JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

Levels and Trends of Polychlorinated Dibenzo-*p*-dioxins/Furans (PCDD/Fs) and Dioxin-like Polychlorinated Biphenyls (PCBs) in Spanish Commercial Fish and Shellfish Products, 1995–2003

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The polychlorinated dibenzo-*p*-dioxin (PCDD), dibenzofuran (PCDF), and polychlorinated biphenyl (PCB) contents of 123 Spanish commercial salmon, tuna fish, sardine, oyster, mussel, and clam samples from 1995 to 2003 were investigated. A significant decrease of dioxin and non-ortho PCB concentrations in the studied species was found over the years. The decrease was greater in the case of dioxins than in that of non-ortho PCBs, especially during the early years of the study. PCB and PCDD/F concentrations in the years 2001−2003 were comparable to those reported in the literature for similar species collected after 1999. Mean PCB concentrations ranged from 3.46 ng/g of fresh weight (fw) in clams to 100 ng/g of fw in tuna fish. PCDD/F mean current levels ranged from 0.62 pg/g of fw in clams to 2.89 pg/g of fw in oysters. Toxic equivalent quantities (WHO-TEQ) ranged from 0.05 pg of $WHO\text{-}TEQ_{PCDD/Fs}$/g of fw in clams to 0.5 pg of $WHO\text{-}TEQ_{PCDD/Fs}$/g of fw in salmon (in the upper bound determination levels). When coplanar PCBs were included, the $WHO\text{-}TEQ_{PCDD/Fs+cop\ PCBs}$ values increased by a range of 1.7 times in oysters to 14.1 times in tuna fish. The decrease in dioxin concentrations suggests that efforts to control dioxin emissions and to reduce human exposure through foodstuffs are succeeding. The high contribution of PCBs to total WHO-TEQs in the fish and shellfish species investigated suggests that it is important to determine PCBs in foodstuffs, and especially in fish products, and they should be included in further research and future legislation.

KEYWORDS: PCDD/Fs; PCBs; temporal trends; commercial Spanish fish products

INTRODUCTION

The term “dioxins” refers to a group of 75 polychlorinated dibenzo-*p*-dioxins (PCDDs) and 135 polychlorinated dibenzofuran (PCDFs) congeners, of which 17 are considered to be biologically active (*1*). Polychlorinated biphenyls (PCBs) are structurally related to dioxins and have similar chemical and physical properties. They were widely manufactured from the 1930s to the 1970s for a range of industrial applications. There are 209 theoretically possible PCB congeners, of which 12 coplanar (non-ortho and mono-ortho chlorinated substituted) congeners exhibit biological activity similar to that of PCDDs and PCDFs and are therefore referred to as “dioxin-like” PCBs (*2*, *3*). In 1998, the World Health Organization (WHO) revaluated the toxicity equivalent factors (TEFs) assigned to dioxins and dioxin-like PCBs for the calculation of the toxic equivalent quantities (TEQs) (*1*).

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For the general population, dietary intake is the main route of PCDD/F and PCB exposure, contributing >90% to the daily exposure (*4*, *5*). Public concern over the adverse health effects of these toxicants at this time has been intensified by a number of dioxin contamination incidents involving food and feedstuffs (*6*−*8*). Recent reports concerning toxicological aspects have led to a revaluation of the tolerable daily intake (TDI) for dioxins and dioxin-like PCBs (*9*) and have forced wide-ranging efforts and the tightening of regulations to reduce dioxin release into the environment (*10*). To prevent the health risk from dioxin and dioxin-like PCB exposure, the European Commission has recently established maximum permissible levels of dioxins in foods (*11*). At the moment these levels refer only to PCDD/Fs, but PCBs will be added soon.

This concern about the human health impact, combined with continuous encouragement from scientific committees to monitor food samples across Europe, has prompted numerous international and regional studies on the concentration of dioxins in particular food items (*12*−*15*). Recently, various food surveys have also been carried out in Spain (*16*−*19*). All of these studies report new data on the background levels of these compounds

10.1021/jf050835z CCC: $30.25
Published on Web 09/14/2005

and highlight the most important food groups in terms of their dioxin and PCB concentrations as well as their contribution to the total daily intake.

Data from various countries show that human dietary intake has been decreasing in recent years (*12*, *15*, 20). Fish and fish products have received special attention due to their widespread consumption by the population and their high dioxin and dioxin-like PCB contents. Most studies report that fish products exhibit higher dioxin levels than any other food category (*13*, *15*, *17*−*19*, *21*). Although current data on PCDD/F levels in individual fish and shellfish species from Europe (*12*, *22*−*24*), including Spain, exist (*16*, *19*, *25*), studies addressing their temporal trends are scarce (*12*, *23*).

We present here the results of the first monitoring program on temporal trends in PCDD, PCDF, and dioxin-like PCB concentrations found in six fish and shellfish species (salmon, sardine, tuna fish, oyster, mussel, and clam) commercially available in Spanish markets from 1995 to 2003. The results were intended to assess whether the measures adopted to reduce levels have really been effective, to ascertain the contribution of coplanar PCBs to the toxic equivalent quantities (WHO-TEQ) in the studied samples, and finally to determine whether the present levels of dioxins and dioxin-like PCBs exceed the maximum permitted levels of dioxins in fish and fish products according to EU Council directive. Finally, the results were compared with those found in other surveys conducted in Spain and other European countries.

MATERIALS AND METHODS

Sampling. A total of 123 fish and shellfish samples [18 salmon (*Salmo salar*), 14 sardines (*Sardina pilchardus*), 24 tuna fish (*Thunnus thynnus*), 22 oysters (*Cassostrea madrasenssis*), 24 clams (*Chamelea gallina* Venus spp.), and 21 mussels (*Mytilus edulis*)] were analyzed in the period 1995−2003. Samples were commercially available and were acquired in Spanish markets.

Once at the laboratory, the nonedible part of the food products was removed and the edible part, skin excluded, was stored in stable conditions, either freeze-dried or frozen at −20 °C, until analysis.

Each sample consisted of pooled individual specimens acquired at the same market location. The aggregate samples were prepared depending on the type of food. For sardines and seafood products, at least 10 individual samples were taken for the pooled samples. In the case of salmon and tuna fish, various subsamples (5−10) of at least 100 g were taken from each individual.

Three time periods were considered in this study. The first period included samples taken between 1995 and 1996, the second samples taken between 1999 and 2000, and the third samples taken between 2001 and 2003.

PCB and PCDD/F Congeners Analyzed. A total of 23 individual PCB congeners were analyzed including the set of 7 indicators (PCBs 28, 52, 101, 118, 138, 153, and 180), PCBs relevant in commercial mixtures (PCBs 95, 132, 149, 183, 170, and 194), and those PCBs that have been assigned a toxic equivalency factor by the WHO (the non-ortho PCBs 77, 126, and 169 and the mono-ortho-substituted PCBs 105, 114, 118, 123, 156, 157, 167, and 189) (*1*), named according to IUPAC nomenclature (*26*). Individual concentrations of the 17 2,3,7,8-substituted toxic congeners were reported for PCDD/Fs.

