The Food and Environment Research Agency

Geographical Investigation for chemical contaminants in fish collected from UK and proximate marine waters

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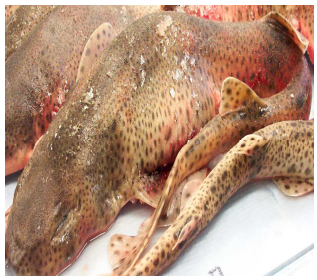


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Geographical Investigation for chemical contaminants in fish collected from UK and proximate marine waters

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Glossary of Main Terms

Term or Acronym	General Meaning Of Term
BFR	Brominated Flame Retardant
PCB	Polychlorinated biphenyl
Ortho-PCB	Ortho-substituted PCB (non planar)
Non-*ortho*-PCB	Non-ortho-substituted PCB (co-planar)
Dioxins	Collective name for chlorinated Dioxins & Furans
PCDD/F	Polychlorinated dibenzo-*p*-dioxin/ polychlorinated dibenzofuran
TEF	Toxic Equivalency Factor – toxicity expressed for each dioxin-like compound relative to 2,3,7,8-TCDD (2,3,7,8-TCDD = 1).
TEQ	Toxic Equivalence – product of the congener concentration and the TEF
Total TEQ	Total of the Sum of all the Toxic Equivalences (TEQs) for each group of compounds
Sum of ICES 6	Sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180
fat weight	Values relevant to the assessed fat content of the sample
whole weight	Values based on the sample as received 'whole' or wet
WHO-TEQ 2005	World Health Organisation - TEQ based on values as set in 2005
HPLC-MS/MS	LC-MS in multiple reaction monitoring mode
Lower bound	assumes values at less than the limit of detection are zero (e.g.<0.01=0)
Upper bound	assumes values at less than the limit of detection are equal to the limit of detection (e.g. <0.07=0.07)
OCPs	Organochlorine pesticides
PBB	Polybrominated biphenyl
PBDD/F	Polybrominated dibenzo-*p*-dioxin/ furan
PBDE	Polybrominated diphenylether
PCN	Polychlorinated naphthalene
PFAS	Perfluoroalkyl substances e.g. perfluorooctane sulphonate - PFOS
PXDD/F, PXB	Polybromo-chloro dibenzo-*p*-dioxin/dibenzofuran, Polybromo-chloro biphenyl
ng/kg	Nanogram per kilogram (part per trillion)
µg/kg	Microgram per kilogram (part per billion)
HPLC-MS/MS	LC-MS in multiple reaction monitoring mode
HRGC-HRMS	High resolution gas chromatography - high resolution mass spectrometry
HRGC-LRMS	High resolution gas chromatography – unit resolution mass spectrometry

Executive Summary

The purpose of this study was to investigate the occurrence of a range of regulated and emerging organic environmental contaminants in commonly consumed marine fish species that are considered to be at the highest risk of non-compliance with existing regulatory limits for contaminants such as dioxins and PCBs. The study provides data on current levels of occurrence, allows the geographical identification of locations that show higher contaminant levels and also facilitates the estimation of human exposure as a result of consumption.

Around 200 fish samples were collected, the majority from UK marine waters, but extending to Norwegian waters in the North and to the Algarve in the South. The main species targeted were sardines, sprats, sea bass, mackerel, herring, grey mullet, but other species such as turbot, halibut, various shark species (dogfish, spurdog), etc. were also included.

All samples were analysed for the regulated environmental contaminants and PBDEs: Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs, dioxins), Polychlorinated biphenyls (PCBs), Potentially Toxic Elements (PTEs), Polybrominated diphenylethers (PBDEs) and Polybrominated biphenyls (PBBs). A sub-set of samples (40 - 60) were also analysed for emerging contaminants: Polychlorinated naphthalenes (PCNs), Polybrominated dibenzo-*p*-dioxins and dibenzofurans (PBDD/Fs), Mixed halogenated dibenzo-*p*-dioxins, dibenzofurans and biphenyls (PXDD/Fs and PXBs), Perfluoalkyl substances (PFAs), Pesticides.

The analytical methodologies used for the analyses of the regulated contaminants were UKAS accredited to the ISO 17025 standard and follow EU Commission regulations for data quality criteria. Similar criteria were used for the other contaminants which use published methodologies that have also been used in previous FSA projects.

All classes of contaminants were detected and although a thorough analysis of the data has not been carried out, concentrations appear to vary depending on species and location. Sea bass, sprats, mackerel, sardines and herring appear to show the highest levels of contamination. Geographically, fish taken from waters around the Southern UK/Northern French coasts and the Irish Sea tend to show higher levels of some contaminants. E.g. samples provided from Northern Ireland tended to show higher concentrations of PCNs, whereas some samples taken from waters off North Western French coasts showed relatively higher concentrations of PCBs. The high frequency of contaminant occurrence combined with the instances of sample concentrations that lie above the regulated limits for some of the contaminants, make it prudent to continue the monitoring of these commonly consumed marine fish species.

1. Study Background

Marine environments are recognised sinks for a range of environmental contaminants, and uptake and bioaccumulation by various fish and shellfish species has been widely documented. Marine shellfish for example, have a recognised potential for bio-accumulating contaminants and some species such as mussels, are commonly used as early indicators of local pollution. Consequently, marine fish and shellfish have been shown to make a significant contribution to human exposure of a range of environmental contaminants.

In an effort to reduce or prevent inputs that could cause pollution, affect human health or adversely impact legitimate uses of the marine environment, the Marine Strategy Framework Directive encourages collaboration and coordination between individual EU Member States with the aim of protecting and preserving marine ecosystems. In the context of the present study, one of the targets for good environmental status under the directive is the limiting of contamination in fish and other seafood along with compliance with maximum contaminant levels established by European Commission regulation, or other relevant standards.

The current investigation however, is not limited to investigating regulated contaminants but includes others that are under review by the European Commission Expert Committee on POPs in Food, or are candidate compounds for listing under the Stockholm Convention and chemicals identified under the Food Standards Agency's own emerging risks programmes.

Fish species available to consumers in the UK are sourced from a variety of locations, but bearing the context of the current study in mind, the main area targeted for investigation were the North Sea and the Greater North Sea sub-region extending up to the Norway, the Irish sea and the Celtic sea sub-regions extending off the North-Western coast of France, and the European coastal North Atlantic regions, including Biscay and extending as far south as the Algarve.

The data from previous studies provide a strong indication that oily fish species such as sardines, sprats, sea bass, mackerel and herring, are likely to show the highest levels of contaminants, and would thus provide a marker for the higher level of the contamination range. However, other species such as turbot, halibut, dogfish etc. were also included.

The contaminants selected for this study represent a wide range of established/regulated, and emerging contaminants that are recognised to be persistent, bio-accumulative and toxic, with the potential to undergo long-range transport.

PCDD/Fs and PCBs are recognised environmental contaminants that are regulated within the EU. The major route of human exposure is through the diet. Among the various different types of foods, fish/shellfish and offal tend to show a relatively higher occurrence of these contaminants. The last Total Diet Study (TDS) that investigated PCDD/Fs and PCBs was reported in 2012 (Fernandes et al 2012) and showed that fish contained the highest contaminant levels among the food groups. It also showed that the decline in contaminant concentrations relative to earlier TDS data, continued, albeit at a slower rate (4.6 ng/kg WHO-TEQ to 3.5 ng/kg WHO-TEQ). The rate may be slower than the figures indicate, since the TEQ calculated in 2012 used WHO-TEF_{2005} (Van den Berg et al 2006) which tend to yield lower TEQ values than the data computed in earlier TDS.

PBDEs are mass produced brominated flame retardants (BFRs) that were incorporated into a number of commonly used commercial materials such as plastics, rubbers, textiles and electronic components. As these are open-ended applications, the BFRs are available to diffuse out of materials into the environment, and this can occur during manufacture, use and disposal of the product. Emerging toxicological data shows that PBDEs can cause liver and neurodevelopmental toxicity and affect thyroid hormone levels. Additionally, they may be particularly harmful during a critical window of brain development during pregnancy and early childhood (Rose and Fernandes 2012). Their occurrence in food has been investigated in studies that also target PBDD/Fs and PBBs (FSA 2006, Fernandes et al 2009) but unlike these contaminants, they show more frequent and abundant occurrence. Fish, and in particular oily fish species, generally tend to show higher levels of contamination than other food types.

