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PRESENCE OF PCDD/FS AND PCBS IN BONITO (SARDA SARDA)

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Introduction

It is well known that about 90-98 % of the human exposure to polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/Fs), as well as polychlorinated biphenyls (PCBs), is estimated to come from diet[1], being fish and seafood one of the major contributors[2]. In these sense, several studies have been focused on assessing the levels of PCDD/Fs and PCBs in specific fish species in order to evaluate the potential impact of its consumption[3,4]. However, more efforts are needed in order to improve the information about the levels of these compounds in this category of food products, particularly in fatty fish species.

In a previous work, we analyzed a large number of fish samples, fatty and non-fatty species, from different fishing areas. It was found that fatty fishes, particularly those from the Mediterranean Sea, presented the highest values of PCDD/F and PCB concentrations[5]. Along with some small or medium sized species, such as sardine or mackerel, bonito (Sarda sarda) showed high levels compared with bigger tuna species.

Spain is one of the main fishery countries of bonito in the European Union (EU), reaching more than 280 KTm of bonito in 2012[6]. The annual consumption of bonito was 25745 kg in 2013. However, information about levels of PCDD/Fs and PCBs for this particular fish is still scarce in the literature. Some authors have already reported high levels of these pollutants in bonito, with concentrations of non dioxin-like PCBs eventually exceeding maximum levels established by the EU[7]. The aim of this work was to determine the concentrations of PCDD/Fs and PCBs in several bonito samples from Spain in order to assess whether they are particularly high and comparable to those included in previous studies.

Materials and Methods

A total of nine bonito (Sarda sarda) samples were obtained from the marked for the analysis of PCDDs/Fs and PCBs, both dioxin-like PCBs (DL-PCBs) and non dioxin-like PCBs (NDL-PCBs).

Once at the laboratory, samples were weighted and measured. General characteristics (fork length and weight) of the samples are presented in Table 1. The non-edible parts of fish and skin were removed, only the muscle meat was analysed. Then, the samples were freeze-dried and homogenized as part of pre-treatment steps. Samples were extracted in a Soxhlet for ~24h with toluene:cyclohexane (1:1) after being spiked with known amounts of mixtures of $^{13}C_{12}$-PCDD/Fs (EPA-1613LCS, Wellington Lab., Guelp, Canada) and $^{13}C_{12}$-DL-PCBs (WP-LCS, Wellington Lab., Guelp, Canada). Next, the extracts were rotary evaporated and kept in the oven overnight (105 ºC) in order to eliminate the solvents prior to gravimetrical fat determination. Afterwards, fat residues were dissolved again in n-hexane. Organic components, fat and other interfering substances were removed by treating the n-hexane extracts with silica gel modified with sulphuric acid (44%). Further sample purification and fractionation were carried out using multilayer silica, basic alumina and carbon columns. Instrumental analysis was based on high resolution gas chromatography coupled to high resolution mass spectrometry (GC-HRMS). All analyses were performed on a 6890N Network GC System Agilent gas chromatograph (Agilent Technologies Inc., Palo Alto, USA) fitted with a DB-5ms fused silica column (J&W Scientific, Folsom, USA) and connected through a heated transfer line kept at 280 ºC to an AutoSpec Ultima NT high resolution mass spectrometer with an EBE geometry (Waters, Manchester, UK). Electron ionization (EI+) mode was used, operating in the selected ion monitoring (SIM) mode at a resolving power of 10000 (10% valley definition). The two most abundant ions of the molecular cluster ions of each homologue group were monitored.

For NDL-PCB analysis, the extraction and purification methodology was similar to that previously described for PCDD/Fs and DL-PCBs. Briefly, freeze-dried samples were spiked with known amounts of

$^{13}C_{12}$-PCBs (MBP-MXE, Wellington Lab., Guelp, Canada) and then extracted in a Soxhlet for ~24h using n-hexane: dichloromethane (1:1). After that, the extracts were rotary concentrated and transferred to n-hexane. Next, purification and fractionation of these extracts were carried out using a silica gel column modified with sulphuric acid (44%) and a basic alumina column. Chromatographic separation was performed using DB-XLB (60m x 0.25mm i.d. x 0.25µm film thickness) column from J&W Scientific (Folsom, USA). Instrumental conditions for NDL-PCB analysis by GC-HRMS were similar to those for PCDD/Fs and DL-PCBs.