Analytical Procedure. The extraction and cleanup methodology has already been described elsewhere (*25*). Briefly, samples, either freeze-dried or fresh previously dried with anhydrous sodium sulfate powder, were homogenized with 1:4 (w/w) silica gel/anhydrous sodium sulfate powder. The mixture was ground to a fine powder, loaded into a column, and spiked with a mixture containing 15 $^{13}C_{12}$-labeled 2,3,7,8-substituted PCDDs and PCDFs and $^{13}C_{12}$-labeled PCBs 77, 126, and 169. Extraction was carried out with 400 mL of a 1:1 (v/v) acetone/hexane mixture. Cleanup steps were carried out using multilayer columns filled with neutral silica, silica impregnated with sulfuric acid (44 and 22%), and silica modified with KOH. Fractionation among the studied compounds was achieved using Supelclean Supelco ENVI-Carb tubes as described elsewhere (*27*). Three fractions were eluted containing ortho-substituted PCBs, non-ortho PCBs, and PCDD/Fs, respectively.

The lipid content was determined by matrix solid-phase dispersion (MSPD) using a chloroform/methanol (1:1, v/v) mixture as elution solvent. The extract was evaporated until dryness, and the lipid content was determined gravimetrically.

Ortho-Substituted PCB Instrumental Analysis. The methodology has been described elsewhere (*28*). Briefly, analyses were performed by GC-μECD (Agilent 6890 series II, Avondale PA), in the splitless injection mode using nitrogen as carrier gas. The GC column was a DB-5 (60 m length, 0.25 mm i.d., 0.25 μm film thickness) from J&W Scientific. 1,2,3,4-Tetrachloronaphthalene (TCN) and PCB 209 were used as internal injection standards. Instrumental method limits of detection (LOD) ranged from 0.04 to 0.4 pg injected. The repeatability and reproducibility, determined by analyzing a standard solution at 10 ng/mL levels of each PCB congener, showed relative standard deviations (RSDs) lower than 8 and 17%, respectively.

Non-ortho PCB and PCDD/F Instrumental Analysis. Purified extracts were analyzed by HRGC-HRMS/EI(+)/SIM on a GC 8000 series (Carlo Erba Instrument, Milan, Italy) coupled to an Autospec Ultima mass spectrometer (MS) (Micromass, Manchester, U.K.) at 10000 resolving power (10% valley definition). A DB-5 GC column (60 m length, 0.25 mm i.d., 0.25 μm film thickness) (J&W Scientific) was used for chromatographic analysis. Helium was used as carrier gas. A 2 μL sample was injected in the splitless mode. The two major ions of the molecular ion cluster were monitored for each compound. In the case of dioxins, the limits of quantification (LOQ) ranged from 0.00001 to 0.34 pg/g of fresh weight (fw), whereas for PCBs they ranged from 0.001 to 0.37 pg/g of fw. Further details on instrumental conditions can be found elsewhere (*16*). The uncertainty, calculated after the repeated determination ($n = 14$) of PCDD/Fs and non-ortho PCBs in a fish certified reference material (specially prepared in an interlaboratory exercise) with a concentration of 1.42 pg of WHO-TEQ/g of fw, was <22% for all congeners.

Quality Control for the Comparability of PCDD/F and Non-ortho PCB Determinations in Fish and Shellfish Products from 1995 to 2003. The quality control for PCDD/F analysis has become stricter year by year. An important issue nowadays is how to compare recent data with data obtained before the 1990s (when food data relating to dioxin first appeared in the literature). To render all data presented in this work comparable for the determination of temporal trends, the following quality control criteria were applied:

All analysis followed quality dioxin analyses such as blanks, recoveries, and parallel analyses and complied with analytical standards (accuracy, precision, recoveries, etc.) as recommended by the EU Commission in the directive for measuring dioxins in food (*30*).

For analyses conducted before 1999, only data with recoveries of >50% and with differences of <40% (based on PCDD/F only) between the upper (ND = LOQ) and lower (ND = 0) bound determination levels were considered.

Moreover, the working group has participated in several international quality control studies for the analysis of PCDD/Fs and PCBs in different food matrices, including fish (*31*−*34*). The results were consistent at all times with the consensus means given by the interlaboratory organizations.

Statistical Analysis. In most cases the distribution of data was highly skewed. The variables did not follow a normal distribution, and nonparametric tests were used for statistical comparisons. The data set was analyzed by nonparametric Kruskal Wallis (χ^2) one-way analysis of variance test to determine significant differences among the PCDD, PCDF, and PCB contents of the three time periods for each species. Differences with $p < 0.05$ were considered to be significant. The Statgraphic Statistical package (version 5.0, STSC Inc., Rockville, MD) was used for the calculations.

RESULTS AND DISCUSSION

PCDD/F and PCB concentrations and WHO-TEQs reported in this survey are expressed in nanograms per gram and

Table 1. Mean (Range) of PCDD/F and Non-ortho PCB Concentrations in Fish and Shellfish from the Spanish Market, in the Upper Bound Determination Level (ND = LOQ) in 1995–1996

	pg/g of fw		pg of WHO-TEQ/g of fw			
	2,3,7,8-PCDD/Fs	non-ortho PCBs	PCDD/Fs	non-ortho PCBs	PCDD/Fs + non-ortho PCBs	% lipid
salmon (10)[a]	22.2 (6.17–66.6)	73.8 (38.1–117)	2.46 (0.65–7.27)	1.7 (1.07–2.84)	4.13 (1.72–10.1)	13.1
sardines (7)	37.6 (4.16–97.5)	152 (75.5–359)	8.33 (1.91–13.3)	3.02 (0.9–5.03)	11.4 (2.81–18.3)	10.2
tuna fish (16)	176 (11.6–488)	48.8 (0.9–292)	9.19 (0.94–30.2)	1.25 (0.02–4.05)	10.4 (0.96–34.3)	8
oysters (11)	367 (0.08–1965)	86.3 (0.23–516)	2.11 (0.01–12.7)	0.67 (0.01–2.33)	2.79 (0.02–15.3)	0.8
clams (10)	4.05 (1.60–9.16)	44.5 (0.45–105)	0.18 (0.11–0.28)	0.10 (0.04–0.20)	0.28 (0.15–0.48)	0.7
mussels (11)	1.86 (0.35–6.42)	6.15 (0.04–0.27)	0.31 (0.04–0.67)	0.08 (0.002–0.27)	0.39 (0.04–0.94)	1.1

[a] Number of samples.

