intake. Based on these calculations, the contributions from other sources than fish were estimated as ng per kg b.w. per day. These estimates were included in the MOE calculation together with the contribution from the fish analyzed in our study, as shown in formula (2).

$$\frac{D_{r,h}}{([BFR_i]\ \text{ng/g} \times \text{Seafood consumed (g/bw/day)} + BFR_{none\ seafood+air\ and\ dust}\ \text{(ng/bw/day)})} = MOE$$

(2)

where $D_{r,h}$ is the estimated chronic human consumption corresponding to the body burden at $BMDL_{10}$. BFR_i is the level of BFRE in the fish species evaluated, $BFR_{none\ seafood+air\ and\ dust}$ is the intake of BFRs from other sources than seafood based on Fromme et al. (2016) and EFSA (2011a, 2011b), and Seafood consumed is based on consumption data from different food surveys.

Here we show the MOEs calculated for Atlantic cod, North Sea (NS) herring (*Clupea harengus*) and farmed Atlantic salmon. Cod and herring are representative of wild lean and fatty fish, respectively, and they are among the 25 main marine species in fisheries worldwide, whereas farmed Atlantic salmon is the most important species farmed in marine and coastal aquaculture (FAO, 2016). The level of BFRs in farmed Atlantic salmon used in this food safety assessment is based on the average over the last four years of measurements and is assumed to be representative of the salmon currently available to consumers (Fig. 3a). We used data from Greenland halibut (*Reinhardtius hippoglossoides*) to calculate a worst-case scenario since this was the fish species with the highest level of ΣPBDEs in the fillet in our study. All other species, and matrices analyzed are shown in Appendix 3.

For comparative reasons, we calculated the maximum intake of each fish matrix compared to the oral reference dose (RfD) set by the US-EPA, and the dose where JECFA expect that adverse effects are unlikely in rodents (JECFA, 2005; US-EPA, 2008a, 2008b, 2008c). For the dose suggested by JECFA we also implemented a commonly used safety factor of 100 (Renwick, 1991), since this value is solely applicable for rodents (JECFA, 2006).

2.7. Statistics

Data presented in this study were left-censored, and therefore not normally distributed. Consequently, left censored data were analyzed as described by Bolks et al. (2014). In brief, the statistical programming language R (version 3.2.3) (R Core Team, 2016) running in RStudio (version 0.99.903; RStudio Team, 2015) ran the script SummaryStatsROS.r to compute summary statistics (mean, median and percentiles) based on robust regression on order statistics (ROS). Confidence interval estimates on the computed means were calculated using the script BootstrapROS.r, which empirically determined 95% confidence limits of the data means through bootstrapping.

Correlation and regression analyses were performed using raw upper bound (UB) data, where levels below LOQ are substituted for the LOQ. Most trend analyses were done on BDE 47 since 99.5% of all measurements were above the LOQ. Further, regression analyses of upper bound (UB) ΣPBDE against BDE 47 showed an r^2 of 0.99 and $p < 0.0001$, while the regression of ΣPBDEs in fillet calculated using ROS generated means for each congener against BDE 47 showed an r^2 of 0.99 and $p < 0.0001$, demonstrating that BDE 47 was a representative marker for ΣPBDE in our data. Correlation and regression analyses were performed using Statistica 13.1 (StatSoft Inc., Tulsa, USA), and Graphpad Prism 5.04 (Graphpad software Inc., San Diego, CA, USA).

3. Results

3.1. Levels of brominated flame retardants in Norwegian seafood

The mean levels of BFRs in selected species of Norwegian seafood are presented in Fig. 2. Overall, 9381 samples of fish were analyzed for BFRs in this study and levels of ΣPBDE in individual fillet samples ranged from the ΣUB LOQs in Atlantic cod fillet to 39.5 µg kg^{-1} in Atlantic halibut I-cut. The concentrations in liver ranged from UB 0.2 µg kg^{-1} to 143 µg kg^{-1}, both extremes measured in samples from Atlantic cod. The levels of HBCD in fillets ranged from concentrations below the LOQ (< 0,002–5 µg kg^{-1}) in several species to 11.4 µg kg^{-1} in Norwegian Spring Spawning herring, while in liver concentrations

