Dietary uptake of dioxins (PCDD/PCDFs) and dioxin-like PCBs in Spanish aquacultured turbot (Psetta maxima)

Sonia Lucía Blanco, Cristina Sobrado, Corina Porro, Santiago Cabaleiro, José Carlos González, Juan Manuel Vieites

Dietary uptake of dioxins (PCDD/PCDFs) and dioxin-like PCBs in Spanish aquacultured turbot (Psetta maxima)

Journal:	*Food Additives and Contaminants*
Manuscript ID:	TFAC-2005-383.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	20-Sep-2006
Complete List of Authors:	Blanco, Sonia; Centro Técnico Nacional de Conservación de Productos de la Pesca (ANFACO-CECOPESCA), Calidad y Seguridad Alimentaria Sobrado, Cristina; Centro Técnico Nacional de Conservación de Productos de la Pesca (ANFACO-CECOPESCA), Calidad y Seguridad Alimentaria Porro, Corina; ANFACO-CECOPESCA, Quality and Food Safety Area; Centro Técnico Nacional de Conservación de Productos de la Pesca (ANFACO-CECOPESCA), Calidad y Seguridad Alimentaria Cabaleiro, Santiago; Centro Tecnológico de la Acuicultura de Galicia (CETGA) González, José Carlos; Centro Técnico Nacional de Conservación de Productos de la Pesca (ANFACO-CECOPESCA), Calidad y Seguridad Alimentaria Vieites, Juan; Centro Técnico Nacional de Conservación de Productos de la Pesca (ANFACO-CECOPESCA), Calidad y Seguridad Alimentaria
Methods/Techniques:	Clean-up, GC/MS
Additives/Contaminants:	Dioxins, Dioxins - TEQs, PCBs
Food Types:	Animal feedingstuffs, Fish and fish products

Dietary uptake of dioxins (PCDD/PCDFs) and dioxin-like PCBs in Spanish aquacultured turbot (*Psetta maxima*)*

Sonia Lucía Blanco[1], Cristina Sobrado[1], Corina Quintela[1], Santiago Cabaleiro[2], José Carlos González[1], Juan M. Vieites[1]

(1) Centro Técnico Nacional de Conservación de Productos de la Pesca (ANFACO-CECOPESCA). Carretera Colexio Universitario nº 16, 36310, Vigo, Pontevedra, Spain
Tel. number: 34 986 469303, Fax nº: 34 986 469269; e-mail: lucia@anfaco.es

(2) Centro Tecnológico de la Acuicultura de Galicia (CETGA). Punta Couso. Aguiño, A Coruña, Spain.

* Presented as a poster at the 2nd International Symposium on 'Recent Advances in Fodo Analysis' held in Prague, Czch Republic, November 2-4th 2005.

Abstract

The human population is exposed to dioxins (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dl-PCBs) mainly through diet; bioaccumulation and biomagnification in aquatic environment results in fishery products and by-products being an important vector to humans. The determination of PCDD/Fs and dl-PCBs in fillets of young turbots (*Psetta maxima)* (0 – 2 years) from aquaculture plant (Galicia, Spain) (N = 21) and in feedingstuffs were carried out, and dietary accumulation values and lipid-normalized biomagnification factors (BMF) relating concentration in fish and in feed were calculated. Levels found in feedingstuffs (0.5 pg TEQ-PCDD/F / g and 1.6 pg TEQ-dl-PCB / g), and turbots (0.13 - 0.27 pg TEQ-PCDD/F / g fresh weight and 0.35 - 1.2 pg TEQ-dl-PCB / g fresh weight) were below maximum permitted levels set by EC. Levels of toxic compounds in feeding stuff are reflected in fish fillets; predominant isomers are 2,3,7,8-TCDF, OCDD, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF and 1,2,3,4,6,7,8-HpCDD, and PCBs 118, 105, 156 and 167. Relevant compounds accounting for total toxicity are the same congeners in feeding stuff and turbots: 2,3,4,7,8- PeCDF; 2,3,7,8-TCDF; 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD, and PCB 126. Higher accumulation efficiency values were obtained for dl-PCBs (30 - 46%); tetra- and penta-chloro substituted PCDD/Fs showed the highest values (27 - 34%) of the PCDD/F group. Biomagnification was shown for these compounds (BMF around 1.5).

Introduction

Turbot aquaculture is a very important activity in Spain, producing fish of remarkable quality due to optimal conditions of sea water of the northwest of the country. According to the United Nations Food and Agriculture Organization (FAO), by the year 2030 more than half of marine products for human consumption will come from aquaculture. Aquaculture is the only way to keep sea products in the daily diet in view of the stagnation of extractive fishing. In this sector, Galicia, in the northwest of Spain, is a world leader thanks to its natural conditions, especially its "rias", and the commitment undertaken by Galicia's companies and Administration. In the year 2002, Galician aquaculture attained an estimated production of more than 267,000 tons, dominating the Spanish market as one of the main suppliers of European markets (Anonymous, 2004). Galician aquaculture experienced continuous growth, in both the volume of its production and first-sale value, between 1994 and 2002, increasing from a value of 93.65 million euros to a total of 193.79 million, turbot representing 14.8% of this economical value (Anonymous, 2004). Many technical advances have been made in breeding turbot in Galicia, the European region where turbot growing is completed in the least time, thanks to the richness and temperature of its waters; at present, there are 17 farms in Galicia, including the largest in the world, in Lira, Carnota (A Coruña).

