LEVELS OF BROMINATED FLAME RETARDANTS, POLYCHLORINATED BIPHENYLS AND ORGANOCHLORINE PESTICIDES IN DIET SAMPLES FROM AVEIRO UNIVERSITY COMMUNITY, PORTUGAL

Coelho SD[1, 2]*, Sousa ACA[1- 4], Nogueira AJA[1]; Isobe T[2] and Tanabe S[2]

[1]Department of Biology & CESAM, University of Aveiro, Aveiro, Portugal; [2]Center for Marine Environmental Studies (CMES), Ehime University, Matsuyama, Japan; [3]Health & Environment Unit, Faculty of Health Sciences, University of Beira Interior, Covilhã, Portugal; [4]Health Sciences Research Center (CICS), University of Beira Interior, Covilhã, Portugal

Introduction

It is well known that food acts as a source of several contaminants to human and wildlife and therefore food ingestion is considered as one of the main human exposure routes to a wide range of compounds, including persistent organic pollutants (POPs). Environmental contamination by POPs is a global concern as they have several toxic properties, they are highly lipophilic and accumulate in animal and human adipose tissues[1], and they are resistant to degradation, thus, they bioaccumulate and persist in the environment[2]. Polybrominated diphenyl ethers (PBDEs), hexabromocyclododecanes (HBCDs), polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCs), such as hexachlorocyclohexanes (HCHs), hexachlorobenzenes (HCBs), chlordanes (CHLs) and dichlorodiphenyl-trichloroethane (DDT) are listed as POPs by the Stockholm Convention. Although their use is already phased out in Europe and other continents, or as in the case of the HBCDs their ban is scheduled, they have been detected worldwide in different types of samples, including human matrices.

The aim of this study is to determine the levels of the above mentioned POPs in duplicated diet samples representative of the daily diet of a group of Portuguese citizens working or studying in the University of Aveiro, Portugal, with the objective to assess the exposure to these contaminants through diet.

Materials and Methods

Sample collection

Twenty one students and researchers from the University of Aveiro (Portugal) participated in this study. The volunteers, while maintaining their regular dietary habits, collected during seven consecutive days, a small portion representative of the dietary products consumed in all meals including snacks and deserts, between May and June 2012. During those seven days all food items consumed were registered in notebooks provided for that purpose. The samples were preserved daily by the participants in their home freezer and at the end of the week delivered to the laboratory. At the laboratory, all samples from the same volunteer were pooled together, homogenized and kept at -20^0C until freeze drying and chemical analysis.

Chemical analysis

PBDEs, HBCDs, OCs and PCBs were quantified according to the method described by Asante, et al. (2013) [3]. An aliquot of about 20 - 30g of the freeze dried samples was homogenized with anhydrous sodium sulfate and extracted using a SE-100 High Speed Solvent Extractor (Acetone/ Hexane; 1:1,v/v). A portion (2 mL) of the obtained extract was used for lipid determination and the other portion was spiked with an internal clean-up spike. As the food samples are a complex matrix, the spiked extract was subjected to an extra clean-up through a multi-layer silica gel column (150 mL of Hexane/ Dichloromethane; 25%,v/v), followed by gel permeation chromatography (hexane/ dichloromethane; 1:1,v/v) for lipid removal. The first obtained fraction was discarded, whilst the second, the lipid-removed fraction containing organohalogen compounds, was subjected to clean-up and fractionation by an activated silica gel column (Wakogel DX).The first fraction contained PBDEs, OCs and PCBs (eluted with 80 mL of hexane/ dichloromethane; 5%, v/v) and the second fraction contained HBCDs (100ml of hexane/ dichloromethane; 25%, v/v). Both fractions were concentrated individually and spiked with the respective internal standards to ensure the recoveries of surrogates. Finally, identification and quantification of PBDEs, OCs and PCBs were performed using a gas chromatograph coupled with a mass spectrometer (GC–MS; Agilent 7980A GC coupled with an Agilent 5975C MS). For HBCDs a liquid chromatography coupled with a tandem mass spectrometer (LC–MS/MS; Acquity UPLC (Waters, Tokyo) equipped with a Quattro Micro API (Waters,Tokyo)) was used.

Results and Discussion

Brominated flame retardants (BFRs) levels (Table 1) were very low and for most of the duplicate diet samples they were even under the limit of detection (LOD). Considering the PBDE congeners of primary interest (BDE-28, 47, 99, 100, 153, 154, 183 and 209) [4], those showing higher detection frequencies and concentrations were BDE-209 >> 47 > 99, even though, for the last two the obtained concentrations were practically below LOD for the 21 samples. When detected, BDE 47, BDE 99 and BDE 209 were the major contributors to the total PBDEs concentrations (∑PBDEs: 250 pg/g ww). Not many studies adressed the levels of PBDEs in these type of diet samples; in Europe, for example, a similar survey was conducted in the University of Antwerp by Roosens, et al. (2009) [5], our results are lower than the ones reported, in 2009, regarding the diet of several students from this belgian university.

Regarding HBCDs, similar results were obtained. The majority of the concentrations of each isomer were below LOD. The highest total HBCDs concentration was 1200 pg/g ww (Table 1), which was higher than the maximum concentration reported for the University of Antwerp study [6].