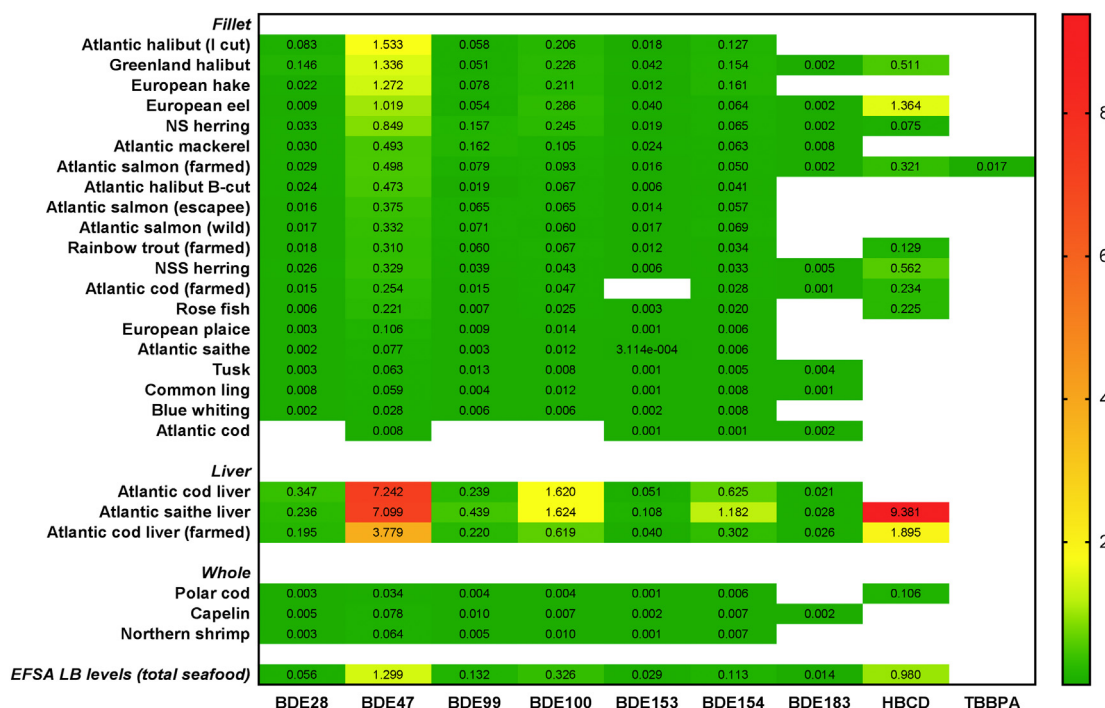|	BDE28	BDE47	BDE99	BDE100	BDE153	BDE154	BDE183	HBCD	TBBPA
Fillet									
Atlantic halibut (I cut)	0.083	1.533	0.058	0.206	0.018	0.127			
Greenland halibut	0.146	1.336	0.051	0.226	0.042	0.154	0.002	0.511	
European hake	0.022	1.272	0.078	0.211	0.012	0.161			
European eel	0.009	1.019	0.054	0.286	0.040	0.064	0.002	1.364	
NS herring	0.033	0.849	0.157	0.245	0.019	0.065	0.002	0.075	
Atlantic mackerel	0.030	0.493	0.162	0.105	0.024	0.063	0.008		
Atlantic salmon (farmed)	0.029	0.498	0.079	0.093	0.016	0.050	0.002	0.321	0.017
Atlantic halibut B-cut	0.024	0.473	0.019	0.067	0.006	0.041			
Atlantic salmon (escapee)	0.016	0.375	0.065	0.065	0.014	0.057			
Atlantic salmon (wild)	0.017	0.332	0.071	0.060	0.017	0.069			
Rainbow trout (farmed)	0.018	0.310	0.060	0.067	0.012	0.034		0.129	
NSS herring	0.026	0.329	0.039	0.043	0.006	0.033	0.005	0.562	
Atlantic cod (farmed)	0.015	0.254	0.015	0.047		0.028	0.001	0.234	
Rose fish	0.006	0.221	0.007	0.025	0.003	0.020		0.225	
European plaice	0.003	0.106	0.009	0.014	0.001	0.006			
Atlantic saithe	0.002	0.077	0.003	0.012	3.114e-004	0.006			
Tusk	0.003	0.063	0.013	0.008	0.001	0.005	0.004		
Common ling	0.008	0.059	0.004	0.012	0.001	0.008	0.001		
Blue whiting	0.002	0.028	0.006	0.006	0.002	0.008			
Atlantic cod		0.008			0.001	0.001	0.002		
Liver									
Atlantic cod liver	0.347	7.242	0.239	1.620	0.051	0.625	0.021		
Atlantic saithe liver	0.236	7.099	0.439	1.624	0.108	1.182	0.028	9.381	
Atlantic cod liver (farmed)	0.195	3.779	0.220	0.619	0.040	0.302	0.026	1.895	
Whole									
Polar cod	0.003	0.034	0.004	0.004	0.001	0.006		0.106	
Capelin	0.005	0.078	0.010	0.007	0.002	0.007	0.002		
Northern shrimp	0.003	0.064	0.005	0.010	0.001	0.007			
EFSA LB levels (total seafood)	0.056	1.299	0.132	0.326	0.029	0.113	0.014	0.980	

Fig. 3. Mean concentrations (µg kg^{-1}) of the different brominated flame retardants analyzed in all species and for each matrix. For samples where all measurements were below LOQ, results are not shown.

Table 2
Main factors affecting the accumulation of BDE 47 in selected wild marine matrices.

Species	Latitude		Fat content		Age		Year of sampling	
	Spearman's rho	p value	Spearman's rho	p value	Spearman's rho	p value	Spearman's rho	p value
NSS herring	−0.47	<0.05	0.3	<0.05	0.75	<0.05		
Atlantic Mackerel	0.03	n.s.	0.08	<0.05	0.15	<0.05		
NS herring	−0.05	n.s.	0.25	<0.05	0.41	<0.05		
Greenland halibut	−0.42	<0.05	0.1	<0.05	0.12	<0.05		
Atlantic cod liver	−0.57	<0.05	−0.05	<0.05	−0.23	<0.05		
Atlantic saithe liver	−0.64	<0.05	−0.33	<0.05	0.44	<0.05		
Polar cod	−0.28	n.s.	0.30	n.s.			−0.58 (0.53)	0.0014
Capelin	−0.77	<0.05	−0.30	n.s.			−0.70 (0.48)	<0.0001
Northern shrimp	−0.37	<0.05	0.23	n.s.			−0.15 (−0.07)	n.s.
Tusk	−0.41	<0.05						
Common ling	−0.19	n.s.						
European hake	−0.50	<0.05	0.46	n.s.				
Atlantic halibut B cut	−0.37	<0.05	0.69	<0.05	0.45	<0.05		
Atlantic halibut I cut	−0.41	<0.05	0.66	<0.05	0.48	<0.05		
European eel	0.54	<0.05	0.13	n.s.				
Wild Atlantic salmon			−0.06	n.s.				
Blue Whiting			0.17	n.s.				
All samples combined			0.68	<0.05				

All correlation analyses were performed on BDE 47 as this was deemed representative of ΣPBDE. All correlations in our study were shown to be monotonic but not linear, hence, spearman rho were used to indicate the goodness of fit. Significant correlations with Spearman rho higher than 0.4 or lower than −0.4 are shown in red (n.s. = not significant). Negative correlation rho's indicates a negative relationship between factors. For years of sampling, arctic samples only are shown in brackets.

ranged from below the LOQ ($< 1\,\mu g\,kg^{-1}$) in farmed Atlantic cod to $28.0\,\mu g\,kg^{-1}$ in Atlantic saithe.

