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Marine Scotland - Science, Marine Laboratory
375 Victoria Road, Aberdeen, AB11 9DB

Summary

Halogenated persistent organic pollutants [chlorobiphenyls (CBs), polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD)], along with total lipid, were measured in the liver and muscle of two species of deep water fish (black scabbard and roundnose grenadier) collected from the Rockall Trough, to the west of Scotland, during 2007. The data was compared with that obtained from a similar survey conducted in 2006, and which has recently been published. CB concentrations (ΣICES7 CBs) greater than 500 µg kg⁻¹ lipid weight were found in 5 of the 30 deep water fish liver samples and 8 of 30 fish muscle samples collected in 2007. Non-ortho CBs (CB81, 77, 126 and 169) were measured in samples with the highest ortho CB concentrations but were not detected in any of the fish muscle or liver samples collected in 2007. Data was assessed using the assessment criteria adopted by OSPAR for use in the 2008 Coordinated Environmental Monitoring Programme (CEMP) assessment and Quality Status Report 2010 and incorporated into a traffic light system. Concentrations were compared to Background Assessment Concentrations (BACs; blue/green transition) and Environmental Assessment Criteriapassive (EACpassive; green/red transition). Concentrations for the individual ICES7 CBs in fish liver were above OSPAR BACs in both species sampled in 2007. The EACpassive was exceeded for CB118 only. As all other CBs were below the EACpassive, CB concentrations in deep water fish were classed as ‘green’. Measured (calculated for the five mono-ortho CBs) and estimated (calculated using published models) Toxic Equivalent (TEQ) concentrations in the fish muscle indicated that consumption of deep water fish muscle is unlikely to represent a risk to human health. PBDEs were detected in both the liver and muscle of the deep water fish collected in 2007. However, concentrations were low with many congeners being below detection limits. HBCD was not detected in any of the Scottish deep water fish collected in 2007.

Halogenated persistent organic pollutants were detected in Scottish deep water fish, confirming that these contaminants are transported to the Scottish deep water environment. However, the CB concentrations found are unlikely to give rise to pollution effects and are not of concern from the perspective of human health. PBDE concentrations could not be assessed, due to the lack of assessment criteria. Furthermore there is only very limited data on PBDEs in deep water fish. However, the concentrations observed in this study were similar to the concentrations recently reported in Mediterranean deep water fish.
Introduction

Contaminant data for fish from offshore areas around Scotland is limited, particularly for fish from the deeper waters to the west of Scotland. There is concern that the deep waters may act as a sink for semivolatile contaminants such as chlorobiphenyls (CBs) and polybrominated diphenyl ethers (PBDEs). Deep water fish have a significant potential for the accumulation of semivolatile, persistent organic pollutants (POPs). This is because many of the deep water species are longer lived and feed at higher trophic levels than shallow water fish. CBs and organochlorine pesticides (OCPs) were investigated in deep sea fish to the west of Scotland, as part of a PhD studentship\(^1\). Black scabbard and roundnose grenadier were collected from the Rockall Trough in the North East Atlantic at depths of 1000 and 2000 m in 1999. Compared to the shallow water species, the deep water species were found to have an increased burden of the more highly chlorinated CBs.

Due to the increasing commercial interest in some deep-water fish, there is a need to obtain pertinent information on the concentration of POPs in such species. Monitoring of hazardous substance is required as part of any assessment of environmental status, and helps towards determining if Scotland is moving towards the Government’s vision of achieving clean, healthy, safe, productive and biologically diverse oceans and seas. The OSPAR Hazardous Substances Strategy requires the progressive reduction of discharges, emissions and losses of hazardous substances to the marine environment with the aim of achieving concentrations of near background for naturally occurring substances or close to zero for man-made chemicals\(^2\). The EU Marine Strategy, which took effect on 17 June 2008 (Directive 2008/56/EC), will require monitoring in more offshore areas. A definition of Good Environmental Status (GES), including chemical status, will be required for regions and sub-regions and will require the establishment of environmental indicators to support decision making. The aim of the Directive is to achieve Good Environmental Status by 2020. The relevant qualitative descriptors for contaminants for determining good environmental status are ‘concentrations of contaminants are at levels not giving rise to pollution effects’ and ‘contaminants in fish and other sea food for human consumption do not exceed levels established by Community legislation or other relevant standards’.

Previous work on deep water fish, collected in 1999, concentrated on legacy contaminants such as CBs\(^1\). However, this work did not include the analysis of the non-\textit{ortho} CBs. The effects of CBs are well understood and include chronic, long-term effects of CBs (and their metabolites) which include endocrine disruption, immunosuppression and vitamin A deficiency\(^3-7\). The most toxic of the 209 CB congeners are the so called ‘dioxin-like’ CBs. These are the four non-\textit{ortho} CBs (CB81, 77, 126 and 169) and eight mono-\textit{ortho} CBs (CB105, 114, 118, 123, 156, 157, 167 and 189) that also have chlorines in both \textit{para} and at least two \textit{meta} positions. The non-\textit{ortho} CBs can obtain a planar configuration and the mono-\textit{ortho} CBs can obtain a near planar configuration. The twelve ‘dioxin-like’ CBs are stereochemically similar to 2,3,7,8-tetrachlorodibenzo\(p\)-dioxin (TCDD) and therefore have similar toxic and biological responses to those of dioxins\(^8,9\) but are normally found at much lower concentrations when compared to the \textit{ortho}-CBs. For this current study 32 CB
congeners were measured, including the non-ortho and five mono-ortho CBs. CB123 and CB167 were not determined because certified standards were not available at the time of method validation for these congeners. CB114 was not determined because it co-elutes, when using an HP5 GC column, with the organochlorine pesticide 2,4'-dichloro-diphenyl-trichloroethane (DDT), and thus cannot be distinguished by GC-electron capture detection (ECD), as currently used by Marine Scotland.

Other contaminant groups such as polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD) and tetrabromobisphenol-A (TBBP-A), have been added to the OSPAR List of Chemicals for Priority Action since the 1999 deep water fish study and there has been increasing interest in these contaminants. Methods for PBDEs, HBCD and TBBP-A have only recently been developed at relevant laboratories, including Marine Scotland-Science. The effects of these contaminants are less well understood compared to CBs, although all were included on the OSPAR list due to their environmental; persistence, potential to bioaccumulate and toxicity\(^{10}\). In addition, PBDEs have recently been included on the OSPAR Co-ordinated Environmental Monitoring Programme (CEMP). Animal studies have shown that PBDEs are endocrine disruptors, affect thyroid hormone functions and can impair the developing central nervous system and brain\(^{11-14}\). PBDEs bioaccumulate with the more highly brominated PBDEs (except for decaBDE) being more likely to bioaccumulate because they are more hydrophobic and therefore have higher octanol water partition coefficients than the lower brominated PBDEs\(^{10,11}\). HBCD has a high octanol water partition coefficient (Log $K_{ow}$ = 5.8) and, the potential to bioaccumulate with an estimated BCF (bioaccumulation factor) of 18,100 l kg\(^{-1}\)\(^{10}\). However, there is very little information available on this compound. The properties of persistence in the environment, its tendency to bioaccumulate and its toxicity to aquatic and terrestrial organisms have not been fully characterised and the risks to human health have not been fully evaluated\(^{16}\). Information on TBBP-A is currently being reviewed by the OSPAR Hazardous Substances Committee and this compound may be removed from the OSPAR List, as the EU environmental risk assessment report for TBBP-A suggest that TBBP-A does not meet the OSPAR criteria for toxicity and bioaccumulation.

Commercial PBDE mixtures are classed as penta-, octa or deca- according to their degree of bromination and were used in furniture and upholstery, and in textiles as flame retardants, up until 2004 when restrictions were put on their use in the EU. An EU ban on the use of deca-BDE in electronics and electrical equipment became effective on 1 July 2008. Historically PBDEs were released to the environment during their production and while manufacturing other products. In the UK, penta and octa formulations of PBDEs were manufactured by the Great Lakes Chemical Company at Newton Aycliffe, from 1996 until 2003, and high PBDE concentrations were found close to the plant\(^{17}\). Since the ban of BDE formulations the main sources will be from the disposal of products containing these chemicals. In addition, PBDEs may continue to leak out of treated material during everyday use of ordinary consumer products, which has been documented by high PBDE concentrations in the indoor environment. PBDEs have been found to concentrate in the Arctic and bioaccumulate in native animals and humans\(^{15}\), indicating long range atmospheric transport of PBDEs. Other possible pathways to the marine environment include direct discharge
from point sources such as storm waters and waste water. However, the most likely source of PBDEs for deep water to the west of Scotland would be from atmospheric deposition.