Polychlorinated naphthalenes (PCNs) also show properties of stability, high bio-accumulative potential and persistence, coupled with a similarity in structural configuration to planar PCDD/Fs. Some congeners can contribute to dioxin-like toxicity and have shown a combination of toxic responses such as mortality, embryotoxicity, hepatotoxicity, immunotoxicity, dermal lesions, teratogenicity and carcinogenicity (Behnisch et al 2003, Blankenship et al 2000). There have been a few recent studies confirming occurrence in food and human exposure, and in particular fish which tends to show higher levels of contamination than other foods (Fernandes et al 2010, Fernandes, 2013).

Brominated dioxins and biphenyls - PBDD/Fs and PBBs have physico-chemical and toxicological properties that are similar to their chlorinated analogues. The PBDD/Fs originate from similar anthropogenic sources as chlorinated dioxins, such as incineration, particularly of bromine containing waste, or chemical manufacture, whereas PBBs were produced commercially as flame retardants chemicals (BFRs) long before the large volume production of the more familiar BFRs such as PBDEs and HBCD. Their occurrence in food in the UK has been confirmed in earlier

studies funded by the FSA, including an investigation on fish carried out in 2005 (FSA 2006A). The study showed a greater frequency of occurrence of PBDFs, whilst PBBs generally showed very low occurrence. A later study on individual foods including fish and shellfish (Fernandes et al 2009) confirmed these findings.

PXDD/F and PXBs are mixed bromo/chloro analogues of PCDD/F and PCBs and share the same sources and toxicological properties as the other analogues, except that PXBs were never intentionally produced. Analysis of these is complex due to the large numbers of possible compounds (4600 PXDD/Fs and 9180 PXBs) and the potential for false positive detection during mass spectrometric measurement, as these compounds share ions with other more abundant and less toxic contaminants. Toxicologically, some PXDD/F congeners are as potent as the most toxic PCDD/Fs and in some cases, more potent. There have been only a few studies carried out to date on the occurrence of these contaminants in foods including fish (Ohta et al, 2008, Fernandes et al 2011, 2014. Zachs et al 2015) and the current study will provide a baseline for the occurrence of these contaminants in fish.

Perfluoroalkyl substances (PFAS) which include the most widely used product, perfluorooctane sulphonate (PFOS) are industrial chemicals that are now understood to be persistent organic pollutants. These compounds were widely used in the production of non-stick coatings, in water repellent and stain resistant coatings for fabrics and furnishings, in fire-fighting foams and other applications. PFAS may bio-accumulate up the food chain through utilisation or disposal routes, or enter directly into food through primary contamination events. PFAS were investigated in a 2004 TDS in the UK which allowed an initial exposure assessment (Food Standards Agency 2006B) and later in 2012 (Fernandes et al 2012). Individual foods have also been investigated (Clarke et al 2010), and all studies report positive identification of PFAS compounds in fish.

Some organochlorine (OC) pesticides are included in the ‘Stockholm 12’ list of persistent organic pollutants (POPs) along with the dioxins, PCB etc. They may also be associated with specific on-farm or more localised industrial use. Other pesticides such as the organophosphurous (OP) class are used (or have been used in the past) for specific applications (such as sheep dipping), but these are not persistent in the environment. Pesticide residues in foods may arise from direct use in wetlands where they may be used to control vector insects, and they may also be used in fish farming, e.g. some pesticides are used to control sea-lice infections of farmed salmon. Pesticides, especially herbicides, can also enter river systems as a result of rainwater and irrigation wash-off from agricultural land into rivers, and then into marine waters. There is then a strong potential for these compounds to bio-magnify and to accumulate in fish and other aquatic and marine biota. The organochlorine pesticides are highly lipophilic and can quickly accumulate in oily fish.

Some trace elements and in particular, heavy metals, are established toxic contaminants. Some elements, such as copper, chromium, selenium and zinc are essential to health but may be toxic at high levels of exposure. Other elements have no known beneficial biological function and long-term, high-level exposures may be harmful to health. Environmental sources are the main contributors to contamination of food which is the major source of the overall exposure of consumers to metals and other elements, although other routes may also be significant (for example, oral exposure via drinking water, inhalation exposure via the occupational setting). Certain food groups naturally accumulate some elements and consequently contain high concentrations of these elements compared to other foods. For example, fish and shellfish are known to accumulate arsenic and mercury and cereals can accumulate cadmium. Metals and other elements may enter marine and aquatic environments and bio-accumulate in species at any point during growth and harvesting. There have been many surveys of sea-fish for trace elements but very few that have been conducted with simultaneous analysis for organic contaminants.
In the UK, the FSA recently conducted a study of metals and other elements as part of TDS (FSA 2009). The results of the study indicated that current population dietary exposures to most of the metals and elements investigated did not raise specific concern for the health of consumers.

2. Experimental

2.1 Sample Collection and Preparation

Approximately 200 samples were collected from UK and proximate marine waters, including the North Sea and the Greater North Sea sub-region extending up to Norway, the Irish sea and the Celtic sea sub-regions extending off the North-Western coast of France, and the European coastal North Atlantic regions, including Biscay and extending as far south as the Algarve. On receipt at the laboratory each prepared sample was given a unique laboratory reference number and the sample details were logged into a database. An overview of the samples is given in Table 1 showing species, fat content and general location, but a full listing is given in Annexe A.

Sample preparation mirrored domestic consumption practices. Depending on the species, samples were dissected to collect edible muscle tissue and exclude skin, bones and organs. However for some species such as sprats, whole fishes were used. The selected tissue (or whole fish) was minced, homogenised by blending and a portion set aside for PFAS and PTE analysis. The remaining sample was freeze-dried. Freeze-dried sample powders were re-homogenised and aliquots of these were used for the other analyses.

2.2 Contaminants measured – Specific Analytes

The following analytes were determined: Regulated contaminants are highlighted in **bold.**
Dioxins **- all 17, 2378-Cl substituted PCDDs and PCDFs.**

Dioxin-like PCBs - **IUPAC numbers 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189.**

Non Dioxin-like PCBs - IUPAC numbers 18, **28**, 31, 47, 49, 51, **52**, 99, **101**, 128, **138**, **153, 180,** 33, 41, 44, 60, 61, 74, 66, 87, 110, 129, 141, 149, 151, 170, 183, 185, 187, 191, 193, 194, 201, 202, 203, 206, 208 and 209.

PBDE congeners: IUPAC numbers 17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 183 and 209.

PBB congeners: IUPAC numbers 15, 49, 52, 77, 80, 101, 126, 153, 169 and 209.

PCNs - PCN-52/60, 53, 66/67, 68, 69, 71/72, 73, 74, & 75.

Brominated dioxins - 2,3,7-T_3BDD, 2,3,8-T_3BDF, and ten, 2,3,7,8-Br substituted tetra – hepta brominated PBDD/F congeners (note that this includes only 1 hexa- and 1 hepta-furan as no standards were available for the other congeners).

Mixed halogenated dioxins and biphenyls (PXDD/F and PXBs) - 13, tri - hexa halogenated DDs/ DFs and 6 coplanar and mono-ortho substituted biphenyls. This includes one tri-brominated compound, 4 di-brominated compounds and 14 mono-brominated compounds (Fernandes et al 2011B).

PFAS - Perfluorooctanesulfonylamide (PFOSA), Perfluorobutane sulfonate (PFBSH), Perfluorohexane sulfonate (PFHxS), Perfluorooctane sulfonate (PFOS), Perfluorooctanoic acid (PFOA), Perfluorononanoic acid(PFNA), Perfluorodecanoic acid (PFDeA), Perfluoroundecanoic acid (PFUnA) and Perfluorododecanoic acid (PFDoA).

