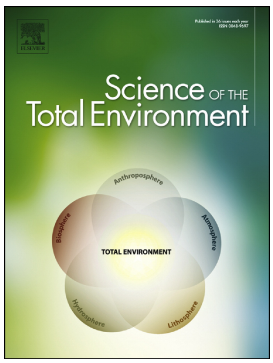

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PII: S0048-9697(20)33436-7

DOI: https://doi.org/10.1016/j.scitotenv.2020.139916

Reference: STOTEN 139916

To appear in: *Science of the Total Environment*

Received date: 2 December 2019

Revised date: 9 March 2020

Accepted date: 1 June 2020

Please cite this article as: G. Diletti, R. Ceci, A. De Benedictis, et al., Polybrominated dibenzo-p-dioxins and furans (PBDD/Fs) in Italian food: Occurrence and dietary exposure, *Science of the Total Environment* (2020), https://doi.org/10.1016/j.scitotenv.2020.139916

Polybrominated Dibenzo-*p*-dioxins and Furans (PBDD/Fs) in Italian Food: Occurrence and Dietary Exposure

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Key-Words – Human exposure, Dioxin-like, Brominated, Toxic equivalence, Relative potency, Tolerable intake

Abstract

Human exposure to polychlorinated dioxins and furans (PCDD/Fs) through the dietary pathway is widely recognised and regulations in some regions of the world help to limit food contamination. Similar information on the analogous polybrominated dioxins and furans (PBDD/Fs) is scarce, partly due to the higher threshold to analytical access and unavailability of some standard materials. The analytical methodology developed here determined twelve planar PBDD/F congeners using ^{13}Carbon labelled PBDD/F surrogates and high resolution mass spectrometric detection, and was extensively validated prior to the analysis of a range of commonly consumed Italian foods. The methodology also allowed simultaneous determination of PCDD/Fs and polychlorinated biphenyls (PCBs). The results show that PBDD/Fs occurred in different foods over a range of concentrations from <0.001 pg/g to 4.58 pg/g in fish. The dietary exposure (upper bound) of different Italian population groups, resulting from these occurrence levels was estimated using the toxic equivalency (TEQ) approach that is commonly used for dioxin-like contaminants and ranged from 0.17 to 0.42 pg TEQ/kg bodyweight/day (lower bound – 0.01 pg TEQ/kg bodyweight/day) depending on the population subgroup. Although precautionary, upper bound values may provide a more realistic estimate of toxicity as not all congeners and foods were measured. As expected, children were more highly exposed than adults due to lower body weight. These exposure levels were between a quarter and a third of that arising from the sum of PCDD/Fs and PCBs (0.61 to 1.38 pg WHO-TEQ/kg bodyweight/day), but they contribute to the dioxin-like toxicity. If this data is considered in view of the revised tolerable dioxin-like dietary intake published by EFSA in 2018, it is evident that the tolerable weekly intake of 2 pg/kg bodyweight/week would be exceeded by some of the assessed population sub-groups, or all sub-groups if the cumulative intake is considered.

1.0 Introduction

Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) are widely recognised and well-studied environmental and food contaminants. They elicit a potent toxic response in animals and humans and consequently, occurrence in environmental compartments and food is regulated in many countries (MEJ, 1999; European Commission, 2011), including a listing in Annex C of the Stockholm Convention which calls for global control on the inadvertent production of these compounds. The halogenated aromatic composition of PCDD/Fs is reflected in the brominated analogues, polybrominated dibenzo-*p*-dioxins and dibenzofurans (PBDD/Fs), which unsurprisingly share many of the characteristics and properties of PCDD/Fs, including toxicity, (Birnbaum et al., 2003; Samara et al., 2009; Wall et al., 2015). However, despite the earliest reports on their occurrence dating back to almost as long as the five decade history of PCDD/Fs, there is relatively little information on PBDD/Fs.

The PBDD/Fs that occur in environmental compartments, foods and humans are primarily of anthropogenic origin, although biogenic formation of some congeners, mediated by marine fauna has been documented (Haglund et al., 2007; Haglund, 2010), and may contribute to the relatively high occurrence levels in shellfish (Fernandes et al., 2008; 2009). Like PCDD/Fs, they are not intentionally produced but are generated inadvertently as by-products in a number of processes and industrial chemicals. Although they can be formed by chemical, photochemical, or thermal reactions from brominated precursors, the principal sources of these compounds arise from combustion processes, such as incineration of waste or as impurities in some chemicals such as brominated flame retardants (BFRs) (Ebert et al., 2003; Ren et al., 2011; Erickson et al., 2012). The thermodynamic formation of PBDD/Fs proceeds through the mechanistic pathway of *de novo* synthesis, catalysed by residual metal ions and influenced strongly by the bromine content of the materials (feedstock) undergoing combustion/incineration (Weber and Kuch, 2003; Soderstrom and Marklund 2004). Occurrence as by-products in mass produced BFRs such as commercial

polybrominated diphenyl ethers (PBDEs) and decabromodiphenylethane (DBDPE) has been documented, and so has formation through the photo-ionisation of chemicals such as PBDE-209 (Soderstrom et al., 2004).