> 50% of the measured BDE 28, 47, 99, 100, 153 and 154 levels were above the LOQ, while only 16% of the measured BDE 183 concentrations were above the LOQ. HBCD values were below the LOQ in approximately 65% of the samples analyzed and TBBPA values were below the LOQ in 98% of the samples. The few samples that had quantifiable levels of TBBPA were within the ranges of the variable LOQ. The quantifiable levels were between 0.03 and $0.06\,\mu g\,kg^{-1}$ and were found in farmed Atlantic salmon. Since most TBBPA levels were below the LOQ, these data are not presented graphically, but are given in [dataset] Appendix data.

The main contributor to the level of sum ΣPBDE in all species was BDE 47, representing about 65% of ΣPBDE. BDE 100 represented 12%, BDE 154 contributed 6%, BDE 99 contributed 5% while BDE 28, 153 and 183 represented < 3.5% each.

The relationship between BDE 47 and UB ΣPBDE showed a significant linear regression with an r^2 of 0.9908, enabling the use of BDE 47 as marker for ΣPBDE. However, linear regression between BDE 47 against levels from each single PBDE-congeners showed greater variability. Particularly, pelagic species showed higher levels of BDE 99 relative to BDE 47 than the benthic species did (Fig. 3c).

3.2. Environmental and biological factors affecting the level of brominated flame retardants in seafood

Factors affecting the accumulation of BFRs in different fish species were assessed to provide for a more accurate risk assessment of seafood. Arctic fish at lower trophic level, i.e. polar cod, capelin and northern shrimp sampled north of the Arctic Circle regularly between 2005 and 2016, were used as indicators for the temporal change of PBDEs in the

Arctic marine environment. The levels of BDE 47 in the pelagic Arctic species capelin and polar cod have decreased significantly over the last decade (2006–2016), while the levels in the hyper-benthic northern shrimp did not change during this time period. The decline in the BDE 47 levels in both capelin and polar cod was monotonic, but not linear, with a Spearman's rank correlation coefficient (Spearman's rho) of −0.70 and −0.58 respectively (Table 2); while the regression coefficients (r^2) were 0.42 and 0.28, respectively (results not shown).

The effect of sampling location on BFR levels was evaluated by analyzing PBDE congeners in livers of cod and saithe. The levels of all PBDEs in liver from fish caught north of the Arctic Circle (66°55′N) were lower than in fish caught south of the Arctic Circle when analyzed using one way ANOVA on ROS generated means (results not shown). Further, significantly higher levels of BFRs in both cod and saithe liver were found in the North Sea and the Skagerrak compared to the Norwegian Sea and the Barents Sea (results not shown). Correlation of latitude against BDE 47 concentration was performed for all samples where coordinates were available, and near-shore samples were included to increase sample size. Latitude of sampling showed a significant negative correlation in eight out of fifteen species, with Spearman rho values from −0.37 to −0.67, showing that BDE 47-levels decreased with increasing latitude. European eel (*Anguilla Anguilla*) was the only species with the opposite pattern with significant correlation (rho of 0.54) (Table 2). Hence, geographical origin can affect the level of BFRs.

Farmed Atlantic salmon were analyzed to evaluate the impact of feed on PBDE accumulation. A decline in levels over years can be observed for both feed and salmon (Fig. 3a). The congener composition of PBDEs in feed and fish fillet was similar. The overall levels of ΣPBDE in both fish feed and farmed salmon have decreased during the last 10 years, and the levels of BDE 47 in farmed Atlantic salmon sampled in

Table 3

Margin of exposure (MOE) calculated for particularly sensitive groups using selected fish species, including BFR from other sources.

Population	Survey	Reference	Fish part of diet	Atlantic cod (wild)								North Sea herring					
				BDE 47		BDE 99[a]		BDE 153		HBCD[a]		BDE 47		BDE 99		BDE 153	
				Mean	p95	Mean	p95	Mean	p95	Mean	p95	Mean	p95	Mean	p95	Mean	p95
Norway																	
Toddlers	Småbarnskost	Kristiansen and Andersen, 2009	Minimum	33.2	33.0	1.3	1.3	5.6	5.6	284.9	145.8	25.2	18.7	1.2	1.1	5.5	5.3
Toddlers	Småbarnskost	Kristiansen and Andersen, 2009	Maximum	77.3	76.5	1.8	1.7	6.6	6.5	309.8	152.0	44.5	27.6	1.6	1.4	6.4	6.2
Europe																	
Toddlers/other children	EFSA	EFSA, 2015	Minimum	33.1	32.9	1.3	1.3	5.6	5.6	259.3	110.2	24.3	16.0	1.2	1.0	5.5	5.2
Toddlers/other children	EFSA	EFSA, 2015	Maximum	77.3	76.0	1.8	1.7	6.6	6.5	279.7	113.7	41.8	22.0	1.6	1.2	6.4	6.0