Feeding stuffs used in cultured fish are made mainly from fish oil and fish meal, being fish fat one of the main components where persistent contaminants such as dioxins and PCBs are accumulated, due to bioaccumulation and biomagnification in the aquatic environment. Due to aerial deposition and diffuse contamination of soil, these contaminants are found in roughages and other types of animal feed of vegetable origin. Contaminated surface waters, sediments and prey lead to dioxins and dl-PCBs in fish, and thus to contamination of fish meal and fish oils used as animal feed. Fishery products and by-products might constitute an important vector of dioxins in humans.

Due to the high toxicity of these compounds, particularly chlorinated dioxins and furans (PCDD/Fs) and dioxin-like PCBs (dl-PCBs), it has been necessary to establish the maximum permitted level (MPL): Directive 2002/32/EC establishes the maximum dioxins and furans content in feeding stuffs, and Regulation (EC) No. 2375/2001 in food. Recently established MPLs for the sum of dioxins/furans and dioxin-like PCBs

have been included in Directive 2006/13/EC (feeding stuffs) and Commission Regulation No. 199/2006 (food).

In order to evaluate the safety of this aquaculture product and the relevance of the feeding stuffs in the final levels of contaminants found in fish, determinations of PCDD/Fs and dl-PCBs in turbots and in feeding stuffs have been carried out in this study.

Materials and methods

Sampling

Two different feedingstuffs were used to feed the turbots: 8 mm diameter (fish younger than 1 year), and 15 mm diameter (fish older than 1 year). Both feedingstuffs showed similar proximal composition: about 20% fat, 53% protein, and 8% moisture. Both feedingstuffs contained a proportion of marine fish oil (data not supplied).

Fish of three different age/size groups (from 0 to 2 years old) of turbots (N=21) (Table 1) were pooled from aquaculture plant in March 2005. Fish were frozen until analysis.

Determinations of PCDD/Fs and dl-PCBs in fish individual samples (N=21) and in feedingstuffs used with them were carried out; analyses were performed in April 2005 - June 2005. Fat content was determined by Soxhlet extraction procedure (AOAC 1990). Dietary accumulation efficiencies were calculated using the equation $\alpha = C_{fish} / (F\ t\ C_{feed})$, where C_{fish} is concentration in fish, C_{feed} is concentration in feed, F is feeding rate and t is time, for each toxic congener in the turbots of 1-2 years old under study. Feed conversion ratio (Kg food consumed / kg weight gain) of 1.1 was applied to calculate the consumed quantity by each individual. This value is applied under conventional turbot aquaculture conditions in the region, and represents the total consumption by a total mass of fish.

Sample pre-treatment

Frozen fish were allowed to thaw (a few hours) and edible parts (fillets) were divided into small pieces and homogenized with a food processor. Fish samples were previously

dried for 24 h using sodium sulphate. Feeding stuffs were grinded and directly processed. Sub-samples of 20 g were taken for analysis.

For sample preparation and purification, open adsorption chromatography columns and Supelco Dioxin Prep System were used, including empty tubes for multi-layer silica gel columns, vacuum manifold, and accessory glassware and connectors. Preparation for PCDD/Fs analysis was carried out as described by Sobrado *et al*. (2004). Sub-samples obtained as described above were previously homogenized, next, they were spiked with known amounts of mixtures of $^{13}C_{12}$-PCDD/Fs and $^{13}C_{12}$-dioxin-like PCBs (EPA-1613LCS and WP-LCS WHO/EPA from Wellington Laboratories) and extracted using adsorption chromatography with n-hexane:acetone (1:1). The extracts were rotary evaporated and transferred to 5 mL n-hexane. In order to remove organic components, fat and other interfering substances, the n-hexane extracts were passed through chromatographic columns packed with silica and acid silica, modified with sulphuric acid, using n-hexane as eluent. After solvent evaporation to 5 mL, the extract is cleaned-up in a multilayer column, packed with silica, acid silica modified with sulphuric acid and basic silica modified with potassium hydroxide, eluted with n-hexane, rotary evaporated to 1 mL, and solid phase-extracted using carbon cartridge Supelclean-Envicarb, from Supelco. Dioxins and furans are retained in carbon structure while interferences are eluted by a direct flow of n-hexane:toluene (99:1 and 75:25 alternatively) and then eluted by reversed flow of toluene. Extract is evaporated to dryness and re-dissolved in 5 µL of nonane and 5 µL of labelled internal standard solution EPA-1613ISS (Wellington Laboratories), prior to injection.

Preparation for dl-PCBs analysis was carried out using adsorption chromatography, following a similar procedure, but introducing the use of Supelco Prep System, by one step method as described by Maeoka *et al.* (2002). This one-step method consists of a multi-layer silica gel column, as described above for dioxins and furans, directly coupled to a dual layer carbon column composed of two different carbon layers in series with distinct binding characteristics, Carboxen 1016 and Carboxen 1000, from Supelco, that retains dioxins and co-planar PCBs which are eluted by reversed flow of toluene. Extract is evaporated to dryness and re-dissolved in 38 µL of nonane and 2 µL of labelled internal standard solution WP-ISS WHO/EPA (Wellington Laboratories), prior to injection.