Table 2. Mean (Range) of PCDD/Fs and Non-ortho PCB Concentrations in Fish and Shellfish from the Spanish Market, in the Upper Bound Determination Level (ND = LOQ) in 1999–2000

	pg/g of fw		pg of WHO-TEQ/g of fw			
	2,3,7,8-PCDD/Fs	non-ortho PCBs	PCDD/Fs	non-ortho PCBs	PCDD/Fs + non-ortho PCBs	% lipid
salmon (6)[a]	11.1 (2.01–18.1)	69.3 (30–51.2)	1.39 (0.36–2.85)	1.66 (0.67–2.42)	3.06 (1.03–5.27)	15.6
sardines (5)	2.17 (1.19–4.15)	31.2 (2.82–35.8)	0.52 (0.26–0.73)	0.77 (0.004–1.55)	1.29 (0.26–2.28)	10.8
tuna fish (5)	0.73 (0.58–1.23)	18.9 (7.92–39.4)	0.16 (0.14–0.17)	0.44 (0.27–0.99)	0.58 (0.41–1.16)	8.8
oysters (5)	3.48 (0.82–10.2)	34.1 (2.14–105)	0.66 (0.19–1.81)	0.07 (0.02–0.25)	0.73 (0.20–2.06)	0.6
clams (6)	1.23 (0.27–2.16)	15.8 (0.03–24.4	0.13 (0.04–0.27)	0.05 (0.001–0.06)	0.18 (0.04–0.33)	0.7
mussels (5)	1.5 (0.74–2.37)	6.75 (0.64–26.3)	0.12 (0.06–0.22)	0.01 (0.001–0.06)	0.13 (0.06–0.28)	0.9

[a] Number of samples.

picograms per gram on a fresh weight basis to avoid seasonal variations of the fat content of fish species, which can vary considerably over one year due to different maturation states (*29*). WHO-TEQs were calculated by multiplying the measured concentration of each 2,3,7,8-substituted PCDD/Fs and dioxin-like (coplanar) PCBs by the appropriate toxic equivalent factor (WHO-TEFs) for humans (*1*). The sum of these products yielded $\text{WHO-TEQ}_{\text{PCDD/Fs}}$ and $\text{WHO-TEQ}_{\text{cop PCBs}}$. PCB 123 was not included in the $\text{WHO-TEQ}_{\text{mono-ortho PCBs}}$ due to coelution problems with PCB 149 in the DB5 GC column. WHO-TEQ values have been calculated at the upper and lower bound determination levels, meaning that the value of nondetectable congeners was taken as equal to the LOQ and zero, respectively (*30*).

Temporal Trends in PCDD/F and PCB Concentrations. The temporal trends study included PCDDs, PCDFs, and non-ortho PCBs (PCBs 77, 126, and 169). The ortho-substituted PCBs were analyzed only after 2000 and are consequently not considered in this section.

The mean concentration and range of the 2,3,7,8-substituted PCDD/Fs and non-ortho PCBs in the foodstuffs studied during the three periods (from 1995 to 2003) are shown in **Tables 1–3**. A decline is observed in the studied samples through the years. The PCDD/F and non-ortho PCB concentrations declined statistically ($p < 0.05$) over the three different periods in almost all cases (**Figure 1**). These variations were greater in the case of dioxins than in the case of dioxin-like PCBs, especially during the first years of the study. This explains the different contribution of PCDDs, PCDFs, and non-ortho PCBs to total WHO-TEQs over the years. PCDD/Fs presented the most important contribution to total WHO-TEQs in the period 1995–1996 (>60%), whereas non-ortho PCBs were predominant in the 2001–2003 period (>58%), except for oyster samples (30%).

Salmon. The mean 2,3,7,8-substituted PCDD/F value declined 2-fold from 1995–1996 (22.2 pg/g of fw) to 1999–2000 (11.1 pg/g of fw) and 5-fold in the period 2001–2003 (2.0 pg/g of fw). These decreases were statistically significant ($p < 0.05$). In the same way, the mean value of non-ortho PCBs declined slightly ($p > 0.05$) from 1995 to 2003.

The mean $\text{WHO-TEQ}_{\text{PCDD/F}}$ content declined ($p > 0.05$) from 1995–1996 (2.46 pg of WHO-TEQ/g of fw) to 1999–2000 (1.39 pg of WHO-TEQ/g of fw) and decreased significantly ($p < 0.05$) in 2001–2003 (0.5 pg of WHO-TEQ/g of fw). The same tendency was observed when non-ortho PCBs were considered.

PCDD/Fs presented the most important contribution to the $\text{WHO-TEQ}_{\text{PCDD/Fs+non-ortho PCBs}}$ values in 1995–1996 (60%), whereas non-ortho PCBs predominated in 1999–2000 (56%) and 2001–2003 (62%).

Sardines. The mean 2,3,7,8-substituted PCDD/F level tended to decrease over the course of this study (1995–2003). A significant decrease ($p < 0.05$) in these values was observed from 1995–1996 (37.6 pg/g of fw) to 1999–2000 (2.17 pg/g of fw); this trend continued in the following years, but in this case the differences were not significant ($p > 0.05$). With regard to non-ortho PCBs, the mean level of 152 pg/g of fw found in 1995–1996 decreased significantly ($p < 0.05$) in 1999–2001 to 31.2 pg/g of fw and then increased slightly to 35.6 pg/g of fw in 2001–2003.

The mean $\text{WHO-TEQ}_{\text{PCDD/F}}$ content clearly declined ($p < 0.05$) from 1995–1996 (8.33 pg of WHO-TEQ/g of fw) to 1999–2000 (0.52 pg of WHO-TEQ/g of fw) and then decreased slightly ($p > 0.05$) in 2001–2003 (0.44 pg of WHO-TEQ/g of fw). When non-ortho PCBs were considered, a significant decrease ($p < 0.05$) was observed in $\text{WHO-TEQ}_{\text{PCDD/Fs+non-ortho PCBs}}$ values from 1995–1996 (11.4 pg of WHO-TEQ/g of fw) to 1999–2000 (1.29 pg of WHO-TEQ/g of fw), whereas in the next years it increased slightly ($p > 0.05$) (2.03 pg of WHO-TEQ/g of fw).

PCDDs presented the most important contribution to the $\text{WHO-TEQ}_{\text{PCDD/Fs+non-ortho PCBs}}$ values in 1995–1996 (42%), whereas non-ortho PCBs, and especially PCB 126, showed the highest contribution (>60%) to the $\text{WHO-TEQ}_{\text{PCDD/Fs+non-ortho PCBs}}$ values after 1999.

Tuna Fish. The mean 2,3,7,8-PCDD/F content decreased significantly ($p < 0.05$) from the period 1995–1996 (176 pg/g of fw) to 1999–2000 (0.73 pg/g of fw), although these values increased slightly ($p > 0.05$) in 2001–2003 (0.77 pg/g of fw).

