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Levels and congener pattern of polychlorinated biphenyl and organochlorine pesticide residues in bluefin tuna (*Thunnus thynnus*) from the Straits of Messina (Sicily, Italy)

G. Di Bella [a,1], P. Licata [a,1], A. Bruzzese [b], C. Naccari [c], D. Trombetta [c], V. Lo Turco [b], G. Dugo [b], A. Richetti [a,1], F. Naccari [a,*]

[a] *Department of Veterinary Public Health, Faculty of Veterinary Medicine, University of Messina, Polo S.S. Annunziata, 98168 Messina, Italy*
[b] *Department of Organic and Biological Chemistry, Faculty of Science, University of Messina, Salita Sperone 31, 98166 Messina, Italy*
[c] *Department of Biologic-Pharmacy, Faculty of Farmacy, University of Messina, Italy*

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Abstract

The aim of this study is to assess the accumulation of OCs and PCBs in *Thunnus thynnus* and to elucidate the suitability of this species as a bioindicator for monitoring contaminations of these compounds in the marine ecosystems of the Straits of Messina. This investigation was conducted on fat, liver and muscle samples of 14 *T. thynnus* collected during April 2004.

Quantitative determination of OCs and PCBs in the various samples examined has been carried out using GC-ECD and GC-MS. The results obtained show the presence of low concentrations of *p,p′*-DDE and PCB congeners (138, 153 and 180) in all fat, liver and muscle samples caught in the Straits of Messina. Concentrations of *p,p′*-DDE and PCB congeners (138, 153 and 180) in all the samples examined were below MRLs (CE n. 97/41, 1999/65 and 1999/71).

Keywords: PCB; Organochlorine pesticide; *Thunnus thynnus*; Straits of Messina

1. Introduction

The Straits of Messina, which are 20 miles long and 2 to 5 miles wide, separate mainland Italy from the island of Sicily and connect the Tyrrhenian Sea with the Ionian Sea. The Straits of Messina are characterized by different water masses which present a complex distribution, as are neighbouring portions of the Ionian and Tyrrhenian Seas. Moreover, this area has very little industrial activity and a medium population density (Salvo et al., 1998).

Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCs) are ubiquitous contaminants in aquatic environments as a result of uncontrolled spillage, stream transport, surface runoff and atmospheric deposition. These compounds show a great chemical stability and persistence and their presence in the environment is a clear indication of anthropogenic pollution (Weatherley et al., 1997; Bayarry et al., 2001; Storelli and Macotrigiano, 2003; Licata et al., 2004). The massive and indiscriminate use of these xenobiotics for industrial and agricultural purposes has caused their widespread diffusion to all environmental compartments including a wide range of organisms such as plankton, fish, marine and land mammals and humans. The bioaccumulation of OCs is a complex phenomenon governed by either physico-chemical properties of these compounds or ecological and biological factors such as feeding behaviours, habit, age, sex, state of health as well as the lipid composition of an animal's tissue or organs (Barlas, 1999; Storelli et al., 2004).

Although the production and use of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCs) have been restricted or banned in most industrialized nations,

* Corresponding author. Department of Veterinary Public Health, Section of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Messina, Polo S.S. Annunziata, 98168 Messina, Italy. Tel./fax: +39 90 3503781.
E-mail address: fnaccari@unime.it (F. Naccari).
[1] Tel./fax: +39 90 3503781.

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Table 1
Biological data of adult *Thunnus thynnus* from the Straits of Messina

Sample code	Sex	Length (cm)	Weight (kg)
1	M	162	55
2	F	235	167
3	F	179	71
4	M	169	64
5	F	162	50
6	M	195	95
7	F	166	75
8	F	194	115
9	F	167	78
10	M	170	80
11	M	189	101
12	M	165	64
13	M	188	100
14	F	167	78

considerable amounts of these persistent compounds are still circulating in the ecosphere (Minh et al., 2000). The solubility in fat and persistence of these compounds contribute to their bioaccumulation and biomagnification in the food chain. In particular, those marine organisms which are top predators of the marine food network accumulate significant amounts of organochlorine because of their longlife-span, to their low biodegradation capacity and the presence of lipid-rich blubber (Tanabe et al., 1987; Kannan et al., 1993a,b, 1994; Colborn and Smolen, 1996; Ueno et al., 2002).

Several studies have shown that these compounds exert various types of toxicity: reproductive deficits, teratogenicity, endocrine toxicity and carcinogenity/tumor promotion (Brown et al., 1991; Ahlborg et al., 1994).

The liver is recognized as the organ where contaminants tend to concentrate, reflecting a short term exposure to pollutants. Moreover, the liver plays an important role in distribution and detoxification or transformation of xenobiotics, and constitutes an important site of pathological effects induced by these contaminants (Evans et al., 1993; Storelli et al., 2004). In our previous studies we investigated the presence of organochlorine pesticides and PCBs in fish living in the Lake of Ganzirri and Straits of Messina using the mullet *Liza aurata* as a biological indicator. No appreciable residues of OCs and PCBs were found in the various samples (muscle and gills) of *L. aurata* (Licata et al., 2003).