Solvent concentration was carried out in a Laborota 4000 Heidolph connected to a Büchi V-500 vacuum pump. Solvent evaporation was carried out under nitrogen in a Reacti-Vap evaporator 18780 coupled to a Reacti-Therm heating module 18790, Pierce. Organic solvents were for residue analysis; sodium sulphate granular was 99+% A.C.S. reagent (Aldrich); silica gel 60 (0.063-0.200 mm) was for column chromatography (Merck).

Determination of PCDD/Fs and dl-PCBs

PCDD/Fs and dl-PCBs were determined using high resolution gas chromatography coupled to an ion trap tandem mass spectrometer (HRGC-MS/MS). Gas chromatograph Varian CP-3800, equipped with an autosampler Varian CP-8400, a VF-5ms capillary column (Factor Four, 60m x 0.25 mm ID, DF = 0.25, Varian); for PCDD/Fs separation, 1 µL of sample is injected in splitless mode at 300º C, followed by column temperature program 90º C (hold 2 min), 20º C min^{-1}, 200º C (hold 1.3 min), 1º C min^{-1}, 230º C (7 min), 10º C min^{-1}, 300º C (hold 20 min); for PCBs separation, injection of 10 µL sample in LVI (large volume injection) mode at 95º C (hold 0.5 min), applying injector temperature program of 100º C min^{-1}, 300ºC (hold 12.55 min), and column temperature program of 60ºC (hold 3 min), 20ºC min^{-1}, 235ºC (hold 10 min), 10ºC min^{-1}, 260ºC (hold 0 min), 20ºC min^{-1}, 300ºC (hold 9 min). Detection was performed with an ion trap mass spectrometer Varian 4000 GC/MS, based on the pattern of fragmentation of the congeners by MS/MS. Quantification was based on the isotope dilution method; calibration was previously performed using native and labelled dioxins/furans solutions from Wellington Laboratories (EPA-1613CVS, CS1-CS-5) and dl-PCBs (WP-CVS WHO/EPA, CS1-CS7) .

High resolution mass spectrometry (HRGC-HRMS) operating in electron ionization (EI) mode and at a resolving power of 10,000 was also applied as confirmation method for PCDD/Fs and dl-PCBs since the values for some congeners obtained in many of the samples were below our detection limits; monitored masses in SIR mode were M and M+2 or M+4.

Quality

All PCDD/F and dl-PCB data were assessed for compliance with the methods performance criteria guidelines laid down in EU Commission Directives 2002/69/EC and 2002/70/EC.

Dioxin, furans and dioxin-like PCBs were quantified by isotopic dilution, based on the linearity of the detector signal of each isomer. The stability of the relative response factor has been checked during successive calibrations, supporting the stability and robustness of the methods.

Recoveries for each labelled congener have been studied in diverse matrix samples. Recovery average values are 60-70%, ranging from 50 to 85%; it is quite constant among congeners and also among tested sample matrices.

Repeatability and reproducibility were checked, using MS/MS method and HRMS as confirmation method.

Certified Reference Materials have been used during the 2004-2006 period (Carp muscle CARP-2, National Research Council Canada NRC-CNRC, Cod liver oil FAPAS test material, Central Science Laboratory). Blanks were also checked, and routinely included in each set of analyses.

HRGC-MS/MS

Identity was confirmed by comparing retention time between native and labelled analogue isomers and the ion ratio is fulfilled between M and M+2 considering an interval ±25% of the theoretical ion ratio. Mass spectra similarity of sample and standard peaks was checked.

The instrumental limit of detection established for each compound was estimated as the concentration providing a signal-to-noise ratio of 3:1. On a fresh-weight basis, the limits of detection were 0.05-0.2 pg/g for tetra- and penta-congeners PCDD/Fs, 0.2-1 pg/g for hexa-, hepta- and octa-congeners PCDD/Fs, and 0.3-1.5 pg/g for dl-PCBs.

HRGC-HRMS

The limits of detection were 0.01-0.02 pg/g for tetra-, penta-, hexa- and hepta-chlorinated congeners PCDD/Fs, 0.10-0.15 pg/g for octa-chlorinated congeners PCDD/Fs.

Results and Discussion

PCDD/Fs and dl-PCBs in Feeding stuffs

Results for the two different feedingstuffs used to feed turbots, 8 mm diameter and 15 mm diameter, are shown figures 1 – 2.