Since the ban on PBDEs, HBCD and TBBP-A are among the most widely used brominated flame retardants (BFRs), with an estimated global use of 16,700 and 119,600 tonnes, respectively\textsuperscript{18}. HBCD has been produced in the UK at Newton Aycliffe, County Durham in the north east of England and high concentrations were found close to the plant\textsuperscript{19}. TBBP-A has not been synthesised in the UK although it is imported. There is only very limited data on HBCD and TBBP-A in the Scottish marine environment. The presence of HBCD in the Arctic indicates that long range atmospheric transport is a possible pathway of this contaminant to the marine environment\textsuperscript{15}. However, there insufficient data on TBBP-A, and where it is analysed concentrations are normally low compared to HBCD. A study of aquatic biota from North Sea food webs reported low concentrations of TBBP-A compared to HBCD\textsuperscript{19}. Due to the lack of data on TBBP-A it is not clear if atmospheric deposition is a major source of TBBP-A to the marine environment\textsuperscript{15}.

In 2006, three species of deep water fish: roundnose grenadier (\textit{Coryphaenoides rupestris}), black scabbard (\textit{Aphanopus carbo}) and black dogfish (\textit{Centroscyllium fabricii}), were collected from the continental shelf slope to the west of Scotland from depths of between 1,000 and 1,500 m (Fig. 1). This data has been published and showed roundnose grenadier to have the highest contaminant concentrations\textsuperscript{20}. Concentrations were above background assessment concentrations but comparison of toxic equivalent concentrations to EU maximum levels showed that there was no risk to human health from consumption of these deep water species.

Additional fish were collected in 2007 (black scabbard and roundnose grenadier). This report presents the organic contaminant data (CBs, PBDEs, and HBCD) in the deep water fish collected from the Rockall Trough to the west of Scotland in 2007 and compares the concentrations to fish collected in 2006 from the Rockall Trough.

**Materials and Methods**

**Sample Collection During 2007**

In September 2007, two species of deep water fish were taken by the research vessel FRV \textit{Scotia} from the Rockall Trough, to the west of Scotland (Fig. 1). The black scabbard (\(n = 10\) individual fish) were caught at 1,000 m. The length of the black scabbard collected ranged from 81 – 110 cm. Black scabbard is a relatively fast-growing species achieving a maximum length of around 150 cm at an age of 8-10 years\textsuperscript{21}. Most fish found at Scottish latitudes are immature and spawning is thought to take place around the island of Madeira. Observation of stomach contents suggests the diet consists of crustaceans, fish and, particularly, squid.
The roundnose grenadier (n = 20 individual fish), were collected at two different locations, corresponding to two different depths (1,000 and 1,500 m). The roundnose grenadier collected in September 2007 ranged from 12 – 16 cm in length (nose to anal fin). The roundnose grenadier is thought to live up to 60 years, maturing around 10 years old\textsuperscript{22,23}. Spawning adults are found at Scottish latitudes and individuals grow to over 100 cm in length. Roundnose grenadier feed on copepods, amphipods and fish.

Whole fish were frozen following collection at sea and dissected on return to the laboratory when the liver and muscle fillets were removed, wrapped separately in aluminium foil and stored at -20 ±5°C.

**Lipid determination**

The total lipid content was determined using the Smedes method\textsuperscript{24}. The biota sample (liver, 0.2 – 0.5 g; muscle, 2 – 5 g) was weighed into a centrifuge tube and iso-propanol (18 ml) and cyclohexane (20 ml) added. The sample was homogenised then de-ionised water (~13 – 22 ml, depending on the moisture content of the sample) added and the mixture homogenised again. Centrifugation was used to separate the organic extract from the particulate material. A second extraction was carried out with 13% (v/v) iso-propanol in cyclohexane. The two extracts were combined and the solvent removed by rotary evaporation before drying in an oven at 80°C (± 5°C) for one hour. The weight of residue was determined and the lipid content calculated.

**Pressurised liquid extraction (PLE)\textsuperscript{25}**

*Ortho* CBs and PBDEs were extracted from the one sample and the extract split in two, one half for PBDE analysis and the other for *ortho* CB analysis. Separate extractions were undertaken for HBCD and non-ortho CBs. For each extraction an appropriate amount of tissue (equivalent to 300 mg lipid) was mixed with sodium sulphate (~20 g). This was spiked with appropriate recovery (ortho CBs - CB112 and CB198; PBDEs –FBDE160\textsuperscript{i}) or labelled internal standard (non-ortho CBs -\textsuperscript{13}C-CB77, \textsuperscript{13}C-CB81, \textsuperscript{13}C-CB126 and \textsuperscript{13}C-CB169; HBCD - \textsuperscript{3}H-α-, β- and γ-HBCD). Samples were then refrigerated overnight before being ground to a fine powder using a mortar and pestle. For CBs (ortho and non-ortho) and PBDEs, solvent washed PLE cells (100 ml) were packed as follows: solvent washed filter paper, pre-washed sodium sulphate (10 g), 5% deactivated alumina (30 g), solvent washed filter paper and the biota/sodium sulphate mixture prepared as above. For HBCD analysis only 5 g of alumina was used. The cell was finally filled to the top with more sodium sulphate then packed down and topped up if required and another filter paper placed on top.

Samples were extracted by PLE using an ASE 300 (Dionex Ltd., Camberley, Surrey, UK) under elevated temperatures and pressures. Fish tissue samples were extracted using an oven temperature of 100°C (CBs and PBDEs) and 60°C (HBCD) at a pressure of 1,500 psi. Five

\textsuperscript{i} A fluorinated PBDE where one of the bromines is replaced with a fluorine
minutes heating was followed by 2 x 5 min static cycles. The cell flush was 50% total cell volume (i.e. 25 % of the cell volume for each flush = 25 ml per flush) with a 120 second purge (using nitrogen) at the end of each sample extraction. The extraction solvent was iso-hexane.

Special precautions are required when analysing PBDEs due to their sensitivity to UV light. Incoming light was minimised in the laboratory by placing UV filters on the windows.

**Extract Clean-Up for Polybrominated Diphenyl Ether (PBDE) and Chlorobiphenyl (CB) Analysis**

Following PLE, the extract was split in two, one half for PBDE analysis and the other for CB analysis. The half for CB analysis was concentrated by Syncore (fitted with flushback module) to ~ 0.5 ml and passed through silica columns. The internal standards (2,4-dichlorobenzyl alkyl hexyl ether with C₆ and C₁₆ alkyl chains) were added to the extract before concentrating using the Syncore system and analysing by gas chromatography with electron capture detection (GC-ECD).

The remaining extract was transferred to Syncore tubes and the volume reduced to ~0.5 ml at 30ºC (Syncore, fitted with flushback module), before transferring, with washings, to pre-weighed crimp top, amber glass GC vials. The extracts were finally concentrated further under a stream of nitrogen to approximately 0.5 ml before analysis of PBDEs by gas chromatography - electron capture negative ionisation mass spectrometry (GC-ECNIMS).

**Preparation of Extract and Clean-Up for Non-ortho Chlorobiphenyls (CBs) Analysis**

A separate sample was extracted, using the same method for ortho CBs, by PLE for the analysis of the 4 non-ortho CBs investigated as part of this study. Extracts were concentrated by Syncore (fitted with flushback module) to ~ 0.5 ml and cleaned-up by silica column chromatography (for removal of co-extracted compounds such as organochlorine pesticides) followed by PYE HPLC (2-(1-pyrenyl) ethyl dimethylsilylated silica; 4.6 x 150 mm column) for the separation of non-ortho and ortho-CBs on the basis of structural polarity.

The cleaned-up extracts were concentrated by Syncore and reconstituted in iso-hexane prior to analysis by gas chromatography-electron impact mass spectrometry (GC-EIMS).

**Preparation of Extract for HBCD**

A separate sample was extracted by PLE for the analysis of HBCD (see above). Labelled internal standards were added prior to extraction (³H-α-, β- and γ-HBCD). Any remaining lipid and other co-extractives were separated from HBCD using gel permeation chromatography (GPC). A guard column and two Phenomenex Phenogel columns (300 x 21.2 mm, 50 Å) were connected in series. The mobile phase used was dichloromethane / iso-hexane (1:1, v/v) with a flow of 5 ml min⁻¹. An aliquot of the iso-hexane extract was injected onto the column via a Rheodyne valve. The first
fraction collected (170 ml) was discarded. The second fraction (170 - 320 ml), containing the HBCD diastereoisomers, was collected and the solvent removed using a rotary evaporator. The exact elution volume was pre-determined by injecting 1 ml of the stock standard (1 µg ml⁻¹) and collecting fractions for analysis of HBCD (sum of diastereoisomers) by GC-ECD. The cleaned-up extract was reconstituted in methanol, mixed using a whirli-mixer and analysed by liquid chromatography-mass spectrometry (LC-MS) using a reverse phase C₁₈ column.