Table 3. Mean (Range) of 2,3,7,8-PCDD/Fs, Mono-ortho PCBs, Non-ortho PCBs, and Total PCB Concentrations (Expressed in Picograms per Gram of Fresh Weight and Picograms of WHO-TEQ per Gram of Fresh Weight) in Fish and Shellfish from the Spanish Market, in the Upper Bound Levels (ND = LOQ) in 2001–2003

	salmon (2)[a]	sardines (2)	tuna fish (3)	oysters (6)	clams (8)	mussels (5)
2378-TCDF	0.79 (0.58–0.99)	0.38 (0.36–0.45)	0.32 (0.01–0.92)	1.33 (0.58–4.67)	0.12 (ND–0.48)	0.27 (0.06–0.51)
12378-PeCDF	0.11 (0.09–0.12)	0.09 (0.09–0.09)	0.04 (0.01–0.12)	0.08 (0.06–0.09)	0.01 (ND–0.05)	0.02 (0.02–0.03)
23478-PeCDF	0.43 (0.28–0.58)	0.42 (0.39–0.45)	0.26 (0.01–0.76)	0.22 (0.17–0.34)	0.03 (ND–0.11)	0.06 (0.03–0.10)
123478-HxCDF	0.03 (ND–0.05)	0.04 (0.03–0.05)	0.01 (ND–0.02)	0.01 (ND–0.03)	0.02 (ND–0.06)	0.015 (ND–0.03)
123678-HxCDF	0.02 (ND–0.03)	0.04 (0.03–0.05)	0.01 (ND–0.03)	0.01(ND–0.03)	0.01 (ND–0.04)	0.013 (ND–0.02)
234678-HxCDF	0.01 (ND–0.03)	0.02 (ND–0.04)	0.02 (ND–0.04)	0.02 (ND–0.05)	0.01 (ND–0.042)	0.013 (ND–0.02)
123789-HxCDF	ND	ND	ND	0.001 (ND–0.007)	0.001 (ND–0.01)	0.002 (ND–0.007)
1234678-HpCDF	0.04 (ND–0.08)	0.04 (0.03–0.04)	ND	0.05 (ND–0.17)	0.02 (ND–0.07)	0.03 (0.02–0.04)
1234789-HpCDF	0.03 (ND–0.06)	ND	ND	ND	0.003 (ND–0.01)	0.008 (ND–0.013)
OCDF	ND	ND	ND	0.04 (ND–0.12)	0.01 (ND–0.02)	0.05 (ND–0.10)
2378-TCDD	0.07 (0.04–0.10)	0.05 (0.04–0.05)	0.02 (0.001–0.04)	0.04 (ND–0.11)	0.003 (ND–0.01)	0.01 (ND–0.023)
12378-PeCDD	0.11 (0.02–0.19)	0.11 (0.10–0.12)	0.04 (ND–0.120)	0.13 (0.06–0.16)	0.01 (ND–0.02)	0.01 (ND–0.020)
123478-HxCDD	0.01 (ND–0.03)	ND	0.006 (ND–0.02)	0.04 (ND–0.06)	0.01 (ND–0.02)	0.01 (ND–0.016)
123678-HxCDD	0.02 (ND–0.04)	0.08 (0.03–0.10)	0.02 (ND–0.05)	0.09 ND–0.13)	0.01 (ND–0.07)	0.03 (0.02–0.06)
123789-HxCDD	0.01 (ND–0.02)	0.07 (0.05–0.09)	ND	0.07 (0.05–0.09)	0.02(ND–0.06)	0.029 (0.02–0.05)
1234678-HpCDD	0.2 (0.13–0.27)	0.22 (0.03–0.30)	0.01 (0.005–0.03)	0.21 (0.18–0.25)	0.05 (ND–0.23)	0.17 (0.04–0.33)
OCDD	0.16 (ND–0.32)	0.37 (0.17–0.57)	0.03 (0.01–0.09)	0.53 (0.37–0.77)	0.30 (ND–1.06)	1.16 (0.11–2.14)
total PCDD/Fs	2.02 (1.14–2.89)	1.92 (1.45–2.39)	0.77 (0.04–2.24)	2.89 (1.46–7.07)	0.63 (0.001–2.35)	1.89 (0.32–3.51)
PCB 77	27.8 (21.4–34.1)	17.3 (14.3–20.3)	19.7 (10.7–36.2)	16.5 (11.2–22.4)	3.28 (0.35–8.29)	14.6 (0.02–41.7)
PCB 126	8.72 (7.7–9.73)	15.6 (14–17.2)	17.8 (5.45–38.9)	1.86 (0.78–2.85)	0.66 (0.12–1.64)	1.27 (0.50–2.50)
PCB 169	1.78 (0.32–3.23)	2.65 (2.23–2.97)	3.34 (1.33–7.24)	0.47 (0.18–0.74)	0.04 (ND–0.16)	0.20 (0.01–0.22)
total non-*ortho* PCBs	38.3 (29.4–47.1)	35.6 (30.5–40.5)	40.8 (17.5–82.4)	18.8 (12.6–26)	3.97 (0.46–8.85)	16.1 (0.53–44.2)
PCB 28 (ng/g)	0.86 (0.002–1.71)	0.31 (ND–0.62)	1.39 (0.06–2.72)	1.53 (0.17–4.67)	0.33 (0.004–1.58)	1.17 (0.05–2.72)
PCB 52 (ng/g)	2.17 (0.47–3.86)	1.00 (0.94–1.07)	3.14 (1.41–5.70)	1.99 (0.06–4.10)	0.41 (0.07–1.93)	0.35 (0.08–0.48)
PCB 95 (ng/g)	2.04 (0.15–3.54)	1.66 (1.50–1.82)	3.21 (1.63–5.42)	2.35 (0.34–4.75)	0.39 (0.02–1.99)	0.60 (0.09–1.25)
PCB 101 (ng/g)	2.63 (1.15–4.10)	0.60 (0.57–0.63)	3.30 (1.66–6.09)	2.27 (0.02–4.59)	0.37 (0.02–2.02)	1.06 (0.24–1.83)
PCBs 123 + 149 (ng/g)	1.42 (0.29–4.89)	4.43 (4.39–4.46)	5.58 (0.7–8.52)	2.28 (0.03–5.28)	0.56 (0.005–3.75)	0.97 (0.04–1.64)
PCB 118 (ng/g)	1.54 (0.73–2.34)	1.02 (0.99–1.05)	2.61 (0.63–3.01)	0.31 (ND–1.15)	0.12 (0.004–0.73)	0.172 (0.04–0.30)
PCB 114 (ng/g)	0.20 (0.06–0.34)	0.07 (0.06–0.08)	0.88 (0.04–2.27)	0.006 (ND–0.04)	0.04 (ND–0.16)	0.003 (ND–0.016)
PCB 153 (ng/g)	3.63 (1.25–5.82)	14.3 (12.9–15.6)	33.5 (2.39–60.7)	3.47 (0.48–7.96)	0.54 (0.016–2.95)	1.242 (0.16–2.55)
PCB 132 (ng/g)	0.22 (ND–1.06)	1.08 (1.03–1.14)	0.05 (0.05–0.05)	0.40 (0.06–0.93)	0.1 (0.003–0.55)	0.122 (0.03–0.23)
PCB 105 (ng/g)	0.28 (0.04–0.44)	0.28 (0.25–0.31)	1.08 (0.09–3.12)	0.04 (ND–0.09)	0.06 (ND–0.21)	0.02 (0.003–0.03)
PCB 138 (ng/g)	3.06(1.06–5.06)	3.23 (0.83–6.71)	21.1 (0.18–24.0)	1.34 (0.06–3.12)	0.40 (0.001–2.25)	0.46 (0.08–0.90)
PCB 183 (ng/g)	0.12 (0.01–0.13)	ND	2.37 (0.11–3.80)	0.04 (ND–0.05)	0.05 (ND–0.305)	0.14 (0.02–0.30)
PCB 167 (ng/g)	1.21 (0.001–2.41)	0.50 (0.21–0.80)	0.60 (0.04–0.89)	0.03 (0.01–0.09)	0.019 (ND–0.07)	0.006 (ND–0.02)
PCB 156 (ng/g)	0.07 (0.07–0.08)	0.28 (0.26–0.30)	0.46 (0.02–1.26)	0.1 (0.004–0.42)	0.019 (ND–0.09)	0.008 (ND–0.03)
PCB 157 (ng/g)	0.02 (0.004–0.03)	0.03 (0.02–0.04)	0.29 (0.01–0.08)	0.05 (ND–0.25)	0.002 (ND–0.001)	0.004 (ND–0.01)
PCB 180 (ng/g)	1.13 (0.64–1.63)	27.7 (19.6–24.0)	18.0 (0.81–35.2)	0.18 (ND–0.77)	0.37 (0.005–2.23)	0.194 (0.05–0.42)
PCB 170 (ng/g)	0.37 (0.11–0.62)	4.95 (4.47–5.45)	2.59 (0.05–7.31)	0.21 (0.02–0.54)	0.08 (0.002–4.08)	0.05 (0.01–0.13)
PCB 189 (ng/g)	0.02 (0.02–0.02)	ND	0.14 (0.06–0.36)	0.05 (0.02–0.10)	0.004 (ND–0.022)	0.002 (ND–0.01)
PCB 194 (ng/g)	0.06 (0.03–0.07)	1.08 (1.0–1.12)	1.72 (0.09–4.89)	0.02 (0.01–0.03)	0.008 (ND–0.012)	0.01 (ND–0.05)
total mono-ortho PCBs (ng/g)	4.75 (1.21–10.6)	6.61 (6.2–7.05)	11.6 (1.59–203)	2.88 (0.06–7.4)	0.96 (0.009–5.02)	1.18 (0.85–2.05)
ΣPCBs (ng/g)	20.9 (6.6–38.2)	56.7 (49–65.2)	100.4 (10–176)	16.8 (1.3–38.9)	3.46 (0.15–24.9)	6.61 (0.88–12.9)
WHO-TEQ (PCDD/Fs)	0.5 (0.45–0.54)	0.44 (0.39–0.50)	0.23 (0.01–0.66)	0.46 (0.37–0.73)	0.05 (0.001–0.16)	0.09 (0.05–0.15)
WHO-TEQ (non-ortho PCBs)	0.89 (0.78–1.01)	1.59 (1.43–1.76)	1.81 (0.56–3.97)	0.19 (0.08–0.30)	0.07 (0.01–0.16)	0.3 (0.05–0.9)
WHO-TEQ (mono-ortho PCBs)	2.34 (0.19–4.06)	0.32 (0.30–0.35)	1.20 (0.12–2.9)	0.14 (0.08–0.68)	0.05 (0.001–0.22)	0.07 (0.02–0.25)
ΣWHO-TEQ	3.63 (1.42–5.6)	2.35 (2.12–2.61)	3.24 (0.69–7.53)	0.79 (0.53–1.71)	0.17 (0.01–0.54)	0.46 (0.12–1.3)
% lipids	13.7	10.8	7.1	1.6	1	1.4

