PCDD, PCDF AND DIOXIN-LIKE PCB LEVELS IN FISH SAMPLES FROM RISK ZONES ALONG THE EBRO RIVER BASIN, SPAIN

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Introduction

This work is part of the research included in the European project AQUATERRA (Integrated modeling of the river-sediment-soil-groundwater system; advanced tools for the management of catchment areas and river basins in the context of global change). Our research is focused on the study of different persistent organic pollutants, such as brominated flame retardants, in different risk zones in the Ebro river basin[1, 2].

The objective of this study was to determine the occurrence of PCDDs, PCDFs and dioxin-like PCBs in three areas considered as risk zones along the Ebro river basin: the Vero, Cinca and Flix areas.

Materials and Method

Sample collection: The study area is located in the north-east of Spain, along the Ebro river basin. Three different risk zones were selected for this study. The first one is located along the Vero River, a tributary of the Cinca River. The sampling point is located 4 Km downstream an area with a textile industry impact. The second risk zone corresponded to the Cinca River. Samples were collected 30 Km downstream Monzón, a heavily industrialized town with a very important chemical industry. And, the third risk area is located in Flix, a small village with two important plants of chlorine and caustic soda production. Previous studies reported high PCB contamination in sediments from this area[1].

Surficial sediments (0-2 cm) were collected at each selected site. Moreover, fish samples were also collected by DC electric pulse (Table 1). Fishes were killed, weighted and the fork length of each fish was measured. Muscle samples were collected from below the dorsal fin and preserved frozen at -20 ºC until analysis. All the samples (sediments and fishes) were sampled in November 2004.

Extraction and cleanup: Among 1 and 4 g of fish samples were weighed and fortified with a known amount of $^{13}C_{12}$-labelled PCDD/F and $^{13}C_{12}$-labelled PCB quantification standard solutions (Wellington Laboratories Inc., Canada), EPA 1613 LCS and WP-LCS respectively. Samples were extracted using a Dionex ASE100 at the following conditions: hexane, 100 ºC, 1500 psi, 90 % flush volume and two static cycles. After extraction, the solvent was removed and, subsequently fat content was determined gravimetrically. Resulting extracts were transferred into a separation funnel and liquid-extracted with concentrated sulphuric acid to remove organic matter. Clean-up stage was then performed in an automated purification Power Prep™ System (FMS, Inc., USA) including acidic silica gel and basic alumina columns for mono-orto PCB purification and an additional carbon column for PCDD/F and co-PCB clean up. Different mixtures of hexane:DCM were used to recover mono-orto PCBs while retaining interfering compounds. PCDD/Fs and co-PCBs were recovered with toluene. The final extracts were concentrated avoiding dryness, spiked with EPA1613-ISS and WP-ISS internal standard solutions (Wellington Laboratories Inc., Canada) and further analysed by GC-MS.

Instrumental analysis:
PCDD/F analyses were performed by a HRGC-HRMS system (Autospec Ultima NT) at 10,000 resolving power using a 30 m chromatographic column (TRB-5MS from Teknochroma). Monitored masses were those proposed

by EPA 1613 method[4]. The program temperature was from 100 ºC (held for 1 min.) to 220 ºC (held for 1 min.) at 20 ºC/min, and finally to 310 ºC (held for 20 min.) at 3 ºC/min. PCB analyses were carried out by GC/MS/MS in a Varian Saturn 2000 workstation equipped with a CP-3800 Gas Chromatograph. A J&W Scientific DB5-MS (40m x 0.18 mm i.d., 0.18 µm film thickness) capillary column was used. Identification and quantification of target species were carried out by following criteria of isotopic dilution technique, allowing high accuracy in the calculation of the final results. The temperature conditions were: 60 ºC (held for 1 min.), 60-235 ºC (held for 21 min.) at 50ºC/min, 235-275 ºC at 20 ºC/min., 275-310 ºC (held for 0,5 min.) at 35 ºC/min. Helium was used as carrying gas at constant flow of 1 ml/min. Temperature of the transfer line was set at 280 ºC and the corresponding in the trap at 250 ºC.