Determination of Chlorobiphenyls (CBs)

Determination of Ortho CBs (including 5 mono-ortho CBs) by Gas Chromatography with Electron Capture Detection (GC-ECD)

The concentration and composition of 28 ortho CB congeners (CB31, 28, 44, 49, 70, 74, 110, 101, 99, 97, 149, 118, 132, 153, 105, 157, 137, 138, 158, 183, 128, 156, 180, 187, 189, 170, 194) were determined by GC-ECD using either a PE Clarus 500 (Perkin Elmer, Beaconsfield, UK) or Agilent 6890 (Agilent, Stockport, UK) GC fitted with a cool, on-column injector. A medium polarity column was used for the analyses (HP 5, 60 m x 0.25 mm, 0.25 µm film thickness; Agilent, Stockport, UK) along with an uncoated pre-column (2.5 m x 0.32 mm i.d.). The carrier gas was hydrogen (1 - 3 ml min⁻¹) and make-up gas nitrogen (30 ± 5 ml min⁻¹). The initial oven temperature was 80°C, which was held for 1 minute. The temperature was raised at 3°C min⁻¹ up to 280°C and held at this temperature for 12 minutes. The data was quantified using the Totalchrom Navigator data system (Perkin Elmer, Beaconsfield, UK). The chromatograph was calibrated using a series of external standards and the two 2,4-dichlorobenzyl alkyl ethers. Seven calibration standards (0.002, 0.005, 0.010, 0.020, 0.050, 0.100 and 0.200 µg g⁻¹), containing all 28 CB congeners, were analysed with each batch of samples and the peak height used to compute the calibration curve. A point to point calibration was used due to the non-linearity of the ECD.

Determination of Non-Ortho CBs by Gas Chromatography – Electron Impact Mass Spectrometry (GC-EIMS)

The concentrations of CB77, 81, 126 and 169 were determined by GC-MSD in electron impact mode using an HP6890 Series gas chromatograph interfaced with an HP5973N MSD, fitted with a cool, on-column injector. A medium polarity column was used for the analyses (HP 5, 30 m x 0.25 mm, 0.25 µm film thickness; Agilent, Stockport, UK). The initial oven temperature was 80°C, which was held for 1 minute. The temperature was raised at 10°C min⁻¹ up to 160°C and held at this temperature for 1 minute. This was followed by a ramp of 3°C min⁻¹ up to a final temperature of 280°C. The MS was set for selective ion monitoring (SIM) with a dwell time of 50 ms. Ions monitored for quantification were m/z 292 (CB81, CB77), m/z 304 (¹³CB81, ¹³CB77), m/z 326 (CB126), m/z 338 (¹³CB126), m/z 360 (CB169) and m/z 372 (¹³CB169). In addition m/z 290, 220

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**ii Those in bold are the dioxin-like mono-ortho CBs**
and 222 (CB81, CB77), m/z 302 (\(^{13}\)CB81, \(^{13}\)CB77), m/z 328, 254 and 256 (CB126), m/z 340 (\(^{13}\)CB126), m/z 362,218 and 220 (CB169) and m/z 374 (\(^{13}\)CB169) were monitored for confirmation.

Six calibration standards containing the 4 non-ortho CB congeners analysed as part of this project and the corresponding \(^{13}\)C-labelled non-ortho CBs as internal standards were analysed with each batch of samples and the peak area used to compute the calibration curve.

**Analysis of PBDEs by Gas Chromatography-Electron Capture Negative Ionisation Mass Spectrometry (GC-ECNIMS)**

The concentration and composition of the PBDEs, specifically BDE28, 47, 66, 85, 99, 100, 153, 154, 183 were determined by GC-ECNIMS using an HP6890 Series gas chromatograph interfaced with an HP5973N MSD, fitted with a cool on-column injector. A Thames Restek STX-500 column (STX-500, 30 m x 0.25 mm i.d., 0.15 µm film thickness, Thames Restek, Buckinghamshire, UK) was utilised, fitted with a Thames Restek Siltek (0.53 mm i.d.) 5 m guard column. The injector temperature was initially 120°C and after 2 minutes it was elevated at 100°C per minute to 300°C where it was maintained to the end of the run. The carrier gas was helium, set at a constant pressure of 15 psi. Methane was used as the reagent gas at a pressure of 1.6 bar. The transfer line was held at 280°C and the ion source at 150°C. Injections were made at 120°C and the oven temperature held constant for 2 minutes. Thereafter the temperature was raised at 15°C min\(^{-1}\) up to 205°C. This was followed by a ramp of 6°C min\(^{-1}\) up to a final temperature of 330°C. The MS was set for selective ion monitoring (SIM) with a dwell time of 50 ms. Ions monitored were m/z 78.9 and 80.9 (ions equating to bromine) for all PBDEs.

**Analysis of HBCD by Liquid Chromatography-Mass Spectrometry (LC-MS)**

A PE Sciex API 150 (Perkin Elmer, Mablesfield, UK) single quadropole mass spectrometer equipped with an electrospray source was utilised for the analysis. The LC mobile phase used was acetonitrile/ water, using ammonium acetate as a modifier. The flow rate was set at 200 µl min\(^{-1}\) using an HP1100 quaternary pump. The run time was 45 minutes. A Luna C\(_18\) column (150 x 2.00 mm i.d., 3 µm; Phenomenex, TOWN, UK) was used.

The MS was set for selective ion monitoring (SIM). An internal standard method using a six-point calibration curve was used for quantification. The ions monitored were m/z 641.0 (HBCD) and 658.2 (\(^{3}\)H-HBCD) with a dwell time of 150 msec.

**Quality Control**

The CB, PBDE and HBCD methods are accredited by the United Kingdom Accreditation Service (UKAS) to ISO 17025. All methods were validated by the replicate analysis of standards and samples, and through spiking experiments or analysis of certified reference materials (CRMs). Limits of detection (LoDs) were determined through the repeat analysis of a low spiked sample and
the LoD calculated from $4.65 \times SD$ (standard deviation) of the mean concentration. LoDs were dependent on the sample size. The replicate analysis of standards on separate days gave coefficient of variation (CV%) of less than 3% for \textit{ortho} CBs. Recoveries of greater than 75% were achieved for CB spiked biota, LoDs were around 0.5 μg kg$^{-1}$ wet weight for the fish liver samples ($\sim 0.5 - 1$ g) and around 0.05 μg kg$^{-1}$ wet weight for fish muscle samples ($\sim 10$ g). For non-\textit{ortho} CBs replicate analysis of standards on separate days by GC-EIMS gave CV% of < 6%. Recoveries of a certified reference material (NIST 1946 lake superior fish tissue) for non-\textit{ortho} CBs were between 80 and 120% of the assigned value for each of the congeners. LoDs for non-\textit{ortho} CBs were in the region of 0.01 μg kg$^{-1}$ wet weight and 0.1 μg kg$^{-1}$ wet weight for fish tissue (10 g sample extracted) and fish liver, respectively. LoDs for the tri- to hepta-BDEs in biota were between 0.05 and 0.07 μg kg$^{-1}$ wet weight (10 g samples) and recoveries were >75%. For HBCD the replicate analysis of standards on separate days by LC-MS gave CV% of <10% and recoveries were >90% for spiked biota. LoDs for HBCD were around 0.3 μg kg$^{-1}$ wet weight for a 10 g sample and 3 μg kg$^{-1}$ wet weight for a 1 g sample.

Internal quality control procedures incorporated the use of a laboratory reference material (LRM) for all determinands, and also a certified reference material (CRM) for CBs, in each batch of samples. Procedural blanks were performed with each batch of samples, and the final concentration adjusted accordingly. The data obtained from the LRM and CRM were transferred onto NWA Quality Analyst and Shewhart charts were produced with warning and action limits being drawn at ± 2 x and ± 3 x the standard deviation of the mean, respectively. CRM data was accepted if recoveries were between 80 and 120% of the certified concentration. Quality assurance was further demonstrated through participation in the QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe) Laboratory Performance Studies.

\textbf{Statistical Analysis}

Analysis of variance (ANOVA) at the 95% confidence level, using Minitab 15, was used to assess significant differences between the different fish species, and compare concentrations between years.

