LEVELS OF DIOXINS (PCDD/Fs) AND DIOXIN-LIKE PCBs IN FISH OIL, FEEDING STUFFS AND FARMED FISH (*Psetta maxima*) FROM GALICIA, SPAIN.

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Introduction

Dioxins (polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans, PCDD/Fs) and dioxin-like polychlorinated biphenyls (dl-PCBs) are persistent organic pollutants (POPs); human population is exposed to them mainly through the diet. Fishery products and by-products constitute an important vector to humans due to bioaccumulation and biomagnification in the aquatic environment.

Fishmeal and fish oil dioxins concentrations show large variations, but products of European fish stocks are more heavily contaminated than those from South Pacific stock [1]. Lack of information regarding contamination of feed materials by dioxin-like PCBs made this analytical task necessary to evaluate the present state of the products. Greatest concerns arise from the use of fishmeal and fish oil of European origin; these are more critical when used in diets for farmed fish and where fishmeal is incorporated in diets of other food-producing animals [1].

Turbot aquaculture (*Psetta maxima*) is a very important activity in Spain, being one of the main suppliers of European markets [2], producing a fish of remarkable quality due to the optimal conditions of the sea water in Galicia, northwest of the country. Persistent pollutants levels in feeding stuffs should be controlled in order to offer safe quality products. Given the potential for accumulation and the occurrence at very low levels of intake in animals, the main health concerns for humans are likely to be associated with long-term intake through food. It is thus desirable to keep dioxins in the food supply as low as possible.

The concern on high toxicity of dioxin-like compounds and the need to reduce levels through food chain has led to establishment of maximum contents of dioxins, furans and, more recently, dioxin-like PCBs in feeding stuffs and food. Directive 2006/13/CE [3] sets maximum levels of contaminants in animal feed, including dioxin-like PCBs. Commission Regulation (EC) No. 199/2006 [4] setting maximum levels for certain contaminants in foodstuffs as regards dioxins and dioxin-like PCBs shall apply from 4 November 2006, so it is of special relevance to evaluate the present state of this aquaculture product. Then, the goal of this paper was to evaluate the levels of dioxins and dioxin-like PCBs in Spanish farmed turbots, and in feedingstuffs and fish oils used as raw materials for animal feed.

Materials and Methods

All the samples were provided by industry or aquaculture plants from Galicia, northwest of Spain, during August 2004 to December 2005. Fish oils (n=22) were obtained from different manufacturers; feedingstuffs for turbots (n=6) and fish (n=23) were obtained from two aquaculture plants during 2005. Determinations of PCDD/Fs in individual samples were carried out as described [5].

Preparation for dl-PCBs analysis was carried out using adsorption chromatography, following a similar procedure as described [6], with minor modifications.

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 300 ºC, followed by column temperature

program 90 ºC (hold 2 min), 20 ºC min^{-1}, 200 ºC (hold 1.3 min), 1 ºC min^{-1}, 230 ºC (7 min), 10 ºC min^{-1}, 300 ºC (hold 20 min); for PCBs separation, injection of 10 µL sample in LVI (large volume injection) mode at 95 ºC (hold 0.5 min), applying injector temperature program of 100 ºC min^{-1} , 300 ºC (hold 12.55 min), and column temperature program of 60 ºC (hold 3 min), 20 ºC min^{-1} , 235 ºC (hold 10 min), 10 ºC min^{-1} , 260 ºC (hold 0 min), 20 ºC min^{-1} , 300 ºC (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; native and labelled dioxins/furans (EPA-1613LCS, EPA-1613ISS) and dl-PCBs (WP-LCS WHO/EPA, WP-ISS WHO/EPA) solutions were used (Wellington Laboratories); calibration was previously performed (EPA-1613CVS, CS1-CS5; 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.

Results are expressed on a wet weight basis as pg / g of sample; only edible portions of fish (fillets) were processed.

Results and Discussion

Levels of PCDD/Fs in analyzed fish oil samples (n = 22) were below the maximum TEQ for animal feed (6 pg/g) and the action limit (5 pg/g) (Figure 1); dioxin-like PCBs levels were higher, from 3 to 15 times the value of dioxins. The total TEQ for fish oil was lower than the recently established limit, 24 pg/g, only one of the 22 samples was above the limit value. Only two of the samples were above the action limit for PCBs (14 pg/g).