Pesticide screen – A selection of samples was screened for a number (60) of commonly used and legacy pesticides (Annexe A), including the following Organo-chlorine compounds – Aldrin, cis- and trans-Chlordane, pp-DDD, pp-DDE, pp- and op-DDT, Endrin, Hexachlorobenzene and Heptachlor.

PTEs - Chromium (Cr), Manganese (Mn), Cobalt (Co), Nickel (Ni), Copper (Cu), Zinc (Zn), Arsenic (As), Selenium (Se), Silver (Ag), **Cadmium (Cd), Mercury (Hg), Lead (Pb).**

2.3 PCDD/Fs, PCBs, PBDD/Fs, PBBs and PBDEs - Analytical Methodology

The method used for the preparation, extraction and analysis of samples has been reported previously (Fernandes et al 2004; 2008) and forms part of the CEN method – EN16215:2012 for PCDD/F and PCB analysis. In brief, samples were fortified with ^{13}C-labelled analogues of target compounds and exhaustively extracted using mixed organic solvents. PBDEs and ortho substituted PCBs/PBBs were separated from non-ortho substituted PCBs/PBBs, PCDD/Fs and PBDD/Fs by fractionation on activated carbon. The two fractions were further purified using adsorption chromatography on alumina. Analytical measurement was carried out using high resolution gas chromatography-high resolution mass spectrometry (HRGC-HRMS) for all analytes apart from the *ortho*-substituted PCBs which were analysed by high resolution gas chromatography-unit resolution mass spectrometry (HRGC-LRMS).

The analysis is accredited (UKAS) to ISO 17025 standards, with the inclusion of an in-house reference material and method blanks which were evaluated prior to reporting of sample data and used to determine the limits of detection. Further quality assurance measures included the successful participation in available international inter-comparison exercises such as Dioxins in Food-2011 to 2014, EURL run PT exercises, etc. on dioxins, dioxin-like PCBs, ICES-6 PCBs and PBDEs. Additionally, quality control evaluation for the accompanying data follows the criteria specified for chlorinated dioxins and PCBs (Commission Regulation 252/2012).

2.4 PCNs - Analytical Methodology

A full description of the reagents, reference standards and procedures used for the extraction and analysis has been reported earlier (Fernandes et al. 2010). In brief, samples were fortified with ^{13}C-labelled analogues of target compounds and exhaustively extracted using mixed organic solvents. PCNs were chromatographically fractionated from potential interferents such as PCBs, using activated carbon. The extract was further purified using adsorption chromatography on alumina. Analytical measurement was carried out using high resolution gas chromatography coupled to high resolution mass spectrometry (HRGC-HRMS). Additional control was provided by the inclusion of methods blanks and a reference material.

The quality control criteria used for evaluating data are very similar to the accredited methodology used for the chlorinated dioxins and PCBs, and validation data including method performance parameters have been reported before (Fernandes et al 2010). There are no available reference materials (RMs) specific to PCNs, but the same material that is used for PCDD/F and PCB analysis (cod liver oil), was analysed during the course of this work with results showing good consistency and agreement with established values.

2.5 PXDD/Fs and PXBs - Analytical Methodology

The analytical methodology for determining PXDD/Fs and PXBs is based on internal standardisation with ^{13}Carbon labelled surrogates, dual carbon column fractionation and analysis by HRGC-HRMS (Fernandes et al 2011). Sample aliquots along with a blank and a reference material were internally standardised, and extracted with dichlroromethane:hexane, on an acid/base modified silica column, eluting directly onto activated carbon. Mono-ortho PXBs were removed from the direct eluate, and the reverse eluted fraction was re-chromatographed on activated carbon to yield the non-ortho PXBs and PXDD/FS. Both fractions were purified by adsorption chromatography on alumina, concentrated, sensitivity standardised and analysed by HRGC-HRMS at a resolution of 13500-15000.

In very general terms, the purification and measurement methodology described here is an extension and a refinement of the methodology previously used for PCDD/Fs, PCBs and PBDD/Fs (Fernandes et al 2004; 2008) and exploits the common physical and chemical properties of these mixed halogenated compounds. The use of these techniques has been accredited and peer-reviewed, and the methodology has been used successfully over many years for the measurement of PBDD/Fs, PCDD/Fs, PCBs and PBDEs, as evidenced by successful participation in international inter-comparison trials (Dioxins in Food, 2011 - 2014). Analytical recoveries typically range from 60-90%, with generally lower recoveries (~ 40%) for the more volatile tri-halogenated compounds. The average method limit of detection (MLOD) is of the order of ~5 fg/g fat which is similar to sensitivities for the measurement of PCDD/Fs and PBDD/Fs. As there are no formal acceptance criteria for data quality for PXDD/Fs and PXBs, the quality control for the accompanying data has followed the criteria currently used for PCDD/Fs and PCBs.

2.6 PFAS - Analytical Methodology

This procedure has been described elsewhere in more detail (Clarke et al 2010). Briefly, replicate portions (5 g) of food were weighed out, into Falcon tubes (50 mL). These were fortified with the appropriate unlabelled standard addition and labelled internal standards ($^{13}C_4$-PFOA, $^{13}C_4$-PFOS, $^{13}C_2$-PFHxA, $^{13}C_4$-PFOA, $^{13}C_5$-PFNA, $^{13}C_2$-PFDeA, $^{13}C_2$-PFUnA, $^{18}O_2$-PFHxS and D_3-n-M-FOSAA), then homogenised in methanol, and made up to 35 mL. Samples were agitated overnight (16 h), centrifuged (15 min, 5,000 rpm). The supernatant methanol extract was dried under a nitrogen stream (80 ^{o}C) to incipient dryness and the residues re-dissolved in aqueous KOH (25 mL, 0.01 M, sonicate 10 min). Aqueous extracts were re-centrifuged (15 min, 5,000 rpm) and the supernatant poured in one continuous gentle movement (to avoid disturbing sediment) into a funnel connected onto the top of a preconditioned SPE cartridge (weak anion exchange). After loading, cartridges were washed with ammonium acetate (2 x 6 mL, 25 mMol, pH 4.5) and eluted with basic methanol (4 mL, 0.1% ammonia). Eluent was dried under nitrogen (30 ^{o}C), until dry and the residues taken up in methanol (400 µL, sonicate 10 min). PFCs were not accurately quantifiable by external

calibration, so a standard addition (SA) approach was used. Six replicate portions were prepared, two unspiked and four over spiked. $^{13}C_4$-labelled PFOS, $^{13}C_2$-PFHxA, $^{13}C_4$-PFOA, $^{13}C_5$-PFNA, $^{13}C_2$-PFDeA, $^{13}C_2$-PFUnA, $^{18}O_2$-PFHxS and D_3-n-M-FOSAA were used as isotope dilution internal standards.

Measurement was undertaken by LC-MS/MS. An Agilent HTS CTC injector and a 1290 LC were coupled to an Agilent 6490 triple quadrupole mass spectrometer. An injection volume (5 µl on a 20 µl loop) was injected onto a Fluorosep RP Octyl phase HPLC column (5 µm, 60 Å, 2.1 x 150 mm, held at 40 ^{o}C) with guard cartridge (C_8). The mobile phase gradient programme (methanol/aqueous ammonium formate, 5 mM, pH 4) started at 10% methanol (0.3 ml/min) increasing by a linear gradient to 100% (10 mins) and returning to 10% (20 mins). The MS detector in MRM mode was used for quantitative analysis using one transition for internal standards and two transitions for the individual PFC analytes.

The use of LC-MS/MS in multiple MRM mode contributes much to the specificity of the measurement process for these compounds. Determination is aided by the use of ^{13}Carbon labelled and deuterated PFC compounds as internal standards. Each food sample was analysed in duplicate throughout the entire extraction method to ensure that advantageous point contamination was not mistaken for the presence of any native PFC. For a specific analyte to be considered present in a sample extract the following criteria must be met: i) the relative retention times of the analyte must be comparable to those of a retention time marker, an internal standard, and to authentic analytical standards of each analyte; ii) the peak must have the correct mass transition, maximising at the correct retention time; iii) the signal to noise ratio of any peak must be greater than 3:1. In order to prove the absence of a given PFC, the internal standard must be present in all extracts, the blank extract must show no signal at the retention time of the target PFC, whilst the overspiked extracts must show a peak for the target PFC at the required retention time.

