Chlorobiphenyls in deep water fish species from the west coast of Scotland

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INTRODUCTION

Deep water (> 1000 m) fish have a high potential for the accumulation of semivolatile, persistent organic pollutants (POPs), such as chlorobiphenyls (CBs), because many deep water species are longer lived and feed at higher tropic levels than shallow water fish. CBs are a group of hazardous substances which are included on the OSPAR List of Chemicals for Priority Action due to their persistence, toxicity and potential to bioaccumulate. Due to concerns about the environmental impact of CBs, production in the UK ceased in the 1970s. Authorisation for use in closed systems continued until 1986 when sales of CB formulations were prohibited in the UK. However, CBs still enter the marine environment following the destruction and disposal of industrial plants and equipment, or from emissions from old electrical equipment (for example from landfill sites). Their semi-volatile character and high environmental half-lives result in long-range atmospheric transport and CBs are, therefore, ubiquitous in the marine environment. There are 209 possible CB congeners of which 20 can attain a planar configuration due to the absence of *ortho*-chlorine substitution of the biphenyl rings. Sterochemically they are similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and therefore these planar (non-*ortho*) CBs have similar toxic and biological responses to those of dioxins. Eight mono-*ortho* CBs (CB105, 114, 118, 123, 156, 157, 167 and 189) are included in the group of so-called 'dioxin-like' CBs, along with the four non-*ortho* or planar CBs (CB81, 77, 126 and 169). Although these compounds are more toxic than those CBs with more than one chlorine in the *ortho*-position they are normally found at much lower concentrations compared to the *ortho*-CBs.

Deep water fish [roundnose grenadier (*Coryphaenoides Rupestris*), black scabbard (*Aphanopus carbo*), and black dogfish (*Centroscyllium fabricii*)] collected on the west of Scotland during 2006 were analysed for halogenated persistent organic pollutants. This report presents the preliminary CB data (including 5 mono *ortho* and 4 planar CBs) in three species of deep water fish.

Materials and Methods

In 2006, the above mentioned three species of deep water fish were taken by the research vessel FRV *Scotia* from the Rockall fishing area, to the west of Scotland (Fig. 1) at depths between 1000 and 1500 m. Liver and fish flesh were sub sampled for contaminant analysis. Fish flesh was not collected from black dogfish. The total lipid content was determined using the Smedes method[1]. The CBs were extracted from the fish tissues by Pressurised Liquid Extraction (PLE). An appropriate amount of tissue, equivalent to a maximum 300 mg lipid, was mixed with anhydrous sodium sulphate (~ 40 g). This was spiked with appropriate recovery standards CB35, CB53, CB112, CB151, CB198 and CB209 and refrigerated overnight before being ground to a fine powder. Thirty (30) g of 5% deactivated alumina was added to the PLE cell as a fat retainer. Samples were extracted using an oven temperature of 60^{o}C and a pressure of 1500 psi. The extraction solvent used was *iso*-hexane. The extract was passed through alumina and silica columns. The internal standards (2,4-dichlorobenzyl alkyl hexyl ether with C_6 and C_{16} alkyl chains) were added to the extract before concentrating using a Syncore system and analysing by gas chromatography with electron capture detection (GC-ECD).

A separate sample was extracted by PLE for the analysis of planar CBs. Extracts were cleaned-up by silica column chromatography followed by PYE HPLC (2-(1-pyrenyl) ethyldimethylsilylated silica; 4.6 x 150 mm column) for the separation of planar and non-planar 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 spectroscopy (GC-EIMS).

The concentration and composition of 28 CB congeners (CB31, 28, 52, 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)[1] were determined by GC-ECD using a Varian 3500 GC fitted with a cool on-column injector. An additional 4 planar CBs were determined by GC-EIMS. A medium polarity column was used for the analyses (HP 5, 60 m x 0.25 mm,

[1] Those in bold are the dioxin-like mono-ortho CBs

0.25 μm film thickness). For planar CBs, the MS was set for selective ion monitoring (SIM) with a dwell time of 50 ms. Ions monitored were *m/z* 292 (CB81, CB77), *m/z* 304 (^{13}CB81, ^{13}CB77), *m/z* 326 (CB126), *m/z* 338 (^{13}CB126), *m/z* 360 (CB169) and *m/z* 372 (^{13}CB169). A Laboratory Reference Material (LRM) and procedural blank were analysed with each batch of samples for both *ortho* and non-*ortho* CBs and the results monitored on Shewhart control charts.