Population	North Sea herring		Greenland halibut								Farmed Atlantic salmon							
	HBCD		BDE 47		BDE 99		BDE 153		HBCD		BDE 47		BDE 99		BDE 153		HBCD	
	Mean	p95	Mean	p95	Mean	p95	Mean	p95	Mean	p95	Mean	p95	Mean	p95	Mean	p95	Mean	p95
Norway																		
Toddlers	817.9	765.2	22.1	14.9	1.3	1.3	5.4	5.0	639.6	468.5	28.0	22.8	1.3	1.2	5.5	5.5	706.7	563.7
Toddlers	1063.0	975.6	35.7	20.1	1.7	1.6	6.2	5.8	780.3	539.8	54.1	37.6	1.7	1.5	6.4	6.4	882.5	670.1
Europe																		
Toddlers/other children	812.1	733.9	21.1	12.3	1.3	1.2	5.3	4.8	616.3	398.2	27.4	20.3	1.3	1.2	5.5	5.5	688.6	497.2
Toddlers/other children	1053.2	925.3	33.0	15.6	1.7	1.6	6.2	5.5	745.9	448.5	51.7	31.3	1.7	1.5	6.4	6.4	854.4	578.2

All MOEs were compared to the threshold MOE at 2.5 as established by EFSA. MOEs below 2.5 are highlighted using underline.

[a] No levels detected above LOQ in fillet from Atlantic cod, maximum LOQ were used as a worst case scenario.

Table 4
Different scenarios of MOE calculations.

MOE scenario	Congener	Parameters	MOE mean (p95)
Risk excluding other sources than fish			
	BDE 47	Atlantic Cod (best case)	95322 (9722)
	BDE 47	Atlantic halibut I cut (worst case)	45 (17)
	BDE 99	Atlantic Saithe (best case)	5771 (589)
Worst-case fish vs best-case fish	BDE 99	Atlantic mackerel (worst case)	10 (4)
	BDE 153	Atlantic Saithe (best case)	143398 (14625)
	BDE 153	Greenland halibut (worst case)	92 (35)
	HBCD	NS herring (best case)	154119 (15719)
	HBCD	European eel (worst case)	732 (278)
Substitution statistics vs regression on order statistics (ROS)[a]	HBCD	Upperbound	1160 (440)
	HBCD	Lowerbound	2535 (963)
	HBCD	ROS	1777 (675)
Total Risk, including all sources[b]			
Total risk	BDE 99	All sources included	1.2 (1.0)
Excluding fish	BDE 99	Excluding fish	1.4 (1.4)
Excluding air and dust	BDE 99	Excluding air and dust	1.3 (1.1)
Excluding other foods than fish	BDE 99	Excluding other foods	8.7 (3.7)
Upperbound versus Lowerbound estimation of dietary intake of other foods than fish[c]	BDE 99	Upperbound	1.2 (1.0)
	BDE 99	Lowerbound	4.2 (2.6)

MOEs are (unless otherwise specified) calculated based on: BDE 99, dietary data from EFSA comprehensive database, for the dietary group of toddlers, using minimum fish part of diet.

MOEs below the 2.5 limit set by EFSA are marked in red letters.

a) HBCD was chosen as example since the LOQ for this have been relative high. Atlantic halibut (I-cut) were chosen as species since this exhibit large variance around LOQ.

b) The fish used in the example is NS herring.

c) Data derived from EFSA report (EFSA, 2011a).

2015 were comparable to the levels in its wild counterpart.

A relatively high analytical LOQ at 0.5–5.0 $\mu g \, kg^{-1}$ for ΣHBCD in feed and salmon in the early years of measurement compared to the LOQ of 0.002–0.01 $\mu g \, kg^{-1}$ in the latest two years, caused many early measurements to be below the LOQ. The relationship between the levels of HBCD in feed and salmon could therefore not be evaluated. No measurements of TBBPA, were above the LOQ in feed, and only six out of 277 measurements of farmed Atlantic salmon samples were above the LOQ. Therefore, no evaluation of TBBPA from feed to fish was feasible.

Correlation analyses were performed to determine a possible relationship between BDE 47 and fat content (Table 2). For all samples combined, including liver, a positive correlation with Spearman rho of 0.68 was observed (Table 2). The relationship between fat content and BDE 47 appears to be monotonic, but not linear, since linear regression showed low goodness of fit ($r^2 = 0.18$) for all samples combined. We observed a large variation in correlation coefficients between different individual tissues, from a significant negative correlation with Spearman rho of 0.33 for saithe liver, to a significant positive correlation with Spearman rho of 0.69 for Atlantic halibut fillet. When seafood samples were divided into three different fat categories the concentration of the BFRs relevant for risk assessment, i.e. BDE 47, 99, 153 and HBCD were significantly higher in the groups with the higher fat content (Fig. 3b).

The relationship between fish age and BDE 47 concentration appears to be monotonic increasing, but not linear, since linear regression showed lower goodness of fit compared to Spearman rho for all samples. Correlation analyses showed a significant relationship with Spearman rho above 0.4 between BDE 47 and fish age for: NSS herring, NS herring, Atlantic saithe liver and both B-cut and I-cut from Atlantic halibut (Table 2).

Sex, spawning, seasonal variation, migration and coast vicinity may contribute to the variation in the data. However, our comprehensive analyses demonstrate that the most important factors determining accumulation of BFR between seafood species were fat contents and geographical origin. Variations within species were mainly determined by fish age, geographical origin and time of sampling.