[a] Number of samples.

The same tendency was found for non-ortho PCB mean concentrations.

The mean WHO-$TEQ_{PCDD/F}$ value clearly declined ($p < 0.05$) from 1995−1996 (9.19 pg of WHO-$TEQ_{PCDD/F}$/g of fw) to 1999−2000 (0.16 pg of WHO-TEQ/g of fw) and showed a slight, nonsignificant increase in 2001−2003 (0.23 pg of WHO-TEQ/g of fw). When non-ortho PCBs were considered, the same tendency was observed.

PCDFs presented the most important contribution to the WHO-$TEQ_{PCDD/Fs+non-ortho\ PCBs}$ values in samples from 1995 to 1996. On the other hand, non-ortho PCBs, mainly PCB 126, showed the highest contribution (>75%) to the WHO-TEQ values in samples from 1999−2000 and 2001−2003 as already described in the sardines analyzed in the same period.

Oysters. The mean 2,3,7,8-substituted PCDD/F level tended to decrease over the years studied. A significant decrease ($p <$ 0.05) in these values was observed from 1995−1996 (367 pg/g of fw) to 1999−2000 (3.48 pg/g of fw), and this trend continued in the following years (2.89 pg/g of fw). In the same way, the mean value of non-ortho PCBs declined ($p > 0.05$) from 1995 to 2003.

The mean WHO-$TEQ_{PCDD/F}$ content declined ($p > 0.05$) from 1995−1996 (2.11 pg of WHO-TEQ/g of fw) to 1999−2000 (0.66 pg of WHO-TEQ/g of fw) and continued to do so ($p >$ 0.05) in 2001−2003 (0.46 pg of WHO-TEQ/g of fw). The same tendency was observed when non-ortho PCBs were considered.

PCDDs contributed the highest percentage (45%) to the WHO-$TEQ_{PCDD/Fs+non-ortho\ PCBs}$ values in samples from 1995−1996, whereas PCDFs were predominant in 1999−2000 (52%) and in 2001−2003 (39%). In this last period the contributions of the three families to the WHO-TEQ were similar.