[Insert Figure 1 about here]

[Insert Figure 2 about here]

Although most abundant isomers in both feeds are 2,3,7,8-TCDF, followed by OCDD and 2,3,4,7,8-PCDF, and PCBs 118, 105, 156 and 167, most contributing to total TEQ are 2,3,4,7,8-PCDF, 2,3,7,8-TCDF, 2,3,7,8-TCDD and 1,2,3,7,8-PCDD, and PCBs 126, 118, 156 and 105. Relatively low levels of PCDD/Fs and dl-PCBs were found in the feeding stuffs (0.52 pg TEQ/g PCDD/Fs and 1.62 pg TEQ/g dl-PCBs), far below maximum levels set by the European Commission (2.25 pg/g TEQ-WHO PCDD/Fs, relative to a moisture content of 12%, and 4.75 pg/g dl-PCBs), and below the action limit (1.75 pg/g TEQ-WHO PCDD/Fs and 3.5 pg/g dl-PCBs) stated by Directive 2006/13/EC.

PCDD/Fs and dl-PCBs in fish

Table 1 shows fat and total WHO-TEQ data in each age/size turbot group.

[Insert Table 1 about here]

Figures 3 – 4 show individual WHO-TEQ for each fish sample.

[Insert Figure 3 about here]

[Insert Figure 4 about here]

The most abundant contaminants in the feedingstuff are the most abundant in the fish, these are: 2,3,7,8-TCDF, OCDD, 1,2,3,7,8-PeCDF, 2,3,4,7,8- PeCDF and 1,2,3,4,6,7,8-HpCDD, and PCBs 118, 105, 156 and 167. The most relevant compounds accounting for the total toxicity in turbots are 2,3,4,7,8- PeCDF, 2,3,7,8-TCDF, 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD, and PCB 126 (figures 3 – 4), the same congeners accounting for the total toxicity in feeding stuff. As reported by other authors in salmon (Lundebye *et al*., 2004), the concentration of dioxins and dl-PCBs in turbots reflect the levels present in the feed.

TEQ PCDD/Fs and dl-PCBs in turbots analysed were below the maximum permitted levels (4 pg/g fresh weight TEQ-WHO PCDD/F and 4 pg/g fresh weight TEQ-WHO PCBs, Regulation EC No. 199/2006) and below the action limit (3 pg/g fresh weight TEQ-WHO PCDD/F and 3 pg/g fresh weight TEQ-WHO PCBs, Recommendation 2006/88/EC).

Other authors have reported similar results in Spanish farmed fish, showing low levels of PCDD/Fs in general, with a pattern characterized by the presence of toxic TCDF, TCDD, PeCDD and PeCDFs (Abad *et al*. 2003), in trout, sea bass, gilthead and turbot. Trouts from French aquaculture showed very similar values (Marchand *et al.* 2004) for the same parameters, with mean values of 0.17 and 0.58 pg/g fresh weight for WHO-TEQ PCDD/Fs and dl-PCBs respectively. Other farmed fish, such as salmon, has been widely analyzed in many European countries for these contaminants, showing higher values of PCDD/Fs and dl-PCBs than wild salmon, associated with the use of fish oil (Karl *et al*. 2004). Levels obtained in salmon are usually higher than values in turbots obtained in this paper, expressed on a wet weight basis, as reported by many authors (Jacobs *et al*. 2002, Karl *et al*. 2004).

Dietary uptake

Correlation coefficients (Pearson) for TEQ levels and fat content were calculated (SPSS 12.0 for Windows, SPSS Inc., 1989-2003), and values obtained showed a significant correlation at the 0.05 level (2-tailed) for tetra- and penta-chlorinated PCDD/F congeners and hexa- and hepta-chlorinated dioxins 1,2,3,6,7,8-HxCDD and 1,2,3,4,6,7,8-HpCDD as well as for total TEQ ($0.5 < R < 0.6$). The 12 dl-PCBs show a significant correlation at the 0.05 level (2-tailed) between mentioned parameters (0.5 <

R < 0.6), showing the expected pattern of accumulation with increasing fat in the organism, for mentioned PCDD/Fs congeners and dl-PCBs. The other PCDD/Fs showed very low levels both in the feeding stuff and fish muscle tissue, under the limit of detection.

Dietary accumulation efficiencies were calculated for each toxic congener in turbots of 1-2 years old under study. Feed conversion ratio (kg food consumed / kg weight gain) of 1.1 was applied to calculate the consumed quantity by each individual. Dietary accumulation values obtained are shown in Table 2. Accumulation efficiency values around 30-46% for the 12 dl-PCBs and 27-34% for PCDD/Fs tetra- and penta-chloro substituted eaten by turbots ended up in the fish fillets. Tetra- and penta-chlorinated PCDD/F congeners show a mean accumulation of 31%, while higher chlorinated PCDD/F congeners show lower efficiencies (< 22 %). Total WHO-TEQ accumulation efficiencies were higher for dl-PCBs than for TCDD/Fs and around 35% of the total ingested TEQ dl-PCBs remain in the organism, versus 30% of TEQ PCDD/Fs.

[Insert Table 2 about here]

These data show that dioxin-like PCBs accumulate more efficiently than PCDD/Fs in the fillets of farmed turbots. Similarly, Isosaari *et al*. (2002) reported higher accumulation efficiency for dl-PCBs in rainbow trout (*Oncorhynchus mykiss*) and Lundebye *et al*. (2004) reported higher retention of dl-PCBs than for PCDD/Fs in cultured Atlantic salmon (*Salmo salar*). The high tendency of the toxic PCB congeners to be retained in fish tissues might explain why PCBs have a high contribution to the total WHO-TEQ in fish tissues.