PBDD/Fs are known to be persistent, although a relatively higher molecular lability compared to PCDD/Fs, is inherent due to the higher stress associated with the carbon-bromine bond which is weaker (bond energy 276 kJ/mol) than the carbon-chlorine bond (397 kJ/mol). This is likely to affect the levels of occurrence in the environment and in foods (Fernandes et al., 2008) and the larger size of the bromine atom and the degree of halogenation is also likely to affect the elicitation of biological effects (Birnbaum et al. 2003). Notwithstanding, these effects are very similar to the chlorinated analogues and include lethality, carcinogenesis, thymic atrophy, teratogenesis, reproductive effects, chloracne, immunotoxicity, enzyme induction, etc. (WHO, 1998). One of the principal effects of both these halogenated groups is the ability of the planar congeners to bind to the aryl hydrocarbon receptor (AhR), a process that sequentially influences the initiation, promotion, and progression of carcinogenesis. An *in vitro* study of several PBDD/F congeners on primary cultured bovine hepatocytes showed a dose - dependent induction in relative mRNA levels for cytochrome P450 1A1 and 1B1. Toxic potencies of PBDD/Fs were ranked in the order of, 2,3,7,8-TBDD > 1,2,3,7,8-PeBDF > 2,3,4,7,8-PeBDF > 1,2,3,6,7,8–HxBDD (Guruge et al., 2009). In rodents, the major overt toxic effects of 2,3,7,8-TBDD are observed on thymus, body weight, and liver, whereas teratogenic effects such as hydronephrosis and cleft palate are identical to that caused by 2,3,7,8-TCDD. Studies indicate that the toxic effects in rodents for 2,3,7,8-TBDD are in the same dose range as that of 2,3,7,8-TCDD, indicating a comparable potency for both congeners. Other possible toxic effects of PBDDs and PBDFs on immune functions and developmental brain functions have recently been studied (Ao et al., 2009; Haijima et al., 2010).

Given the reported occurrence of PBDD/Fs in environmental compartments, biota and foods it is abundantly clear that humans are exposed to these chemicals. The reported occurrence in human

milk and adipose tissue confirms this exposure (Colles et al., 2008; Kotz et al., 2005; Pratt et al., 2013; Aylward et al., 2016). For the general population, the major pathway to exposure is likely to be through dietary intake. Although there is not the extent and volume of occurrence data that exists for PCDD/Fs, some studies have reported on the occurrence of PBDD/Fs in food. Some of these are targeted at particular foods or marine biota (Haglund et al., 2010; Fernandes et al., 2008; 2009; 2018), while others have examined a wide range of commonly consumed foods (Mortimer et al., 2013; Fernandes et al 2014). The observations from these studies indicate that the distribution of PBDD/F congener occurrence in foods differs at least in part to PCDD/F occurrence. In general, most, and sometimes all PCDD/F congeners occur in food. On the other hand, PBDFs predominate in most foods but some PBDD congeners such as hexa-brominated compounds are frequently not detectable (Fernandes et al., 2009). These observations however are from selected countries and while the observations are indicative, the small volume of data makes it difficult to generalise the patterns of occurrence which could be influenced by local parameters.

This study investigates the occurrence of PBDD/Fs in commonly consumed foods of animal origin that were produced in different regions of Italy. Based on the well-validated methodology that has been successfully used for many years (Scortichini et al., 2004; Diletti et al., 2007) for the analysis of PCDD/Fs, a method for the analysis of PBDD/F s was developed, validated and successfully applied to the determination of PBDD/Fs, PCDD/Fs, and PCBs, in 60 food samples, including eggs, meat, milk, fish and mussels. The occurrence data generated from this study was used to assess human exposure by estimating mean dietary intakes for children, adolescents and adults in Italy. The data generated will additionally provide a baseline for future monitoring of the levels of these contaminants in Italian food.

2.0 Experimental

2.1 Chemicals

Solvents such as hexane, ethyl ether, absolute ethanol, petroleum ether, dichloromethane, toluene and isooctane were analytical grade (Honeywell Burdick & Jackson, Seezle, Germany). Ultra-pure water was generated within the laboratory by means Purelab option-Q system (ELGA LabWater, High Wycombe, United Kingdom). Other reagents included anhydrous sodium sulphate, concentrated sulphuric acid and sodium chloride, all at reagent grade (Honeywell Burdick & Jackson, Seezle, Germany).