The objectives of this study are to assess the accumulation of OCs and PCBs in bluefin tuna (*Thunnus thynnus*) and to elucidate the suitability of this specie as a bioindicator for monitoring contaminations of these compounds in the marine ecosystems of the Straits of Messina (Ostapczuk et al., 1997; Inagaki et al., 2001).

This research is part of a monitoring program focused on persistent organic pollution in several edible marine species from the Straits of Messina.

Table 2
Congener compositions of PCBs

PCBs	Structure	Name
PCB-28	$C_{12}H_7Cl_3$	2.4.4′-trichlorobiphenyl
PCB-52	$C_{12}H_6Cl_4$	2.2′.5.5′-tetrachlorobiphenyl
PCB-101	$C_{12}H_5Cl_5$	2,2′,4,5,5′-pentachlorobiphenyl
PCB-118	$C_{12}H_5Cl_5$	2.3′.4.4′.5-pentachlorobiphenyl
PCB-138	$C_{12}H_4Cl_6$	2.2′.3.4.4′.5′-hexachlorobiphenyl
PCB-153	$C_{12}H_4Cl_6$	2.2′.4.4′.5.5′-hexachlorobiphenyl
PCB-180	$C_{12}H_3Cl_7$	2.2′.3.4.4′.5.5′-heptachlorobiphenyl

Table 3
Detection limits of OC pesticides and PCB congeners

OC pesticides	Detection limits (μg/g)	PCBs	Detection limits (μg/g)
α-BHC	0.5	PCB-28	0.219
β-BHC	1.0	PCB-52	0.235
γ-BHC	0.5	PCB-101	0.172
Heptachlor	0.5	PCB-118	0.178
Aldrin	0.5	PCB-138	0.167
Heptac.epoxide	0.5	PCB-153	0.097
Dieldrin	1.0	PCB-180	0.170
4,4′-DDE	1.0		
2,4′-DDD	1.5		
Endrin	1.5		
4,4′-DDD	1.5		
2,4′-DDT	1.5		
4,4′-DDT	1.5		

2. Materials and methods

2.1. Sampling

The present investigation was conducted on bluefin tuna (*T. thynnus*), collected during April 2004 from the Straits of Messina. The study was carried out on several samples (fat, liver and muscle) of 14 bluefin tuna, of different age and sex: 7 females between 162 and 235 cm long and weighing between 50 and 190 kg, 7 males between 162 and 195 cm long and weighing 55–101 kg (Table 1). Samples were stored at − 20 °C under nitrogen until analysis.

Table 4
Retention times and characteristic fragments of OC pesticides and PCB congeners

OC pesticides	Retention times (min)	*m/z*	PCBs	Retention times (min)	*m/z*
α-BHC	17,086	219,183,217,181	PCB-28	21,654	258,256,186
β-BHC	18,663	181,183,219,217	PCB-52	25,259	292,290,220
γ-BHC	19,149	181,183,217,219	PCB-101	32,751	326,254,324
Heptachlor	24,247	100,270,272	PCB-118	34,678	254,324,326,328
Aldrin	26,889	265,261,263,66	PCB-138	42,777	360,362,290,358
Heptac. epoxide	30,125	357,355,353,81	PCB-153	39,444	360,362,290,358
Dieldrin	35,285	79,263	PCB-180	48,633	394,324,254
4,4′-DDE	35,541	318,316,248,246			
2,4′-DDD	36,196	235,237,165,199			
Endrin	37,035	243,263,265			
4,4′-DDD	39,099	235,237,165,199			
2,4′-DDT	39,388	235,237,165,199			
4,4′-DDT	42,813	235,237,165,199			

Table 5
Mean values±S.D. and relative standard deviation of the spike recoveries of OC pesticides

OC pesticides	mv±S.D. %	RSD %
α-BHC	95.00±3.1	3.3
β-BHC	96.70±2.8	2.9
γ-BHC	93.50±3.9	1.9
Heptachlor	98.65±3.6	3.7
Aldrin	96.00±4.0	3.9
Heptac.epoxide	95.98±2.9	4.1
Dieldrin	97.40±3.2	2.7
4,4′-DDE	92.75±1.9	2.6
2,4′-DDD	99.00±5.0	3.8
Endrin	97.50±2.9	2.0
4,4′-DDD	94.90±3.7	2.9
2,4′-DDT	94.60±3.0	3.4
4,4′-DDT	97.30±2.1	2.8

2.2. Determination of organochlorine pesticides and polychlorinated biphenyls

The extraction and the clean-up procedures of the samples were carried out using the procedures of Di Bella et al. (1986) and Giuffrida et al. (1994).

Following the Ministry of Health guideline seven congeners of PCB were determined on the basis of the representative standard of the compounds compared to the total profile of contamination by polychlorinated biphenyls. The seven congeners studied were: PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153, PCB-180.