Accumulation values reported by Isosaari *et al*. (2004) in salmon were higher both for PCDD/Fs and PCBs: 57-64% of the total TEQ PCDD/Fs and 86-94% TEQ dl-PCBs. These values have been obtained from whole fish,adult salmon, while ours correspond to the edible portion of fish, the fillets, of young turbots up to 2 years old; we have to consider that maturation takes place at the age of 5 years (natural environment), but aquaculture industry usually produce turbots of around 2 Kg (2-3 years old).

According to data from these authors, approximately 30% of both pollutants (WHO-TEQ) found in fish were located in skinned fillet (Isosaari *et al*. 2004), which is concordant with accumulation efficiency values obtained for turbot in this study.

Log K_{ow} are in the range 6.6 – 8.0 for tetra- to octa-substituted PCDD/F congeners, and increase with increasing number of chlorine atoms (Rittler *et al*., 1996). Correlation between log K_{ow} and bioaccumulation of several hydrophobic compounds shows that the highest bioconcentration factors of non-ionic compounds and the longest half-lives of several hydrophobic organochlorine compounds occur at log K_{ow} values of approximately seven (Fisk *et al*., 1998; Meylan *et al*., 1999). This is concordant with the higher accumulation mean values in tetra- and penta-substituted congeners (27.6 - 34.0%) obtained in present study.

Log K_{ow} for PCBs indicate the potential for bioaccumulation varies between different congeners with the more chlorinated PCBs having a much higher potential for bioaccumulation than less-chlorinated congeners (5.6 - 6.5 for tetra-, 6.2 – 6.5 for penta-, 6.7 – 7.3 for hexa-chlorobiphenyl isomers) (Mackey *et al*., 1992; Ritter *et al*., 1996), although our results do not support this observation, tetra-substituted congeners (77 and 81) showing similar accumulation values to higher chlorinated PCBs (see Table 2). Isosaari *et al*.(2004) have obtained preferential accumulation of tetrachlorinated biphenyls in salmon, finding no significant differences in the accumulation efficiencies among PCBs with five to eight chlorines.

Biomagnification factors

Lipid-normalized biomagnification factors (BMFs) were calculated and results are shown in Table 2. Congener-specific differences in biomagnification factor were similar to the trends observed in accumulation efficiencies, which is concordant because both parameters were based on the same fish fillets concentrations. Biomagnification (> 1) was shown for tetra- and pentachlorinated PCDD/Fs (mean BMF 1.5), but not for all the higher-chlorinated congeners. Biomagnification factors were similar for all dl-PCB congeners, with the same mean of 1.5 as for tetra- and pentachlorinated PCDD/Fs, and all the measured PCB congeners biomagnified, except PCB 169. Similar results have been obtained for whole salmon (Isosaari *et al*. 2004), although higher BMF for all dl-PCB congeners than for PCDD/Fs were obtained by these authors, who have described

that the lipid-normalized concentration ratios of salmon fillet to whole fish showed an equal partitioning of these contaminants.

Dietary intakes of PCDD/Fs and dl-PCBs recently calculated in Spanish population (Fernández *et al*. 2004) show that fish and seafood accounted for 11% of the intake, being dl-PCBs an important component in the total WHO-TEQ, up to 80% of this value in fish food. Considering the more efficient accumulation of PCBs in fish, that might explain the high contribution to the total TEQ in fish tissues (Isosaari *et al*. 2004), and the relatively important contribution of fish to dietary intake, it will be necessary to ensure the use of uncontaminated fish feed in aquaculture industry.

Conclusions

These preliminary results for young turbots may be helpful to approach the pattern of dioxins and related contaminants retention from the diet in aquaculture fish, showing that the levels of toxic compounds in feed are reflected in fish fillets and biomagnification for relevant compounds accounting for total toxicity takes place, the dl-PCBs being more efficiently accumulated. Considering the biomagnification of the most toxic PCDD/Fs and dl-PCBs and the more efficient accumulation of PCBs in fish, as well as the relatively important contribution of fish to dietary intake of total WHO-TEQ, it will be necessary to ensure the use of uncontaminated fish feed in aquaculture industry. The inclusion of dl-PCBs in this study provides valuable information for forthcoming risk assessments and contributes to establish a relationship between dioxins and similar contaminants in farmed fish meat and diet, to help assure consumer confidence on farmed fish.

Acknowledgements

This work was funded with Grant CAL 01-031 from National Institute for Agriculture and Nutrition Research and Technology, Spanish Ministry of Science and Technology (INIA-MCYT). Authors are grateful to the contributions of Ana I. Bernardino and Gerardo Fernández.

References

Abad, E., Abalos, M., Calvo, M., Nombela, J., Caixach, J., Rivera, J., 2003, Surveillance program on dioxins and dioxin-like PCBs in fish and shellfish consumed in Spain. *Organohalogen Compounds*, **62**, 49-52.

Anonymous, 2001, Council Regulation (EC) Nº 2375 / 2001 of 29 November 2001 amending Comission Regulation (EC) No. 466 / 2001 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Communities L321, 6.12.2001,* pp. 1-5.

