Bull. Environ. Contam. Toxicol. (2002) 69:800–807

DOI: 10.1007/s00128-002-0131-5

Bulletin of Environmental Contamination and Toxicology

Investigation of Polychlorobiphenyls and Organochlorine Pesticides in Tissues of Tuna (*Thunnus Thunnus Thynnus*) from the Mediterranean Sea in 1999

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Received: 1 March 2002/Accepted: 18 July 2002

Polychlorobiphenyls and organochlorine pesticides are among endocrine – disrupting chemicals (ECDs). They can cause adverse effects in organisms involving disrupting of the endocrine system and the reprodution in wildlife and humans (Fossi et al. 2001, Taylor and Harrison 1999, Tanabe et al. 1987). Organochlorine (OC) pesticides and polychlorinated biphenyls (PCBs) are distributed world – wide and are highly persistent organic pollutants; their presence in marine environments, especially in the Mediterranean Sea, has been reported by several authors (Fowler and Elder 1980; Mckenzie et al. 1999; Storelli and Marcotrigiano 2000).
The Mediterranean Sea is a completely land – locked water body which is polluted by extensive anthropogenic activities from industrialized hinterland and so it is particularly exposed by this type of contamination.

Many of the organochlorine compounds, such as PCBs and OC pesticides are highly lipophilic and persistent, and, consequence, they are capable of bioaccumulating in aquatic organisms, especially, those occupying the top of the food chain (Olsson et al. 2000; Ruus et al. 1999; Law et al. 2001; Tanabe et al. 1993). Biomagnification of lipophilic organochlorine substances has been shown to occur in aquatic food chains (Borga et al. 2001, Zaranko et al. 1997).

This research is part of a large monitoring program supported with funds of the Italian Ministry of Environment to measure the levels of contaminants in top marine predators (*Xiphies gladius, Thunnus Thynnus thynnus*) and to develop sensitive biomarkers for estimation of toxicological risk in these species.
In this paper we have reported the values of the contamination in 25 specimens of tuna.

MATERIALS AND METHODS

In November – December 1999 several tuna fish has been fished along the Sicilian coast (Strait of Messina). Immediately after being caught, the biometrical data was recorded and after the dissection, the samples of some tissues and organs were stored in a deep freezer (-20°C) until analyses. One sample of muscle, liver, gonad, blubber was taken from each fish, and separately analysed.

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Table 1. Details of Tuna fished along the Sicilian coast.

code	sex	Weight (kg)	Length (cm)
T1	M	250	250
T2	M	54	138
T3	F	50	146
T4	M	65	155
T5	M	45	140
T6	M	48	146
T7	M	68	156
T8	M	58	
T9	F	78	156
T10	F	54	153
T11	F	56	146
T12	M	50	141
T13	M	82	156
T14	M	40	135
T15	M	61	146
T16	M	65	
T17	M	51	147
T18	M	52	141
T19	M	63	157
T20	F		128
T21	M		142
T22	M	65	156
T23	M	46	138
T24	M		152
T25	M	56	140

In table 1 details of 25 specimens of Tuna studied are reported.
An amount of sample between 2 and 10 g was weighed in a 250 ml centrifuge tube and was extracted with light petroleum + acetone (100 ml; 1+1, v+v) homogenizing in Ultra Turrax T25 for 2 min at ca. 9500 r.p.m. A 5 % w/v sodium chloride solution (100 ml) was added. The mixture was manually shaken for 2 min, and phases allowed tot separate.
The organic phase was pipetted and passed through a column of sodium sulphate (25 g in a glass tube, 200x20 mm. i.d.). The solvent was collected into a 250 ml Erlenmeyer flask (weighed at ± 0.01 g). The extraction was repeated with 2 x 50 ml portions of light petroleum 40 - 60 ° C, which were in their turn passed through sodium sulphate.
The combined solvent phases were concentrated to a small volume (ca. 1-2 ml) by rotary evaporator and to dryness by manually rotating the flask. The lipid residue was weighed. The analytes were separated from the lipids by a combination of solid-matrix partition and C18 chromatography. The solid - matrix partition was carried out by dispersing an n-hexane solution of the lipid residue into an Extrelut-NT 3 (E. Merck, Darmstadt, Germany, Cat. No 1.15095.0001).