2.7 Pesticides

A sub-sample was extracted with ethyl acetate, prior to clean-up using high performance gel permeation chromatography (HPGPC). The extract was further purified using an alumina clean-up procedure and subsequent determination using a gas chromatograph fitted with a Tandem Mass Spectrometer (GC-MS/MS). The determination step involved use of matrix matched, multi-level, bracketed calibration solutions. This process was further strengthened by the use of representative stable isotope labelled internal standards. The analytical procedure was carried out to meet the requirements of DG SANCO guidelines (SANCO 2014).

2.8 PTEs – Analytical methodology

1 g (fresh weight) of each sample was weighed into allotted digestion vessels and a mixture (4:1) of nitric acid and hydrochloric acid added (5.0 ml). The vessels were capped and the contents digested using a single reaction chamber microwave digestion system ('Ultraware' Milestone). Reagent blanks, certified reference materials and a spiked sample were also taken through the procedure. The resulting solutions were transferred to pre-marked acid-clean plastic test tubes and diluted to 10 ml with deionised water (18MΩ).

Seven calibration standards, in an acid matrix to match that of the samples, were prepared from certified stocks, to cover the expected concentration range for each element. The digest solutions and standards were diluted further with internal standard (indium or rhodium) in dilute nitric acid (1% v/v). Measurements were made using an Agilent 7700 ICP-MS instrument. The element concentrations in the diluted samples were calculated from the response curve of the standards at the beginning of each run. The concentrations of 12 elements were determined (Cr, Mn, Co, Ni, Cu, Zn, As, Se, Ag, Cd, Hg and Pb).

The analytical procedure is accredited to the ISO17025 standard. The criteria used to assess data included checks on instrument drift, spike recovery, replicate agreement, limits of detection and certified reference material values. The LOD was defined as three times the standard deviation of the signal from reagent blanks (which had been taken through the entire analytical procedure) when subsequently corrected for sample weight and dilution. The LOQ was defined as ten times the standard deviation of the signal from reagent blanks (which were taken through the entire analytical procedure) when subsequently corrected for sample weight and dilution.

Analyses included re-measurement of a calibration standard at the end of each ICP-MS run. In order to pass this check, the re-measured standard had to be within ± 20 % of the initial value. Data were accepted if the recovery of spike for each analyte was within the range 60 to 140 % with at least 75 % of these recoveries lying within the range 80-120 %. Replicate values for a given sample had to have a relative standard deviation of <20 % or a standard deviation of <LOQ, whichever was greater. Results for reference materials had to lie within the certified range, or 25% of the quoted value, whichever was greater. Where indicative values were shown on certificates, measured concentrations had to be within a factor of 2 of the quoted value. Data were accepted if results for at least two of the three reference materials passed the criteria above.

Table 1: Overview of samples (for full listing see Annexe A)

Species	Sardines	Mackerel	Herring	Grey Mullet	Sprats
Sample number	n=16	n=41	n=19	n=26	n=25
General Location	England- South Coast, Northern Brittany, Algarve	Norwegian Coast, Scotland, England-South Coast, Irish Sea, Northern Brittany, Algarve	Scotland, England-South Coast, Irish Sea, Northern France	England- South Coast, East Coast, Welsh Coast, Northern France, Western Mediterranean	Scotland, England-South Coast, East Coast, Irish Sea, Welsh Coast
Average fat content (%)	13	10	10	3	10

Species	Sea Bass	Turbot	Shark (various spp)	Other spp - Halibut, Haddock, Plaice, Lemon sole, Witch, Megrim, Monkfish (inc. Liver)
Sample number	n=25	n=16	n=14	n=10
General Location	England- South Coast, East Coast, Welsh Coast, Northern France	Scotland, England-South Coast, East Coast, Irish Sea,	England- South Coast, East Coast, Irish Sea,	England, East Coast, NE-Atlantic, Holland
Average fat content (%)	3.3	1.4	4	1.3

3. Results

The volume of data generated for this project is very large (~ 30,000 results) so these have been included in Annexe A. However a summary of the results for each analyte group has been presented in Tables 3.1 to 3.8. All samples were measured for the regulated contaminants (PCDD/Fs, PCBs and PTEs) and for PBDEs, while smaller sub-sets of samples (covering the more susceptible species - sardines, mackerel, herring, grey mullet, sprat, sea bass and turbot) were measured for the other emerging contaminants (PCNs, PBDD/Fs, PXDD/Fs and PXBs, PFAS) and pesticides. Concentration units reflect current convention either as required by regulation, or as reported in recent literature for the emerging contaminants. The reporting limits (quoted as "<") for most analytes are estimated as a dynamic parameter incorporating current method blanks and are therefore the limits of determination that prevail during measurement. For the regulated contaminants, PCDD/Fs, PCBs, and metals, the limits are consistent with the requirements of EU regulations, but for all reported contaminants, the limits are generally either better than or similar to those reported in current literature. Data on the reference materials that were analysed concurrently with the samples and for the regulated contaminants are also presented in Annexe A. In general the results of analysis of available reference materials for analytes such as PCNs, PBDEs, PTEs, etc. were within established acceptable limits. Typical measurement uncertainty estimates are included in the concentration tables for most of the different contaminant groups.

In addition to the concentration of individual congeners, the dioxin-like toxicity of the samples arising from PCDD/Fs and dioxin-like PCBs has also been reported as a toxic equivalent (WHO-TEQ), using the 2005 toxic equivalent factors (TEF_{2005} - Van den berg et al 2006), as required by regulation. Additionally as per the requirements of Regulation 1259/2011, the sum of the ICES-6 PCBs is also provided. WHO-TEQ and other summed concentration values have generally been reported on a whole weight basis unless specified in the individual tables. TEQ values have also been included for the PBDD/F and non-ortho PBB analytes. As in previous studies, TEFs associated with the analogous chlorinated compounds have been used to compute these TEQs, as there is no universally recognised TEF scheme for these compounds.

A summary of PCDD/F and PCB concentrations in the fish samples is given in Table 3.1, for all the major fish species studied. PCDD/Fs and PCBs were detected in all fish samples at varying concentrations, ranging from 0.03 to 12.5 ng sum WHO-TEQ/kg whole weight, with an average value of 1.4 ng WHO-TEQ/kg whole weight. The sum of ICES-6 PCBs ranged from 0.1 to 145 µg/kg whole weight. However some species (Sea Bass, Sprats, Sardines) showed a greater tendency to bio-accumulate these contaminants with average sum WHO-TEQ values of 2.5, 2.0 and 2.0 ng/kg respectively. These concentrations are lower than those reported (Fernandes et al

2009B) for fish sampled in the UK about a decade ago with sum WHO-TEQ values of 3.7 and 4.3 ng/kg for sea-bass and sprat respectively. However it should be noted that the historical data would have been calculated using TEF_{1998} factors which tend to yield higher WHO-TEQ values. According to current regulation the maximum limits for fish muscle are 3.5 ng/kg for PCDD/F WHO-TEQ and 6.5 ng/kg for summed PCDD/F and PCB WHO-TEQ (European Commission 2011). Two samples (21357 sea-bass and 21368 mackerel both from waters off Northern France), showed sum WHO-TEQ values of 12.5 and 7.5 ng/kg respectively (Annexe A). The sea-bass sample also returned an ICES-6 PCB concentration of 145 µg/kg whole weight against a maximum limit of 75 µg/kg whole weight. In general, the pattern of occurrence of PCDD/Fs and PCBs in fish tissues was consistent with historical data, with higher PCB-TEQ relative to PCDD/F TEQ.