2.2 Analytes

Thirteen PBDD/F congeners with (twelve with 2,3,7,8-bromine substitution) were selected for analysis, based on current toxicological knowledge, chemical configuration, and degree of halogenation, and perhaps most practically because of the availability of reference standards. The selection targeted the planar configuration arising from lateral halogenation of the diaromatic substrate that is known to induce toxic effects in similar compounds such as PCDD/Fs, PCBs and PCNs (Fernandes et al., 2017). Practically however, the selection was limited by the availability of reliable reference standards, and twelve planar compounds were obtained from either Wellington Laboratories Inc. Ontario, Canada or from Cambridge Isotope Labs, MA, USA, along with an additional tetra-brominated (non-2,3,7,8-substituted) furan. The compounds measured were 2,3,7,8-Tetrabromodibenzo-*p*-dioxin (**2,3,7,8-TBDD**), 1,2,3,7,8-Pentabromodibenzo-*p*-dioxin (**1,2,3,7,8-PeBDD**), 1,2,3,4,7,8-Hexabromodibenzo-*p*-dioxin (**1,2,3,4,7,8-HxBDD**), 1,2,3,6,7,8-Hexabromodibenzo-*p*-dioxin (**1,2,3,6,7,8-HxBDD**), 1,2,3,7,8,9-Hexabromodibenzo-*p*-dioxin (1,2,3,7,8,9-HxBDD), 1,2,3,4,6,7,8-Heptabromodibenzo-*p*-dioxin (**1,2,3,4,6,7,8-HpBDD**), 1,2,3,4,5,6,7,8-Octabromodibenzo-*p*-dioxin (**OBDD**), 2,3,7,8-Tetrabromodibenzofuran (**2,3,7,8-TBDF**), 2,4,6,8-Tetrabromodibenzofuran (2,4,6,8-TeBDF), 1,2,3,7,8-Pentabromodibenzofuran (1,2,3,7,8-PeBDF), 2,3,4,7,8-Pentabromodibenzofuran (**2,3,4,7,8-PeBDF**), 1,2,3,4,7,8-Hexabromodibenzofuran (**1,2,3,4,7,8-HxBDF**), 1,2,3,4,6,7,8-Heptabromodibenzofuran (**1,2,3,4,6,7,8-HpBDF**), 1,2,3,4,5,6,7,8-Octabromodibenzofuran (**OBDF**). Those congeners listed in bold above, were

also acquired as $^{13}C_{12}$ labelled analogues and were used as surrogates for the internally standardised quantitation procedure.

Additionally, the seventeen 2,3,7,8-chloro substituted PCDD/Fs and twelve dioxin-like PCBs (PCBs 77, 81, 126, 169, 105, 114, 118, 123, 156, 157, 167 and 189) were also determined simultaneously in the same samples as those investigated for PBDD/F occurrence.

2.3 Samples

Food samples that represented commonly consumed items of the Italian diet were selected. As halogenated dioxins and PCBs are lipophilic compounds and are known to accumulate in fatty animal tissues, this study concentrated on foods of animal origin, although it is recognised that these contaminants may also occur, albeit at lower concentrations in other vegetable, fruit and cereal based foods. A total of 60 samples including eggs, meat, milk, fish and mussels were collected randomly between January 2014 and February 2016 by the regional veterinary services, covering all areas of Italy.

In order to select fresh produce, samples were taken directly from slaughterhouses, individual breeders, farms, and production and processing sites. Cuts of meat were taken directly from the butcher, without removal of fat, and using the same cut for different types of meat, so that the lipid data provided for each food type (Table 1) reflects typical fat contents. Sample preparation involving homogenisation for all samples has been described earlier (Diletti et al., 2007), and aliquots containing between 1-5 g of fat were used for extraction. Fish samples were collected from the Mediterranean Sea off the coast of Sicily, Abruzzo and Apulia. Samples of mussels were collected from aquaculture farms located near the industrial area of Taranto, an area that was previously known to be contaminated by PCDD/Fs and PCBs.

2.4 Analytical Methodology - Extraction and purification

All equipment was cleaned and thoroughly rinsed with dichloromethane prior to use. Vials were kept capped while flasks and concentration tubes were covered with cleaned aluminium foils to avoid direct sunlight and airborne contamination of containers.

The methodology for the analysis of PCDD/Fs and PCBs has been described in detail in earlier reports (Scortichini et al., 2004; Diletti et al., 2007; Visciano et al., 2015) and the description given here will concentrate on the procedures specific to PBDD/Fs. Prior to analysis, all sample aliquots along with procedural blanks and reference materials were fortified with the internal standard solution (20 pg/ul for tetra-penta-substituted congeners, 50 pg/ul for hexa- and hepta-substituted congeners and 150 pg/ul for OBDD and OBDF).

Apart from milk, all samples were extracted by accelerated solvent extraction (ASE) using an ASE 350 Thermo Scientific Dionex (Sunnyvale, California, USA) instrument with a mixture of n-hexane and acetone 80:20 (v/v) as the extraction solvent. Milk samples were mixed with ethyl alcohol and ammonia solution and then liquid/liquid exchanged against diethyl ether and petroleum ether 1:1 (v/v). Lipid content was determined gravimetrically for all samples after evaporation of the solvent. The extracted fat was dissolved in hexane prior to purification that was carried out in two stages. In the initial stage acid hydrolysis was performed using a double liquid-liquid partitioning process with sulphuric acid to exclude the lipid component. In the second stage, the resulting extract was dissolved in hexane and purified using an automated a Power-Prep™ system (Fluid Management System, Massachusetts, USA) using disposable columns (multilayer silica, alumina and carbon). The details of the purification procedure are described earlier (Diletti, 2018). The fraction containing PBDD/Fs was eluted from the carbon column using 80 ml toluene. This fraction was concentrated, first under vacuum and then under a gentle stream of nitrogen to incipient dryness. At this stage the internal sensitivity standards ($^{13}C_{12}$ labelled 1,2,3,7,8-PeBDF and $^{13}C_{12}$ labelled 1,2,3,7,8,9-HxBDD) were added and the final volume of the extract was adjusted to 20 ul, using isooctane as the solvent.