\textbf{Assessment Criteria}

The OSPAR Background Concentration (BC) is ‘the concentration of a contaminant at a ‘pristine’ or ‘remote’ site based on contemporary or historical data’. For man-made synthetic substances such as CBs, OSPAR has adopted a BC of zero. The Background Assessment Concentration (BAC) is ‘a value for testing whether concentrations at a site are at or close to background’. Observed concentrations are said to be ‘near background’ if the mean concentration is statistically significantly below the corresponding BAC (Table 3). A traffic light system was recently proposed for use in assessments of contaminant data. A ‘green’ assessment for a particular contaminant would mean that the environmental concentrations are satisfactory in that they present little or no risk. The first transition point (blue/green, $T_0$) for a particular contaminant would require
concentrations for that contaminant to be at, or close to, background concentrations and, therefore, the BACs were selected as $T_0$ for CBs in fish liver. Environmental Assessment Criteria (EACs) are currently under consideration and ICES recommend that the draft OSPAR EACs for CBs in fish liver should not be used in data assessments. Therefore an alternative solution had to be found for the green to red transition ($T_1$). Recent work on the bioavailability of hydrophobic contaminants in sediment using silicone rubber passive samplers has generally shown the potential for all the burden of CBs in sediments to be mobilised into the sediment pore water, i.e. to be potentially bioavailable. Therefore, partitioning theory can be reliably applied to calculate the concentrations of CBs in lipid in biota that would be in equilibrium with the CBs in the sediment. The biota sediment accumulation factor (BSAF) can be expressed as the ratio between the contaminant concentration in sediment (expressed on the basis of organic carbon) and the concentration in biological material. In cases where the total concentration of a contaminant in sediment is potentially bioavailable, the value of BSAF is close to unity. Therefore, EACs ($T_1$) for CBs in sediment were used to calculate concentrations of CBs in fish liver (on a lipid weight basis) in equilibrium with sediment containing CB concentrations equal to the EACs in sediment and it was proposed that these calculated values (termed EAC$_{\text{passive}}$) be used as the green-red boundary ($T_1$) for CBs in fish liver (Table 3). Formally, BACs and EACs are designed for use with data collected under the OSPAR annual trend monitoring programme, but it is still instructive to compare the concentrations observed in this study to BACs and EACs.

**Results and Discussion**

**Total Lipid Content**

The total lipid content of the muscle and liver of the deep water fish was determined using the Smedes method (Table 1 and 2). The mean lipid content in the livers of the black scabbard collected in 2007 was 15.7%, which was not significantly different to the lipid content of the black scabbard collected in 2006 (mean = 13.7%) (Table 1). As found in 2006, the lipid content of the roundnose grenadier was significantly higher compared to the black scabbard with a mean of 73.0% in 2007 (Table 1). Black dogfish were not collected in 2007; however the mean lipid content of the black dogfish liver samples collected during 2006 was 5.7%. The CB and PBDE concentrations in the liver were positively correlated with the lipid content and with each other.

The mean lipid content for the muscle samples collected during 2007 was 2.26% and 1.02% for black scabbard and roundnose grenadier, respectively (Table 2). The CB concentration in the fish muscle was positively correlated with the lipid content.

**Chlorobiphenyls (CBs)**

**Ortho CBs**
CB concentrations were normalised to the lipid content (%) to take into account the different lipid content of the liver of the three species (Table 1, Fig. 2a). CB concentrations for the sum of 28 ortho CBs (ΣCB28 ortho) in liver from black scabbard in 2007 was 600 μg kg⁻¹ lipid weight (SD = 241 μg kg⁻¹ lipid weight, n = 10) whilst in 2006 a mean of 731 μg kg⁻¹ lipid weight (SD = 353 μg kg⁻¹ lipid weight, n = 9) was obtained (Table 1). There was no significant difference in CB concentrations between the two years (p > 0.05). Concentrations in liver from roundnose grenadier were significantly lower in 2007 compared to 2006 (p = 0.017) with ΣCB28 ortho mean concentrations of 441 μg kg⁻¹ lipid weight (SD = 351.1 μg kg⁻¹ lipid weight, n = 21) in 2007 compared to 1,231 μg kg⁻¹ lipid weight (SD = 1,380 μg kg⁻¹ lipid weight, n = 18) in 2006. In 2007 roundnose grenadier were collected further north than in 2006 (Fig. 1), however, they are likely to be from the same fish population. The size distribution in both years was similar and there was no significant difference in the fish weight. Therefore, there is no obvious reason for the different concentrations in roundnose grenadier collected in 2006 and 2007. Black dogfish were only caught in 2006 and gave a mean of 270 μg kg⁻¹ lipid weight (SD = 112 μg kg⁻¹ lipid weight, n = 4). Only one of the 39 fish samples, a black scabbard collected in 2007, gave concentrations for ΣCB28 ortho > 1,000 μg kg⁻¹ lipid weight compared to nine in 2006 (seven roundnose grenadier and two black scabbard). There was no significant difference in the CB concentrations between roundnose grenadier and black scabbard (p > 0.05, ANOVA) collected in 2007. Only the roundnose grenadier were collected at different locations, corresponding to two depths (1,000 and 1,500 m), however, no significant difference was detected in the CB concentrations in the muscle or liver from these locations (p > 0.05, ANOVA). Concentrations in the black dogfish (sampled 2006 only) were significantly lower than black scabbard, sampled in 2006 and 2007 (p = 0.019).

CB concentrations were previously reported in roundnose grenadier and black scabbard liver collected in 1999 at different depths from Rockall (1,000 – 1,974 m) although only 24 CB congeners were measured¹. Mean concentrations for sum of 24 congeners (ΣCB24 ortho) in roundnose grenadier liver ranged from 772 μg kg⁻¹ lipid weight at 1,000 m to 2,130 μg kg⁻¹ lipid weight at 1,974 m, with concentrations increasing with depth. The 2006 and 2007 concentrations for ΣCB24 ortho were compared to the 1999 data; concentrations were significantly lower in 2007 (p<0.001) but there was no significant difference with the 2006 data (p>0.05). In black scabbard liver, collected in the 1999 study, concentrations ranged from 231 μg kg⁻¹ lipid weight (850 – 900 m) to 645 μg kg⁻¹ lipid weight (1250 – 1400 m), concentrations were marginally lower in 2006 (p = 0.033), but there was no significant difference with the 2007 CB concentrations. In contrast to the study by Mormede et al.¹ this study showed no increase in CB concentration with depth, however, fish were collected from two depths only.

Concentrations for ΣCB28 ortho in the black scabbard muscle were similar in 2006 and 2007, with means of 893 μg kg⁻¹ lipid weight (SD = 682 μg kg⁻¹ lipid weight, n = 5) and 1,001 μg kg⁻¹ lipid weight (SD = 508 μg kg⁻¹ lipid weight, n = 10), respectively (Table 2). As for the liver, mean concentrations in roundnose grenadier muscle were lower in 2007 compared to 2006 with means of 901 μg kg⁻¹ lipid weight (SD = 913 μg kg⁻¹ lipid weight, n = 10) in 2006 and 510 μg kg⁻¹ lipid weight (SD = 292 μg kg⁻¹ lipid weight, n = 20) in 2007 (Table 2, Fig. 3a). However, there was no significant
difference (p > 0.05) in the CB concentrations in roundnose grenadier muscle between the two years. There was no significant difference in the CB concentrations in muscle between the two species (p> 0.05, ANOVA).

Concentrations in the 2006 and 2007 black scabbard were also compared to black scabbard muscle collected in 1999 at different depths from Rockall (1,000 – 1,974 m) CBs were not measured in roundnose grenadier muscle collected in 1999. In the 1999 black scabbard muscle, concentrations for \( \Sigma \text{CB}_{24\text{ortho}} \) ranged from 356 \( \mu \text{g kg}^{-1} \) lipid weight at 2,025 m to 1,150 \( \mu \text{g kg}^{-1} \) lipid weight at 900 m and were not significantly (p > 0.05) different compared to the CB concentrations in 2006 and 2007.

The ICES7 CBs were recommended by the European Union Community Bureau of Reference; these CBs were selected as indicators due to their relatively high concentrations in technical mixtures and their wide chlorination range. The ICES7 CBs are the most frequently measured CB grouping and are often used as indicators in environmental monitoring. Concentrations for \( \Sigma \text{ICES7} \) CBs follow the same pattern as concentrations for \( \Sigma \text{CB}_{28\text{ortho}} \) and were approximately two thirds the \( \Sigma \text{CB}_{28\text{ortho}} \) concentration. For black scabbard the mean concentration in 2007 was 380 \( \mu \text{g kg}^{-1} \) lipid weight (SD = 156 \( \mu \text{g kg}^{-1} \) lipid weight, n = 10) compared to a mean of 463 \( \mu \text{g kg}^{-1} \) lipid weight (SD = 193 \( \mu \text{g kg}^{-1} \) lipid weight, n = 9) in 2006. In roundnose grenadier mean concentrations were 284 \( \mu \text{g kg}^{-1} \) lipid weight (SD = 235 \( \mu \text{g kg}^{-1} \) lipid weight, n = 18) in 2007 whilst in 2006 the mean was 792 \( \mu \text{g kg}^{-1} \) lipid weight (SD = 893 \( \mu \text{g kg}^{-1} \) lipid weight, n = 18) (Table 1, Fig. 2b). Five deep water fish (3 black scabbard and 2 roundnose grenadier) collected in 2007 gave concentrations for \( \Sigma \text{ICES7} \) CBs of > 500 \( \mu \text{g kg}^{-1} \) lipid weight. Previous work on CBs in shallow water fish liver has shown that concentrations for \( \Sigma \text{ICES7} \) CBs are generally <500 \( \mu \text{g kg}^{-1} \) lipid weight at sites remote from industrial and urban activity. However, deep water fish have been shown to accumulate higher concentrations of contaminants, as they tend to be longer lived and can feed at a higher trophic level.