RESULTS AND DISCUSSION

The mean % lipid in the livers of the black dogfish, black scabbard and roundnose grenadier was 5.7%, 13.7% and 57.8%, respectively. The roundnose grenadier liver had the highest lipid content, although it was very variable, ranging from 28.0 to 83.0%. The CB concentrations in the liver were positively correlated with the lipid content. Muscle tissue from the black scabbard and roundnose grenadier were also collected for analysis. The lipid content in the fish muscle of these two species was similar with means of 1.07 and 0.92% for the black scabbard and roundnose grenadier, respectively. There was no correlation between lipid content and CB concentrations in the fish muscle.

CB concentrations were normalised to the % lipid to take into account the different lipid content of the three species. Mean concentrations for the ∑ICES7[2] CBs in liver were 369 μg/kg lipid weight (SD = 410 μg/kg lipid weight, n = 5), 463 μg/kg lipid weight (SD = 193 μg/kg lipid weight, n = 9) and 792 μg/kg lipid weight (SD = 893, n = 18) for black dogfish, black scabbard and roundnose grenadier, respectively (Fig. 2). Previous studies have shown that concentrations for ∑ICES7 CBs in fish (plaice) liver at offshore sites away from point sources are < 500 μg/kg lipid weight with higher concentrations being found at sites close to point sources such as at the former sewage sludge dump site (Garroch Head) in the Clyde[2]. Ten liver samples (six roundnose grenadier, three black scabbard and one black dogfish) gave concentrations for ∑ICES7 CBs of > 500 μg/kg lipid weight. The black scabbard and roundnose grenadier muscle gave mean ICES7 CB concentrations > 500 μg/kg lipid weight with concentrations of 583 μg/kg lipid weight (SD = 405 μg/kg lipid weight, n = 5) and 610 μg/kg lipid weight (SD = 607 μg/kg lipid weight, n = 10), respectively (Fig. 2).

Planar CBs were also measured in selected livers (those with CB concentrations 500 μg/kg lipid weight) and flesh from 6 roundnose grenadier, 3 black scabbard and 1 pool of 5 black dogfish livers. CB81 was not detected in any of the deep water fish liver samples. CB126 was not detected in the roundnose grenadier or black scabbard samples but was detected in the pooled black dogfish sample (13.8 μg/kg lipid weight). The more highly chlorinated CB169 was detected in all but one fish liver sample from a roundnose grenadier. Concentrations for this congener ranged from 0.6 μg/kg lipid weight to 5.0 μg/kg lipid weight. Planar CBs were < limit of quantification (LoQ) in the fish muscle samples.

OSPAR Background Concentrations (BCs) are used to assess chemical monitoring data and identify areas of potential environmental concern. For xenobiotic substances such as CBs, OSPAR has adopted a BC of zero. Observed concentrations are said to be 'near background' if the mean concentration is statistically significantly below the corresponding Background Assessment Concentration (BAC). CB BACs (μg/kg wet weight) were exceeded for the liver of all three species and were more than 100 times the BACs for the more chlorinated CBs in roundnose Grenadier. 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. Overall toxic equivalent (TEQ) concentrations for the nine dioxin-like CBs measured at FRS were calculated by summing the individual TEQs. CB118 and CB105 had the greatest contribution towards the overall TEQ (mainly >60%). Where measured, planar CBs (CB81, 77, 126 and 169) accounted for <1% of the 'dioxin-like' CBs, however depending on which planar CBs were present this could result in a large increase in the TEQ. TEQs were highest in the roundnose grenadier. TEQs are normally used to assess if fish or fishery products are safe to eat, with the Commission Regulation (199/2006/EC) setting a maximum TEQ concentration for the sum of dioxins and dioxin-like CBs of 8 pg/g wet weight in the muscle meat of fish and fishery products. TEQs for the dioxin-like CBs in the deep water fish muscle ranged from 0.02 to 0.365 pg/g wet weight. TEQs were also estimated using published models

[2] Seven CB congeners selected by the International Council for the Exploration of the Seas (ICES) as indicators due to their relatively high concentrations in technical mixtures and wide chlorination range

to predict the overall TEQ using total CB[3, 4] or indicator CB (CB138, CB153 and CB118)[4] concentrations. TEQs calculated for the dioxin-like CBs measured, and estimated TEQs calculated using published models, indicated that the CB concentrations found in deep water fish are unlikely to represent a risk to human health.

The relationship between the ICES7 CB concentrations and TEQs were also investigated. Concentrations for the ICES7 CBs were positively correlated with concentrations for the calculated and estimated TEQs. Ratios for the ICES7 CB against the calculated TEQs were relatively consistent and were similar for the three species (Fig. 3). The mean ratios of the calculated TEQs (pg/g wet weight) to the ICES7 CB concentrations (µg/kg wet weight) were 0.018 (SD = 0.0017, n = 5), 0.021 (SD = 0.007, n = 9) and 0.026 (SD = 0.007, n = 18) in the liver of black dogfish, black scabbard and roundnose grenadier, respectively. Therefore it may be possible to use the ICES7 CB concentrations to estimate the 'dioxin-like' CB concentrations or the TEQs from these CBs.