2.5 Instrumental Analysis and Quantitation

Analysis was performed using gas chromatography and high resolution mass spectrometry (GC-HRMS) using a Trace Series 1310 GC, coupled to a DFS (Thermo Fisher Scientific, USA) as described in greater detail in Diletti, 2018. Injections of 1 µL of the final sample extracts were made with a split/splitless injector operated in surge splitless mode with a pressure of 100 kPa applied for the 4.0 minutes injection (splitless) time. The injector temperature was set at 280°C. The GC/MS interface was set to 280°C. PBDD/Fs were separated on a DB-5 MS capillary column (15 m x 0.25 mm, 0.10 µm film thickness; J&W Scientific, California, USA). The oven temperature control programme was as follows: initial hold at 100°C for 4 min, ramped to 200°C at 40°C/min, held at 200°C for 3.5 min, ramped to 320°C at 10°C/min, held at 320°C for 2 min. Helium (1 ml/min) was used as the carrier gas. The DFS HRMS system was tuned to a resolution of 10,000 and the mass axis was calibrated using perfluorokerosene (PFK). Mass filtering was carried out with electron ionisation (EI) at 50 eV, in the selected ion monitoring (SIM) mode. The two most intense ions in the molecular ion cluster were measured for each ^{13}C-labelled PBDD/F and native PBDD/F homologue group.

Mass Spectrometric data was imported to Excel™ spreadsheets where PBDD/F amounts in each of the samples was calculated and converted to concentration data.

2.6 Quality Control and Method performance parameters

There are no specific guidelines for analytical method validation for PBDD/Fs, but given the obvious similarities in properties and behaviour to PCDD/Fs, the validation requirements in EU Commission Regulation (EC) No 1883/2006 on PCDD/F and PCB analysis were used to establish performance of the method. The regulation requires the use of HRMS, control of analytical recovery, use of ^{13}C labelled surrogates/internal standards and provides specifications on sensitivity, accuracy, precision and measurement uncertainty. The calibration curves for individual congeners were evaluated for linearity of response over a working concentration range of 0.4-500 ng/ml and showed coefficients of determination (R^2) ranging from 0.987 to 1.000. The instrument limit of detection (ILOD) for PBDD/Fs was in a similar range to levels reported in other studies (Fernandes et al., 2008; 2009;

2017) with tetra-brominated congeners showing better response than OBDD/F. Practically for food samples, the limits of quantitation (LOQs) ranged from 0.04 pg TEQ/g for fish, 0.012 pg TEQ/g for milk, 0.048 pg TEQ/g for eggs and 0.024 pg TEQ/g for meat.

The use of $^{13}C_{12}$ labelled surrogates contributes to a better level of reproducibility of measurement and the precision achieved in this study ranged from 7.3% for 2,3,7,8-TBDF to 19.6% for OBDD. The accuracy of measurement was evaluated using replicate food samples that were fortified with native PBDD/F congeners. The analysis of these replicates showed excellent accuracy of measurement ranging from a minimum of 93.4% for 2,3,7,8-TBDD to a maximum of 110 % for 1,2,3,7,8,9-HxBDD. $^{13}C_{12}$ labelled surrogates were also used to estimate analytical recoveries and these ranged from 72 % to 96 % for the automated extractions and from 80 - 90% for the liquid-liquid partition procedure used for the milk samples. Expanded measurement uncertainties, estimated at a confidence level of 95% and obtained by determining the potential sources of uncertainty in the method according to EU guidelines, were found to be in a similar range as those achieved for dioxins and PCBs with values ranging from 28.6% for 1,2,3,4,6,7,8-HpBDF and 46.1% for OBDD, at measurement concentrations of 1 to 4 pg/g.

2.7 Dietary Intake Estimation

Dietary intakes of PBDD/F TEQ were estimated for three different age groups (children, adolescents and adults) of the Italian population. Food consumption data were taken from the European Food Safety Authority (EFSA) Comprehensive European Food Consumption database (EFSA, 2015) which is based on information on the dietary habits of consumers in the 2005–2006 Italian national food consumption survey INRAN-SCAI (Leclercq et al., 2009).

The population groups were classified according to the age category as follows: a) children 3–10 years, average body weight 26.1 ± 8.3 kg; b) adolescents 10–18 years (average body weight 52.6 ±

12.5 kg); c) adults 18–65 years (average body weight 69.7 ± 13.5 kg). Consumption amounts for each food group and age group were extracted from truncated normal distributions (with mean and standard deviation derived from consumption surveys). The intake estimation assumes independent consumption among different food categories, but in this case excludes vegetable, cereal and fruit based foods as these were not analysed. The average cumulative intake, expressed as pg TEQ/kg body weight per day, was estimated applying the deterministic approach. In order to calculate daily intake, the following parameters were taken into consideration: mean value of the contamination level; average consumption per food category and age group, and mean weights of the different age groups (children, adolescents, and adults). More detailed information on the methodology used for estimation is described and discussed elsewhere (Diletti et al., 2018).