PBDEs were observed in all samples with all measured congeners being detected apart from BDE-126 (Annexe A). A summary of the data is presented in Table 3.2 which provides descriptive statistics for the sum of all (17) measured PBDEs, as well the sum of the PBDEs specified in the latest EU recommendations (European Commission 2014), for each of the major fish species. There are only minor differences between the average values for both the sum of the 17 congeners and the EU sum, which confirms a more informed choice of congeners for the EU list. The concentrations range from 0.04 µg/kg to 8.87 µg/kg whole weight for the sum of all measured PBDE congeners (0.04 µg/kg to 8.63 µg/kg for the EU listed PBDEs). The highest average values were observed for herring, sea bass, mackerel and sprat (2.08, 2.0, 1.45 and 1.27 µg/kg respectively). PBBs were detected less frequently and at lower concentrations (Annexe A), confirming a trend observed in other studies (Fernandes et al 2008, 2012) The highest value observed was 0.65 µg/kg for BB-52 for grey mullet from France. In general, most of the higher positive values for PBBs were observed for samples taken from French waters and from the southern coast of England. This may reflect a higher utilisation of PBBs in France relative to the UK where PBBs are generally not detected or occur at very low concentrations in foods.

PCNs were measured in 76 samples covering 7 species (Table 3.3). The sum of the 12 reported PCNs ranged from 0.7 ng/kg whole for a sample of turbot to 265 ng/kg whole for a sample of sprats. Mackerel and sprats showed the highest concentrations with average values of 68 ng/kg whole and 67 ng/kg whole respectively. An earlier study on individual UK foods (Fernandes et al 2010) showed an average of 20 ng/kg whole for individual fish samples (salmon, herring, sprats, eels, trout, etc.), and the concentration in the fish group in the last TDS (Fernandes et al 2012) was 6.6 ng/kg whole. The composition of the TDS fish group would include both oily and white fish as well as shellfish, as compared to the mostly oily species targeted in this study. Although a majority of the higher concentrations observed in this study arose from the Southern and Eastern

UK coasts and Northern France, the highest values were seen in samples received from Northern Ireland.

The occurrence profile for the PBDD/Fs confirm observations from earlier studies on these contaminants, with a greater frequency of detection for the PBDFs relative to the PBDDs. The penta- and hexa-brominated dioxins were not detected in any of the samples (Annexe A). The PBDD/Fs occur to a much lower extent than the chlorinated analogues. The PBDD/F concentration data converted to TEQs, range from 0.001 to 0.04 ng/kg TEQ whole weight (Table 3.4) which is comparable to the concentration in the fish group in the last TDS (Fernandes et al 2012) at 0.02 ng/kg whole. As in previous studies, TEFs associated with the analogous chlorinated compounds have been used to compute these TEQs, as there is no universally recognised TEF scheme for these compounds.

As for the PBDD/Fs, PXDFs were detected more frequently than the PXDDs. Apart from a couple of sea bass samples, at least one PXDD/F congener was detected in all of the 59 analysed samples (Annexe A), albeit at low concentrations relative to PCDD/Fs. Data summarised for the most commonly analysed species are presented in Table 3.5. PXBs were detected in all samples with highest concentrations being observed in mackerel, sprats and sea-bass. In general, the frequency of detection was similar to that observed in an earlier study on foods (Fernandes et al 2014) and followed the order PXBs>PXDFs>PXDDs. The concentration range reported in the earlier study for a set of 40 fish was <0.005 to 1.12 ng/kg fat for PXDD/Fs and <0.005 to 14.7 for the PXBs. The range in the current study was similar for PXDD/Fs (<0.005 to 1.62 ng/kg fat), but the upper end of the range for PXBs (<0.005 to 42 ng/kg fat) was approximately a factor of 3 higher than the earlier study. The higher concentrations for the PXBs were generally associated with samples from Northern France/Southern UK waters and the Irish Sea.

PFAS were detected in all of the 50 samples measured in this study. In general higher concentrations were observed in sardines, sprats and sea bass, with PFOS, PFOSA and PFOA often showing the highest concentration levels (Table 3.6). Higher concentrations tended to be seen more frequently in samples from Southern UK waters and the Irish Sea. Comparison with earlier studies in the UK (e.g. Food Standards Agency 2006B) is limited because of the very different reporting levels, which resulted in most analytes remaining undetected in the earlier work. However, data for the fish group in the last TDS study which showed a total concentration of 12.6 µg/kg whole, was comparable to the range (0.64 to 15.3 µg/kg whole) observed for the sum of the 9 compounds measured in this study.

A set of 50 fish samples comprising of sardines, herring, mackerel, mullet, sea bass and sprats were analysed for a range of pesticides (60 compounds, Annexe A). Only 5 compounds – pp-DDD,

pp-DDT, pp-DDE dieldrin and hexachlorobenzene (HCB) were present above the limits of detection, ranging from 0.2 µg/kg for pp-DDD and HCB to 12 µg/kg for pp-DDE (Table 3.7). These tended to occur at relatively higher levels in mullet, sea bass and herring which originated from Southern UK/ Northern France waters and the Irish Sea.

PTEs were measured in all fish muscle samples and concentrations in mg/kg of whole weight tissue are given in Annexe A, with a summary for cadmium, lead and mercury given in Table 3.8. Some metals such as manganese, zinc, copper, arsenic, selenium and mercury were detected in all or most of the samples, irrespective of species. In general, silver, nickel, chromium and lead showed the lowest frequency of detection. Mercury is regulated by the EC (Commission Regulation EC 1881/2006 as amended by 629/2008) with a general limit of 0.5 mg/kg for fish. Eight samples of sea bass and one of dogfish showed mercury concentrations that were above this limit. One sample of dogfish was above the general regulated limit for cadmium (0.05 mg/kg) and a sample of mackerel was above the higher cadmium limit set for this species (0.1 mg/kg - EC (Commission Regulation EC 1881/2006 as amended by 488/2014). Most of these samples were from Southern UK/ Northern France waters and the Irish Sea. Two mullet samples that were above the limit set for lead (0.3 mg/kg), originated from Wales.

4. Conclusions

This study has characterised a comprehensive range of environmental contaminants in a number of commonly consumed fish species, taken from marine waters around the UK and from other proximate fishing areas from which retail fish in the UK is commonly sourced.

All classes of contaminants were detected and although a thorough analysis of the data has not been carried out, concentrations appear to vary depending on species and location. Sea bass, sprats, mackerel, sardines and herring appear to show the highest levels of contamination. Geographically, fish taken waters around the Southern UK/Northern French coasts and the Irish Sea tend to show higher levels of most contaminants. For example samples provided from Northern Ireland tended to show higher concentrations of PCNs, whereas some samples taken from waters off North Western French coasts showed relatively higher concentrations of PCBs.

Although a small reduction in concentration levels is evident for some contaminants such as PCDD/Fs and PCBs, it is more difficult to discern a trend for other contaminants. This may be due to a slower rate of decline or perhaps because some of the data are unique (e.g. there are no earlier data for PCNs or PXDD/Fs, PXBs in grey mullet) and in these cases, the study provides a useful concentration baseline from which future studies can be assessed. In all cases however, the data would be useful in allowing risk assessment from the consumption of these species.

The high frequency of contaminant occurrence combined with the instances of samples that lie above the regulated limits for some of the contaminants, make it prudent to continue monitoring of these commonly consumed marine fish species, from the point of view of public health.