Table 1. Characteristics of fish samples collected in this study.

Site	Fish specie	Code	Length (cm)	Weight (g)
Vero	Barbel (*Barbus graellsii*)	B3V3	24.5	206
		B4V3	26.5	230
		B5V3	26.6	237
		B6V3	28.8	284
		B7V3	32.6	417
		B8V3	33.5	464
Monzón	Southwestern nase	M1C4	15.1	40
	(*Chondrostoma toxostoma*)	M2C4	15	43
		M3C4	16.8	65
Flix	Barbel (*Barbus graellsii*)	B1E2	51	1600
		B2E2	48	1500
		B6E2	17	62.8
		B8E2	18	74.9
		B345E2*	16.5, 16.9, 16.5	55, 55, 55
	Carp (*Cyprinus carpio*)	C2E2	49	2500
		C3E2	50	1900
		C4E2	29	514
	Red Roach (*Rutilus arcasii*)	R1E2	22	155.1
		R2E2	20	125
		R4E2	19.5	116.5
		R7E2	21	156
		R356E2*	20, 18.7, 20.1	117.6, 102, 128.9
	Wels Catfish (*Silurus glanis*)	S1E2	81	3400
		S2E2	63	1600
	Largemouth Bass	P15E2*	15, 16	50, 57
	(*Micropterus salmoides*)	P234E2*	15, 15.5, 16	50, 50, 58

* These samples corresponded to pool samples (2 or 3 specimens)

Results and Discussion

Total TEQ values, including PCDDs, PCDFs and PCBs, for sediment samples from Vero, Cinca and Flix were 7.42, 9.92 and 189 pg/g dry weight (dw), respectively. Whereas Vero and Cinca sediments presented levels below the safe sediment value (20 pg TEQ/g dw), Flix sample exceeded by far this safe value. Moreover, dioxin-like PCB contribution to total toxicity of the Flix sediment was the highest (40%, 60% and 69% for Vero, Cinca and Flix, respectively), confirming the PCB problem in this area.

Table 2 shows the PCDD/F and dioxin-like PCB levels found in the different fish samples. Total levels ranged from 11 to 962 pg WHO-TEQ/g dw. As expected due to the sediment contamination, the highest levels were

found in fishes from Flix. It should be pointed out that, whereas PCDD/F contamination was similar at the three risk zones, the highest values of Flix samples are attributed to the highest contamination of dioxin-like PCBs. In Flix samples, the PCB contribution to the total TEQ values ranged from 77 to 98 %, whereas this contribution ranged from 17 to 79 % and 42 to 51 %, for Vero and Monzón areas, respectively.

From the different fish species analysed in the Flix area, the most contaminated samples corresponded to wells catfish (*Silurus glanis*), followed by carps (*Cyprinus carpio*), largemouth bass (*Micropterus salmoides*), red roach (*Rutilus arcasii*), and finally, the lowest values were detected in barbels (*Barbus graellsii*). It should be pointed out that wells catfish and carps were the species with the highest length and weight.

Table 2. PCDD/F and dioxin-like PCB levels (expressed in pg WHO-TEQ/g lipid weight) in fish samples.