Anonymous, 2002, Directive 2002 / 32 / EC of the European Parliament and the Council of 7 May 2002 on undesirable substances in animal feed. *Official Journal of the European Communities L 1 40, 30.5.2002,* pp. 10-21.

Anonymous, 2002, Comission Directive 2002 / 69 / EC of 26 July 2002 laying down the sampling methods and the methods of analysis for the official control of dioxins and the determination of dioxin-like PCBs in foodstuffs. *Official Journal of the European Communities L209, 6.8.2002,* pp. 5-14.

Anonymous, 2002, Comission Directive 2002 / 70 / EC of 26 July 2002 establishing requirements for the determination of levels of dioxins and dioxin-like PCBs in feedingstuffs. *Official Journal of the European Communities L209, 6.8.2002,* pp. 15-21.

Anonymous, 2004, Fishing – Aquaculture: the future of the sector. Xunta de Galicia. Servicio de Información Pesquera de la Consellería de Pesca, 2002. http:/www.xunta.es/galicia 2004/es/07_09.htm

Anonymous, 2006, Council Directive 2006 / 13 / EC of 3 February 2006 amending Anexes I and II to Directive 2002/32/EC of the European Parliament and of the Council

on undesirable substances in animal feed as regards dioxins and dioxin-like PCBs. *Official Journal of the European Communities L32, 4.2.2006,* pp. 44-53.

Anonymous, 2006, Comission Regulation (EC) Nº 199 / 2006 of 3 February 2006 amending Regulation (EC) No 466 / 2001 setting maximum levels for certain contaminants in foodstuffs as regards dioxins and dioxin-like PCBs. *Official Journal of the European Communities L32, 4.2.2006,* pp. 34-38.

Anonymous, 2006, Commission Recommendation of 6 February 2006 on the reduction of the presence of dioxins, furans and PCBs in feedingstuffs and foodstuffs. 2006 / 88 / EC. *Official Journal of the European Communities L42, 14.2.2006,* pp. 26-28.

AOAC 1990. Helrich, K. (editor), Oficial Methods of Analysis, Vol I and II. Association of Official Analytical Chemists, Arlington, VA.

Fernández, M.A., Gomara, B., Bordajandi, L.R., Herrero, L., Abad, E. Abalos, M., Rivera, J., González, M.J., 2004, Dietary intakes of plychlorinated dibenzo-*p*-dioxins, dibenzofurans and dioxin-like polychlorinated biphenyls in Spain. *Food Additives and Contaminants*, **21**, 983-991.

Fisk, A.T., Norstrom, R.J., Cymbalisty, C.D., Muir, D.C.G., 1998, Dietary accumulation and depuration of hydrophobic organochlorines: Bioaccumulation parameters and their relationship with the octanol/water partition coefficient. *Environ Toxicol Chem*, **17**, 951-961.

Isosaari, P., Vartiainen, T., Hallikainen, A., Ruohonen, K., 2002, Feeding trial on rainbow trout: comparison of dry fish feed and Baltic herring as a source of PCDD/F and PCBs. *Chemosphere*, **48**, 795-804.

Isosaari, P., Kiviranta, H., Lie, Ø., Lundebye, A.-K., Ritchie, G., Vartiainen, T., 2004, Accumulation and distribution of polychlorinated dibenzo-p-dioxin, dibenzofuran, and polychlorinated biphenyl congeners in atlantic salmon (*Salmo salar*). *Environmental Toxicology and Chemistry*, **23**, 1672-1679.

Jacobs, M., Ferrario, J., Byrne, C., 2002, Investigation of polychlorinated dibenzo-*p*-dioxins, dibenzo-*p*-furans and selected coplanar biphenyls in Scottish farmed Atlantic salmon (*Salmo salar*). *Chemosphere*, **47**, 183-191.

Karl, H., Ruoff, U., Schwind, K-H.,Jira, W., 2004, Dioxins, dioxin-like PCBs and organochlorine pesticides in farmed salmon of various origins. *Organohalogen compounds*, **66**, 1684-1690.

Lundebye, A.-K., Berntssen, M.H.G., Lie, Ø., Ritchie, G., Isosaari, P., Kiviranta, H., Vartiainen, T., 2004, Dietary uptake of dioxins (PCDD/PCDFs) and dioxin-like PCBs in Atlantic salmon (*Salmo salar*). *Aquaculture Nutrition*, **10**, 199-207.

Mackay, D., W.Y. Shiu and K.C. Ma., 1992, Illustrated Handbook of Physical Chemical Properties and Environmental Fate for Organic Chemicals. Volume II: Polynuclear Aromatic Hydrocarbons, Polychlorinated Dioxins, and Dibenzofurans. Lewis Publishers, New York NY.

Maeoka, M., Inoue, I., Shimono, H., Morita, N., 2002, Quick Pretreatment Prep Study for Dioxin Analysis. *11th Symposium on Japan Environmental Chemistry* (June 3-5), 521- 532.

Marchand, P., Matayron, G., Gade, C., LeBizec, B., Andre, F., 2004, PCDD/F, dioxin-like and markers PCBs in trouts from French aquaculture. *Organohalogen Compounds*, **66**, 1932-1939.