3.0 Results and Discussion

As per convention for PCDD/F occurrence in foods of animal origin, PBDD/F concentrations in this study are reported in units of pg/g (equivalent to ng/kg). As human exposure through dietary intake is also being estimated, the concentrations are given on a whole weight (ww) basis. From the point of view of food safety, and given the known AhR receptor active responses (Olsman et al., 2007; Samara et al., 2009; Van den Berg et al., 2013; Wall et al., 2015), these concentrations have also been converted to toxic equivalents (TEQs) using a similar approach to PCDD/Fs and dioxin-like PCBs (Van den berg et al., 2006). The TEQ values were calculated using REP values which are analogous to the PCDD/F toxic equivalent factor (TEF) values, as have been used in other studies (Fernandes et al., 2009; 2011; 2018; 2019; Pratt et al., 2013; Zacs et al., 2016). It is important to note that using this scheme to assess risk to humans is an interim measure, due to the small number of studies on sub-chronic exposure and differences in REPs for some congeners such as 2,3,7,8-TeBDF and 1,2,3,7,8-PeBDF which reportedly show higher relative potencies than their chlorinated analogues (Van den Berg et al., 2013; Frawley et al., 2014). A wide margin of uncertainty on the use of these values

should be assumed. Notwithstanding these concerns, there is sufficient evidence to conclude that concentrations of 2,3,7,8-substituted PBDD/Fs in food contribute to the cumulative burden of the total TEQ.

3.1. Occurrence levels in food

Apart from poultry tissue and cow's milk, PBDD/Fs were detected in all other types of food although the frequency and magnitude of detection varied depending on the type. In general, there was a low incidence of detection in foods from terrestrial animals where only a few samples showed positive detection and in most cases only one congener was detected. In poultry muscle tissue and cow's milk, no positive detections were made at the LOQ of the method (typically 0.02 to 0.05 pg/g depending on the congener and the matrix). On the other hand, marine foods such as mussels and fish showed a greater number of detectable congeners with up to seven of the thirteen measured congeners being detected in a sample of mussels. The sum of measured congeners ranged from <0.31 pg/g in sheep milk to 12.3 pg/g in mussels. The most commonly detected congeners were tetra- and hepta-brominated furans and hepta-brominated dioxins, but in general, the frequency of detection was greater for PBDFs than for PBDDs (e.g. six out of seven PBDF congeners were detected in a sample of mussels compared to one out six PBDD congeners in the same sample). This higher frequency (and often higher magnitude) of PBDF occurrence relative to PBDFs in food and marine biota has also been confirmed by other studies (Ashizuka et al. 2008; Fernandes et al., 2009; 2018; 2019; Zacs et al 2016; Bjurlid et al 2018) as has the non-detection of pentaBDD and hexaBDDs. In the present study, the occurrence of other non-2,3,7,8 bromo-substituted congeners was also observed in the foods, although due to the lack of reference standards (except for 2,4,6,8-TBDF) it was not possible to quantify these compounds.

When these concentrations are converted to toxic equivalents, the upper bound PBDD/F contamination levels in fish ranged from 0.025 to 0.093 pg TEQ/g ww and from 0.022 to 0.036 pg

TEQ/g ww in mussels. For food from terrestrial animals, levels ranged from 0.013 to 0.045 pg TEQ/g ww in meat, from 0.011 to 0.023 pg TEQ/g ww in milk and from 0.024 to 0.048 pg TEQ/g ww in eggs.

A summary of the occurrence levels in the measured foods is presented as lower, medium and upper bound averages and upper bound range in Table 1 and also includes the corresponding data for PCDD/Fs and PCBs. In general, apart from fish and shellfish, the occurrence levels for PCDD/Fs and PCBs are relatively low in comparison to other recent studies on foods. (Diletti et al., 2018; Lee et al., 2016; Shen et al., 2017) which report levels up to 4.7 pg TEQ /g and 1.24 pg TEQ/g in fish and a median value of 4.1 pg TEQ/g in pig liver, respectively. The PBDD/F TEQ values show a significant difference between the lower and upper bound concentrations (lower bound ignores the contribution from non-detected congeners, medium bound assumes that these are present at half the level of the LOQ and upper bound TEQs includes detected congeners and non-detected congeners at the level of the LOQ . This is the same principle as applied to the reporting of PCDD/F data) which reflects the low frequency of detection of the PBDD/F congeners in these samples and in particular the PBDDs. In this context it is noteworthy that the LOQ values achieved in this study were low and within the same range as would be expected for sensitive PCDD/F analysis in food. It is debatable as to which of the three reporting modes (lower, medium and upper) provide a better representation of the TEQ given the fact that all contributing congeners are not included as well as the uncertainty surrounding the recommended TEF values. Dietary exposure assessments, in general, follow the precautionary approach and tend to use the upper bound values.

3.2 Reported occurrences of PBDD/Fs in foods and marine biota

There are very few reports on the occurrence of PBDD/Fs in food or biota, and most of these have focussed on occurrence in marine foods and biota. In 2005, PBDDs were identified (di- tri- tetra and penta-brominated dioxins) in blue mussels (*mytilus edulis*) from the Baltic Sea (Malmvärn et al., 2005). The summed concentration of tri-BDD congeners was estimated to be 170 ng/g fat weight. Continuing research within the same group, Haglund et al., 2007, measured PBDDs in marine fish,