3.3. Risk assessments of BFRs

BDE 47, 99, 153 and HBCD were selected for MOE evaluation of fillet samples from Atlantic cod, NS herring, Greenland halibut and farmed Atlantic salmon (Table 2). Risk assessments of other fish species and other tissues are presented in Appendix 3. Liver is the matrix with the highest level of BFRs in our study. However, as consumption of cod and saithe liver is near to negligible, the calculated MOEs were high for this food (Appendix 3). The MOEs for BDE 99 for all fish species assessed were below the MOE threshold of 2.5 set by the EFSA. The other BFRs were not below the threshold, albeit BDE 153 showed MOEs around 5. The MOE for HBCD for toddlers were well above 100, and hence suggested low risk even though other exposure than fish were derived from the group "other children" which could lead to an overestimation. The worst case vs the best case fish in terms of risk are shown in Table 4 for each BFR evaluated.

The difference between the European dietary intake surveys and the Norwegian dietary surveys did not reveal large differences in the MOE for toddlers exposed to BDE 99. For the other compounds the Norwegian surveys showed either similar or slightly higher MOEs (Table 5).

When the fish constituted a minor part of the diet in toddlers, the MOEs were higher than when fish constituted a larger part of total diet (Table 3). MOE calculations excluding fish also revealed MOE below 2.5 (Table 4). The highest impact on the MOE were observed when other foods than fish were excluded from the MOE scenario (fig MOE scenario), while removal of air, dust and seafood showed lower impact on final MOE. Calculation of MOE based on lowerbound (LB) data for food sources compared to upperbound (UB) data showed that UB were below the threshold set by EFSA, while the LB were above. The impact of using ROS in food data were also exemplified for HBCD (Table 4), where LB, UB and ROS statistics were compared. The LB showed the highest MOE (2535), the UB showed the lowest MOE (1160), and the ROS resulted in a MOE in between (1777). The lowest MOE observed when all factors were included was 1.0 for BDE 99 at the 95th percentile calculated for North Sea herring and Atlantic mackerel (*Scomber scombrus*) consumption using the European dietary surveys (Fig. 4).
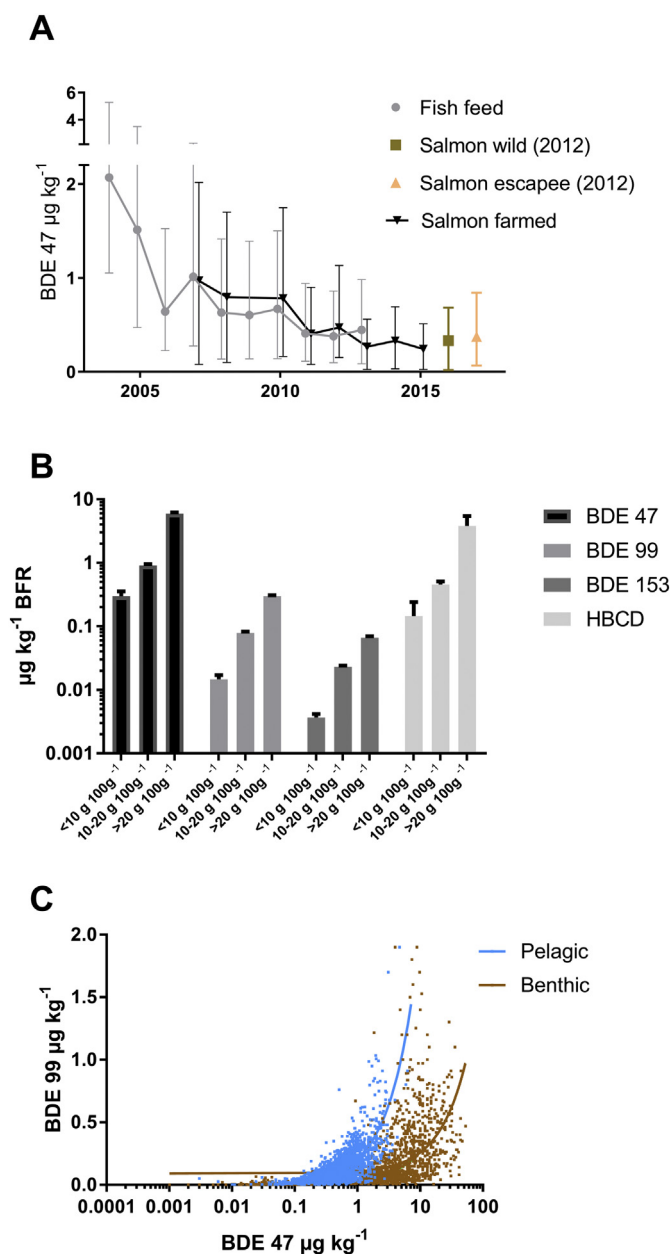
Fig. 4. Factors affecting the level of BFRs in Norwegian seafood. A) The level of BDE 47 in feed and farmed Atlantic salmon from 2004 to 2015, as well as wild caught salmon and escaped farmed salmon from 2012. Data are presented as mean ± CI. B) BFR per fat category across all species. C) Plot of BDE 47 against BDE 99 in the pelagic species blue whiting, capelin, mackerel, NS herring, NSS herring and polar cod (blue dots), and in the demersal species Atlantic halibut, Atlantic cod, common ling, cusk and European plaice (brown dots). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

MOEs were also calculated for all species excluding other sources than the seafood reported in this study, for all dietary age groups (Appendix 3). None of the seafood analyzed revealed MOEs lower than the threshold of 2.5 set by the EFSA (EFSA, 2011a; EFSA, 2011b). The lowest MOEs were found for BDE 99 from fatty fish species, such as NS herring, mackerel and farmed Atlantic salmon. The sensitivity to BDE 99 exposures was: toddlers > infants > adults > adolescents for the Norwegian survey, while for the European food survey it was: toddlers > adolescents > adults > infants.