A maximum of 1g of fat was applied to the Extrelut - NT 3 cartridge. The n-hexane solution was let drain into the cartridge and 10 min were allowed to obtain an even distribution of the solution into the filling material. The solvent was removed by passing a stream of nitrogen through the cartridge at 0.5 L/min for 30 min, from bottom to top.
An Extrelut NT - 1 (Cat.No.1.15094.0001 E. Merck, Darmstadt, Germany) cartridge was emptied so as to leave only 1 cm height of the Extrelut material. C18 material (0.36 g; 40 - 60 mesh, International Sorbent Technology, Part No. 9221 - 1000, obtained through Stepbio - Bologna, Italia) and 1.5 cm of Extrelut material was added. The C18 cartridge was located downstream to the Extrelut-NT 3 and the system of combined cartridges was eluted with 4x5 ml portions of acetonitrile. The combined solvent was concentrated by rotary evaporator (bath temp. 50 °C; reduced pressure) to a small volume (ca. 1 ml) and then to dryness by manually rotating the flask.
Extracts from Extrelut partition system or portion thereof, containing no more than 50 mg lipid residue were cleaned up and fractionated by adsorption chromatography over activated Florisil (2.5 g; 60 - 100 mesh, Supelco, Bellefonte, PA, USA, cat. N. 2 - 0280, activated at 130 ° C overnight).

PCBs and some OC pesticides, such as HCB (hexachlorobenzene), DDEs, *trans* - Nonachlor were eluted from the column with 30 ml of n-hexane (1st fraction) and most of the OC pesticides with a fraction of 25 ml n-hexane + toluene (80 + 20, v + v) (2nd fraction), while endrin, dieldrin, HEPO (eptachlor epoxide with 30 ml n-hexane + toluene + ethyl acetate (180 + 19 + 1, v + v + v) (3rd fraction).
The fractions were separately collected and analysed.
A 1 ml of isooctane (as "keeper") was added to the 1st fraction only. The fractions were concentrated to a small volume (ca. 1 mL) by rotary evaporator (bath temperature 50 °C; reduced pressure). The internal standard solution (PCB 209) was added before injecting into a GC/ECD apparatus.
PCBs and OC pesticides were determinated using a capillary gas chromatograph (GC) equipped with twin splitless injectors and twin electron capture detectors. The gas chromatograph used was an HP 5890 Series II equipped with two fused silica capillary columns, DB- XLB and SP - 1701, both 30 m x 0.25 mm i.d. x 0.25 μm film thickness and operated under the following conditions: helium carrier gas, flow rate 1.5 ml/min supplied through Electronic Pressure Control (EPC) in constant flow mode, oven temperature programming :60 °C, 2 min hold, ramp to 160 °C at a rate of 10 °C/min, 160 °C to 250 °C at a rate of 2 °C/min, with a final hold of 10 min, injection 1 μl into a split/splitless injector used in (splitless mode , with vent time 1 min) equipped with a dual – tapered deactivated liner using a HP-7673 autosampler.
The detection limit of the analysed organochlorine compounds were 0.05 ng g^{-1} for PCB and 0.01 ng g^{-1} for OC pesticides. The recovery values from spiked samples were in the range 75% - 90 % and 80 – 90 % for PCBs and OC pesticides, respectively. Precision was estimated from multiple analyses of spiked samples (RSD < 10%).
The concentrations of individual PCB congeners and OC compounds were

Table 2 – List of the organochlorine pesticides (OC) and PCBs investigated.