Table 3.1 Summary of PCDD/F & PCB WHO-TEQ, and ICES-6 PCB concentrations (upper bound)

PCDD/F and PCB	Sardines (n=16)				Mackerel (n=41)				Herring (n=19)				Mullet (n=26)			
	MIN	**MED**	**MEAN**	**MAX**	**MIN**	**MED**	**MEAN**	**MAX**	**MIN**	**MED**	**MEAN**	**MAX**	**MIN**	**MED**	**MEAN**	**MAX**
WHO-TEQ Whole weight ng/kg																
PCDD/F	0.13	0.36	0.40	1.20	0.04	0.26	0.43	1.62	0.34	0.55	0.64	1.55	0.02	0.10	0.14	0.51
non ortho-PCB	0.47	1.10	1.48	3.16	0.06	0.63	0.90	5.56	0.23	0.40	0.56	1.27	0.07	0.32	0.47	1.91
Ortho-PCB	0.03	0.06	0.09	0.33	0.01	0.04	0.07	0.37	0.02	0.04	0.05	0.12	0.01	0.04	0.06	0.22
Sum WHO-TEQ	**0.63**	**1.51**	**1.97**	**4.37**	**0.10**	**1.05**	**1.40**	**7.51**	**0.64**	**1.00**	**1.24**	**2.78**	**0.11**	**0.48**	**0.67**	**2.36**
Sum ICES-6 PCB, µg/kg	**5.41**	**12.35**	**16.62**	**54.89**	**0.86**	**6.73**	**10.59**	**63.64**	**3.76**	**7.68**	**8.49**	**17.84**	**0.89**	**6.92**	**12.16**	**43.76**
	Sprat (n=25)				Sea Bass (n=25)				Turbot (n=16)				Shark-various spp (n=14)			
	MIN	**MED**	**MEAN**	**MAX**	**MIN**	**MED**	**MEAN**	**MAX**	**MIN**	**MED**	**MEAN**	**MAX**	**MIN**	**MED**	**MEAN**	**MAX**
WHO-TEQ Whole weight ng/kg																
PCDD/F	0.13	0.87	0.91	2.55	0.09	0.34	0.44	1.34	0.02	0.14	0.17	0.44	0.02	0.08	0.12	0.30
non ortho-PCB	0.09	1.13	1.02	2.25	0.23	1.26	1.92	10.38	0.05	0.42	0.47	1.37	0.01	0.08	0.14	0.46
ortho-PCB	0.01	0.08	0.07	0.15	0.02	0.09	0.14	0.84	0.01	0.02	0.03	0.10	0.01	0.03	0.07	0.21
Sum WHO-TEQ	**0.23**	**2.14**	**2.00**	**4.35**	**0.35**	**1.65**	**2.50**	**12.49**	**0.07**	**0.66**	**0.67**	**1.91**	**0.03**	**0.22**	**0.32**	**0.93**
Sum ICES-6 PCB, µg/kg	**1.35**	**11.49**	**11.07**	**28.32**	**2.76**	**12.87**	**22.16**	**144.92**	**0.52**	**3.97**	**4.98**	**17.20**	**0.11**	**1.97**	**9.82**	**33.97**

Table 3.2 Summary of PBDE concentrations µg/kg whole weight

PBDE Concentrations, µg/kg whole weight	Sardines (n=16)				Mackerel (n=41)				Herring (n=19)				Grey Mullet (n=26)			
	MIN	MEDIAN	MEAN	MAX	MIN	MEDIAN	MEAN	MAX	MIN	MEDIAN	MEAN	MAX	MIN	MEDIAN	MEAN	MAX
Sum measured PBDEs	**0.14**	**0.39**	**0.50**	**2.18**	**0.15**	**1.24**	**1.45**	**3.86**	**0.61**	**1.14**	**2.08**	**8.87**	**0.09**	**0.58**	**1.10**	**5.41**
***Sum PBDEs (EU list)**	**0.13**	**0.38**	**0.49**	**2.12**	**0.14**	**1.16**	**1.35**	**3.65**	**0.58**	**1.10**	**2.00**	**8.63**	**0.08**	**0.57**	**1.08**	**5.36**
	Sprat (n=25)				Sea Bass (n=25)				Turbot (n=16)				Shark-various spp (n=14)			
	MIN	MEDIAN	MEAN	MAX	MIN	MEDIAN	MEAN	MAX	MIN	MEDIAN	MEAN	MAX	MIN	MEDIAN	MEAN	MAX
Sum measured PBDEs	**0.33**	**1.09**	**1.27**	**4.59**	**0.28**	**1.75**	**2.00**	**5.71**	**0.07**	**0.33**	**0.37**	**0.84**	**0.04**	**0.13**	**0.54**	**2.02**
***Sum PBDEs (EU list)**	**0.31**	**1.05**	**1.23**	**4.56**	**0.27**	**1.73**	**1.97**	**5.64**	**0.06**	**0.31**	**0.35**	**0.79**	**0.04**	**0.12**	**0.51**	**1.91**

*- Sum BDE-28, 47, 49, 99, 100, 138, 153, 154, 183 and 209 (EU recommendation 2014/118/EU)

Table 3.3 Summary of PCN concentrations

Species (number)		Sum PCNs lower bound	Sum PCNs upper bound	Sum PCNs lower bound	Sum PCNs upper bound
		ng/kg whole weight		ng/kg fat weight	
	MIN	5.1	5.4	21	22
Sardines	MEDIAN	16.6	16.6	171	172
12	MEAN	19.7	19.8	289	291
	MAX	63.1	63.1	1215	1215
	MIN	10.0	10.1	109	112
Mackerel	MEDIAN	50.3	50.5	450	451
14	MEAN	67.9	68.0	647	648
	MAX	243.0	243.0	1653	1654
	MIN	18.3	18.3	141	141
Herring	MEDIAN	29.5	29.7	231	231
6	MEAN	38.5	38.7	429	431
	MAX	89.5	89.5	1342	1342
	MIN	4.2	4.2	122	125
Grey mullet	MEDIAN	12.2	12.4	553	554
9	MEAN	14.6	14.7	1291	1293
	MAX	33.5	33.5	7564	7572
	MIN	29.4	29.4	138	138
Sprat	MEDIAN	46.0	46.0	335	335
15	MEAN	66.4	66.5	680	680
	MAX	264.5	264.8	2387	2390
	MIN	13.7	14.2	301	302
Sea Bass	MEDIAN	28.6	29.2	840	848
13	MEAN	29.3	29.4	995	999
	MAX	48.5	48.5	3081	3084
	MIN	0.7	0.7	128	132
Turbot	MEDIAN	3.4	3.5	234	246
6	MEAN	5.3	5.3	338	343
	MAX	15.5	15.5	828	828

Table 3.4 Summary of PBBD/F concentrations - fat weight basis

Species (number)		PBDD/F TEQ lower bound	PBDD/F TEQ upper bound	PBDD/F TEQ lower bound	PBDD/F TEQ upper bound
		ng/kg whole weight		ng/kg fat weight	
	MIN	<0.001	0.012	<0.001	0.130
Sardines	MEDIAN	0.003	0.019	0.055	0.263
7	MEAN	0.006	0.022	0.063	0.265
	MAX	0.021	0.042	0.234	0.435
	MIN	<0.001	0.010	<0.001	0.072
Mackerel	MEDIAN	0.003	0.015	0.060	0.176
17	MEAN	0.004	0.015	0.076	0.332
	MAX	0.012	0.031	0.320	2.138
	MIN	<0.001	0.014	0.002	0.103
Herring	MEDIAN	0.002	0.016	0.009	0.146
7	MEAN	0.005	0.019	0.034	0.153
	MAX	0.013	0.034	0.099	0.219
	MIN	<0.001	0.008	<0.001	0.115
Grey Mullet	MEDIAN	0.003	0.012	0.088	0.226
8	MEAN	0.005	0.013	0.103	0.304
	MAX	0.017	0.021	0.275	0.523
	MIN	<0.001	0.007	<0.001	0.089
Sprats	MEDIAN	0.002	0.016	0.033	0.146
11	MEAN	0.004	0.016	0.077	0.234
	MAX	0.012	0.026	0.579	0.950
	MIN	<0.001	0.010	<0.001	0.124
Sea Bass	MEDIAN	0.002	0.012	0.062	0.482
15	MEAN	0.003	0.014	0.116	0.510
	MAX	0.010	0.022	0.521	0.971
	MIN	<0.001	0.001	<0.001	0.318
Turbot	MEDIAN	<0.001	0.008	0.018	0.919
6	MEAN	0.002	0.008	0.097	1.056
	MAX	0.008	0.013	0.433	1.999