In 2007 the \( \Sigma \text{ICES7} \) CB mean concentration in black scabbard muscle was 599 \( \mu \text{g kg}^{-1} \) lipid weight (SD = 298 \( \mu \text{g kg}^{-1} \) lipid weight, n = 10). In 2006 the mean was 583 \( \mu \text{g kg}^{-1} \) lipid weight (SD = 405 \( \mu \text{g kg}^{-1} \) lipid weight, n = 4). For roundnose grenadier collected in 2007 a mean of 331 \( \mu \text{g kg}^{-1} \) lipid weight (SD = 293 \( \mu \text{g kg}^{-1} \) lipid weight, n = 20) was obtained, compared to 610 \( \mu \text{g kg}^{-1} \) lipid weight (SD = 607 \( \mu \text{g kg}^{-1} \) lipid weight, n = 10) in 2006 (Table 2, Fig. 3b). CB concentrations in the muscle were correlated with the corresponding concentrations in the liver (correlation coefficient = 0.68, p <0.001). Eight individual fish muscle samples (2 round nose grenadier and 6 black scabbard) gave concentrations for \( \Sigma \text{ICES7} \) CB of > 500 \( \mu \text{g kg}^{-1} \) lipid weight, five of these also gave high concentrations (>500 \( \mu \text{g kg}^{-1} \) lipid weight for \( \Sigma \text{ICES7} \) CB) in the liver.

**Non-ortho CBs**

Four non-ortho CBs (CB81, 77, 126 and 169) were measured in selected deep sea fish liver and muscle collected in 2007 (those with \( \Sigma \text{ICES7} \) CB concentrations > 500 \( \mu \text{g kg}^{-1} \) lipid weight).
Concentrations were below the detection limit in all 2007 samples. In 2006 non-ortho CBs were detected in 4 roundnose grenadier and 3 black scabbard (liver only). Concentrations were very low compared to the mono-ortho CBs accounting for less than 1% of the ‘dioxin-like’ CB concentration.

**Comparison with Assessment Criteria**

The data obtained was compared with the assessment criteria adopted by OSPAR for use in the Quality Status Report 2010 (Table 3). The mean CB concentrations in the deep water fish and approximate upper 95% confidence limit on the mean (mean + (t_{df,0.95} \times SE^{iii})) were calculated to enable comparisons to BACs (μg kg⁻¹ wet weight) and the EAC\textsubscript{passive} (μg kg⁻¹ lipid weight) to be made (Fig. 4a and 4b). Where CB concentrations were below the LoD, half the LoD value was used in the calculation. As for the 2006 liver samples\textsuperscript{20}, the mean concentrations in the black scabbard and roundnose grenadier were above BACs for all CBs, with upper confidence bound concentrations in the roundnose grenadier for some congeners (more chlorinated CBs) being more than one hundred times the BAC (Fig. 4a). Black dogfish were not collected in 2007, but in 2006 upper confidence bound concentrations for CB28 and CB101 were below BACs in the black dogfish and above for CB52, 118, 138, 153 and 180\textsuperscript{20}. CB28 was below the LoD in all black dogfish samples (Fig. 4a). CB concentrations being above BACs is not unexpected; CB concentrations in fish liver even from offshore sites away from point sources, such as Colonsay and Broad Bay (used as reference sites for the Clyde trend monitoring programme), are rarely below BACs\textsuperscript{26, 27}.

All three fish species in both years exceeded the EAC\textsubscript{passive} for only C118 (Fig 4b). Shallow water fish from Scottish waters were also found to exceed the EAC\textsubscript{passive} for CB118, even in areas remote from industrial and urban activity and with Σ\textsubscript{ICES7} CB concentrations < 500 μg kg⁻¹ lipid weight\textsuperscript{26, 27}. To aggregate results for CB congeners, the rule that was adopted was that if 2 congeners were classed as ‘red’ then the overall CB classification would be ‘red’. This approach, used by OSPAR, minimises the potential of unusual or outlying data from misleadingly affecting assessments. As only CB118 was classed as ‘red’, the aggregated assessment for CBs in deep water fish was ‘green’.

The EU Marine Strategy Framework Directive requires that ‘contaminants in fish and other seafood for human consumption do not exceed levels established by Community legislation or other relevant standards’. Therefore, the CB concentrations in fish liver were also examined in terms of their potential human health risk. Toxic Equivalency Factors (TEFs), the toxicity of a CB congener relative to the most toxic dioxin, 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD), are available for the twelve ‘dioxin-like’ CBs\textsuperscript{32}. An overall toxic equivalent (TEQ) concentration can be calculated by summing the TEQ (sum of the product of the concentration of each congener x TEF) for individual dioxins (when measured) and the twelve dioxin-like CB congeners. The dioxin-like CBs normally have the greatest contribution towards the overall TEQ, due to their greater concentrations in fish relative to the actual dioxins present. TEQs are normally used to assess if fish or fishery products

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\textsuperscript{iii} Standard error of the mean
are safe to eat, with the Commission Regulation (1881/2006/EC) setting a maximum TEQ concentration for the sum of dioxins and dioxin-like CBs of 8 pg g\(^{-1}\) wet weight in the muscle meat of fish and fishery products. On 1 July 2008 limits were also set for fish liver (amendment 565/2008) with a maximum TEQ concentration for the sum of dioxins and dioxin-like CBs of 25 pg g\(^{-1}\) wet weight in fish liver.

TEQs were calculated for the five mono-ortho CBs (TEQ\(_{\text{calc}(5 \text{ congeners})}\)) in the deep water fish muscle and liver collected in 2007. Non-ortho CBs were also measured in the 2007 muscle or liver samples with concentrations for \(\Sigma\)ICES7 CBs > 500 \(\mu\)g kg\(^{-1}\) lipid, but were not detected. Although the TEQ\(_{\text{calc}(5 \text{ congeners})}\) does not take into account all mono-ortho CBs (CB114, 123 and 167 were not analysed because calibration standards were not available and due to co-elution problems during the analysis) or dioxins, these congeners are likely to have the highest concentrations and thus will have a significant contribution to the overall toxicity arising from the exposure to dioxins and ‘dioxin-like’ CBs.

Mean TEQ\(_{\text{calc}(5 \text{ congeners})}\) were highest in the roundnose grenadier liver (Fig. 5a). The TEQ\(_{\text{calc}(5 \text{ congeners})}\) was below the Commission’s maximum level for dioxins and dioxin-like CBs in fish liver of 25 pg g\(^{-1}\) wet weight for all 2007 liver samples. Three deep water fish livers collected in 2006, all roundnose grenadier, gave TEQ\(_{\text{calc}(5 \text{ congeners})}\) greater than the Commission’s maximum level for dioxins and dioxin-like CBs in fish liver of 25 pg g\(^{-1}\) wet weight (outliers in Fig. 5a).

The fish muscle TEQ\(_{\text{calc}(5 \text{ congeners})}\) are shown in Fig. 5b, and were well below the Commissions maximum level of 8 pg g\(^{-1}\) wet weight, the highest being 1.18 pg g\(^{-1}\) wet weight (outlier in Fig. 5b) in a black scabbard collected in 2007. As the 5 mono-ortho CBs are likely to contribute significantly to the overall TEQ, and TEQ\(_{\text{calc}(5 \text{ congeners})}\) in the deep water fish muscle in most cases were more than a factor of 10 lower than Commissions maximum level of 8 pg g\(^{-1}\) wet weight, consumption of Scottish deep water fish muscle is unlikely to represent a risk to health.

