LOW LEVELS OF PCDDs, PCDFs AND PCBs IN GREEK FARMED FISH

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Introduction

Fish is one of the most wholesome components of human nutrition, since it is an excellent source of nutrients such as proteins, vitamins, minerals and fatty acids. Fish lipids contain high levels of polyunsaturated omega-3 fatty acids, whose beneficial effects on human health are well known and documented and include prevention of coronary heart disease, lower risk of stroke, age-related macular degeneration, and promotion of normal brain development [1, 2]. A regular fish consumption of 1 - 2 servings per week has been recommended by WHO for the prevention of coronary heart disease and ischaemic stroke[3]. Studies worldwide focus on identifying the balance between health benefits from fish consumption (especially heart-protective action) and health risks due to environmental pollutants that are present in fish tissues[1, 4, 5, 6, 7]. Farmed fish represent an affordable alternative to wild caught fish for many consumers in Greece and worldwide. Due to the environmental conditions and morphology of its shoreline, Greece has numerous aqua-culture facilities that produce approximately 50% of the total global production of Mediterranean fish species. Therefore, Greek aquaculture is a very important sector of the national economy, as it produces one of the most significant food export products, with a high annual export turnover. Major cultivated species in marine culture are fresh Sea Bream and Sea Bass. More specifically, Gilthead Sea Bream (Sparus aurata) and European Sea Bass (Dicentrarchus labrax) account for over 98% of the total annual production of maricultured fish. For freshwater aquaculture the dominant product is rainbow trout (Oncorhynchus mykiss). The Mass Spectrometry and Dioxin Analysis Laboratory, during its 12 years of operation has analyzed 81 fish samples of the above species, produced by the major fish farms in Greece. Analyses included measurement of PCDD/Fs, dioxin-like PCBs (non-ortho and mono-ortho) and non dioxin-like (indicator) PCBs. Results are presented in the current study, in order to evaluate safety of Greek farmed fish for public health, from the point of view of these environmental pollutants. Results from analyses of wild-caught fish of several species, collected during the same time period, are also presented for comparison.

Materials and methods

Collection of samples

All samples were collected by the Hellenic Food Authority within the national food safety control programmes for the years 2002-2012, from major fish farms all around Greece for aqua-cultured species and local markets for wild caught fish.

Lipid extraction and clean-up

The lipid extraction and clean-up method applied have been described in detail elsewhere [8]. Quantification standards (^{13}C-labelled solutions of PCDD/Fs (Wellington) in toluene) were added to each sample prior to extraction. Extraction of lipids was achieved using Soxhlet apparatus.

Further clean-up was performed by successive steps of active carbon chromatography (Carbosphere 80/100 mesh, Alltech) and basic alumina chromatography. The carbosphere column has the advantage of high capacity for at least ten grams of fat. Moreover, it has a low affinity for lipids [9].

Final eluates were evaporated to dryness and re-dissolved in 50 μL of the appropriate ^{13}C-labelled injection standard.

Instrumental analysis

The quantification of PCDD/Fs was performed on a DB5-MS column (30m, 0.25mm, 0.1μm, J&W) by HRGC-HRMS (EI) in MID mode on a Trace GC mass chromatograph (ThermoFinnigan) coupled to a MAT-95 XP mass spectrometer (ThermoFinnigan) equipped with a CTC A 200S autosampler at 10000 resolving power (10% valley definition). The quantification was carried out by the isotopic dilution method. Instrumental conditions and purity control criteria are according to EPA 1613B method and Regulation 252/2012/EC. The quantification was carried out by the isotopic dilution method. For TEQ calculations both the WHO-98 [10] and WHO-2005 [11] toxic equivalency factors (TEF) were applied. The limit of detection (LOD) for each congener was determined as the concentration in the extract which produced an instrumental response at two different ions to be monitored

with a signal to noise ratio of 3:1 for the less sensitive signal. Values below LOD were assumed to be equal to LOD (upperbound concentrations).

Quality control

A method blank and a quantitative control sample (reference) were included in the study. It should be added that the laboratory has been participating successfully in international interlaboratory studies since 2003, it is accredited according to ISO/IEC 17025/2005 and it is the European Reference Laboratory for the control of dioxins and dioxin-like compounds for Greece and Cyprus.

Results and discussion

Concentrations of PCDD/Fs, dioxin-like PCBs and non-dioxin-like PCBs in farmed and wild fish samples studied are presented in Table 1. Mean values as well as min-max values are shown. Upperbound TEQ values were calculated by multiplying with the appropriate WHO-TEF 1998 and also upperbound values were determined by application of the revised WHO-TEF 2005, which are currently required for TEQ estimations by European legislation (Commission Regulation 1259/2011). Both calculations are presented, since the WHO-TEFs 1998 values enable comparisons with data from previous studies, while levels calculated by current TEFs allow comparisons with maximum levels set by the European Union in Commission Regulation 1259/2011 which is currently in force.

