# Chlorinated dibenzo-dioxins, -furans and biphenyls in tissues of wild and farmed bluefin tuna (Thunnus thynnus) from the Western Mediterranean Sea: accumulation patterns, toxic equivalents and risk for human consumption

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## Introduction

The widespread contamination in the Mediterranean is increasingly threatening the environment and the health of both wildlife and humans. The production of PCBs in France, Italy and Spain between 1973 and 1980 reached 100,000 metric tonnes[1]. Such burden is even more threatening considering that although production is now banned, the rate at which PCBs were produced has far outstripped by the rate at which they are converted back into harmless chemicals and that average turnover time of Mediterranean Seas is 100 years. Ecotoxicological studies were carried out on the presence of persistent organic pollutants (POPs) in the bluefin tuna *Thunnus thynnus*. This species shows interesting and peculiar biological features. Since the time they are juvenile, tuna live in uniformed-sized schools which can be found in the Mediterranean Sea throughout the year.[2] Adult tuna are less gregarious during the inter-genetic period (end of Summer – beginning of Spring: erratic period), and they group in schools only during the reproductive period (mid May – mid June: gregarious period).[3] Tuna migrate out of the Mediterranean Sea and spend an unknown number of years in the Atlantic Ocean before coming back to the Mediterranean, when they start to reproduce. They are very voracious during their inter-genetic period and they store energy as lipid reservoir; meanwhile, they start to produce gametes. When gonads are mature (May), both males and females start to starve and they gather in schools.[2,4] They are top predators and feed on a variety of fish species, crustaceans and cephalopods.[5] Tuna are extremely interesting for POP distribution studies given the fact that they accumulate lipids, migrate and are distinguished as being top predators.

Tuna samples were tested for polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB) and dichlorodiphenyldichloroethylene (*p,p'*-DDE). Data were used to evaluate both the dioxin toxic equivalents (TEQs) and the Tolerable Weekly Intake (TWI), which gives insight into the safety of a product intended for human consumption[6,7].

## Materials and Methods

***Collection of samples***. Muscle, liver, gonad, gill and blood tissue samples were analyzed to detect the presence of POP residues. Bluefin tuna specimens were caught off the Sicilian coast between May 26 and July 9, 2003 by long-line fishing (n = 35, weight = 145±72, length = 192±43; adult 8-12 years old = wild giants) and the centuries-old *mattanza* technique (n = 21, weight = 32±3, length = 126±7; 4-6 year-old juveniles = the wild young).[4] Some of these specimens were then put inside pens and recaptured between October and November 2003, in order to satisfy the market demand. They were fed with a herring-based diet; samples were collected from dead carcasses (n = 8, weight = 232±77, length = 246±21).

***Analytical methods for chlorinated chemicals*.** PCB congeners and pesticides were analyzed following the method described elsewhere, with some modifications.[8,9] HCB, *p,p*'-DDE, PCBs, PCDDs and PCDFs were identified and quantified using a gas chromatograph (Perkin Elmer mod. Autosystem) equipped with $^{63}$Ni electron capture detector (GC-ECD; capillary column coated with DB-5 (Supelco Inc.) and GCQ plus ion trap mass spectrometer from ThermoFinnigan.[10] Blanks were analyzed throughout the analytical procedure. Recoveries and detection limits were previously described and validated.[8] PCB congeners are represented by their IUPAC numbers throughout the text. Results are given on a wet weight basis (wet wt).