BDE 47, 99 and 153 have also been evaluated by the JECFA and the US-EPA, in addition to the EFSA. The fish species included in the

present study were therefore, for comparative reasons, also evaluated according to the RfD set by the US-EPA, as well as doses suggested by JECFA at which adverse effects were unlikely in rodents plus a safety-margin of 100. The RfD is an estimate of the daily exposure to a potential hazard that is likely to be without risk of deleterious effects during a lifetime (US-EPA, 2011). These results are shown in Appendix 2. The results showed that none of the fish species or tissues in our study can be considered high-risk in terms of PBDE levels.

4. Discussion

In this study, we have analyzed the levels of several PBDEs, HBCD and TBBPA in > 9000 samples of seafood and fish feed, and assessed factors affecting BFRs levels in these. The health risk of BFRs for this representative selection of North East Atlantic seafood have been evaluated following the latest risk assessments from the EFSA. Further, several key factors influencing the outcome of risk assessments have been scrutinized, including variation in PBDE levels among, and within, species. Also, the impact of statistics and impact of dietary surveys on risk assessment have been assessed.

4.1. Environmental and biological factors affecting levels of brominated flame retardants

The PBDE levels in seafood presented in this study were comparable to previous studies (Pardo et al., 2014; Voorspoels et al., 2007; de Wit et al., 2010). The small differences found may be due to different sampling location, tissue measured, detection limits and/or other environmental and biological factors which affect the BFR levels. The fat content of the individual species and the individual tissues strongly affected the levels of BFRs as demonstrated by using fat categories. Still, the weak positive correlation between fat content and BDE 47 concentration, and the large variation among individual species, suggests a role of additional factors. The octanol-water partition coefficients (logKow) for the PBDEs analyzed in this study are generally high (from around 6.5 to 8.3 depending on congener), indicating that the bioaccumulation potential for these compounds is high (US-EPA, 2010b). Hence, the total levels of PBDE may not decrease even if the fish were to be emaciated. In agreement with this, and with an earlier study (Vuorinen et al., 2012), we demonstrated a correlation between age and the level of BDE 47 in several, but not all species.

Due to the ban on the use of many BFRs, Arctic species may serve as indicators of temporal change in the BFR levels in the environment. Temporal evaluation of areas distant from emission sources illustrates effects of long-range transport, and may better reflect the total load of BFRs in the global environment. In agreement with a previous study (Jenssen et al., 2007), we observed that the levels of BFRs in most species tended to decrease with increasing latitude in the North east Atlantic. In our study, polar cod, capelin and Northern shrimp were sampled north of the Arctic Circle regularly over the last decade. During this period, the levels of BDE 47 decreased in the pelagic species polar cod and capelin, but not in the hyper-benthic species Northern shrimp. The difference may be related to their feeding habits since Northern shrimp feed on benthic organisms, detritus and on zooplankton, while the two fish species are plankton feeders. PBDE deposited in the sediment may be remobilized as described by Josefsson et al. (2010), and then taken up by shrimp feeding on the benthos. The decreased levels of BDE 47 in the two pelagic species are in line with the recent temporal assessments of BFRs in the environment (Law et al., 2014). This may reflect a decrease in the total load of long range transported BFRs, and possibly decreased global release. However, due to the extended use of deca BDE, the congener BDE 209 may continue to be a threat to seafood safety (Law et al., 2014). Further, the occurrence of novel and emerging BFRs already observed in biota (Sahlström et al., 2015), may pose a challenge for future risk analyses of BFRs as a group.

When comparing species in the open seas, a decrease in BDE 47

Table 5
Margin of exposure (MOE) calculated for selected species based on different food consumption surveys.

Population	Survey	Reference	Atlantic cod (wild)								North Sea herring						
			BDE 47		BDE 99[a]		BDE 153		HBCD[a]		BDE 47		BDE 99		BDE 153		HBCD
			Mean	p95	Mean	p95	Mean	p95	Mean	p95	Mean	p95	Mean	p95	Mean	p95	Mean
Norway																	
Infants	Spedkost	Øverby et al., 2009	16,282	5163	167	53	7627	2418	662	210	161	51	21	7	393	125	26,325
Toddlers	Småbarnskost	Kristiansen and Andersen, 2009	10,186	4257	104	44	4771	1994	414	173	101	42	13	6	246	103	16,468
Adolescents	Ungkost	Hansen et al., 2015	24,973	7097	256	73	11,698	3325	1016	289	247	70	33	9	603	171	40,376
Adults	Norkost3/Fisk og vilt	Meltzer et al., 2002	22,362	5908	229	61	10,475	2768	909	240	221	58	29	8	540	143	36,155
Europe																	
Infants	EFSA	EFSA, 2015	95,322	9722	977	100	44,651	4554	3877	395	943	96	124	13	2303	235	154,119
Toddlers	EFSA	EFSA, 2015	8185	3108	84	32	3834	1456	333	126	81	31	11	4	198	75	13,233
Adolescents	EFSA	EFSA, 2015	21,415	6643	219	68	10,031	3112	871	270	212	66	28	9	517	160	34,624
Adults	EFSA	EFSA, 2015	29,919	9174	307	94	14,015	4297	1217	373	296	91	39	12	723	222	48,373