References

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Figure 1 West of Scotland deep water *FRV* Scotia trawl sites (♦)

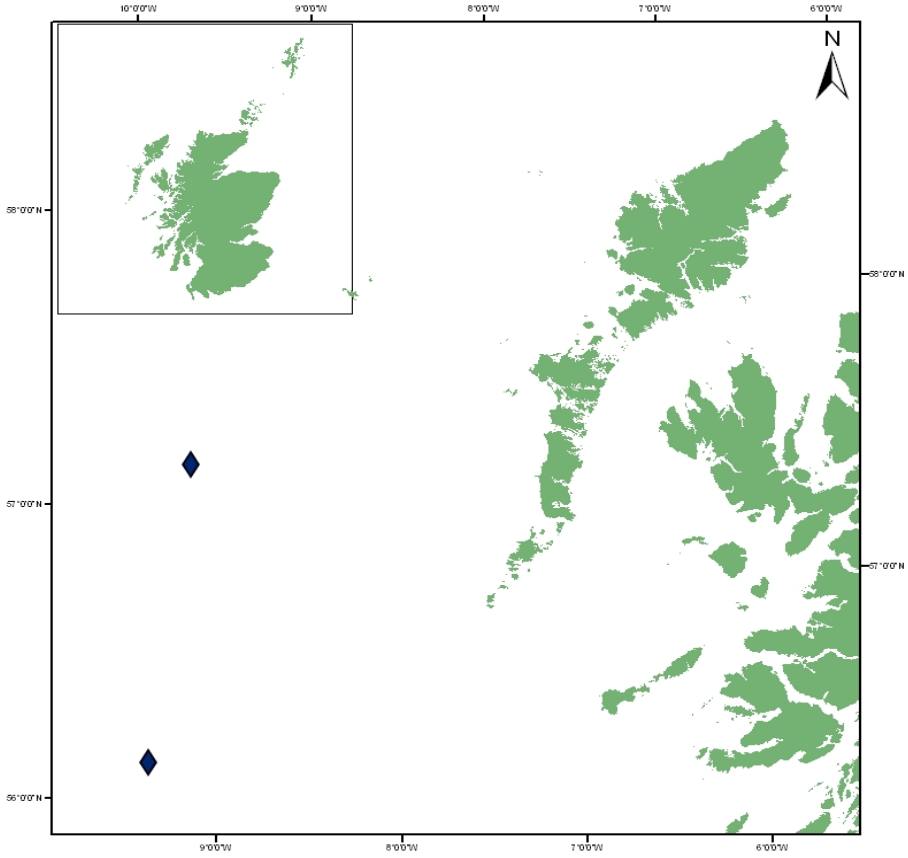

Figure 2 Concentrations (µg/kg lipid weight) for the ∑ICES7 CBs in the liver and flesh of deep water fish, collected from fishing area Rockall, to the west of Scotland, in 2006. The circle is the mean concentration and asterisks are outliers. Concentrations of < 500 µg/kg lipid weight for the ∑ICES7 CBs have previously been found in plaice liver collected at remote, reference sites.

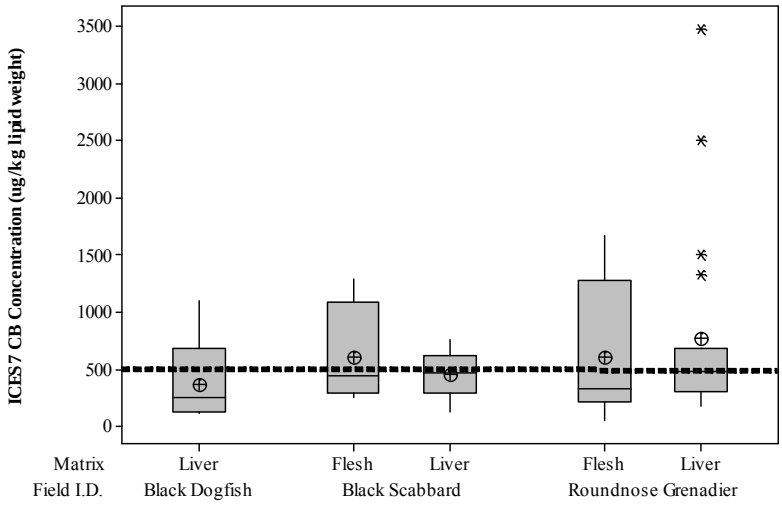

Figure 3 Plot showing the relationship between the calculated Toxic Equivalence (TEQ) concentrations from the 'dioxin-like' CBs measured and **(a)** the ICES7 CB concentration and **(b)** the estimated TEQs derived from published models.

(a) (b)