Results and Discussion

In general, WHO-TEQ results for PCDD/Fs were far below the limit value of 3.5 pg WHO-$TEQ_{PCDD/Fs}$/g fresh weight (fw) indicated at the EU Regulation8. The isomer distribution of toxic congeners was mostly characterized by the presence of the lowest chlorinated compounds, particularly 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF and 1,2,3,7,8-PeCDF. Congeners with the highest chlorinated content (8 Cl) were also observed, while most of HxCDD/F and HpCDD/F congeners were in minor proportions. A common DL-PCB profile distribution for biotic samples was also observed in all the cases, being dominated by PCB 118 followed by PCB 156, PCB 105 and PCB 167, with non-ortho PCBs among those with the lowest concentrations (Figure 1).

Concentrations of individual PCDD/F and DL-PCB congeners, as well as total results expressed in WHO-TEQs, are shown in Table 1. The S1 sample showed the highest concentrations for these compounds, followed by samples S6 and S7. In these three cases, the sum of PCDD/F and DL-PCBs, in terms of WHO-TEQ (21.5, 16.3 and 17.1 pg WHO-$TEQ_{PCDD/Fs+DL-PCBs}$/g fw, respectively), clearly exceeded the limit established at the EU Regulation for these products (6.5 pg WHO-$TEQ_{PCDD/Fs+DL-PCBs}$/g fw).

In addition, NDL-PCB concentrations, expressed as the sum of the 6 congeners analysed, were in agreement with WHO-$TEQ_{PCDD/Fs+DL-PCBs}$ levels in the samples. Particularly, very high NDL-PCB values were observed in these three specific samples (S1, S6 and S7). The S6 and S7 samples showed levels around two times higher than the maximum limit allowed (75 ng/g fw)[8]. The S1 sample exhibited an extraordinary high concentration (938 ng/g fw). It has to be noted that S1 bonito was the largest size fish of the nine samples considered in this study, with more than double the mean weight and 32% greater fork length than all the rest measured fish (see Table 1).

The extremely high levels in the biggest fish can be explained taking into account that the food chain constitutes the main source for bioconcentration and bioaccumulation of these pollutants in exposed organisms. Bonito (Sarda sarda) is a predator that commonly preys on sardine, anchovy, mackerel and other pelagic fishes as part of its diet, which are fatty fish showing high levels of PCDD/Fs and PCBs. In addition, the environmental contamination levels in the fishing areas and/or the geographical areas were these wild fish are usually located could also affect the concentration of PCDD/Fs and PCBs observed in the fish.

Data reported in this study highlights the need to perform further studies on the presence of PCDD/Fs and PCBs in bonito (Sarda sarda), and probably in other big/fatty fish species, in order to know the potential health impact on regular consumers of this kind of fish products. Moreover, similar studies performed on wild eel (Anguilla anguilla) and wild spiny dogfish (Squalus acanthias) have already given to amend the EU regulation. The maximum levels of DL-PCBs and NDL-PCBs have been increased for these particular species[8,9]. In this sense, similar findings might be observed in other fishes for which still scarce data are available related to the content of these pollutants. From the results showed in this work, bonito (Sarda sarda) appears to be one of these potential fish candidates to be included in a monitoring program.