Mean upperbound concentrations (assuming concentration equal to LOD for non-detected congeners) were preferred, since these values represent the highest possible amount of a contaminant according to analytical results, which is important when examining human exposure to toxic compounds. Moreover, according to EU legislation, analytical results are expressed as upperbound level. Table 1 contains results from farmed sea bass, sea bream and trout and from wild caught fish. Various Mediterranean fish species are contained in the wild fish sample population. Mean values for this study group are presented so as to serve as an indication of PCDD/Fs and PCBs levels in farmed versus wild-caught fish. A more extensive and species-specific study is necessary for a reliable conclusion on PCDD/Fs and PCBs levels in Greek wild-caught fish.

As shown in table 1, mean levels (calculated with current TEF 2005) of farmed sea bream, farmed sea bass and farmed rainbow trout are far below maximum levels set by the European Union in Commission Regulation 1259/2011, which are 3.5 pg/g w.w. for WHO-PCDD/F-TEQ and 6.5 pg/g w.w for WHO-PCDD/F-PCB-TEQ. Although direct comparisons between farmed and wild caught fish cannot be performed and statistical criteria should not be applied, because wild fish study population comprises various fish species, it is probable that there is no significant difference between Greek farmed sea bass and rainbow trout and wild-caught fish regarding dioxin and dioxin-like compound levels.

Higher levels were measured in farmed sea bream, probably due to its higher lipid content (mean % lipid 14.27, versus 8.41 in sea bass, 8.52 in rainbow trout and 4.75 in wild fish), but these levels are also much lower than maximum levels set by the European Union. As in most relevant studies, major TEQ contribution is from PCBs.

Higher WHO-PCDD/F-TEQ and WHO-PCDD/F-PCB-TEQ values were calculated with the application of TEF 1998, as shown in Table 1. This finding is in accordance to a reported study on the influence of the new WHO-TEF on TEQ-based results of food samples [12].

Similar levels in farmed sea bass and sea bream have been reported in a study in fish farms in Turkey (WHO-PCDD/F-TEQ 0.14-0.31 for sea bass and 0.29-0.70 for sea bream, WHO-PCDD/F-PCB-TEQ 0.60-1.91 for sea bass and 1.87-5.22 for sea bream, values were calculated by WHO-TEFs 1998) as reported by Çakıroğulları et al.[14]. Levels ranging from 0.032 to 1.60 pg/g w.w. have been measured for WHO-PCB-TEQ in farmed sea bass in Italy [13]. Mean levels of 0.07 pg/g w.w. WHO-PCDD/F-TEQ and 0.39 pg/g w.w. WHO-PCDD/F-PCB-TEQ were found in farmed rainbow trout in Poland [7].

Apart from dioxin-like PCBs, our study includes results for non-dioxin-like PCBs for the farmed fish samples collected after 2007. These PCB congeners, usually referred to as indicator or marker PCBs, are the most abundant in the environment, and their toxicological actions include neurological, immunological and carcinogenic effects (http://www.efsa.europa.eu/en/efsajournal/doc/284.pdf). Indicator PCBs do not contribute to the total WHO-PCDD/F-PCB-TEQ value (no TEF value has been assigned to them). However, the European Union has recently set maximum levels for these compounds in its latest Regulation that is currently in force (Commission Regulation 1259/2011). Mean and min-max values for the sum of indicator PCBs in farmed and wild-caught fish shown in Table 1 are far below EU limit of 75 ng/g w.w.

The maximum values of non-dioxin-like PCBs were observed in sea bream, perhaps due to its higher lipid content. These are in agreement with levels reported by other authors for farmed sea bass and sea bream [13, 14, 15, 16].

Table 1 Average results of PCDDs, PCDFs dioxin-like PCBs (pg/g fresh product) and non-dioxin-like PCBs (ng/g fresh product) in cultured sea bream, sea bass and trout, calculated as upper bound concentrations.

	Sea bream (n=42)	**Sea bass (n=32)**	**Trout (n=7)**	**Wild fish (n=34)**
Lipid content (%)	14.27 (3.21-23.54)	8.41 (1.10-16.56)	8.52 (5.44-12.39)	4.75 (0.77-24.09)
PCDD/F WHO TEQ 1998	0.26 (0.02-1.12)	0.15 (0.01-0.35)	0.11 (0.04-0.33)	0.17 (0.00-0.93)
PCDD/F WHO TEQ 2005	0.22 (0.02-0.91)	0.13 (0.01-0.28)	0.10 (0.04-0.28)	0.13 (0.00-0.72)
PCB WHO TEQ 1998	0.86 (0.01-1.75)	0.68 (0.17-1.69)	0.37 (0.07-0.87)	0.43 (0.01-1.20)
PCB WHO TEQ 2005	0.66 (0.01-1.65)	0.55 (0.11-1.35)	0.33 (0.13-0.79)	0.35 (0.01-1.01)
PCDD/F-PCB WHO TEQ 1998	1.12 (0.03-2.71)	0.83 (0.18-1.99)	0.48 (0.15-0.98)	0.60 (0.01-1.70)
PCDD/F-PCB WHO TEQ 2005	0.88 (0.03-2.56)	0.68 (0.12-1.61)	0.43 (0.18-0.89)	0.49 (0.01-1.31)
sum of indicator PCBs	8.02 (3.24-15.46)	5.24 (2.63-9.18)	2.90 (0.76-4.78)	4.58 (0.14-15.11)