## Results and Discussion

***HCB, p,p'-DDE and ΣPCBs*.** The ∑PCB, *pp*'-DDE and HCB concentrations were of the same order of magnitude in the tissues of both the wild and farmed tuna (Table 1). However, the wild tuna showed values slightly more elevated with respect to the farmed tuna, likely in relation to body size. Concentrations in wild young tuna were unexpectedly higher then in wild giants; tuna can spend years in the Atlantic Ocean, that is less polluted than the Mediterranean Sea. Moreover, the starvation during the gametes production period (time of samplings) may contribute to a mobilization of stored POPs. The concentration pattern resulted in ∑PCBs > *pp*'-DDE > HCB, in accordance with the results found by other authors regarding both Mediterranean tuna and fish as well as bluefin tuna from Japanese coasts.[8,11,12] For both the wild and farmed tuna, the brain was the most contaminated tissue. The elevated POP levelsin the brain, muscle and liver may be due to the nature of lipid components in these tissues. HCB was very high in the gills of all the specimens, likely due to the elevated exchange rate with the environment through the gills.[13] Different POP accumulation patterns in wild and farmed fish may be due to the different lipid composition of tissues, because of captivity. This is important in relation to the human consumption of tuna muscle and gonads. In the tissues of both the wild and farmed tuna, the isomer concentration pattern was hexa-CBs > hepta-CBs > penta-CBs > octa-CBs > tetra-CBs > nona-CBs (Figure 1). Concentrations of hexa-CBs, the most abundant class, varied from a minimum of 9.5 ng/g in the blood of farmed tuna to a maximum of 1209 ng/g in the brain of wild tuna. The hexa-CBs represented about 44-51% of the total PCB residue in the various tissues and, when examined together with the hepta-CBs, represented about 70%. The most abundant PCB congeners in all the tissues of both the wild and farmed tuna were the IUPAC numbers 153 > 138 > 180 > 118 > 170 > 187. Adding up together the partial concentrations of these congeners, they represented 60% respect to the PCB residue. This pattern agreed with those reported by Kannan *et al*. and Corsolini *et al*. in Mediterranean bluefin tuna.[9,12] PCB levels found in muscle of Southern bluefin tuna (mean body wt = 36 kg) farmed along the Australian coasts were lower than the concentrations found in our samples (31.87 ng/g wet wt).[14] PCBs 153, 138, 180 and 118 were predominant in various species of fish, mollusks and crustaceans from the Mediterranean basin.[9,12,15-17]Those congeners have a chlorine atom in the 2, 4, 5 positions of one or both the biphenyl ring; invertebrates and fish are not able to metabolize such chemicals.[18,19]

The slightly decreasing levels of POP in farmed tuna with respect to length might be due to dilution, due to body growth. In fact, those specimens were fed very intensively with a herring-based diet to reach market size.

***PCDDs and PCDFs*.** Analyses in order to determine the presence of dioxins and furans were carried out on samples of muscular tissue, the edible part of bluefin tuna. The highest ∑PCDD and ∑PCDF levels were found in the wild specimens rather than in those that were farmed: 25±22, 11±14 and 17±22 pg/g of dioxins and 36±23, 24±18 and 17±12 pg/g of furans in wild young and giants and farmed tuna, respectively (Table 2). PCDD/Fs concentrations in wild tuna from the Ionian Sea were of the same order of magnitude.[9] PCDD/Fs congener concentrations were similar in all samples, with the exception of 2,3,7,8-TCDD, 1,2,3,4,6,7,8,9-OCDD, 1,2,3,7,8-PCDF and 1,2,3,4,6,7,8-HpCDF (Table 2). Herrings from the Baltic showed the following patterns: 1,2,3,6,7,8-HxCDD 1,2,3,7,8-PCDD > 2,3,7,8-TCDD > 1,2,3,4,6,7,8,9-OCDD and 2,3,4,7,8-PCDF > 2,3,7,8-TCDF > 1,2,3,7,8-PCDF.[20] As these giant farmed tuna were fed with herrings, they might accumulate the congeners 1,2,3,4,7,8-HxCDD, 1,2,3,4,6,7,8,9-OCDD, 1,2,3,7,8-PCDF, 2,3,4,7,8-PCDF and 1,2,3,4,7,8-HxCDF; those congeners made up 80%, 60% and 30% of the residue in the farmed tuna, respectively. Penta-CDDs/Fs predominated in farmed Southern bluefin tuna from Australia[14].

***TEQs and TWI.*** The TEQ values in the muscle tissue, the edible part of tuna, were attributed to PCDDs (68%) in the wild tuna and to PCDFs (58%) in the farmed tuna (Table 2). The highest TEQ value, equal to 8.562 pg/g, was found in the wild tuna while the TEQ value for farmed tuna was equal to 3.91 pg/g, even if concentrations were of the same order of magnitude. TEQ values were much lower than those evaluated in farmed Southern bluefin tuna from Australia; Padula et al. (2004) calculated 0.531 pg $TEQ_{PCDD/F}$/g wet wt.[14]

The Scientific Committee on Food (SCF) has recommended a Tolerable Weekly Intake (TWI) of 14 $pg_{TEQ}$/kg/body weight per week. This figure is in line with the provisional Tolerable Monthly Intake of 70 $pg_{TEQ}$/kg body weight/month established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).[21,22] The values calculated, based on a weekly 200g filet portion of the edible part (muscle) of wild young, wild giants and farmed giants, were 24, 15, and 11 $pg_{TEQ}$/kg body weight, respectively, for men (body wt=70 kg) and 32, 21, 17 $pg_{TEQ}$/kg body weight, respectively, for women (body wt=50 kg). However it important to note that the sample number of the farmed giants was less than a third that of the wild giants, and the standard deviation between the samples was large (Table 1). With this in mind, with the exception of males who consume farmed tuna filet, TWI values were higher than the limits

recommended by the WHO. [21]

In general, our results highlighted that several factors can affect the POP accumulation and distribution in bluefin tuna: migration habits, diet and reproductive period. Besides, captivity may have an influence on POP accumulation and distribution in the body.