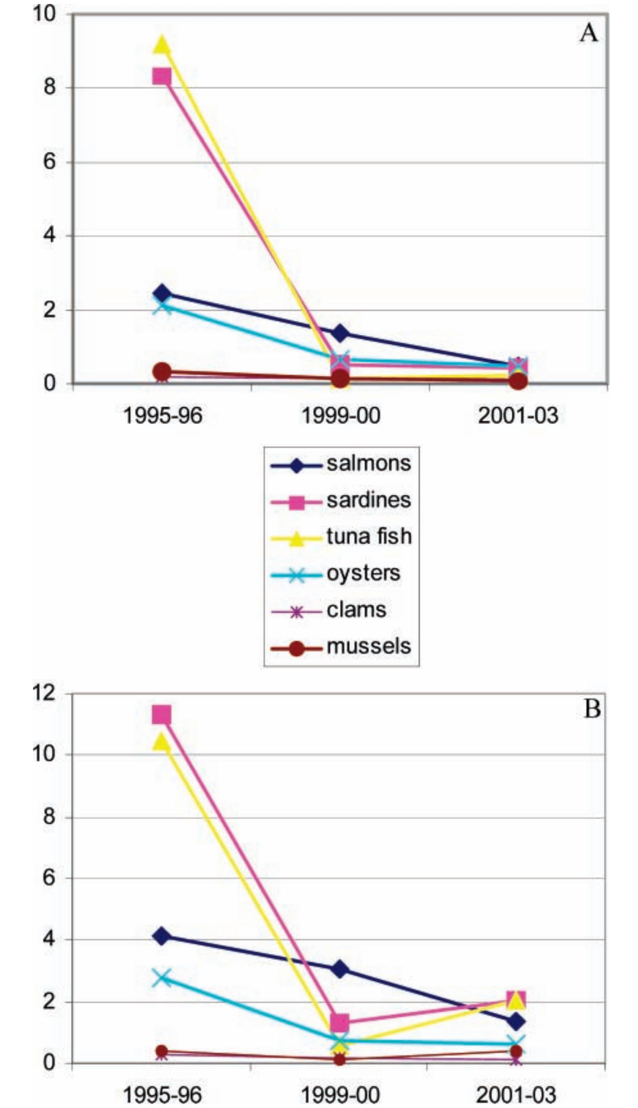

Figure 1. Decline of PCDD/Fs and PCDD/Fs and non-ortho PCBs in Spanish commercial fish between 1995 and 2003, expressed in (**A**) picograms of WHO-TEQ$_{PCDD/Fs}$ per gram of fresh weight and (**B**) picograms of WHO-TEQ$_{PCDD/Fs+non-ortho\ PCBs}$ per gram of fresh weight.

Clams. The mean 2,3,7,8-substituted PCDD/F level found in 1995−1996 was 4.05 pg/g of fw. This value decreased ($p > 0.05$) to 1.23 pg/g of fw in 1999−2000. There was a further slight decrease ($p < 0.05$) in the following years, reaching a mean value of 0.63 pg/g of fw in 2001−2003. A similar tendency was observed for non-ortho PCBs, the mean level of which decreased ($p < 0.05$) over the studied years.

The mean WHO-TEQ$_{PCDD/F}$ content declined ($p > 0.05$) from 0.18 pg of WHO-TEQ/g of fw) in 1995−1996 to 0.13 pg of WHO-TEQ/g of fw in 1999−2000 and decreased significantly ($p < 0.05$) in 2001−2003 to 0.05 pg of WHO-TEQ/g of fw. When non-ortho PCBs were considered, a similar and significant ($p < 0.05$) decrease was observed in the WHO-TEQ $_{PCCD/Fs+non-ortho\ PCBs}$ mean values.

The contribution of the three families to the WHO-TEQ$_{PCCD/Fs+non-ortho\ PCBs}$ content was similar in 1995−1996, with values between 32 and 35%. PCDFs presented the most important contribution to the WHO-TEQs in samples from 1999 to 2000, and non-ortho PCBs, mainly PCB 126, presented the most important contribution (>50%) to the WHO-TEQ values in samples included in the last period studied.

Mussels. The mean 2,3,7,8-substituted PCDD/F value found in the period 1995−1996 was 1.86 pg/g of fw. This value decreased ($p > 0.05$) to 1.5 pg/g of fw in 1999−2000 and then increased slightly ($p > 0.05$) in the following years, reaching a value of 1.89 pg/g of fw in 2001−2003. The non-ortho PCB concentrations increased over the studied years. Nevertheless, the mean WHO-TEQ $_{PCDD/F}$ content declined almost 3-fold, from 0.31 to 0.12 pg of WHO-TEQ$_{PCDD/Fs}$/g of fw between 1995−1996 and 1999−2000, and almost 4-fold in 2001−2003 to 0.09 pg of WHO-TEQ$_{PCDD/F}$/g of fw. When non-ortho PCBs were included, WHO-TEQ$_{PCCD/Fs+non-ortho\ PCBs}$ contents decreased almost 2-fold between the two first periods studied and increased in 2001−2003 to reach a mean value of 0.39 pg of WHO-TEQ/g of fw. The differences found among the three periods of time were not statistically significant ($p > 0.05$).

PCDDs presented the most important contribution to the WHO-TEQ$_{PCCD/Fs+non-ortho\ PCBs}$ values in samples from 1995−1996 (58%) and from 1999−2000 (65%), whereas non-ortho PCBs were predominant in 2001−2003 (76%).

Present PCDD/F and PCB Concentration (2001−2003). Mean concentrations of total PCBs, mono- and non-ortho PCBs, 2,3,7,8-substituted PCDD/Fs, and individual congeners are shown in **Table 3**. They are expressed in nanograms per gram or picograms per gram and in picograms of WHO-TEQ per gram of fresh weight in the upper bound limit of determination (assuming that nondetected values are equal to their corresponding limit of quantification). Fat percentage of the samples is also included in **Table 3**.

Total PCB concentrations (as the sum of the 41 congeners analyzed) in the six fish and shellfish species presented large and significant variations. Thus, tuna fish had the highest mean value (100 ng/g of fw), followed by sardines (56.7 ng/g of fw), salmon (20.9 ng/g of fw), oysters (16.8 ng/g of fw), mussels (6.61 ng/g of fw), and finally clams (3.46 ng/g of fw).

2,3,7,8-Substituted PCDD/F concentrations presented less variation than PCBs. The highest levels were found in oysters with a mean of 2.89 pg/g of fw followed by salmon (2.02 pg/g of fw), sardines (1.92 pg/g of fw), mussels (1.89 pg/g of fw), tuna fish (0.77 pg/g of fw), and clams (0.63 pg/g of fw).