Regarding the congener profile of PCDDs, PCDFs and PCBs, for PCDD/Fs the dominant congeners are 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF, OCDD and OCDF. For non-ortho PCBs the most abundant congener is PCB-77, followed by PCB-126 and for mono-ortho PCBs the dominant congener is PCB-118 followed by PCB-105. PCB-138 and 153 are the most abundant indicator PCB congeners. These observations are in agreement with previously published relevant findings for farmed and wild sea bass[13, 15], and for farmed sea bass and sea bream [14]. Congener profile is schematically presented in figure 1 for PCDDs and PCDFs, in figure 2 for dioxin-like PCBs (non-ortho and mono-ortho) and in figure 3 for indicator PCBs, where levels are presented in pg/g fat to enable comparisons between species. As shown in figures 1 and 3, farmed fish have lower concentrations per gram of fat of TCDF, PeCDF, OCDD, OCDF, PCB-138, PCB-153 and PCB-180 than wild caught fish.

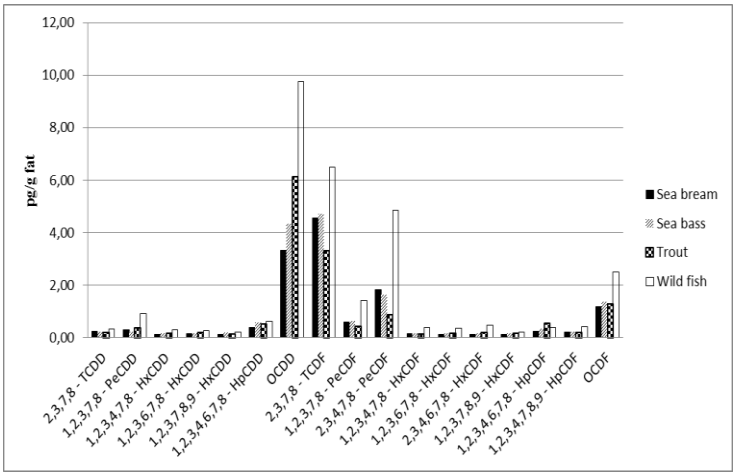

Figure 1 Congener profile of PCDD/Fs (concentration pg/g fat)

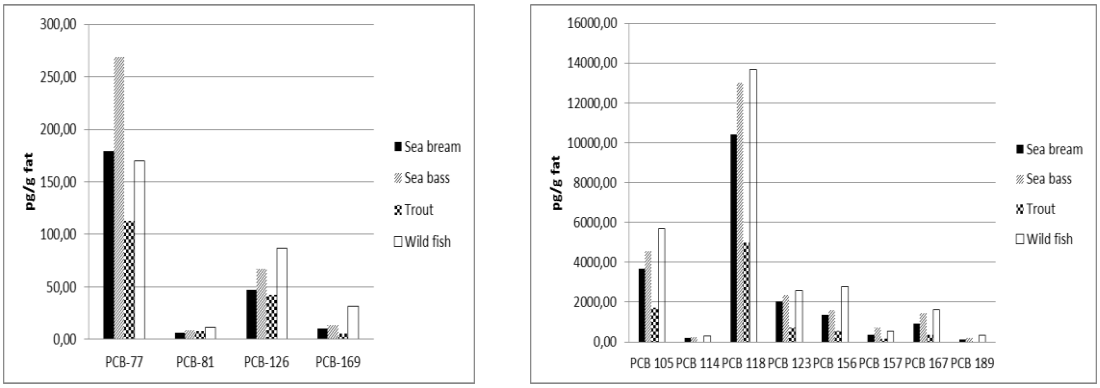

Figure 2 Congener profile of non-ortho and mono-ortho PCBs (concentration pg/g fat)

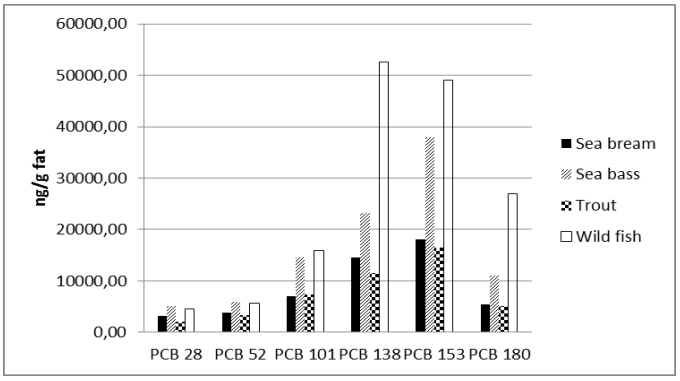

Figure 3 Congener profile of indicator PCBs (concentration pg/g fat)

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