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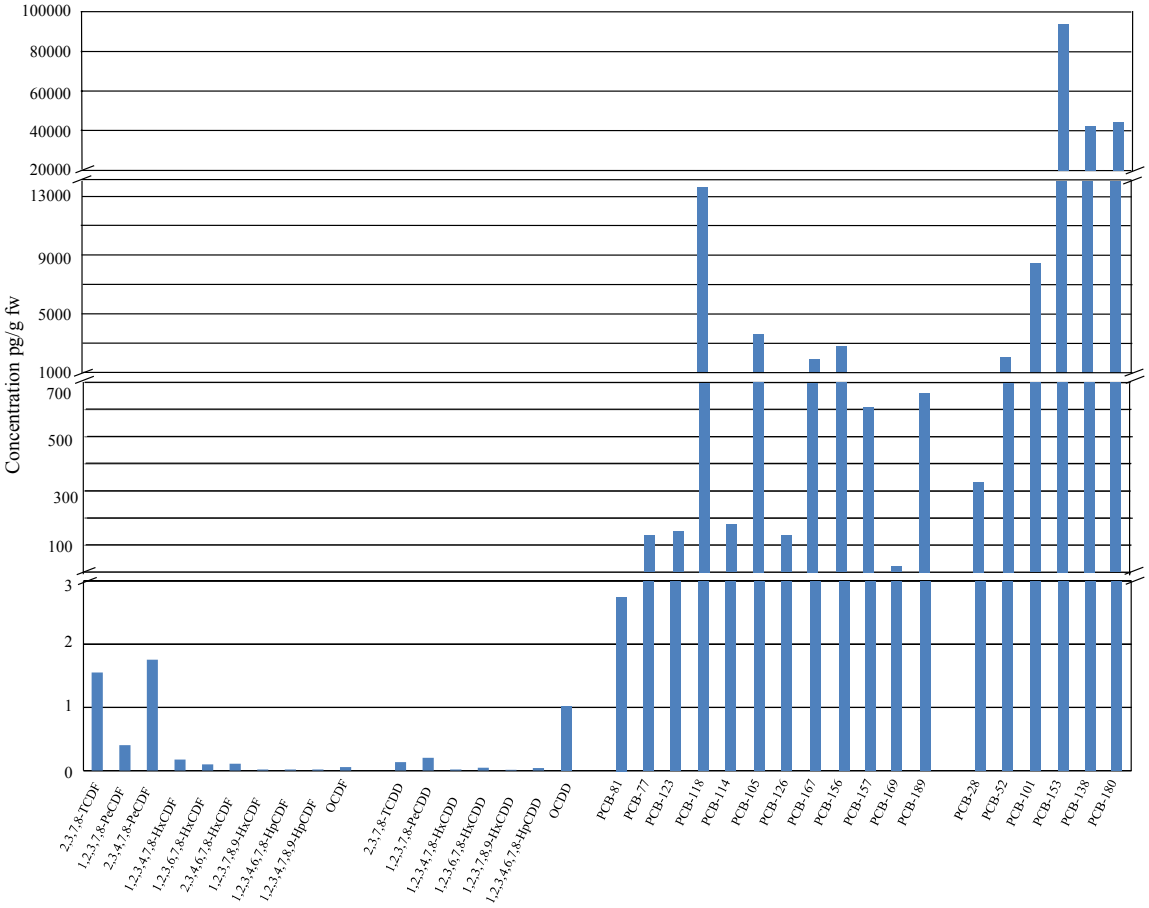

Figure 1. Individual concentrations of PCDD/F, DL-PCB and NDL-PCB (expressed in pg/g fw) in sample S6.

Table 1. Concentrations of individual PCDD/F and PCB congeners (pg/g fw for PCDD/Fs and DL-PCBs and ng/g fw for NDL- PCBs) as well as WHO-TEQ values (upperbound) (pg WHO-TEQ/g fw) in bonito samples.