PCB profiles indicate some similarities and differences among the fish and shellfish species (**Table 3**). PCBs 153 and 138 were the major contributing congeners in all of the species investigated (fish and shellfish), each accounting for >6% of the total PCBs (between 6 and 33%). PCBs 118, 170, and 180 were in the majority for fish species (between 2 and 38% of the total PCBs), whereas the less chlorinated PCBs (PCBs 28, 52, 95, 101, and 123 + 149) were in the majority for shellfish species, contributing between 5 and 18% of the total PCBs. In all cases the mono-ortho PCBs 114, 167, 156, 157, and 189 and the non-ortho PCB congeners PCBs 77, 126, and 169 presented a lower contribution (<2%). With regard to the PCDD/F profiles, 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF, HpCDD, and OCDD predominated in all cases.

The highest mean concentration of WHO-TEQ$_{PCDD/Fs}$ was found in salmon (0.5 pg of WHO-TEQ/g of fw), followed by oysters (0.46 pg of WHO-TEQ/g of fw), and sardines (0.44 pg of WHO-TEQ/g of fw). The lowest value was found in tuna fish with 0.23 pg of WHO-TEQ/g of fw, mussels (0.09 pg of WHO-TEQ/g of fw), and clams (0.05 pg of WHO-TEQ/g of fw).

None of the fish and shellfish studied exceeded the maximum levels, in the upper bound determination level, set by the EU for this kind of product (4 pg of WHO-TEQ$_{PCDD/F}$/g of fw) (*11*).

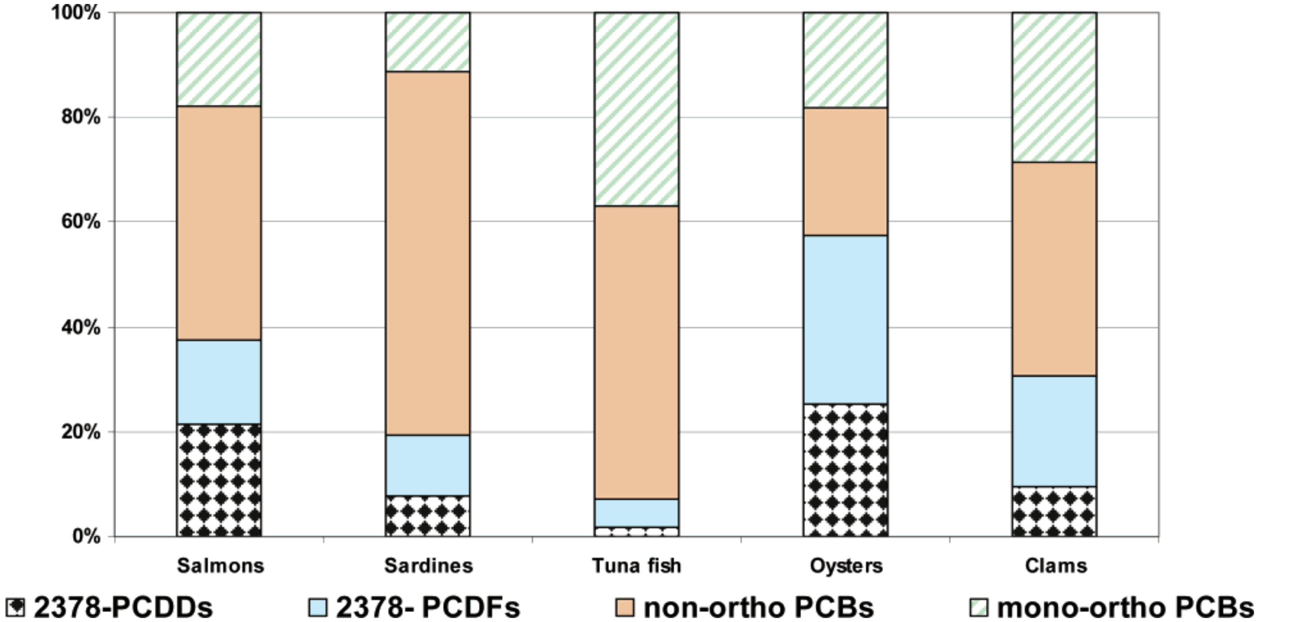

Figure 2. Contribution (in percentage) of PCDD/Fs and PCBs to the total WHO-TEQ$_{PCCD/Fs+cop\ PCBs}$ in Spanish commercial fish and shellfish (2001–2003).

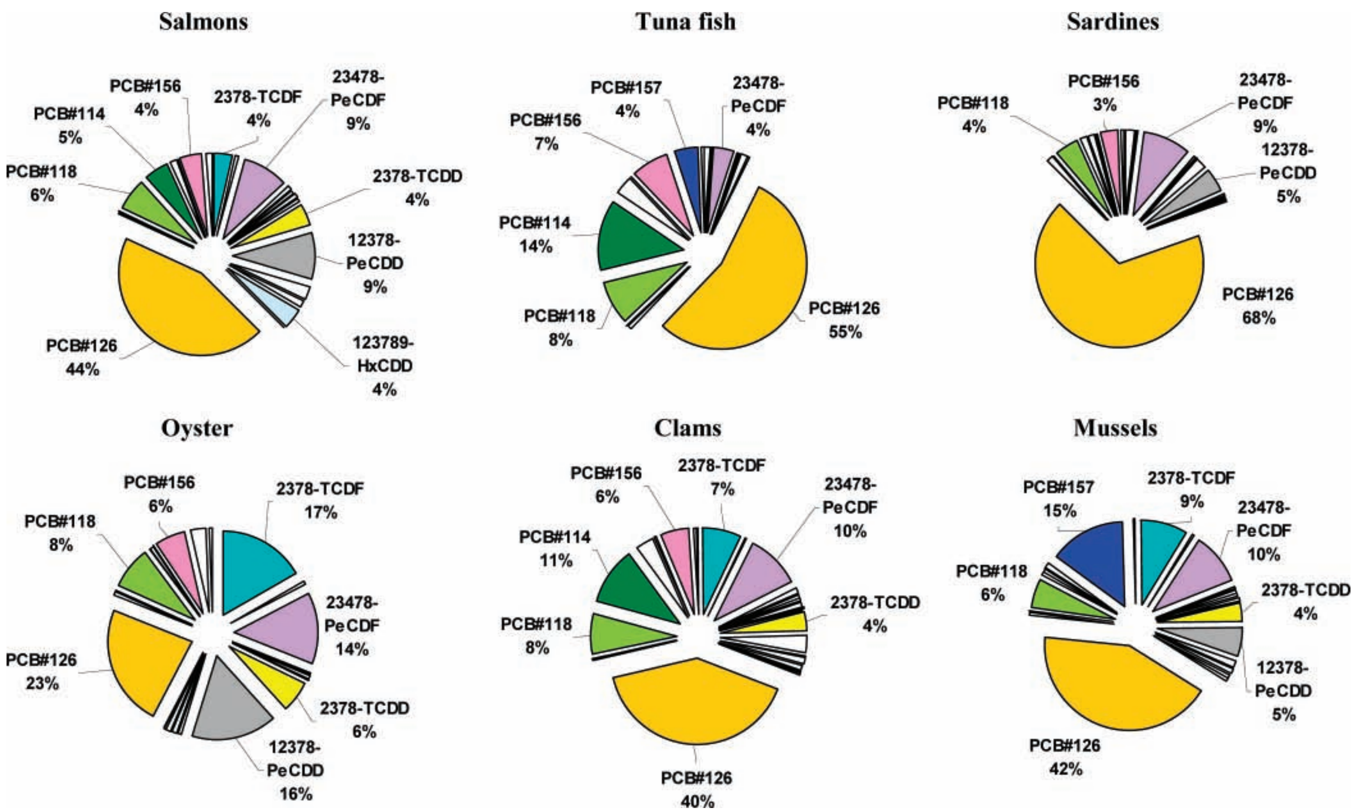

Figure 3. Percentage contribution to the total WHO-TEQ of mono-, non-ortho PCB, and PCDD/F congeners in Spanish commercial fish and shellfish (2001–2003).