mussels, and shellfish from Bothnian Bay and the Bothnian Sea, the West Coast of Sweden, and the Baltic Proper. This study reported that the levels of PBDDs were higher in mussels than in other species, and there was an increasing temporal trend of PBDDs in mussels with an average annual increase of 11% from 1995 to 2003. Investigating the occurrence in shellfish destined for retail and export markets, Fernandes et al., 2008, analysed PCDD/Fs, PBDD/Fs, PCBs, and other compounds in mussels, oysters, and scallops in Scotland. This study provided the first evidence that although levels of tri- and tetra-PBDDs were high in marine shellfish, the occurrence of PBDF congeners predominated over brominated dioxins. The study also showed variation in occurrence between species with mussels and oysters generally showing relatively higher levels of contamination than scallops, and geographically, that levels of contamination in Southern coasts were greater than those in the North and Northwest, which was consistent with Scottish industrial activity levels (Fernandes et al., 2008). These finding were confirmed in a later study that extended to the southern areas of the UK coastline (Fernandes et al., 2009). The PBDD/F TEQ ranged from 0.01 to 0.22 pg TEQ/g ww, the upper range of which is almost an order of magnitude greater than that reported in this study. Ashizuka et al., measured PBDD/Fs in different fish samples from three regions in Japan. 1,2,3,4,6,7,8-HpBDF was the most abundant congener of PBDFs with concentrations of 0.10 - 25.6 pg/g ww (Ashizuka et al., 2008). PBDD/F concentrations in the full range of foods was reported (Fernandes et al., 2014; Mortimer et al., 2013) in Total Diet (TDS) and individual food studies in the UK over nine years where PBDD/Fs were detected in all food groups and the predominance of PBDFs over PBDDs also extended to all food groups. Although a decline over time in PBDD/F concentrations was observed when upper bound data was evaluated, this was not supported by the lower bound data which showed increasing trends for some foods. It was noted that the observed decline in the upper bound data may simply be a measure of the improvement in sensitivity of measurement (Fernandes et al., 2014).

3.3 Comparative toxic content

As mentioned in section 2.2, PCDD/Fs, and PCBs were analysed simultaneously in the same samples. Given the common mechanism of toxicity between these contaminants and the additive nature of the individual responses (van den Berg et al., 2013), it is instructive to compare the relative proportions of PCDD/F and PBDD/F TEQ, whilst bearing in mind the provision made earlier regarding the use of analogous TEF or REP values for the PBDD/Fs. Additionally, not all PBDD/F congeners that contribute to TEQ were measured in this study. Given the very different origins of these contaminants (PCDD/Fs and PBDD/Fs are mostly derived from combustion sources or as minor by-products in industrial chemicals whereas PCBs were manufactured as bulk chemicals) it may also be relevant to compare the relative contributions of these to the cumulative TEQ from all three classes of contaminant. The first observation though, when making this comparison, is the rather low levels of contamination of both PCDD/Fs and PBDD/Fs for the terrestrial foods. This can be seen in the differences between lower, medium and upper bound TEQs in Table 2.

As noted elsewhere (Fernandes et al., 2014) differences between lower and upper bound TEQs for PBDD/Fs do arise (in this study, they are also evident for PCDD/Fs in some samples) and in order to observe the effects of reducing individual congener concentration data to TEQs, all three comparisons are presented. The comparison given in Table 2 indicates that PBDD/F TEQ is generally smaller than PCDD/F TEQ, particularly on the lower bound data.

On a lower bound basis the PBDD/F to PCDD/F TEQ ratio is smallest for shellfish, poultry, milk and hens eggs (<0.01 to 0.03), while the higher order animal tissues show higher ratios, up to 0.11 (Table 3.2). The proportion of PBDD/F TEQ to the cumulative TEQ from PCDD/Fs and PCBs is considerably smaller because of the high levels of PCBs in all samples, but particularly the fish and shellfish. Other studies have also noted this relatively lower contribution from PBDD/Fs to TEQ (Fernandes et al., 2008; Mortimer et al., 2013) but it is unclear as to whether, this is a result of the current occurrence levels that are to be expected given the greater lability of PBDD/Fs or whether environmental levels are still stabilising or increasing (Fernandes et al., 2014). The comparisons in this study are indicative

at best because of the high proportion of non-detected values and also due to the relatively small number of samples. There is however no doubt that for some sample types there is a significant contribution to the cumulative TEQ arising from the occurrence of PBDD/Fs.

3.4 Dietary Intake

The primary pathway to non-occupational human exposure to dioxin-like contaminants is the dietary route, and the food occurrence data obtained in this study was used to estimate the intake of PBDD/F TEQ by different population groups and also to compare it to the cumulative body burden of dioxin-like human exposure arising from PCDD/F and PCB intake as estimated earlier (Diletti et al., 2018). This was derived from a contemporaneous but much larger set of samples (2600 compared to 60 in this study) which would provide a more robust representation of the PCDD/F and PCB content of widely consumed Italian food. The comparisons were made by taking into consideration the same food groups analysed in both studies: 1) meat and meat products, 2) fish and other sea food, 3) milk and other dairy products, 4) eggs and poultry. The estimates were based on mean consumption data from the Italian Food Consumption Database and were derived for the three principal age groups Given the differences observed in upper and lower bound TEQ levels because of the low incidence of detection, of (particularly) the PBDDs, the estimates have also been given as lower, medium and upper bound values (Table 3).