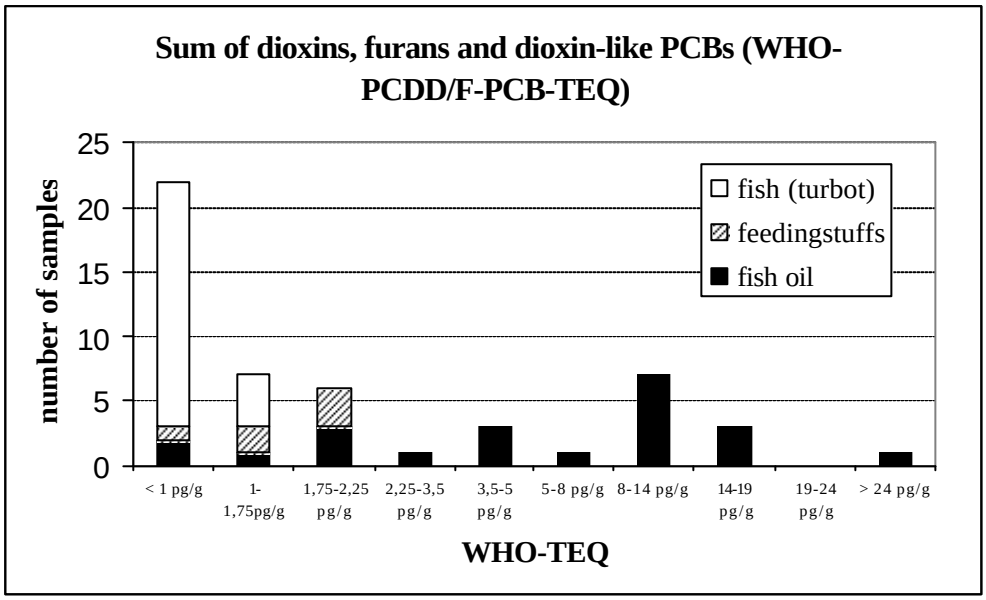

Figure 1: Total WHO-TEQ in fish oil samples, feeding stuffs for fish turbot (*Psetta maxima*) and fish turbot fillets.

Values in feedingstuffs for fish (turbot) analyzed (n = 6) were far below the maximum TEQ content (7 pg/g) (Figure 1); dioxins were below the action limit of 1.75 pg/g in all feedingstuffs, and PCBs were below the action limit of 3.5 pg/g, accounting for a total content below 2.25 pg/g in all the samples.

Data obtained in aquaculture turbots of different ages (n = 23) show that levels are far below the maximum TEQ of 8 pg/g in fish for human consumption (Figure 1). All the samples having total TEQ values higher than 1 pg/g showed PCBs content below the action limit 3.0 pg/g established by present regulation for human consumption, and any of them was over 1.5 pg/g TEQ PCBs; dioxin values were lower, below 0.6 pg/g TEQ PCDD/Fs in all samples.

We found that most abundant contaminants in the feeding stuff 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 [7]. Fish oils show a similar pattern, with higher levels of the most abundant isomers, being also abundant PCBs 123 and 189. Concentrations of PCBs are shown in Figure 2 (mean data and error bars for each group of samples).

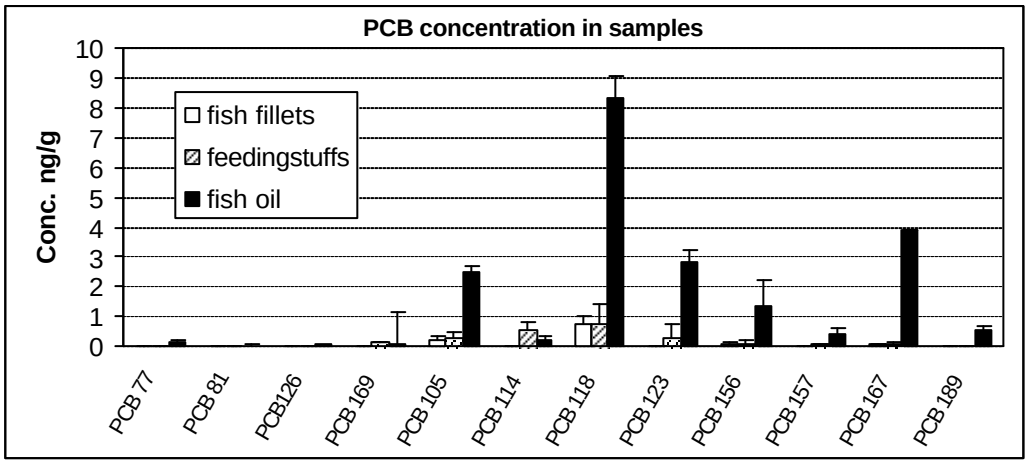

Figure 2: Dioxin-like PCBs concentration (mean values with error bars) in fish fillets (turbot, *Psetta maxima*), feedingstuffs for fish and fish oil.

Other authors have reported similar results in Spanish farmed fish (trout, sea bass, gilthead and turbot), showing low levels of PCDD/Fs in general, with a pattern characterized by the presence of toxic TCDF, TCDD, PeCDD and PeCDFs [8], as well as reported values in trouts from French aquaculture [9].

The inclusion of dioxin-like PCBs in this study provides valuable information for forthcoming risk assessment and the present state of a product coming from Spanish aquaculture, as well as the materials used to feed them, related to recently revised European regulations. In summary, farmed fish from Galicia accomplish present regulations, although it is of greatest interest to evaluate safety of raw materials in order to prevent a rise in toxicity.

Acknowledgements

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