Due to the expense of high resolution GC-MS a number of papers have been published looking at alternative methods to predict the total TEQs (for dioxins and ‘dioxin-like’ CBs) in fish tissue, using total CB concentrations or indicator CBs (ortho). These models have previously been applied to Marine Scotland CB data for fish and have been described in detail previously\(^{20}\). In summary, Bhavsar et al. proposed that the total CB (Aroclor equivalent) concentration determined by GC-ECD could be used to estimate the total TEQ for dioxin and ‘dioxin-like’ CBs\(^{33}\). The relationship between the TEQ and total CB concentration was determined and found to be: 

\[
\text{TEQ}_{(\text{Bhavsar})} = 2.56 \times 10^{-5} \times \text{Total CB concentration}
\]

Lasrado et al. looked at four models to predict TEQs using the US Environment Protection Agency fish tissue study\(^{34}\). The authors concluded that the analysis of selected compounds or total CBs could be used to estimate total TEQs and proposed the following models:

\[
\text{TEQ}_{(\text{Lasrado})} = 0.95 + 0.21[\text{CB138}] - 0.08[\text{CB153}] + 0.27[\text{CB118}]
\]
$\text{TEQ (Lasrado total CBs)} = 1.24 + 0.02[\text{total CB}]$

Concentration units for TEQs are pg g$^{-1}$ wet weight and for the CBs µg kg$^{-1}$ wet weight.

All three models were applied to this study to estimate total TEQs (TEQ$_{\text{est}}$). Concentrations for the TEQ$_{\text{calc}(5\text{ congeners})}$ were correlated with the three TEQ$_{\text{est}}$ with correlation coefficients of 0.99 for all ($p < 0.001$). The Lasrado et al. model using the indicator CBs gave the highest TEQ$_{\text{est}}$ and were almost double the TEQ$_{\text{calc}(5\text{ congeners})}$. The TEQ$_{\text{est}}$ for each species (muscle and liver) is shown as boxplots in Figure 5. For the 2007 liver samples, only one roundnose grenadier exceeded 25 pg g$^{-1}$ wet weight. This was when using the Lasrado model (Fig. 5a). As found in 2006, all TEQ$_{\text{est}}$ for all deep water fish muscle collected in 2007 were below the European Commission’s maximum levels of 8 pg g$^{-1}$ wet weight, using all three models (Fig. 5b), confirming the earlier conclusion based on the measured congeners that there is no risk to human health from consumption of these deep water fish.

**CB Sources**

By examining the distribution of CBs it can be possible to distinguish CB sources. Uptake of CBs via food is likely to result in similar absorption for the various congeners present in the food; hence the pattern will be determined by the diet. Sedentary, bottom dwelling fish, such as plaice, should accumulate higher contaminant levels compared to pelagic species since their prey, mainly small benthic organisms, and habitat may be more highly contaminated. CBs can be metabolised by the cytochrome P450 group of enzymes. The age, sex and species of fish can affect the extent to which CBs are metabolised. Most of the less chlorinated CB congeners (tri- and tetra-chloro) and some of the more highly chlorinated CBs can be metabolised by fish. The proportion of higher chlorinated CBs ($\geq 6$ chlorines) increases through the food chain as they are less volatile, more lipophilic and more resistant to metabolic and microbial degradation. Hope et al. have shown a high percentage of the hexa- and hepta-CBs in fish from Midway Atoll (North Pacific Ocean), with 67 - 80% of the total CB concentration being accounted for by these compounds. Storelli et al. also reported the CB profile in the liver of the benthic angler fish to be dominated by the higher chlorinated CBs, with the hexa- and hepta-CBs accounting for 69.6 and 16.0% of the total CB concentration, respectively.

As was found for the 2006 samples, a greater proportion of the higher chlorinated CBs was observed in black scabbard and roundnose grenadier liver and flesh collected in 2007 with the hexa-CBs (CB132, CB137, CB149, 138, 153, 128, 156, CB157, 158) accounting for 30 to 43% followed by the penta-CBs (CB99, CB97, CB101, CB110, CB105, CB118) and hepta-CBs (CB170, 180 and CB189) which accounted for 18 – 41% and 5 – 23%, respectively (Fig. 6). There was little difference between the muscle and liver. Mormede also reported a high proportion of the hexa- and hepta-CBs in black scabbard muscle and liver from Rockall, in this case accounting for 30 – 60% and 20 - 30%, respectively. A similarly higher proportion of more chlorinated CBs was found in deep water fish, including black dogfish, collected from the west coast of Greenland. The
black dogfish from this 2006 study contained 52.3% hexa-CBs, 32.8% penta-CBs and 9.1% hepta-CBs\(^{39}\). The high proportion of the more chlorinated CBs in deep water fish compared to shallow water species is attributed to the vertical transport of contaminants associated with sinking particles in the water column\(^{1,39}\).

**Brominated Flame Retardants**

There are 209 possible PBDE congeners. However, PBDE technical mixtures contain only a limited number of these congeners (~20). Nine of these (BDE28, BDE47, BDE66, BDE100, BDE99, BDE85, BDE154, BDE153 and BDE183) were selected, taking into account their occurrence in the environment and their toxicity, to be routinely determined as part of the OSPAR CEMP. The sum of these nine PBDEs (\(\Sigma\) PBDE\(_9\)) in the liver of black scabbard collected in 2007 was 25.8 µg kg\(^{-1}\) lipid weight (SD = 8.2 µg kg\(^{-1}\) lipid weight, \(n = 10\)). Concentrations were not significantly different compared to the 2006 black scabbard (mean = 25.5 µg kg\(^{-1}\) lipid weight, SD = 17.7 µg kg\(^{-1}\) lipid weight, \(n = 10\)) (Table 4). As for the CBs, PBDE concentrations were significantly lower (\(p = 0.009\)) in the liver from roundnose grenadier collected in 2007 compared to those collected in 2006, with mean concentrations for \(\Sigma\) PBDE\(_9\) of 46.0 µg kg\(^{-1}\) lipid weight (SD = 36.6 µg kg\(^{-1}\) lipid weight, \(n = 18\)) in 2006 and 21.1 µg kg\(^{-1}\) lipid weight (SD = 16.9 µg kg\(^{-1}\) lipid weight, \(n = 20\)) in 2007 (Table 4, Fig. 7a). Concentrations in the black dogfish (2006 only) were significantly lower (\(p < 0.05\), ANOVA) than in the black scabbard with a mean of 8.8 µg kg\(^{-1}\) lipid weight (SD = 5.5 µg kg\(^{-1}\) lipid weight, \(n = 5\)) (Table 4, Fig. 7a).

PBDE concentrations in the fish muscle were low with concentrations being below the LoD for most congeners. Mean \(\Sigma\) PBDE\(_9\) concentrations in black scabbard muscle were 42.5 µg kg\(^{-1}\) lipid (SD = 26.4 µg kg\(^{-1}\) lipid weight, \(n = 10\)) in 2007 whilst in 2006 the mean was 30.5 µg kg\(^{-1}\) lipid weight (SD = 28.4 µg kg\(^{-1}\) lipid weight, \(n = 5\)) (Table 4, Fig. 7b). For roundnose grenadier, concentrations were again lowest for the 2007 samples with a mean of 33.6 µg kg\(^{-1}\) lipid weight (SD = 36.0 µg kg\(^{-1}\) lipid weight, \(n = 9\)) in 2006 and 11.6 µg kg\(^{-1}\) lipid weight (SD = 7.4 µg kg\(^{-1}\) lipid weight, \(n = 12\)) in 2007 (Table 4, Fig. 7b). There was no significant difference in the PBDE concentrations between the two species.

The PBDE profile in both the muscle and liver was typical of other studies with BDE47, 99 and 100 dominating the profile\(^{15,17}\). BDE17, 75, 71, 77 119, 183 and 190 were detected in only a few instances.

Currently there is only very limited published data on PBDEs in deep water fish. Ten different species of deep water fish (whole fish) from the Sulu Sea, western Pacific, collected at depths of up to 1,015 m were analysed for organohalogen contaminants, including PBDEs. Concentrations of all contaminants were very low with PBDE concentrations (sum of 14 congeners) ranging from 0.85 to 2.1 µg kg\(^{-1}\) lipid weight\(^{40}\). More recently PBDE concentrations were reported in the liver of two deep sea fish species (hollowsnout grenadier and roughsnout grenadier) from the Mediterranean\(^{41}\). Concentrations were similar to the concentrations found in the Scottish deep water fish from this
study. Concentrations for the sum of 8 congeners (BDE28, 49, 47, 66, 100, 99, 154, 153) ranged from 11.8 – 27.3 µg kg\(^{-1}\) lipid weight (n = 6) in hollowsnout grenadier and from 3.2 – 7.0 µg kg\(^{-1}\) lipid weight (n = 9) in roughsnout grenadier\(^{41}\). Higher concentrations of PBDEs have been found in the liver of four different populations of Atlantic salmon, with mean total PBDE concentrations (BDE47, 99 and 100) ranging from 50 to 263 µg kg\(^{-1}\) lipid weight\(^{42}\).