Organochlorine		Polychlorobiphenyl	
HCB	α- Endosulfan	Tri CB - 28	Hexa CB – 128
α- HCH	β- Endosulfan	Tri CB – 31	Hexa CB – 132
β-HCH	Metoxychloro	Tetra CB – 44	Hexa CB – 138
δ-HCH	Mirex	Tetra CB – 47	Hexa CB – 151
γ-HCH	Quintozene	Tetra CB – 49	Hexa CB - 153
trans-Nonachlor	o,p'DDT	Tetra CB – 52	Hexa CB – 156
Oxychlordane	p,p'DDT	Tetra CB – 66	Hexa CB – 169
α-chlordane	o,p'DDE	Tetra CB – 70	Hepta CB – 170
γ-chlordane	p,p'DDE	Tetra CB – 74	Hepta CB – 180
α- chlordene	o,p'TDE	Tetra CB – 81	Hepta CB – 183
γ-cholrdene	p,p'TDE	Penta CB – 82	Hepta CB – 185
Aldrin		Penta CB – 87	Hepta CB – 187
Endrin		Penta CB – 101	Hepta CB – 188
Dieldrin		Penta CB – 104	Hepta CB – 189
Heptachlorepoxide(HEPO)		Penta CB – 105	Octa CB – 194
Heptachlor		Penta CB – 110	Octa CB – 198
Octachlorstirene		Penta CB – 118	Nona CB – 206
Endosulfan Sulphate		Penta CB – 119	

In table 2 are reported the PCB congeners and OC pesticides analysed by this method.

RESULTS AND DISCUSSION

The mean and range of concentrations of Σ PCBs (the sum of 35 congeners), ΣDDTs (p,p'-DDE, o,p'-DDE, p,p'-DDT, o,p'-DDT, p,p'-DDD and o,p'-DDD), Σ Chlordane (oxychlordane, α-chlordane, γ-chlordane, α-chlordene, γ-chlordene and *trans*-nonachlor), Σ HCH (α-HCH, β-HCH, δ-HCH, γ-HCH), mirex, HCB and dieldrin in muscle, liver, gonad and blubber of Tuna are reported in table 3. The lipid contents were quite variable, ranging from 2.86 % in muscle samples to 90.56 % for blubber samples. Liver and gonad present intermediate values (11.17 % and 8.47 %, respectively).
Differences in the lipid content of the tissues and organs into this marine predator may be an important variable governing contaminant concentrations.
In fact, the level of contamination observed in tissues and organs analysed is related to the lipid content of tissue. In fact, the values of contamination decrease with the same trend from blubber to muscle.
The levels of contamination decreased in the order Σ PCBs > ΣDDTs > Σ Chlordane in all tissues investigated.

The PCBs were detected in all individual Tuna fishes. The mean level of Σ PCBs was highest in blubber (2860.95 ng/g wet weight), followed in decreasing order by liver (262.46 ng/g wet weight), gonad (174.29 ng/g wet weight) and muscle (74.91 ng/g wet weight).

The relative percentages of the main congeners of PCBs in different tissue are shown in figure 1. No significant differences were observed in different tissues. PCB profiles were dominated by congeners 138, 153, 118, 170, 180, 183 and 187. The first two are the most abundant up to 26.4 % and 25.4 % respectivaly of $\sum$ PCBs in liver samples.
As shown in figure 1, the major congeners were penta -, hexa – and heptachlorobiphenyls, including the highly persistent and hydrophobic congeners 138 and 153.
Several highly chlorinated congeners, including 185, 189, 194 and 206 were consistently near detection limits in these samples.
The mean concentration of this study is similar to that found in Tuna's liver from Catalan mediterranean coast (Porte and Albaiges 1993) whereas the mean concentration of PCBs detected in muscle was higher than the values found by Porte and Albaiges (1993).

$\sum$ DDT were the second – most prevalent group of contaminats and were detected in all tissues of all tuna fishes. The highest concentration was again found in blubber (2728.51 ng/g wet weight), followed by liver (178.11 ng/g wet weight), gonad (142.53 ng/g wet weight) and muscle (52.04 ng/g wet weight).
Levels of DDTs in blubber were markedly lower than those found in blubber of Risso' Dolphin from Mediterranean Sea (7493 ng/g wet weight) by Storelli and Marcotrigiani (2000) and by Tanabe et al. (1993) from the Bay of Bengal, South India. The level found in this tropical area is up to 35000 ng/g wet weight; this trend highlights the actual agricultural usage of technical DDT in India.
Figure 2 reports the relative percentages of the isomers of DDT and its metabolites. Analysis of DDTs in Tuna samples shows that p, p'- DDE was present in the greatest concentrations, making up > 85 % of total DDT in the majority of samples. The observed DDT component pattern in most samples was p, p'- DDE >> p, p'- DDT $\approx$ p, p'- DDD. The same trend is observed for o, p' – DDT and its metabolites.
This pattern is similar to that observed in most top predators by Law et al. (2001) of the coast of England and Wales.