As observed elsewhere (Fernandes et al., 2008; 2010; 2014B; Mortimer et al., 2013) for similar dioxin-like contaminants such as mixed halogenated dibenzo dioxins and furans (PCDD/Fs, PXDD/Fs) and polychlorinated naphthalenes (PCNs) as well as for PBDD/Fs, the highest levels of intake are for young children who are more strongly impacted as a result of lower body weight. The lower bound estimates for PBDD/F intake are around a third of the upper bound estimates, this difference being mostly attributable to the non-occurrence of those (mostly) PBDD congeners, which are reported to show higher REP values. This contrasts with PCDD/F and PCB occurrence where in general the differences are less marked. Notwithstanding, these differences, the estimated intake from PBDD/Fs

reflects the relative concentrations and medium and upper bound intakes are significant when compared to the PCDD/F intake. The PBDD/F contribution is considerably lower than the summed intake from all three classes of contaminant but there are currently some uncertainties surrounding the contribution to TEQ from PCB 126 (EFSA, 2018) which may be overestimated. The TEF value for this compound is currently being re-evaluated with a new study. However, in light of the re-evaluation of the tolerable weekly intake (TWI) of dioxin-like toxicity which now stands at 2pg/kg bw/week (EFSA, 2018) the contribution from PBDD/Fs becomes a significant addition to cumulative intake. It is clear from the table however, that even when PBDD/F toxicity in food is considered on its own, young children will exceed the new TWI when the upper bound level is considered. Given that all dioxin-like PBDD/F congeners were not included in this estimation as well as the uncertainty surrounding the TEF values for PBDD/Fs (van den Berg et al., 2013) consideration of the medium or even upper bound levels may provide a more realistic measure of intake. Also as noted earlier, vegetable based foods and cereals were not included in the study due to the relatively low levels of PBDD/F contamination that are expected to be found in these foods (as have been reported in other studies (e.g. Mortimer et al., 2013; Fernandes et al., 2014). This may give a small measure of underestimation to the dietary intakes. The daily intake estimates are similar, if perhaps lower than those estimated by an earlier total diet study (Mortimer et al., 2013) which reported mean upper bound intakes of 0.67 to 0.95 pg/kg bw/day for 1.5 to 10 year old children and 0.29 pg/kg bw/day for adults. This compares to 0.42 and 0.17 pg/kg bw/day children and adults respectively, estimated in this study (Table 3). Although the earlier study includes a contribution from polybrominated biphenyls, this was considered to be negligible due to very low (the majority of congeners were below the LOQ) occurrence levels in the total diet samples. The difference is more likely to arise from the higher occurrence levels of PBDD/Fs as well as a contribution from other components of the diet (cereals, bread, processed meat, preserves, etc.) that were not included in the current study.

4.0 Conclusions

The occurrence of PBDD/F congeners in a range of different and commonly consumed foods has been confirmed by well validated methodology. In common with the limited number of studies reported in the literature, the methodology covers most, but not all of the relevant planar, or 2,3,7,8-bromine substituted PBDD/Fs that contribute to dioxin-like toxicity. The observed occurrence varied between the studied food groups, with terrestrially raised food often showing low or non-detectable levels of contamination compared to fish and shellfish. However, levels of PCDD/Fs measured in the same foods were also low, which provides an indication of the generally low contamination of the foods that were analysed in this study. When expressed as toxic equivalents, the PBDD/F TEQ was comparable to PCDD/F TEQ on a medium/upper bound basis for most foods apart from fish and shellfish. The levels were considerably lower when compared to DL-PCB TEQ although the ongoing re-evaluation of TEF values for PCBs may alter this comparison. Estimates of dietary exposure from PBDD/F toxicity vary depending on the way the data is evaluated. When lower bound toxicity is considered, all population groups are well below the new TWI of 2pg/kg bw/week. However when upper bound TEQs are considered, young children would exceed this TWI based on the foods examined in this study. As it was not possible to measure all TEQ contributing PBDD/Fs and all food types in this study as well as the uncertainty surrounding the REP values, the medium or upper bound intake may represent a more realistic value, and also support the precautionary approach to estimating human dietary exposure.

It is important to note that this first study on the occurrence of PBDD/Fs in Italian foods and the resulting dietary exposure to the Italian population provides an estimate that is based on a limited amount of data and should therefore be viewed primarily as a confirmation of PBDD/F occurrence in the food chain and of the additional contribution to the cumulative dioxin-like toxicity burden through the diet. The continuing use of bromine in consumer products and the mechanisms of PBDD/F formation and transfer make it likely that the observed occurrences in food will continue.

ACKNOWLEDGEMENTS

This research was financed by the Italian Ministry of Health (Grant: MSRCTE 08/13). The authors thanks: Professor Dario Compagnone, faculty of bioscience and technology for food, agriculture and environment – University of Teramo (Italy) and Dr. Giampiero Scortichini, Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche (Italy) for their scientific support of the PhD study and related research.