OSPAR adopted a BC of zero for man-made synthetic substances such as PBDEs. Due to the lack of data on PBDEs in the marine environment, BACs have not yet been established for PBDEs. Furthermore, EACs are not currently available for PBDEs. Therefore the environmental significance of the PBDE concentrations found in the deep water fish cannot be assessed.

The presence or otherwise of HBCD was also investigated in the deep water fish samples collected in 2007. Concentrations were below the LoD (0.3 µg kg\(^{-1}\) for a 10 g sample and 3 µg kg\(^{-1}\) for a 1 g sample). Few publications give method LoDs. Morris et al. report limits of quantification of 1.2 µg kg\(^{-1}\) (biota and sediment) based on the extraction of 1 g and an injection volume of 15 µl for analysis by LC-MS\(^{19}\). This is around half of the Marine Scotland LoD for a similar sample size.

HBCD has been detected in the European, Asian and Arctic environments\(^{15, 43}\). HBCD has been found in biota from a number of published studies, although if detected concentrations are low\(^{43 - 48}\). The environmental occurrence of HBCD was investigated in Sweden\(^{45}\). HBCD was found in a range of matrices (water, soil, sediment, biota) with the highest total concentrations (determined by GC-MS) found in pike (27 µg kg\(^{-1}\) lipid weight) and in sediment (25 µg kg\(^{-1}\) dry weight). HBCD was measured in the Scheldt estuary in mysid shrimp and sediment\(^{46}\). HBCD (sum of \(\alpha\), \(\beta\), \(\gamma\)-HBCD) was detected in both sediment (14 – 71 µg kg\(^{-1}\) dry weight) and mysid (562 - 727 µg kg\(^{-1}\) lipid weight). Morris et al. investigated HBCD in sediment and biota from North Sea estuaries and rivers, including the River Clyde in Scotland\(^{19}\). The highest \(\Sigma\)HBCD concentrations in sediment were from the River Skerne, County Durham, close to the Newton Aycliffe plant, with concentrations of 1.7 mg kg\(^{-1}\) dry weight. HBCD was also detected in Clyde sediment at concentrations (7 to 187 µg kg\(^{-1}\) dry weight) lower than found close to the Newton Aycliffe plant but higher than found in other rivers such as the Mersey (22 µg kg\(^{-1}\) dry weight) and Humber (6 µg kg\(^{-1}\) dry weight). HBCD is most commonly detected in marine mammals and has been investigated as part of food chain studies. HBCD concentrations were measured in different trophic levels of the North Sea food web\(^{45}\). Most samples contained \(\alpha\)-HBCD, typically the dominant congener in biota. Concentrations for \(\Sigma\)HBCD ranged from 2.1 to 6.8 mg kg\(^{-1}\) lipid weight in liver and blubber of harbour porpoises and seals with some evidence of biomagnification through the food web. Zegers et al. investigated HBCD concentrations in harbour porpoises and dolphins from western European Seas\(^{47}\). The highest HBCD concentration was in harbour porpoises stranded on the Irish and Scottish coasts of the Irish Seas (2.9 mg kg\(^{-1}\) lipid weight) and on the north west coast of Scotland (5.1 mg kg\(^{-1}\) lipid weight). Concentrations in other areas were < 1.5 mg kg\(^{-1}\) lipid weight. \(\alpha\)-HBCD was the only diastereoisomer detected. HBCD was found in harbour porpoise sampled from Scottish waters between 2001 and 2003\(^{48}\). Concentrations for \(\Sigma\)HBCD ranged from 393.3 to 9,592 µg kg\(^{-1}\) lipid weight (mean = 2,354 µg kg\(^{-1}\) lipid weight). Although HBCD has been detected in
Scottish waters, this has mainly been in marine mammals. HBCD was investigated in plaice liver from the Clyde (Garroch Head and Holy Loch, Marine Scotland unpublished data) but was not detected in any sample. Therefore, as HBCD was not detected in any of the deep water fish collected to the west of Scotland, or in fish liver from the Clyde, HBCD may not be an issue for Scotland.

Conclusions

1. High CB concentrations (greater than 500 μg kg\(^{-1}\) lipid weight for \(\Sigma\)ICES7 CBs) were found in 5 of the 30 deep water fish liver samples and 8 of 30 fish muscle samples, collected in 2007 from the Rockall Trough with the profile being dominated by the more highly chlorinated CBs. CB concentrations in the liver were correlated with the CB concentrations in the muscle.

2. BACs (μg kg\(^{-1}\) wet weight) were exceeded for the liver of black scabbard and roundnose grenadier sampled in 2007, and were more than 100 times the BACs for the most chlorinated CBs in roundnose grenadier. The EAC\(^{\text{passive}}\) was only exceeded for CB118, for this congener the upper bound confidence limit was greater than the EAC\(^{\text{passive}}\). As only one congener was above the EAC\(^{\text{passive}}\), the overall classification for CBs in Scottish deep water fish was ‘green’ and CB concentrations found are unlikely to give rise to pollution effects.

3. Toxic Equivalent (TEQ) concentrations were calculated for the 2007 liver samples using the five mono-ortho (‘dioxin-like’) CBs and were below the Commission’s maximum level for dioxins and dioxin-like CBs in fish liver of 25 pg g\(^{-1}\) wet weight. Estimated total TEQs, calculated using published models, were > 25 pg g\(^{-1}\) wet weight in only one of the roundnose grenadier livers collected in 2007.

4. Both calculated and estimated TEQs for the deep water fish muscle were below the Commission Regulation (1881/2006/EC) maximum TEQ concentration for the sum of dioxins and ‘dioxin-like’ CBs in the muscle meat of fish and fishery products (8 pg g\(^{-1}\) wet weight). Therefore, consumption of the muscle from these deep water fish is unlikely to represent a risk to human health.

5. Polybrominated diphenyl ethers (PBDEs) were found in the liver and muscle of the deep water fish with BDE47, 99 and 100 dominating the profile. However, concentrations were low, often below LoDs. The significance of these concentrations could not be assessed due to the lack of suitable assessment criteria. However, concentrations were similar to those found in Mediterranean deep water fish.

6. HBCD was not detected in either the liver or muscle tissue of the deep water fish collected in 2006 and 2007. To date HBCD has not been detected in any Scottish fish liver samples.
(including samples from the Clyde) and, therefore, this contaminant may not be an issue for Scotland.

References


29. C. F. Moffat, J. Pijnenburg and T. Trass (eds), 2004. OSPAR/ICES Workshop on the evaluation and update of Background Reference Concentrations (B/RCs) and Ecotoxicological Assessment Criteria (EACs) and how these assessment tools should be used in assessing contaminants in water, sediment, and biota. Report of the joint OSPAR/ICES Workshop, The Hague, Netherlands 9 – 13 February 2004.


TABLE 1

Lipid content (%) and concentrations for $\Sigma$CB$_{28}$ ortho and $\Sigma$ICES7 CBs (µg kg$^{-1}$ lipid weight) in liver from three deep water fish species collected from Rockall during September 2006 and 2007.

<table>
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<th>Median</th>
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<td>Lipid Content (%)</td>
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<td></td>
<td></td>
<td>$\Sigma$CB$_{28}$ ortho Concentration (µg kg$^{-1}$ lipid weight)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>black dogfish</td>
<td>2006</td>
<td>159 – 378.6 (n = 4)</td>
<td>270</td>
<td>271</td>
<td>112.0</td>
</tr>
<tr>
<td>black scabbard</td>
<td>2006</td>
<td>163 – 1,244 (n = 9)</td>
<td>731</td>
<td>748</td>
<td>353</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>221 – 914 (n = 10)</td>
<td>600</td>
<td>555</td>
<td>241</td>
</tr>
<tr>
<td>roundnose grenadier</td>
<td>2006</td>
<td>233 – 5,326 (n = 18)</td>
<td>1,231</td>
<td>714</td>
<td>1,380</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>62 – 1,641 (n = 20)</td>
<td>441</td>
<td>364</td>
<td>351</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\Sigma$ICES7 CB Concentration (µg kg$^{-1}$ lipid weight)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>black dogfish</td>
<td>2006</td>
<td>111 – 260.4 (n = 4 pools)</td>
<td>188</td>
<td>191</td>
<td>79.2</td>
</tr>
<tr>
<td>black scabbard</td>
<td>2006</td>
<td>129.6 – 760 (n = 9)</td>
<td>463</td>
<td>470</td>
<td>193</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>154.7 – 605.5 (n = 10)</td>
<td>380</td>
<td>345</td>
<td>156</td>
</tr>
<tr>
<td>roundnose grenadier</td>
<td>2006</td>
<td>177 – 3,475 (n = 18)</td>
<td>792</td>
<td>472</td>
<td>893</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>44.4 – 1,106 (n = 20)</td>
<td>284</td>
<td>235</td>
<td>235</td>
</tr>
</tbody>
</table>
TABLE 2

Lipid content (%) and concentrations for $\Sigma$CB$_{28}$ ortho and $\Sigma$ICES7 CBs (µg kg$^{-1}$ lipid weight) in fish muscle from two deep water fish species collected from Rockall in September 2006 and 2007.