	S1	S2	S3	S4	S5	S6	S7	S8	S9
Fork length (cm)	74	54	49	56	55	52	50	53	52
Weight (g)	5433	2436	1695	2482	2563	2117	1931	2086	1967
Compounds									
2,3,7,8 - TCDD	0.13	0.01	0.02	0.03	0.06	0.13	0.11	0.03	0.03
1,2,3,7,8 - PeCDD	0.16	0.02	0.03	0.08	0.11	0.20	0.18	0.08	0.07
1,2,3,4,7,8 - HxCDD	<0.01	<0.01	<0.01	0.01	0.01	0.02	0.02	0.01	<0.01
1,2,3,6,7,8 - HxCDD	0.03	0.01	<0.01	0.03	0.04	0.05	0.06	0.02	0.02
1,2,3,7,8,9 - HxCDD	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.02	0.01	<0.01
1,2,3,4,6,7,8 - HpCDD	0.06	0.02	<0.02	0.03	0.04	0.04	0.05	0.04	0.03
OCDD	1.8	0.099	0.08	0.15	0.16	1.03	0.36	0.31	0.21
2,3,7,8 - TCDF	1.0	0.10	0.59	0.84	1.3	1.5	1.7	0.87	0.98
1,2,3,7,8 - PeCDF	0.56	0.03	0.09	0.18	0.29	0.40	0.64	0.20	0.14
2,3,4,7,8 - PeCDF	1.5	0.098	0.33	0.74	0.85	1.8	1.1	0.95	0.68
1,2,3,4,7,8 - HxCDF	0.02	<0.01	0.04	0.09	0.11	0.17	0.06	0.11	0.04
1,2,3,6,7,8 – HxCDF	0.03	0.01	<0.01	0.09	<0.01	0.10	<0.02	0.08	0.02
1,2,3,7,8,9 – HxCDF	<0.01	<0.01	0.02	0.05	0.10	0.11	0.12	0.05	0.03
2,3,4,6,7,8 – HxCDF	<0.01	<0.01	<0.01	<0.02	<0.01	<0.02	<0.02	0.01	<0.01
1,2,3,4,6,7,8 - HpCDF	<0.02	<0.02	<0.02	0.05	0.04	<0.02	0.02	0.04	0.03
1,2,3,4,7,8,9 - HpCDF	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
OCDF	0.03	0.04	0.02	0.04	0.05	0.05	0.16	0.12	0.03
pg WHO-TEQ/g fw (PCDD/F)	0.88	0.08	0.22	0.45	0.60	1.1	0.86	0.52	0.42
PCB-81	6.8	<0.43	<1.9	<1.3	1.5	2.8	2.2	1.2	1.1
PCB-77	76.3	6.8	24.3	40.5	88.1	139	199	37.9	44.2
PCB-123	463	8.7	33.1	70.2	106	151	141	57.8	107
PCB-118	26130	692	1875	3301	7388	14098	16881	3461	3358
PCB-114	459	9.4	28.9	51.6	99.2	179	195	43.4	41.7
PCB-105	6583	191	440	787	1799	3635	4067	775	838
PCB-126	183	5.1	22.1	40.4	60.7	138	145	41.2	49.6
PCB-167	6015	123	345	732	1214	2081	2462	689	825
PCB-156	7986	137	440	917	1710	2902	3488	823	1034
PCB-157	1295	33.6	82.4	177	310	616	691	162	212
PCB-169	23.3	0.86	4.1	8.1	10.3	20.7	27.0	7.6	11.4
PCB-189	1767	34.8	105	256	337	664	945	229	352
pg WHO-TEQ/g fw (DL-PCB)	20.6	0.58	2.4	4.5	6.8	15.2	16.2	4.5	5.5
pg WHO-TEQ/g fw (PCDD/F + DL-PCB)	21.5	0.67	2.7	4.9	7.4	16.3	17.1	5.1	5.9
PCB-28	0.55	<0.33	<0.33	<0.33	<0.33	<0.33	<0.33	<0.33	<0.33
PCB-52	2.6	<0.33	0.35	0.53	1.0	2.0	1.5	0.46	0.33
PCB-101	38.9	1.3	1.7	3.0	5.7	8.5	7.7	2.4	3.1
PCB-153	439	10.9	17.1	38.2	64.0	94.0	95.1	24.0	45.7
PCB-138	179	4.6	7.6	18.3	31.4	42.2	38.7	10.0	18.4
PCB-180	276	4.9	7.6	17.6	32.0	44.3	42.8	12.1	23.2
∑NDL-PCB (ng/g fw)	938	22.3	34.8	78.0	134	191	186	49.4	91.1