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Table 1: Summary of PBDD/F, PCDD/F and PCB occurrence in analysed food groups. (Lower (LB), Medium (MB) and Upper (UB) bound concentration in whole weight basis)

Food (No. of samples)	**PBDD/Fs (pg TEQ/g)**			**PCDD/Fs (pg WHO-TEQ/g)**			**PCBs (pg WHO-TEQ/g)**		
[% Lipid Mean and Range]	**Lower Bound**	**Medium Bound**	**Upper Bound (Range)**	**Lower Bound**	**Medium Bound**	**Upper Bound (Range)**	**Lower Bound**	**Medium Bound**	**Upper Bound (Range)**
Beef (n=6) [10.6, 3.6 - 26]	0.0011	0.014	0.029 (0.020 – 0.045)	0.010	0.015	0.02 (0.027 – 0.051)	0.084	0.085	0.086 (0.013 – 0.23)
Poultry meat (n=5) [11.2, 2.7 - 17]	<0.0001	0.008	0.016 (0.013 – 0.019)	0.013	0.014	0.015 (0.004 – 0.043)	0.014	0.015	0.015 (0.002 – 0.039)
Pork (n=6) [19.3, 6.4 - 30]	0.0004	0.010	0.021 (0.016 – 0.026)	0.004	0.007	0.01 (0.002 – 0.021)	0.012	0.013	0.013 (0.001 – 0.054)
Cow milk (n=6) [3.6, 3.2 - 4.6]	<0.0001	0.008	0.017 (0.012 – 0.023)	0.003	0.004	0.004 (0.003 – 0.006)	0.022	0.022	0.022 (0.005 - 0.049)
Sheep milk (n=4) [5.8, 3.5 – 7.2]	0.0004	0.007	0.014 (0.011 – 0.020)	0.013	0.014	0.015 (0.006 – 0.023)	0.081	0.081	0.081 (0.059 – 0.12)
Mussels (n=8) *[-, 0.5 – 1.8]	0.0056	0.016	0.027 (0.022 – 0.036)	0.770	0.79	0.81 (0.25 – 2.3)	2.0	2.0	2.0 (0.95 – 5.4)
Fish (n=19) *[-, 0.4 – 11]	0.0092	0.029	0.048 (0.025 – 0.093)	0.10	0.10	0.1 (0.003 – 0.37)	0.89	0.9	0.9 (0.022 – 3.5)
Hens Eggs (n=6) [10.3, 9.3 - 12]	0.0007	0.017	0.034 (0.024 – 0.048)	0.028	0.03	0.032 (0.008 – 0.07)	0.046	0.047	0.049 (0.005 – 0.19)

* Typical range for species studied.

Table 2: Ratios of PBDD/F TEQ to PCDD/F TEQ and summed TEQ (PBDD/Fs, PCDD/Fs and PCBs)

Food groups (n. samples)	PBDD/Fs-TEQ : PCDD/Fs-TEQ			PBDD/Fs-TEQ : Sum (PCDD/F, PBDD/F, PCB)TEQ		
	LB	MB	UB	LB	MB	UB
Beef meat (n=6)	0.11	0.93	1.45	0.01	0.13	0.22
Poultry meat (n=5)	<0.01	0.58	1.07	<0.01	0.22	0.35
Pig meat (n=6)	0.10	1.43	2.00	0.03	0.33	0.48
Cow milk (n=6)	<0.03	2.15	3.95	<0.01	0.25	0.40
Sheep milk (n=4)	0.03	0.50	0.93	0.01	0.07	0.13
Mussels (n=8)	0.01	0.02	0.03	<0.01	0.01	0.01
Fish (n=19)	0.09	0.29	0.48	0.01	0.03	0.05
Hens Eggs (n=6)	0.03	0.57	1.06	0.01	0.18	0.31

Table 3. Daily and weekly intake estimates based on the deterministic approach in the different groups of the Italian general population. Mean intake calculated in lower, medium and upper bound concentrations.

Population groups	PBDD/Fs			PCDD/Fs*	PCDD/Fs + DL-PCBs*
	Daily Intake (pg WHO_{05}-TEQ/kg bw per day)				
	Lower	Medium	Upper	Upper*	Upper*
Children (3-10 y)	0.013	0.22	0.42	0.43	1.38
Adolescents (10-18 y)	0.008	0.12	0.24	0.26	0.80
Adult (18-65 y)	0.006	0.09	0.17	0.19	0.61
	Weekly Intake (pg WHO_{05}-TEQ/kg bw per week)				
Children (3-10 y)	0.09	1.54	2.94	3.02	9.66
Adolescents (10-18 y)	0.06	0.84	1.68	1.79	5.60
Adult (18-65 y)	0.04	0.63	1.19	1.33	4.27

* Values extrapolated from Diletti et al., 2018 taking under consideration the following food groups: 1) meat and meat products, 2) fish and other sea food, 3) milk and other dairy products, 4) eggs and egg products

Credit authorship contribution statement

Gianfranco Diletti, Conceptualization, Resources, Methodology, Funding acquisition, Formal analysis, Data curation, Writing - review & editing.

Roberta Ceci: Analysis, Data curation, Investigation Review & editing.

Giacomo Migliorati: Conceptualization, Resources, Methodology, Funding acquisition

Alfonso De Benedictis, Manuela Leva, Luigi Pirito, Ilaria Vairano: Methodology, Formal analysis, Data curation

Alwyn R. Fernandes: Conceptualization, Data curation, Writing - original draft, Writing - review & editing.

Conflict of Interest Statement

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Highlights

- First study on PBDD/Fs in Italian foods shows occurrence in most food types
- PBDD/F TEQ is generally lower than PCDD/F and PCB TEQ
- The new TWI for dioxin-like toxicity may be exceeded for children's dietary intake of PBDD/Fs