Meylan, W.M., Howard, P.H., Boethling, R.S., Aronson, D., Printup, H., Gouchie, S., 1999, Improved method for estimating bioconcentration/bioaccumulation factor from octanol/water partition coefficient. *Environ Toxicol Chem*, **18**, 664-672.

Ritter, L., K.R. Solomon, J. Forget, M. Sternoff and C.O. Leary, 1996, Persistent Organic Pollutants. An Assessment Report on: DDT-Aldrin-Dieldrin-Endrin-Chlordane Heptachlor-Hexachlorobenzene Mirex-Toxaphene Polychlorinated Biphenyls Dioxins and Furans. Prepared for the International Programme on Chemical Safety.

Sobrado, C., Quintela, C., Blanco, L., Cabaleiro, S., González, J.C., Vieites, J.M., 2004, Suitability of an ion trap GC/MS/MS method for routine analysis of PCDD/Fs in fishery products and by-products. *Organohalogen Compounds*, **66**, 369-375.

Figure 1

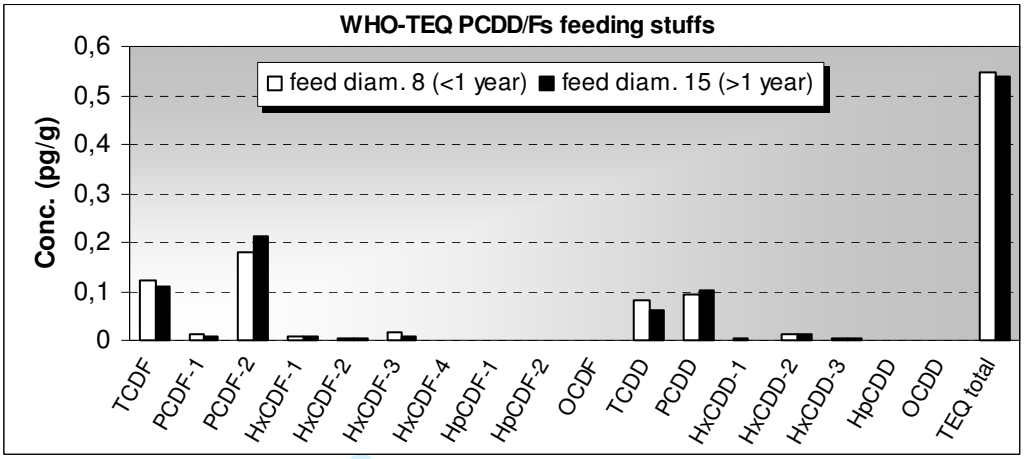

Figure 2

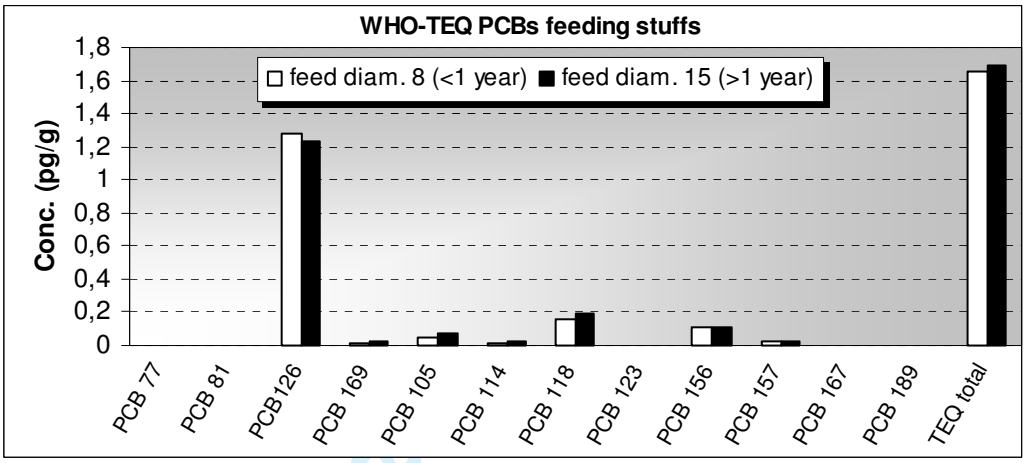

Figure 3

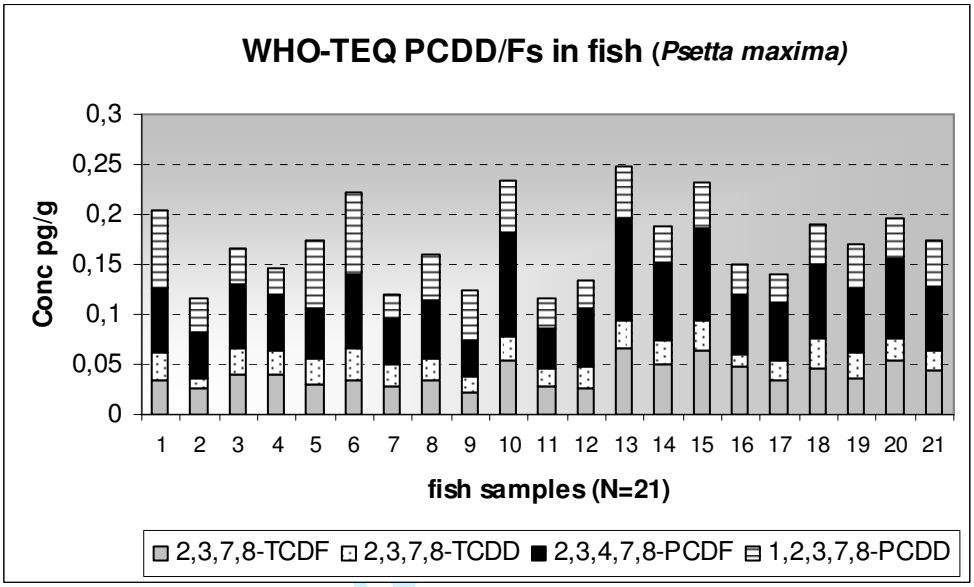

Figure 4

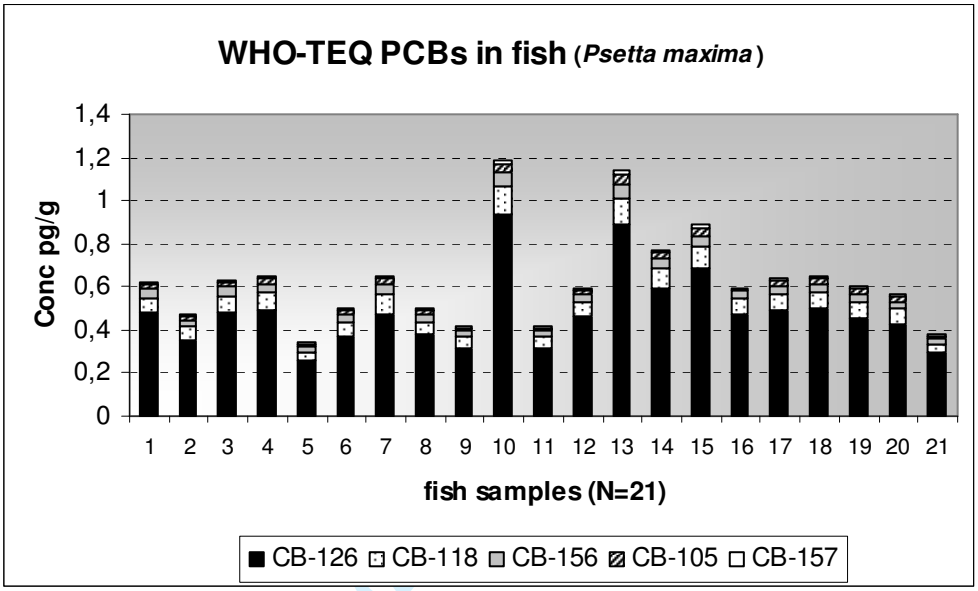

Table 1

Age (years)	Size (cm)	Weight (Kg)	Fat		TEQ PCDD/Fs		TEQ PCBs		Sum TEQ	