Table 3.5 Summary of PXDD/F and PXB concentrations – ng/kg fat

PXDD/Fs	Sardines n=7	Mackerel n=13	Sprats n=13	Sea bass n=15	Turbot n=4
	Range ng/kg fat weight				
2-B-7,8-CDD	<0.01 - <0.145	<0.018 - 0.097	<0.009 - 0.199	<0.005 - <0.197	<0.027 - 0.186
2-B-3,7,8-CDD	<0.006 - <0.033	<0.008 - 0.078	<0.009 - 0.134	<0.005 - <0.16	<0.007 - <0.071
2,3-B-7,8-CDD	<0.005 - <0.074	<0.008 - <0.03	<0.005 - <0.07	<0.005 - 0.101	<0.007 - <0.067
1-B-2,3,7,8-CDD	<0.005 - <0.093	<0.008 - <0.046	<0.005 - <0.073	<0.005 - <0.111	<0.011 - <0.106
2-B-1,3,7,8-CDD	<0.006 - <0.076	<0.006 - <0.035	<0.006 - <0.049	<0.005 - <0.097	<0.007 - <0.061
2-B-3,6,7,8,9-CDD	<0.006 - <0.092	<0.009 - <0.064	<0.008 - <0.122	<0.005 - <0.191	<0.008 - <0.085
2-B-7,8-CDF	<0.014 - <0.075	<0.012 - 0.083	<0.01 - <0.094	<0.007 - 0.231	<0.011 - 0.133
3-B-2,7,8-CDF	<0.005 - <0.056	<0.017 - 0.09	<0.008 - 0.134	<0.005 - <0.172	<0.015 - 0.091
2-B-6,7,8-CDF	<0.005 - <0.05	0.051 - 0.508	0.036 - 1.627	<0.005 - <0.241	<0.006 - 0.3
2,3-B-7,8-CDF	<0.005 - <0.704	<0.014 - <0.19	<0.009 - 0.619	<0.011 - 1.267	<0.025 - <0.172
1-B-2,3,7,8-CDF	<0.005 - <0.1	<0.006 - <0.066	<0.005 - <0.061	<0.005 - <0.134	<0.005 - <0.06
4-B-2,3,7,8-CDF	<0.011 - 0.175	<0.014 - <0.101	<0.015 - 0.257	<0.005 - 0.255	<0.02 - <0.093
1,3-B-2,7,8-CDF	<0.005 - <0.089	<0.005 - <0.037	<0.005 - <0.039	<0.005 - <0.185	<0.006 - <0.082
PXBs					
4'-B-3,3',4,5-CB (PXB126)	0.033 - 0.495	0.081 - 0.517	0.04 - 0.529	0.008 - 0.192	0.178 - 0.532
3,4-B-3',4',5'-CB (PXB126 di-Br)	<0.005 - 0.069	<0.005 - 0.078	<0.005 - 0.062	<0.005 - 0.084	0.006 - 0.05
3',4',5'-B-3,4-CB (PXB126 tri-Br)	<0.005 - <0.05	<0.005 - <0.048	<0.005 - <0.047	<0.005 - 0.225	<0.007 - <0.1
4'-B-2,3',4,5-CB (PXB 118)	0.567 - 9.428	1.639 - 14.582	0.842 - 17.673	2.13 - 42.032	2.376 - 7.606
4'-B-2,3,3',4-CB (PXB 105)	0.201 - 2.804	0.601 - 4.939	0.317 - 9.159	0.684 - 9.705	0.783 - 3.103
4'-B-2,3,3',4,5-CB (PXB 156)	0.101 - 1.407	0.286 - 2.853	0.118 - 2.753	0.302 - 6.567	0.056 - 1.275

Table 3.6 Summary of PFAS Concentrations - µg/kg whole weight

	Species	Sardines n=8	Mackerel n=12	Herring n=9	Mullet n=7	Sprat n=9	Sea Bass n=5
		µg/kg whole weight					
PFOA	Range	0.06 - 0.92	0.06 - 0.35	0.08 - 1.17	0.01 - 0.26	0.13 - 3.82	0.05 - 0.24
	Mean	(0.34)	(0.2)	(0.34)	(0.13)	(1.48)	(0.13)
PFNA	Range	0.01 - 0.27	0.04 - 0.23	0.02 - 0.45	0.02 - 0.19	0.05 - 0.69	0.04 - 0.16
	Mean	(0.16)	(0.1)	(0.1)	(0.07)	(0.26)	(0.07)
PFDeA	Range	0.04 - 0.94	0.07 - 1.07	0.02 - 0.87	0.14 - 0.58	0.05 - 0.45	0.06 - 0.33
	Mean	(0.37)	(0.4)	(0.3)	(0.27)	(0.25)	(0.18)
PFUnA	Range	0.04 - 2.29	0.13 - 1.89	0.06 - 0.58	0.15 - 0.84	0.22 - 1.09	0.12 - 0.59
	Mean	(0.78)	(0.4)	(0.16)	(0.39)	(0.51)	(0.3)
PFDoA	Range	0.02 - 0.51	0.01 - 2.04	0.03 - 0.64	0.13 - 1.34	0.05 - 0.64	0.02 - 0.48
	Mean	(0.26)	(0.35)	(0.17)	(0.42)	(0.25)	(0.17)
PFBSH	Range	0.03 - 0.35	0.01 - 0.1	0.01 - 0.6	0.02 - 0.15	0.02 - 0.5	0.01 - 0.08
	Mean	(0.07)	(0.02)	(0.12)	(0.08)	(0.11)	(0.04)
PFHxSH	Range	0.01 - 0.12	0.01 - 0.14	0.04 - 0.06	0.01 - 0.08	0.02 - 0.15	0.01 - 0.1
	Mean	(0.03)	(0.02)	(0.02)	(0.02)	(0.08)	(0.03)
PFOS	Range	0.78 - 3.59	0.22 - 4.92	0.16 - 1.84	0.37 - 12.83	1.51 - 9.44	1.28 - 10.79
	Mean	(2.18)	(1.12)	(0.59)	(2.58)	(3.94)	(3.82)
PFOSA	Range	0.06 - 3.4	0.04 - 0.39	0.02 - 0.89	0.29 - 0.67	0.08 - 3	0.43 - 2.13
	Mean	(0.92)	(0.22)	(0.38)	(0.36)	(0.85)	(0.84)

Table 3.7 Summary of Pesticide concentrations

Species		DDD-pp	DDE-pp	DDT-pp	dieldrin	HCB
		µg/kg whole weight				
Sardine (n=7)	Range	0.3 - 0.72	1.0 - 2.98	0.3 - 0.91	1.5 - 2	0.4 - 2.2
	Mean	0.47	1.71	0.56	1.83	1.07
Herring (n=7)	Range	0.4 - 2.6	2.0 - 6.98	0.4 - 1.78	<2 - 4.9	0.5 - 1.8
	Mean	1.07	4.19	0.79	2.98	0.96
Mackerel (n=10)	Range	0.3 - 5.8	1.3 - 5.7	0.4 - 1.2	1.5 - 3.1	0.3 - 1.7
	Mean	1.81	3.11	0.69	2.06	0.95
Sea Bass (n=9)	Range	0.3 - 2.0	1.3 - 8.2	0.4 - 1.2	1.1 - 2.8	0.2 - 0.92
	Mean	0.85	4.83	0.58	1.59	0.45
Mullet (n=4)	Range	0.4 - 5.3	0.9 - 12	<0.5 - 2.57	<1 - 2.5	0.3 - 0.5
	Mean	2.22	6.06	1.14	1.91	0.45
Sprats (n=13)	Range	0.2 - 6.9	0.78 - 4.7	0.4 - 1.2	1.2 - 3.2	0.3 - 1.97
	Mean	1.62	2.46	0.75	2.30	1.14

Table 3.8 Summary of Cadmium, Mercury and Lead concentrations

Species		Cadmium (Cd)	Mercury (Hg)	Lead (Pb)
		mg/kg whole weight		
Sardines (n=16)	Range	0.005 - 0.06	0.034 - 0.073	0.005 - 0.07
	Mean	0.028	0.053	0.029
Mackerel (n=41)	Range	0.003 - 0.162	0.03 - 0.351	<0.002 - 0.018
	Mean	0.029	0.072	0.005
Herring (n=19)	Range	0.004 - 0.017	0.013 - 0.075	<0.002 - 0.064
	Mean	0.009	0.051	0.010
Gr Mullet (n=26)	Range	<0.002 - 0.005	0.01 - 0.117	<0.002 - 0.901
	Mean	0.002	0.038	0.066
Sprat (n=25)	Range	0.004 - 0.023	0.009 - 0.061	0.005 - 0.226
	Mean	0.012	0.032	0.033
Sea Bass (n=25)	Range	<0.002 - 0.007	0.095 - 0.737	<0.002 - 0.157
	Mean	0.002	0.395	0.018
Turbot (n=16)	Range	<0.002	0.018 - 0.263	<0.002 - 0.028
	Mean	<0.002	0.130	0.004
Shark (various sp.) (n=14)	Range	<0.003 - 0.055	0.061 - 1.008	<0.002 - 0.009
	Mean	0.009	0.435	0.004

5. References

Behnisch, P.A., Hosoe, K., Sakai, S., 2003. Brominated dioxin-like compounds: in vitro assessment in comparison to classical dioxin-like compounds and other polyaromatic compounds. Environ. Int. 29, 861-877.