When non-ortho PCBs were considered, tuna fish and sardines exhibited the highest levels among the species studied, with mean values of ∼2 pg of WHO-TEQ$_{PCDD/Fs+non-ortho\ PCBs}$/g of fw, followed by salmon (1.39 pg of WHO-TEQ/g of fw), oysters (0.65 pg of WHO-TEQ/g of fw), mussels (0.39 pg of WHO-TEQ/g of fw), and clams (0.12 pg of WHO-TEQ/g of fw).

When mono-ortho PCBs were included, the total WHO-TEQ$_{PCDD/Fs+cop\ PCBs}$ values increased, and in this case, salmon exhibited the highest mean value (3.63 pg of WHO-TEQ/g of fw), followed by tuna fish (3.24 pg of WHO-TEQ/g of fw), sardines (2.35 pg of WHO-TEQ/g of fw), oysters (0.79 pg of WHO-TEQs/g of fw), mussels (0.46 pg of WHO-TEQs/g of fw), and finally clams (0.17 pg of WHO-TEQ/g of fw).

Some individual fish (salmon and tuna fish) with a high lipid content (13.7 and 7.1%, respectively) exhibited levels over those permitted by the current legislation (at present only for dioxins) (4 pg of WHO-TEQ/g of fw) (*11*). Most probably, these levels will decrease when coplanar PCBs are included in the legislation. In fact, the results of this study indicate that PCB concentrations have fallen in the past few years, although this decrease has not been as evident as the decrease of dioxins.

It is of particular interest to assess the contribution of PCDD/Fs and dioxin-like PCBs to the total WHO-TEQs. Many studies in the past did not include the determination of dioxin-like PCBs, but they have recently started to do so because it has been stated that they dominated the TEQ content in many foodstuffs (*18*, *19*), mainly fish products. In this study, the relative abundance of coplanar PCBs with respect to total WHO-TEQs in the fish and shellfish was very high in all cases, accounting for >40% (**Figure 2**). This contribution is higher in fish (between 62 and 90%) than in shellfish (between 42 and 70%). As shown in **Figure 2**, the largest contribution was for non-ortho PCBs, even larger than that of mono-ortho PCBs. The results reported from similar surveys have been comparable (*16*–*19*, *35*) and altogether point to the importance of their determination in foodstuffs, especially fish products, and support the need of their inclusion in further studies and legislation.

Regarding the contribution of PCB and PCDD/F congeners to total WHO-TEQ, PCB 126 seemed to be the most important among the PCBs (**Figure 3**), with a contribution ranging from 23% in the case of oysters to 68% in the case of sardines. Concerning 2,3,7,8-PCDD/Fs, the less chlorinated PCDD/F congeners, such as 2,3,7,8-TCDD, 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF, and 1,2,3,7,8-PeCDD, were the main contributors to the total WHO-TEQ.

The results of this study and other similar studies (*23*, *36*) show that levels of these contaminants in fish have decreased considerably over the past years. For that reason, only data published after the year 1999 were considered for level comparisons.

PCDD/F and PCB concentrations found in fish and shellfish species (**Table 3**) were lower or in the same range as those reported in the literature for similar species taken in the same period of time. The ΣPCBs found in this study are rather difficult to compare with those found in previous studies due to the fact that the numbers of PCBs included are not the same in each particular study. Making allowances for this limitation, it was found that PCB mean levels (Σ7 congeners) in mussels and clams from the Adriatic Sea ranged between 3.43 and 13.5 ng/g of fw (*37*, *38*) and reached 42.4 ng/g of fw in samples collected in Catalonia (Spain) (*39*). PCB concentrations were between 12.5 and 18.4 ng/g of fw (Σ7 congeners) in sardines from the Adriatic Sea (*37*). Commercial salmon (farmed and wild) collected in Belgium and Scotland had PCB concentrations (Σ59 congeners) ranging from 8.10 to 93.8 ng/g of fw (*40*). These values are higher than those reported in this study for mussels, clams, sardine, and salmon.

There are few references in the literature to PCDD/F concentrations in individual fish and shellfish species, except for salmon and mussels, because most refer to pools of various species (*17*, *21*). PCDD/F concentrations in commercial salmon ranged from 2.3 to 7.8 pg/g of fw in Sweden (*41*) and had a mean value of 2.95 pg/g of fw in Scotland (*35*). On the other hand, PCDD/F concentrations ranged from 7.27 to 34.9 pg/g of fw in mussels from Catalonia (Spain) (*16*) and from 62 to 128 pg/g of fw in mussels from Norway (*23*). These values are similar to or higher than those found for mussels and salmon in this study.

The levels of $WHO\text{-}TEQ_{PCDD/Fs}$ found in the fish and shellfish species in this study were similar to those included in an estimation of dioxin dietary intake for the Spanish population conducted between 2000 and 2003 (*19*). In addition, PCDD/F concentrations found in salmon from the present survey (ranging from 0.45 to 0.54 pg of WHO-TEQ/g of fw) were between the values provided from farmed salmon (ranging from 1.2 to 3 pg of WHO-TEQ/g of fw) and wild salmon (ranging from 0.2 to 0.5 pg of WHO-TEQ/g of fw) from Europe and North America (*42*). The mean concentrations of dioxins and dioxin-like PCBs found in the muscle of wild and farmed fish products (including salmon and sardines but excluding fish from the Baltic Sea) from an EU monitoring program were 0.49 pg of $WHO\text{-}TEQ_{PCDD/F}$/g of fw and 1.79 pg of $WHO\text{-}TEQ_{PCCD/Fs+cop\ PCBs}$/g of fw (in the upper determination level) (*14*): that is, they were comparable to the results of this study.

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Received for review April 13, 2005. Revised manuscript received August 8, 2005. Accepted August 10, 2005. We thank the Spanish Ministry of Health, the CSIC, and the Junta of Andalucía for their financial support.

JF050835Z