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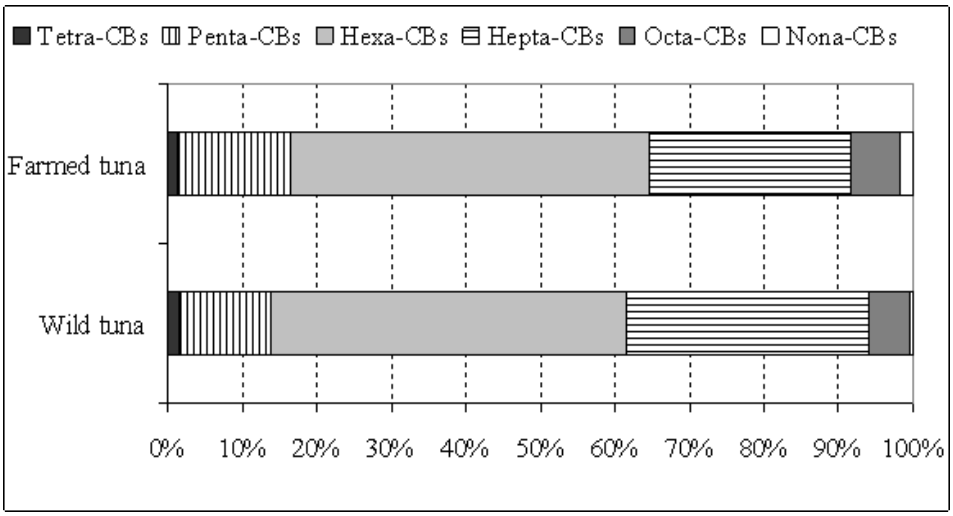


Figure 1: PCB class of isomer patterns of wild and farmed tuna muscle.

Table 1: ΣPCB, HCB and *p,p'*-DDE average concentrations (±S.D.), minimum and maximum values (in brackets; ng/g wet wt) in wild and farmed tuna tissues.

| | | n | Lipid % | HCB | *p,p'*-DDE | SPCBs |
|---|---|---|---|---|---|---|
| muscle | wild young | 21 | - | 0.2±0.1 | 98.9±40.0 | 766.3±611.7 |
| | | | | (0.0-0.4) | (24.4-130.8) | (143.4-1819.3) |
| | wild giants | 35 | 2.9-5.3 | 0.9±0.8 | 64.7±42.9 | 732.2±675.0 |
| | | | | (0.2-2.4) | (29.5-133.8) | (279.6-2023.6) |
| | farmed giants | 8 | 39 | 4.4±4.4 | 46.5±46.5 | 481.2±481.2 |
| | | | | (2.0-9.0) | (21.1-97.8) | (238.4-1071.9) |
| blood | wild young | 2 | - | 0.1±0.1 | 3.9±2.8 | 28.4±13.6 |
| | | | | (0.02-0.1) | (1.9-5.9) | (18.8-38.0) |
| | wild giants | 30 | - | 0.4±0.2 | 2.3±1.6 | 24.5±12.8 |
| | | | | (0.1-0.6) | (0.1-5.6) | (7.5-46.8) |
| | farmed giants | 8 | - | 0.6±0.1 | 1.4±0.9 | 21.5±12.5 |
| | | | | (0.4-0.7) | (0.5-3.1) | (7.3-37.1) |
| gill | wild young | 21 | - | 26.0±5.1 | 0.1±0.1 | 4.5±1.0 |
| | | | | (19.5- | (0.01-0.4) | (2.6-5.4) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | 34.6) | | |
| | wild giants | 32 | - | 19.7±8.0 | 0.1±0.1 | 1.9±1.0 |
| | | | | (8.9-33.5) | (0.01-0.2) | (1.0-3.4) |
| | farmed giants | 6 | - | 63.5±57.6 | 0.5±0.3 | 5.6±5.6 |
| | | | | (6.8-138.8) | (0.1-0.8) | (0.6-12.2) |
| liver | wild young | 19 | - | 0.6±0.3 | 150.9±121.6 | 842.3±741.1 |
| | | | | (0.4-0.8) | (64.9-236.9) | (318.3-1366.3) |
| | wild giants | 34 | 3.9-11.5 | 1.6±2.0 | 76.5±93.6 | 510.3±523.4 |
| | | | | (0.2-3.9) | (17.1-184.4) | (146.9-1110.2) |
| | farmed giants | 8 | - | 2.4±1.8 | 29.6±6.2 | 273.8±92.3 |
| | | | | (0.8-4.4) | (22.4-33.5) | (167.6-334.8) |
| brain | wild young | 21 | - | 0.6±0.4 | 205.9±51.6 | 1952.6±983.2 |
| | | | | (0.3-0.9) | (169.4-242.3) | (1257.3-2647.8) |
| | wild giants | 32 | - | 3.2±3.3 | 228.5±194.4 | 2722.8±2515.4 |
| | | | | (0.9-7.0) | (98.8-452.0) | (1005.6-5610.2) |
| | farmed giants | 8 | - | 3.3±2.0 | 112.6±76.0 | 1017.6±703.6 |
| | | | | (2.1-5.6) | (61.4-199.9) | (446.2-1803.5) |