Mean concentrations of $\sum$ Chlordane and other OC pesticides found in this study were significantly lower in liver and muscle samples in comparison with an other top predator like the beluga whale from Hendrickson Island, Artic coast (Metcalfe et al. 1999). It is probably that the chlordane profile reported for artic predator is indicative of consistent inputs of atmospheric contaminants.
The differnces in patterns of persistent organic contaminants in muscle, liver, gonad and blubber may reflect differences in the contaminant metabolism, the content and composition of lipids, or the degree of blood perfusion in the various tissues and organs.

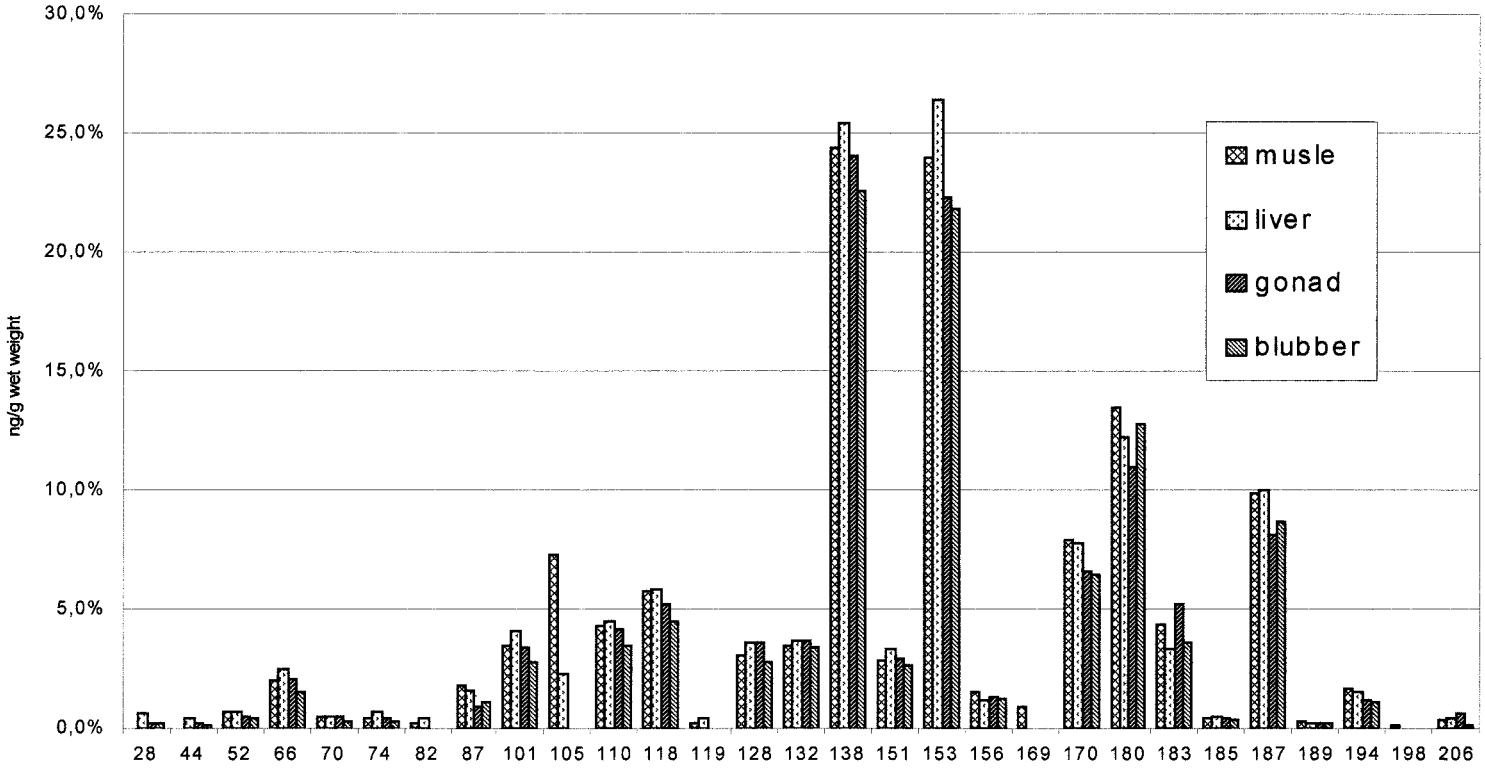

Figure 1. Relative percentages of each congener to the total PCBs found in some tissues of the Tuna

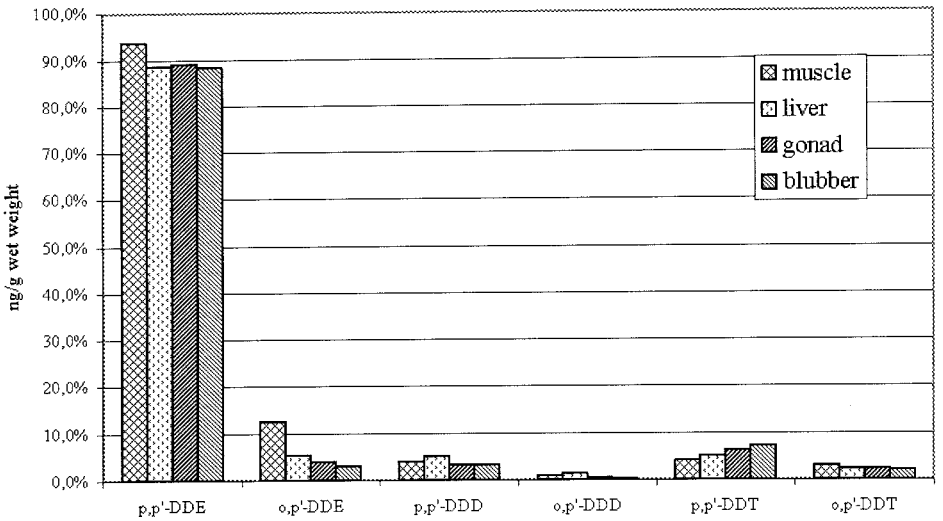

Figure 2. Relative percentages of DDT and its metabolites to the total DDT found in some tissues of tuna