Table 1: BFRs concentrations in the 21 duplicate diet samples expressed as pg/g ww; 2 significant digits. (*Sum of the 8 BDE congeners).

	Range
BDE 28	< LOD
BDE 47	< LOD - 14
BDE 99	< LOD - 10
BDE 100	< LOD
BDE 153	< LOD
BDE 154	< LOD
BDE 183	< LOD
BDE 209	< LOD - 220
∑PBDEs*	31 - 250
α-HBCD	< LOD - 920
β-HBCD	< LOD - 9.4
γ-HBCD	< LOD - 230
∑HBCD	8.4 - 1200

Table 2: PCB concentrations in the 21 duplicate diet samples expressed as pg/g ww; 2 significant digits. (** Sum of the 18 PCB congeners).

	Range
PCB 28	< LOD
PCB 52	< LOD - 12
PCB 101	< LOD - 33
PCB 138	0.86 - 170
PCB 153	< LOD - 240
PCB 180	< LOD - 110
PCB 77	< LOD
PCB 81	< LOD
PCB 105	< LOD
PCB 114	< LOD
PCB 118	< LOD - 43
PCB 123	< LOD
PCB 126	< LOD
PCB 156	< LOD
PCB 157	< LOD
PCB 167	< LOD
PCB 169	< LOD
PCB 189	< LOD - 25
∑PCBs**	17 – 660

The concentrations of dioxin-like PCBs (77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189) and non-dioxin-like PCBs (28, 52, 101, 138, 153 and 180) are summarized in Table 2. The most abundant PCB congeners were PCB-153 > 138 > 180 > 118 > 101 demonstrating a great dominance by indicator PCBs (non-dioxin-like PCBs plus PCB-118). Similar results (higher prevalence of congeners PCB-153, 138 and 180) were found in a previous study on the levels of PCBs in several food items in four European countries, including Portugal [7].

Considering the levels of some highly persistent chlorinated pesticides (Table 3), the frequency was DDTs > CHLs > HCHs > HCBs. Although these OCs agricultural use has been banned, they were detected in all the analyzed samples, and only few (HCB and CHLs) had concentrations below the LOD. DDTs frequency and

range (110 – 730 pg/g ww) showed their leading presence in the studied duplicated diet samples regardless the fact that their use as a pesticide was banned in the European Union in 1986 [8].

There are few duplicate diet studies performed in order to assess POPs concentrations and the influence of diet on POPs intake, most probably because the BFRs, PCBs and OCs distribution varies among foodstuff. The generally low levels of POPs found in our duplicate diet samples are possibly associated with intrinsic characteristics of the collection procedure. For example, by homogenising similar portions of complete meals, the detection of POPs in food can be compromised as the quantities of low contaminated ingredients exceeded the high contaminated ingredients, such as meat, fish and dairy products, reported as important contributors to total dietary intake of POPs by several authors [9].

Table 3: OCs concentrations of 21 duplicate diet samples expressed as pg/g ww; 2 significant digits.

	Range
HCH	15 - 160
HCB	< LOD - 62
CHLs	< LOD – 1100
DDTs	110 - 730

Table 4: Intake of POPs from food ingestion (ng/day/kg) considering the highest detected concentrations; 2 significant digits.

	Daily intake
BDE-47	0.38
BDE-99	0.28
BDE-209	5.9
∑PBDEs	6.7
∑HBCD	31
PCB 101	0.87
PCB 118	1.2
PCB 138	4.4
PCB 153	6.3
PCB 180	3.0
∑PCBs	18
HCH	4.2
HCB	1.7
CHLs	28
DDTs	20

In order to understand the human exposure through food, the daily intakes (Table 4) were estimated considering 1870 g/inhab/day as the daily edible per capita in 2012 (year when the samples were collected), reported by the National Institute of Statistics, Portugal[10], and a mean adult weight of 70 kg. By simulating the worst case scenario (the highest obtained concentrations) the estimated daily intakes are 6.7 and 31 ng/day/kg for PBDEs and HBCD, respectively. The daily dietary intake of PCBs was 18 ng/day/kg, for the highest concentration detected, and this value is far below the tolerable daily intake (TDI) established by FAO/ WHO, 1 µg/day/kg[11]. The same situation occurs with the OCs, for the highest concentrations in food the estimated daily intakes (table 4) were far below the TDIs established by WHO, which are 8, 0.17, 0.5 and 20 µg/day/kg for HCH, HCB, CHLs and DDTs, respectively[11-13]. This evaluation is impossible in the case of BFRs, as the TDIs of PBDEs and HBCD are still not established due to the uncertainties and deficiencies in their toxicological databases[4, 14].

Overall, the levels in our samples are relatively low; however we were able to detect POPs in several samples, confirming their persistency in the environment. Based on the supplied duplicate diet, our results demonstrate that the daily ingestions of the selected POPs are far below the tolerable daily intake and thus population risk is negligible. Nevertheless it should be once again highlighted that in the supplied samples people tended to put much more quantity of carbohydrates than fish, meat or dairy products. Therefore we suggest performing a complementary total diet study or market basket study, with the objective to describe more accurately the levels in foodstuff, and also to understand which are the most contaminated items, in the Portuguese diet.

Acknowledgements

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