Table 2: PCDD, PCDF and mono-*ortho* PCBs concentrations (±S.D., minimum and maximum values in brackets; pg/g wet wt), and TEQ (pg/g wet wt) in bluefina tuna mucles (wild youngs = wy, wild giants= wg, farmed giants=fg).

| | Concentration±S.D. (min-max) | | | TEQ (pg/g) | | |
|---|---|---|---|---|---|---|
| | Wild young | Wild giants | Farmed giants | wy | wg | fg |
| 2,3,7,8-TCDD | 5.7±5.7 | 2.5±4.9 | <0.5±0 | 5.728 | 2.491 | 0.500 |
| | (<0.5-11.7) | (<0.5-12) | | | | |
| 1,2,3,7,8-PCDD | <0.5±0 | <0.5±0 | <0.5±0 | 0.500 | 0.500 | 0.500 |
| 1,2,3,4,7,8-HCDD | <0.5±0 | <0.5±0 | 1.1±1.1 | 0.250 | 0.250 | 0.567 |
| | | | (<0.5-2.4) | | | |
| 1,2,3,6,7,8-HCDD | <0.5±0 | <0.5±0 | <0.5±0 | 0.005 | 0.005 | 0.005 |
| 1,2,3,7,8,9-HCDD | <0.5±0 | <0.5±0 | <0.5±0 | 0.005 | 0.005 | 0.005 |
| 1,2,3,4,6,7,8,9-OCDD | 17.7±19 | 6.4±14.5 | 14±23 | 0.002 | 0.001 | 0.001 |
| | (<0.5-41) | (<0.5-36) | (<0.5-40) | | | |
| *ΣPCDDs* | *25±22* | *11±14* | *17±22* | *6.489* | *3.252* | *1.578* |
| | *(3-55)* | *(3-38)* | *(3-42)* | | | |
| 2,3,7,8-TCDF | <0.5±0 | <0.5±0 | <0.5±0 | 0.025 | 0.025 | 0.025 |
| 1,2,3,7,8-PCDF | 13±1.7 | 14±3.8 | 11±11 | 0.654 | 0.727 | 0.570 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | (11-15) | (11-21) | (<0.5-23) | | | |
| 2,3,4,7,8-PCDF | 1.7±2.9 | 1.8±3.1 | 2.9±4.2 | 0.848 | 0.880 | 1.450 |
| | (<0.5-7.7) | (<0.5-8.1) | (<0.5-7.7) | | | |
| 1,2,3,4,7,8-HCDF | 0.9±0.9 | <0.5±0.7 | 0.8±<0.5 | 0.086 | 0.054 | 0.077 |
| | (0.4-2.6) | (0.01-1.9) | (<0.5-1.3) | | | |
| 1,2,3,4,6,7,8-HpCDF | 20±21 | 6.6±15 | <0.5±0 | 0.199 | 0.066 | 0.005 |
| | (<0.5-45) | (<0.5-37) | | | | |
| 1,2,3,4,6,7,8,9-OCDF | <0.5±0 | <0.5±0 | <0.5±0 | 0.001 | 0.001 | 0.001 |
| *ΣPCDFs* | *36±23* | *24±18* | *17±12.1* | *1.812* | *1.752* | *2.127* |
| | *(13.8-61)* | *(14-61)* | *(3-26)* | | | |
| PCB 118 | 46545±39000 | 44999±37614 | 33429±22081 | 0.233 | 0.225 | 0.167 |
| | (1110-11660) | (6051-111629) | (14747-75178) | | | |
| PCB 156 | 4376.56±3953 | 3932±3443 | 3136±2270 | 0.022 | 0.020 | 0.016 |
| | (1091-11431) | (569-11431) | (1620-7524) | | | |
| PCB 189 | 1211±1519 | 3876±8505 | 3428±3540 | 0.006 | 0.019 | 0.017 |
| | (149-4263) | (149-35698) | (426-10055) | | | |
| *mono-ortho ΣPCBs* | *5213*±44749 | *12185*±15901 | *7775*±7328 | *0.261* | *0.264* | *0.200* |
| | (12344-132322) | (1809-58559) | (2195-21841) | | | |
| ΣTEQs | | | | 8.562 | 5.268 | 3.905 |