Site	Fish specie	Code	PCDD/F	DLPCBs	TOTAL
Vero	Barbel (*Barbus graellsii*)	B3V3	24.0	9.42	33.4
		B4V3	7.25	4.00	11.3
		B5V3	87.1	17.9	105
		B6V3	3.25	12.5	15.8
		B7V3	54.6	11.5	66.1
		B8V3	22.1	58.3	80.4
		Mean	*33.1*	*18.9*	*52.0*
		Median	*23.1*	*12.0*	*49.8*
Monzón	Southwestern nase (*Chondrostoma toxostoma*)	M1C4	34.2	34.9	69.1
		M2C4	16.0	11.5	27.5
		M3C4	14.1	11.3	25.4
		Mean	*21.4*	*19.2*	*40.7*
		Median	*16.0*	*11.5*	*27.5*
Flix	Barbel (*Barbus graellsii*)	B1E2	9.42	86.9	96.3
		B2E2	12.1	303	315
		B6E2	52.6	18.1	70.7
		B8E2	7.35	61.1	68.5
		B345E2	6.33	120	126
		Mean	*17.6*	*118*	*135*
		Median	*9.42*	*86.9*	*96.3*
	Carp (*Cyprinus carpio*)	C2E2	28.6	243	272
		C3E2	43.8	299	343
		C4E2	79.7	325	405
		Mean	*50.7*	*289*	*340*
		Median	*43.8*	*299*	*343*
	Red Roach (*Rutilus arcasii*)	R1E2	44.5	213	257
		R2E2	32.8	135	168
		R4E2	65.4	276	341
		R7E2	37.5	179	217
		R356E2	42.7	163	206
		Mean	*44.6*	*193*	*238*
		Median	*42.7*	*179*	*217*
	Wels Catfish (*Silurus glanis*)	S1E2	21.4	940	962
		S2E2	15.2	682	698
		Mean/Median	*18.3*	*811*	*830*
	Largemouth Bass (*Micropterus salmoides*)	P15E2	49.9	167	217
		P234E2	36.5	231	267
		Mean/Median	*43.2*	*199*	*242*

Another interesting question is the comparison of our results with the European limit of PCDDs, PCDFs and PCBs in fish for human food, set at 3 pg TEQ/g wet weight. Fishes collected at these risk zones presented values between 0.29 to 7.13 pg TEQ/g wet weight (Figure 1). From the 26 samples analysed, 4 exceeded the European limit: one sample corresponded to the Monzón area, whereas the rest were collected at the Flix area.

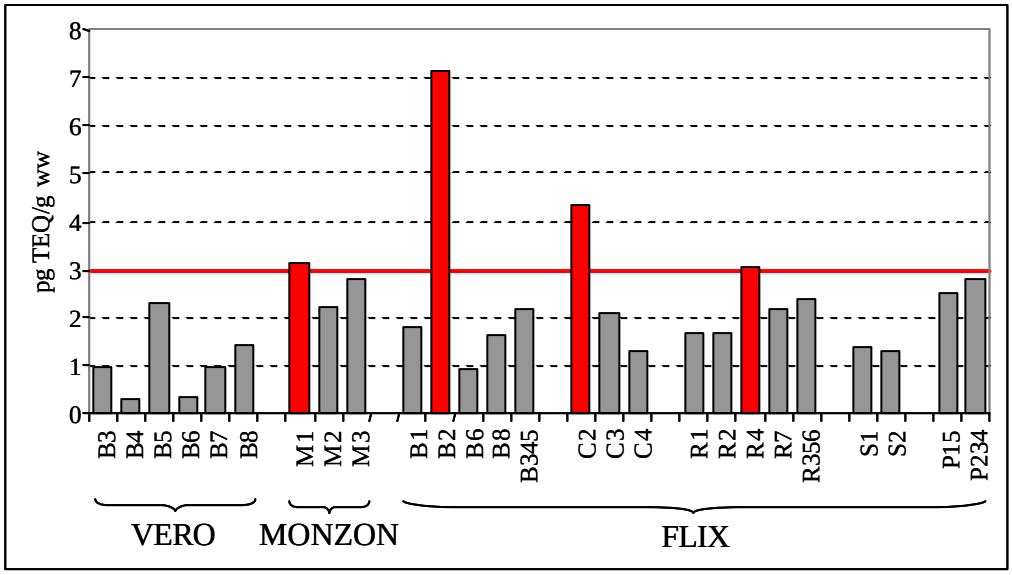

Figure 1. Total (PCDDs+PCDFs+DLPCBs) TEQ values, expressed in pg/g wet weight (ww) in different fish samples.

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