Population	North Sea herring	Greenland halibut									Farmed Atlantic salmon							
	HBCD	BDE 47		BDE 99		BDE 153		HBCD		BDE 47		BDE 99		BDE 153		HBCD	
	p95	Mean	p95	Mean	p95	Mean	p95	Mean	p95	Mean	p95	Mean	p95	Mean	p95	Mean	p95
Norway																		
Infants	8347	102	32	66	21	183	58	3950	1253	274	87	42	13	490	155	6180	1959
Toddlers	6883	64	27	41	17	114	48	2471	1033	172	72	27	11	306	128	3866	1616
Adolescents	11,475	157	45	100	29	280	80	6059	1722	421	120	65	19	751	213	9478	2694
Adults	9553	141	37	90	24	251	66	5425	1433	377	100	58	15	673	178	8487	2242
Europe																		
Infants	15,719	599	61	384	39	1069	109	23,12-7	2359	1607	164	249	25	2867	292	36,178	3690
Toddlers	5025	51	20	33	13	92	35	1986	754	138	52	21	8	246	93	3106	1180
Adolescents	10,741	135	42	86	27	240	74	5196	1612	361	112	56	17	644	200	8128	2521
Adults	14,832	188	58	120	37	335	103	7259	2226	504	155	78	24	900	276	11,355	3482

[a] No levels detected above LOQ in fillet from Atlantic cod, maximum LOQ were used as a worst case scenario.

levels with increasing latitude was observed. Lower levels of BDE 47 were detected in samples from the Barents Sea and the Norwegian Sea than in samples from Skagerrak and the North Sea. In general, lower levels of BDE 47 were observed in biota farthest from the main emission sources of the more populated central Europe. The spread of PBDEs may be driven by the main oceanic currents passing Norway, such as the North Atlantic Current and the Norwegian Coastal Current, which is basically northbound all along the coast of Norway (Barentswatch, 2017). Some spread of BFR to the Arctic has also been shown to occur by atmospheric long-range transport (de Wit et al., 2006). One species showing the reversed pattern of increasing BDE 47 with increasing latitude was the European eel. However, this species were exclusively sampled within the coastal border in southern Norway, and showed relative high levels of ΣPBDE which could suggests an impact of local pollution. This further visualizes the need to use open sea pelagic species as markers of global BFR distribution in order to minimize the noise from local pollution.

Analyses of farmed Atlantic salmon and fish feed showed that the composition and amount of both PBDEs and HBCD in the fish feed corresponded with the levels in the fish fillet. Levels of PBDE in farmed salmon vary extensively among studies in the literature (EFSA, 2011a; Hites et al., 2004; Montory et al., 2012; Pardo et al., 2014; Schecter et al., 2010; Trabalón et al., 2017; van Leeuwen and de Boer, 2008), ranging from 0.06 µg kg^{-1} w.w. in salmon sampled at a local supermarket (Pardo et al., 2014) to a mean level of 1.21 µg kg^{-1} w.w. BDE 47 reported by EFSA (EFSA, 2011a). Different sampling techniques, and year of sampling, can contribute to differences in reported BFR levels in farmed Atlantic salmon. In this study, Norwegian Quality Cut (NQC) was sampled according to Norwegian standard NS 9401 (1994). The fat distribution in a salmon fillet is not homogenous, but the NQC gives a good estimate of the fat content in Atlantic salmon fillets (Zhu et al., 2014), inferring that the level of lipid-soluble contaminants such as BFRs are representative of total muscle from this cut.

Here we show that the mean levels of BDE 47 in farmed Atlantic salmon declined over the last decade. The samples from years preceding 2012 had higher level of ΣPBDE than wild salmon which is in agreement with Hites et al. (2004), but due to the change in feed composition, the levels of PBDEs in farmed and wild salmon were comparable in 2012. The feeds used in salmon production in 2012–2013 typically contained only 10% fish-oil and 18% fish meal, the major contributors of PBDE to fish feed (Ytrestøyl et al., 2015).

4.2. Risk assessment of brominated flame retardants in North East Atlantic seafood

In this study we used the MOE approach, described by the EFSA (EFSA, 2011a; EFSA, 2011b; EFSA, 2011c), to evaluate seafood safety. Human exposure to BFRs originates both from food and non-food sources, such as indoor dust, but their relative contribution is debated (Ni et al., 2012; Sahlström et al., 2015; US-EPA, 2010a, 2010b, 2010c). Calculation of the MOE for BDE 99 using the exposure levels reported by the EFSA and Fromme et al. (2016), without including seafood, resulted in a MOE of 1.4 for mean intake, instead of 1.2 when NS herring represented total fish intake. A study in the US determined that intake of red meat and poultry contribute substantially to the PBDE body burdens in the US (Fraser et al., 2009). Although, levels in the US are substantially higher than in Europe (Fromme et al., 2016; Hites, 2004), food from other sources than fish contributed considerably to the overall exposure. Removing air and dust from our calculations still resulted in a MOE of 1.3, further suggesting total food intake is a large contributor to BDE 99 exposure. Therefore, we performed calculations both excluding and including other sources of BFR than seafood.

When other sources of BFR than seafood was excluded, we did not find any risk to the average consumer or the highest 95 percentile consumers, compared to the threshold set by the EFSA. However, the seafood described in this study derives from Northern Europe, which

may contain lower BFR levels since this study shows that concentrations decrease with increasing latitude. It is therefore possible that seafood harvested further south may contain higher levels of BFR. The lower MOEs calculated for BDE 99 exposure compared to the MOEs for BDE 47 exposure, demonstrated that the low concentration of the more toxic BDE 99 is of far greater importance than the higher concentration of the less toxic BDE 47 in risk assessments. In this context, it is noteworthy that the US-EPA has set the RfD of both BDE 47 and BDE 99 at 100 ng kg^{-1} b.w.