Table 3. Mean and range of concentration levels of PCBs, HCHs, DDTs, Chlordanes on wet weight basis (ng/g) in muscle, liver, gonad and blubber tissues of Tuna fishes.

	Muscle	Liver	Gonad	Blubber
n. analysed sample	24	24	24	10
Lipid %	2.86	11.17	8.47	90.56
ΣPCBs	74.91 (21.39 – 324.54)	262.46 (13.15 – 602.88)	174.29 (9.12 – 539.67)	2860.95 (1520.85 – 5409.15)
ΣDDTs	52.04 (0.95 – 299.58)	178.11 (12.33 – 382.36)	142.53 (2.34 – 404.349)	2728.51 (1576.33 – 4707.76)
ΣHCHs	0.48 (0.06 – 2.38)	0.59 (0.07 – 1.01)	0.36 (0.08 – 1.24)	2.29 (1.88 – 6.53)
ΣChlordane	1.37 (0.30 – 4.7)	4.80 (0.99 – 32.5)	1.85 (0.12 – 4.83)	36.79 (25.05 – 49.97)
HCB	0.22 (<0.01 – 0.92)	0.84 (<0.01 – 4.44)	0.81 (<0.01 – 4.42)	6.12 (<0.01 – 7.72)
Mirex	0.34 (<0.01 – 1.15)	1.03 (<0.01 – 3.41)	0.53 (<0.01 – 1.15)	9.22 (<0.01 – 15.19)
Dieldrin	0.39 (<0.01 – 0.97)	1.04 (<0.01 – 2.09)	0.10 (<0.01 – 0.27)	7.14 (<0.01 – 10.72)

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