<table>
<thead>
<tr>
<th>Species</th>
<th>Year</th>
<th>Range</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lipid Content (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>black scabbard</td>
<td>2006</td>
<td>0.44 – 2.12 (n = 5)</td>
<td>1.07</td>
<td>0.80</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>0.59 – 6.4 (n = 10)</td>
<td>2.26</td>
<td>1.35</td>
<td>1.96</td>
</tr>
<tr>
<td>roundnose grenadier</td>
<td>2006</td>
<td>0.39 – 2.70 (n = 10)</td>
<td>0.92</td>
<td>0.73</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>0.52 – 4.2 (n = 20)</td>
<td>1.20</td>
<td>0.85</td>
<td>0.98</td>
</tr>
<tr>
<td>$\Sigma$CB$_{28}$ ortho</td>
<td></td>
<td>Concentration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>black scabbard</td>
<td>2006</td>
<td>367 – 2,069 (n = 5)</td>
<td>893</td>
<td>684</td>
<td>682</td>
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<tr>
<td></td>
<td>2007</td>
<td>312 – 1,905 (n = 10)</td>
<td>1,001</td>
<td>933</td>
<td>508</td>
</tr>
<tr>
<td>roundnose grenadier</td>
<td>2006</td>
<td>62 – 2,626 (n = 10)</td>
<td>901</td>
<td>473</td>
<td>913</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>113 – 1,229 (n = 20)</td>
<td>510</td>
<td>483</td>
<td>292</td>
</tr>
<tr>
<td>$\Sigma$ICES7 CB</td>
<td></td>
<td>Concentration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>black scabbard</td>
<td>2006</td>
<td>251 – 1,285 (n = 5)</td>
<td>583</td>
<td>497</td>
<td>405</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>185 – 1,131 (n = 10)</td>
<td>599</td>
<td>581</td>
<td>298</td>
</tr>
<tr>
<td>roundnose grenadier</td>
<td>2006</td>
<td>52 – 1,671 (n = 10)</td>
<td>610</td>
<td>325</td>
<td>607</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>65.4 – 846 (n = 20)</td>
<td>331</td>
<td>322</td>
<td>293</td>
</tr>
</tbody>
</table>
TABLE 3

Summary of OSPAR assessment criteria for CBs in fish liver. $T_0$ is the first transition point (blue/green transition) which is defined by the BAC while $T_1$ is the second transition point (green/red transition) which is defined by the EAC$^{\text{passive}}$. Concentrations less than the BAC result in a blue traffic light while concentrations less than the EAC$^{\text{passive}}$ but greater than the BAC result in a green traffic light. A red traffic light occurs when concentrations are greater than the EAC$^{\text{passive}}$. BC, background concentration; LC, low concentration; BAC, background assessment concentration; EAC$^{\text{passive}}$, environmental assessment criteria derived from the EAC for sediment using data from passive sampling. It should be noted that the units for $T_0$ and $T_1$ are different with $T_0$ being reported on the basis of ($\mu$g kg$^{-1}$ wet weight) and $T_1$ on the basis of ($\mu$g kg$^{-1}$ lipid weight).

<table>
<thead>
<tr>
<th>Congener</th>
<th>BC/LC</th>
<th>Assessment Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_0$ ($\mu$g kg$^{-1}$ wet weight)</td>
<td>$T_1$ ($\mu$g kg$^{-1}$ lipid weight)</td>
</tr>
<tr>
<td>CB28</td>
<td>0.0/0.05</td>
<td>0.6</td>
</tr>
<tr>
<td>CB52</td>
<td>0.0/0.05</td>
<td>0.2</td>
</tr>
<tr>
<td>CB101</td>
<td>0.0/0.05</td>
<td>1.9</td>
</tr>
<tr>
<td>CB118</td>
<td>0.0/0.05</td>
<td>1.3</td>
</tr>
<tr>
<td>CB138</td>
<td>0.0/0.05</td>
<td>0.2</td>
</tr>
<tr>
<td>CB153</td>
<td>0.0/0.05</td>
<td>0.2</td>
</tr>
<tr>
<td>CB180</td>
<td>0.0/0.05</td>
<td>0.5</td>
</tr>
</tbody>
</table>
TABLE 4

Concentration for the sum of 9 PBDE congeners (μg kg⁻¹ lipid weight) in fish liver and muscle from three deep water fish species collected from Rockall in September 2006 and 2007. LoQ, limit of quantification.

<table>
<thead>
<tr>
<th>Species</th>
<th>Year</th>
<th>Range</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>black dogfish</td>
<td>2006</td>
<td>2.0 – 14.6 (n = 5)</td>
<td>8.8</td>
<td>11.4</td>
<td>5.5</td>
</tr>
<tr>
<td>black scabbard</td>
<td>2006</td>
<td>3.7 – 51.4 (n = 10)</td>
<td>25.5</td>
<td>23.4</td>
<td>17.7</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>13.7 – 40.0 (n = 10)</td>
<td>25.8</td>
<td>26.6</td>
<td>8.2</td>
</tr>
<tr>
<td>roundnose grenadier</td>
<td>2006</td>
<td>3.4 – 151.7 (n = 18)</td>
<td>46.0</td>
<td>39.4</td>
<td>36.0</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>3.8 – 59.8 (n = 20)</td>
<td>21.1</td>
<td>11.6</td>
<td>16.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Muscle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>black scabbard</td>
<td>2006</td>
<td>&lt;LoQ – 60.0 (n = 5)</td>
<td>30.5</td>
<td>29.3</td>
<td>28.4</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>15.0 – 99.0 (n = 10)</td>
<td>42.5</td>
<td>34.1</td>
<td>26.4</td>
</tr>
<tr>
<td>roundnose grenadier</td>
<td>2006</td>
<td>&lt;LoQ – 217.36 (n = 9)</td>
<td>33.6</td>
<td>39.4</td>
<td>36.0</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>&lt;LoQ – 26.7 (n = 12)</td>
<td>11.6</td>
<td>11.6</td>
<td>7.4</td>
</tr>
</tbody>
</table>
Figure 1  Location of deep water fish sites on the west of Scotland sampled in September 2006 and September 2007 from the FRV Scotia.
Figure 2 Concentrations (µg kg\(^{-1}\) lipid weight) for (a) \(\Sigma CB_{28\text{ ortho}}\) and (b) \(\Sigma ICES7\) CBs in the liver of deep water fish collected in 2006 and 2007 from the Rockall Trough to the west of Scotland. Concentrations of < 500 µg kg\(^{-1}\) lipid weight for the \(\Sigma ICES7\) CBs have previously been found in plaice liver collected at remote, reference sites. The circle is the mean concentration and asterisks are outliers.
Figure 3 Concentrations (µg kg\(^{-1}\) lipid weight) for (a) ΣCB\(_{28\;ortho}\) and (b) ΣICES7 CBs in the flesh of deep water fish collected in 2006 and 2007 from the Rockall Trough to the west of Scotland. The circle is the mean concentration.
Figure 4 Interval plots of ICES7 CBs in fish liver for three species of deep water species (BD, black dogfish; BSC, black scabbard; RNG, roundnose grenadier) collected in 2006 and 2007. (a) Individual congener concentrations reported as $\mu$g kg$^{-1}$ wet weight; the blue hashed line represents the BAC and (b) individual congener concentrations reported as $\mu$g kg$^{-1}$ lipid weight; the green hashed line represents the EAC$^{\text{passive}}$ for CB118. (Note: the EAC$^{\text{passive}}$ was above the scale of the axis for all other congeners).
Figure 5 Boxplot of the sum of the calculated and estimated toxic equivalence (TEQs) concentrations (pg g⁻¹ wet weight) in deep water fish (a) liver and (b) muscle collected from Rockall in 2006 and 2007. The central line represents the median concentration and the circle the mean concentration. The hashed line at 25 pg g⁻¹ wet weight is the EC limit for fish liver. The EC limit for muscle is 8 pg g⁻¹.
Figure 6 CB homolog % composition in the liver and muscle of deep water fish collected from the Rockall fishing area during 2007.
Figure 7 Concentrations (μg kg⁻¹ lipid weight) for the sum of 9 PBDE congeners in the (a) liver and (b) muscle of deep water fish. The fish were collected from the Rockall fishing area, which is to the west of Scotland, in September 2006 and September 2007 from depths of 1000 or 1500 m. The circle is the mean concentration and asterisks are outliers.