Blankenship, A., Kannan, K., Villalobos, S., Villeneuve, D., Falandysz, J., Imagawa, T., Jakobsson, E., Giesy, J.P., 2000. Relative potencies of Halowax mixtures and individual polychlorinated naphthalenes (PCNs) to induce Ah receptor-mediated responses in the rat hepatoma H4IIE-Luc cell bioassay. Environ. Sci. Technol. 34(15), 3153-3158.

CEN method – EN16215:2012 - Determination of dioxins and dioxin-like PCBs by GC/HRMS and of indicator PCBs by GC/HRMS

Dioxins in Food (2011) Norwegian Institute of Public Health Report Available at: http://www.fhi.no/dav/4be109b07d.pdf

Dioxins in Food (2012) Norwegian Institute of Public Health Report. Available at: www.fhi.no/ilc

Dioxins in Food (2013) "Interlaboratory Comparison on dioxins in food – Fourteenth round Norwegian Institute of Public Health Report. -Available at: www.fhi.no/ilc

Dioxins in Food (2014) "Interlaboratory Comparison on dioxins in food – Fifteenth round - Norwegian Institute of Public Health Report. Available at: http://www.fhi.no/dokumenter/2c9eab2243.pdf

Clarke D, Bailey V, Routledge A, Lloyd A, Mortimer D and Gem M.(2010) Dietary intake estimate for perfluorooctanesulphonic acid (PFOS) and other perfluorocompounds (PFCs) in English retail foods following determination using standard addition LC-MS/MS. *Food Additives and Contaminants 27(4) 530-545*

Council Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs *Official Journal of the European Union*, L364/5-24, 20.12.2006

European Commission (2011) Commission Regulation (EU) No 1259/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non dioxin-like PCBs in foodstuffs.

European Commission (2012). Commission Regulation (EU) No 252/2012 of 21 March 2012 laying down methods of sampling and analysis for the official control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EC) No 1883/2006 *Official Journal of the European Union*, L84/1, 23.3.2012

European Commission (2014). Commission Recommendation 2014/118/EU of 3 March 2014, on the monitoring of traces of brominated flame retardants in food. *Official Journal of the European Union*, L65/39, 5.3.2014

European Commission (2014) Commission Regulation (EU) No 488/2014 of 12 May 2014 amending Regulation (EC) No 1881/2006 as regards maximum levels of Cadmium in foodstuffs. *Official Journal of the European Union*, L138/75, 13.05.2014

Fernandes A, White S, D'Silva K and Rose M (2004), Simultaneous Determination of PCDDs, PCDFs, PCBs and PBDEs in Food, *Talanta*, 63, 1147-1155.

Fernandes, A., Dicks, P., Mortimer, D., Gem, M., Smith, F., White, S., Rose, M., 2008. Brominated and Chlorinated Dioxins and Brominated Flame Retardants in Scottish Shellfish: Methodology, Occurrence and Human Dietary Exposure.Mol. Nutr. Food Res. 52(2), 238-249.

Fernandes A, Smith F, Petch R, Panton S, Carr M, and Rose M (2009). Investigation into the occurrence of brominated contaminants in selected foods. Report to the Food Standards Agency, Fera Report FD 08/07, 2009

Fernandes A Mortimer D.N., Rose, M., Knowles, T.G & Gem, M. (2009B) The Occurrence of Dioxins (PCDDs, PCDFs) and PCBs in Wild, Farmed and Processed Fish, and Shellfish *Food Additives and Contaminants B,* **2(1)**, 15-20

Fernandes, A., Mortimer, D., Gem, M., Smith, F., Rose, M., Panton, S., Carr, M., 2010. Polychlorinated Naphthalenes (PCNs): Congener specific analysis, occurrence in food and dietary exposure in the UK. Environ. Sci. Technol. 44, 3533

Fernandes, A., Rose M, Mortimer, D., Carr, M Panton, S., Smith, F., (2011) Mixed Brominated/Chlorinated Dibenzo-p-Dioxins, dibenzofurans and Biphenyls: Simultaneous Congener-selective Determination in Food Journal of Chromatography A, 1218, 9279-9287(2011)

Fernandes A, Rose M, Smith F and Holland M (2012). Organic Environmental contaminants in the 2012 Total Diet Study samples. Report to the Food Standards Agency, Fera Report FD 12/04, 2012

Fernandes A (2013) in "Persistent organic pollutants and toxic metals in foods" M Rose and A Fernandes , Eds. P 367, ISBN 978-0-85709-245-8 Woodhead Publishing Ltd. Cambridge UK (2013)

Fernandes, A, Mortimer, D, Wall R, Bell D, Rose, M, Carr, M, Panton, S, and Smith, F. (2014) Mixed Halogenated Dioxins/Furans (PXDD/Fs) and Biphenyls (PXBs) in Food: Occurrence and Toxic Equivalent Exposure using Specific Relative Potencies. Environment International, 73, 104-110

Food Standards Agency (2006). Brominated chemicals: UK dietary intakes. Food Surveillance Information Sheet 10/06, FSA, London.

Food Standards Agency (2006A) Brominated chemicals in farmed and wild fish and shellfish and fish oil dietary supplements Food Survey Information Sheet 04/06 Available at: http://www.food.gov.uk/news/ science/surveillance/fsisbranch2006/fsis0406

Food Standards Agency (2006B). Fluorinated chemicals: UK dietary intakes. . Food Surveillance Information Sheet 10/06 available at http://www.food.gov.uk/multimedia/pdfs/fsis1106.pdf

Food Standards Agency (2009) Survey on measurement of the concentrations of metals and other elements from the 2006 UK total diet study. Food Surveillance Information Sheet No. 01/2009, FSA, London

Ohta, S.; Tokusawa, H.; Nakao, T.; Aozasa, O.; Miyata, H.; Alaee, M.(2008) Global contamination of coplanar PBCBs in the market fishes from Japan. *Chemosphere*, **2008**, *73,* 531-538.

Rose M and Fernandes A (2012) in “Chemical contaminants and residues in food”. Ed. D Schrenk. ISBN 0 85709 058 5. Woodhead Publishing Ltd. Cambridge UK.

SANCO 2014 Method Validation & Quality Control Procedures for Pesticide Residues Analysis in Food & Feed. Guidance Document SANCO/12571/2013Available at: http://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=727

Van den Berg, M, Birnbaum, L.S, Denison, M, De Vito, M, Farland, W, Feeley, M, Fiedler, H, Hakansson, H, Hanberg, A., Haws, L., Rose, M., et al (2006). The 2005 World Health Organization Re-evaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-like Compounds. Toxicol. Sci. 93, 223–241.

Van den Berg M., Birnbaum L., Bosveld A.T.C., Brunström B., Cook P., Feeley M., Giesy J.P., Hanberg A., Hasegawa R., Kennedy S.W., Kubiak T., et al (1998). Toxic Equivalency Factors (TEFs) for PCBs PCDDs and PCDFs for humans and wildlife. *Environmental Health Perspectives*, 106, 775-792.

Zacs D, Rjabova J, Fernandes A, Evans E and Bartkevics V (2015) Characterization of brominated (PBDD/DFs and PBDEs), chlorinated (PCDD/DFs and PCBs) and mixed brominated/chlorinated (PXDD/DFs and PXBs) persistent organic pollutants in European eels (*Anguilla anguilla*) from Latvian lakes. Submitted: Env. Sci. Tech. (2015)