Developmental neurotoxicity is a major concern related to potential adverse health effects of BFRs (Costa and Giordano, 2007). Neurodevelopment in infants (Herbstman et al., 2010), and adolescents has also been shown to be subtly affected by PBDEs (Kicinski et al., 2012). Children are particularly vulnerable, and risk assessment of BFR exposure from seafood alone indicates that toddlers are the most sensitive group. This is due to a higher total food intake, including fish, relative to the low bodyweight in this group (Kristiansen and Andersen, 2009; Totland et al., 2012). PBDEs, already at current exposure, have been associated with subtle cognitive and behavioral changes in 4–7 year old children (Chevrier et al., 2016).

4.3. Limitations on current risk assessments and statistical considerations

Only four congeners, BDE 47, BDE 99, BDE 153 and HBCD were included in the risk assessments in this study. Levels of TBBPA were mainly below the LOQ providing limited data for calculating a MOE. Still, comparing the levels detected in a small number of samples and LOQ with the levels assessed by EFSA in their previous risk assessment, suggests that TBBPA poses a low risk (EFSA, 2011c). However, as our dataset on TBBPA mainly describes levels in farmed salmon, we cannot conclude on seafood in general. In our study the congener BDE 209 has not been analyzed, and there is consequently no risk assessment of BDE 209. This render the risk assessment incomplete compared to those congeners assessed by EFSA (EFSA, 2011a). However, it can be assumed that the factors affecting the congener levels presented in the study would also apply to BDE 209, nevertheless BDE 209 should be included in future risk assessments. Interaction effects between PBDEs and other halogenated compounds have previously been observed (Fitzgerald et al., 2012), suggesting that in the presence of other contaminants PBDE could be considered more toxic. However, it is difficult to separate possible effects of other halogenated compounds from the effects of PBDE in epidemiological studies. Although it is of great interest to investigate mixture toxicity among PBDE congeners and in mixtures with other contaminants, EFSA concluded that epidemiological studies on PBDEs were inconsistent (EFSA, 2011a). It is beyond the scope of this study to evaluate possible interaction effects, and derived BMDLs are therefore derived from studies based on individual congeners of PBDE (EFSA, 2011a).

The use of "fish" or "seafood" as food categories in risk assessments overlooks the large variation that exists between and within species, as we have demonstrated in this study. For future risk assessments, more data is needed on the levels of BFRs, and the consumption of individual species for better estimation of risk. Such data could also be used to develop a "representative fish" for more accurate risk assessments as exemplified by Tachovsky et al. (2010).

Left censored data are normally dealt with using the substitution method described by the WHO European Programme for Monitoring and Assessment of Dietary Exposure to Potentially Hazardous Substances (GEMS/Food-EURO, 1995). Although this is a simplistic method providing a range that contains the true level, it may cause difficulties interpreting the risk related to certain compounds. As an example, EFSA concluded that toddler high-risk consumers might be at risk due to the estimated UB intake level for BDE 99 of 2.99 ng kg^{-1} in toddlers, whereas the lower bound (LB) level was 0.58 ng/g suggesting no risk (EFSA, 2011a). In our risk assessment including other sources than fish, all species had a MOE of < 2.5 for BDE 99. This exemplifies

the need for a more accurate determination of the level of compounds actually present in different food. Indeed, the EFSA has stated that if the difference between LB and UB is not negligible, more refined methods should be implemented (EFSA, 2010). Hence, we propose that regression on order statistics (ROS) is a suitable statistical tool for use in risk assessment of food to better illustrate means and spreads in large surveillance data. In previous left censored simulation studies, ROS have been shown to provide better results than other methods (substitution, parametric maximum likelihood estimation (MLE), and non-parametric Kaplan-Meier (KM)) at almost all samples sizes and at almost all censoring rates (Tekindal et al., 2017).

It has previously been argued that the MOE threshold set by EFSA at 2.5, is too low due to large gaps in knowledge regarding kinetics and adverse effects of PBDEs (Lyche et al., 2015). Adding to this the uncertainties related to risk assessments addressed in this study, a re-assessment of the current exposure and toxicity of PBDEs including other BFR should be instigated. A number of aspects, particularly regarding exposure sources, congener composition of food, use of statistics and dietary surveys, complicate risk assessments of BFRs.

5. Conclusion

Risk assessment based on an extensive dataset of seafood, the most relevant dietary surveys, and the use of ROS statistics (for estimating mean levels for seafood), indicates that there is low risk related to exposure to BDE 47, BDE 99, BDE 153 and HBCD from consumption of seafood from the North East Atlantic. However, in our study, a risk of BDE 99 for toddlers was observed when all exposure sources were included at upper bound levels. A future re-evaluation of BFRs is warranted to take into account the great variation in MOEs which can be caused by: choice of seafood, other sources than seafood and the statistical interpretation of surveillance data. Taking such steps could improve the accuracy of future risk assessment.

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Declarations of interest

None.

Contributions from each author

OJN has collected data from previously performed surveillance, performed statistical analyses, and been in charge of writing the manuscript.

BMN has contributed with data for fillet samples from Greenland halibut and Atlantic halibut and for liver samples from saithe.

RH has contributed with data on farmed fish.

JDR contributed to the statistical analysis of the data.

AM has led overall work on collecting and monitoring wild fish for contaminants including brominated flame retardant.

AD has contributed with data on NS herring.

SFR has contributed with data on NSS herring, Northeast Atlantic mackerel, capelin, polar cod and Northern shrimp.

MS has contributed with data on fish feed and feed ingredients.

BMN, AM, AD, AKL, RH, MS, SFR, JDR and LM participated in the

preparation of the manuscript. All authors have participated in discussions and interpretation of the data.

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