			%	SD	Mean	SD	Mean	SD	Mean	SD
0 - 1	21 - 28	0 - 0.5	4.9	1.4	0.17	0.05	0.57	0.12	0.77	0.11
1 - 2	30 - 36	0.5 - 1	5.0	1.9	0.19	0.06	0.74	0.33	0.93	0.39
	36 - 38	1 - 1.5	5.2	1.3	0.20	0.03	0.63	0.15	0.83	0.17

Table 2

PCDD/Fs	Accumulation efficiency		Biomagnification Factor	
	%	SD		SD
TCDF	32.7	9.4	1,55	0,36
PCDF-1	29.0	7.2	1,41	0,31
PCDF-2	31.4	8.3	1,26	0,27
HxCDF-1	15.0	4.8	0,75	0,24
HxCDF-2	28.1	9.8	1,29	0,50
HxCDF-3	13.0	3.3	0,77	0,26
HxCDF-4	-	-	-	-
HpCDF-1	19.1	8.5	1,69	0,55
HpCDF-2	-	-	-	-
OCDF	-	-	-	-
TCDD	27.6	5.6	1,58	0,51
PCDD	34.0	7.9	1,48	0,44
HxCDD-1	-	-	-	-
HxCDD-2	21.0	6.3	0,98	0,23
HxCDD-3	42.2	12.8	1,14	0,16
HpCDD	25.3	8.0	1,40	0,39
OCDD	11.3	2.7	0,81	0,33
TEQ total	30.5	7.1		
dl-PCBs				
PCB 77	42.3	16.2	1,42	0,38
PCB 81	38.0	11.3	-	-
PCB126	35.2	13.1	1,60	0,44
PCB 169	30.0	14.1	0,74	0,22
PCB 105	38.7	18.9	1,34	0,54
PCB 114	40.4	14.9	1,44	0,40
PCB 118	38.7	13.8	1,51	0,41
PCB 123	46.5	17.1	1,46	0,38
PCB 156	32.2	11.1	1,48	0,42
PCB 157	33.0	11.7	1,51	0,44
PCB 167	30.6	10.4	1,53	0,44
PCB 189	26.2	9.5	1,62	0,52
TEQ total	35.5	13.1		