2.3. Reagents and chemicals

- *N*-hexane for pesticides Carlo Erba reagent, Rodano (Mi),
- silica gel MERCK, Darmstad Germany, kieselgel 60 (0.063–0.200 mn) activated at 130 °C for 8 h and then deactivated with 25% of bi-distilled water,
- standard mix of pesticides SUPELCO (Bellefonte, USA) made by: α-BHC 25 ng/ml, β-BHC 100 ng/ml, γ-BHC 25 ng/ml, heptachlor 25 ng/ml, aldrin 50 ng/ml, heptachlor epox. 80 ng/ml, dieldrin 120 ng/ml, *p*,*p*′-DDE 100 ng/ml, *o*,*p*′-DDD 200 ng/ml, endrin 200 ng/ml, *p*,*p*′-DDD 190 ng/ml, *o*,*p*′-DDT 225 ng/ml, *p*,*p*′-DDT 260 ng/ml,
- seven congeners of PCB (Dr. Ehrenstorfer, Augsburg Germany): PCB-28; PCB-52; PCB-101; PCB-118; PCB-138; PCB-153; PCB-180, as reported in Table 2.

2.4. Gas chromatography-electron capture detector analysis

The extract of each sample, obtained using this procedure, was dried by means of a flow of inert gas (N_2) and, after addition of 0.2 ml of the internal standard (bromophos-methyl) at a concentration of 500 μg/ml, the extract was subjected to GC-ECD analysis using a 17-A gas chromatograph bitted with electron capture detector and Restek RTX-5 (30 m×0.32 mm i.d.) in the splitless mode; helium was used as a carrier gas at a constant flow rate of 36 cm/s.

The injector temperature was maintained at 250 °C. The temperature of the detectors was 280 °C. The column oven was temperature programmed from an initial value of 50 °C (2 min hold) to 150 °C at a rate of 25 °C/min and then to 270 °C at 4 °C/min (20 min hold).

Quantitative evaluation of the organochlorine residues was performed by the internal standard method (bromophos-methyl) and the calibration curves were obtained by comparing the value of the areas of the specific peaks with their concentration. Good laboratory practice (GLP) was applied throughout and procedural blanks (without the addition of sample) were analyzed to exclude any risk of interference in the gaschromatography plots. Congeners of PCB were evaluated with the same analysis. The detection limits for organochlorine pesticides and for PCB determined according to regulations fixed by the Council of Europe Pharmacopoeia are reported in Table 3.

Table 6
Total mean±S.D. and relative standard deviation (RSD) of the spike recoveries of PCB congeners

PCBs	mv±S.D. %	RSD %
PCB-28	96.5±3.1	3.21
PCB-52	95.4±2.8	2.93
PCB-101	97.8±2.7	2.76
PCB-118	96.9±2.8	2.88
PCB-138	98.2±2.1	2.14
PCB-153	95.6±3.2	3.35
PCB-180	93.5±3.4	3.64

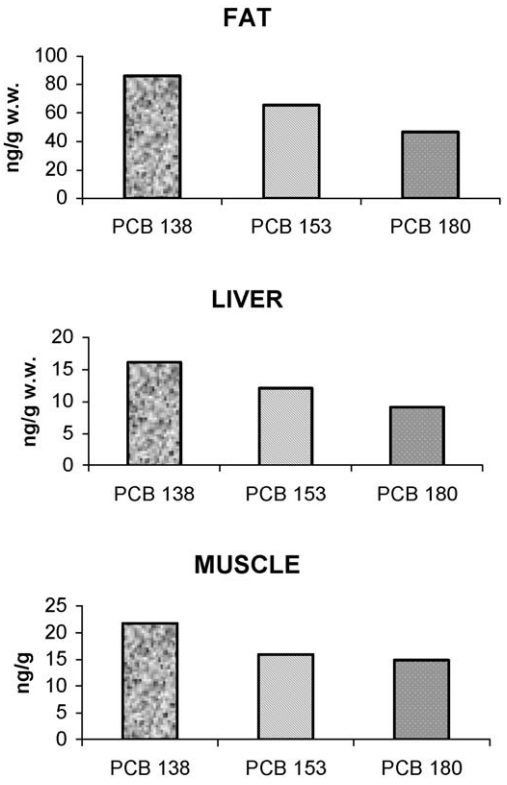

Fig. 1. Mean concentrations of PCB congeners in fat, liver and muscle samples (ng/g wet wt.) of *Thunnus thynnus* from the Straits of Messina.

2.5. Gas Chromatography–Mass Spectrometry (GC-MS)

Confirmation of residues was performed by GC-MS using a Shimadzu QP5050 and SPB-5MS (5% biphenyl–95% methyl polysiloxane) (30 m×0.25 mm; 0.25 μm film thickness); the pressure at the head of the column was 72.1 KpA; helium was used as a carrier gas at a rate of 30 cm/s. The injector temperature was maintained at 250 °C; the temperature of interface was 230 °C. The column oven was temperature programmed from an initial value of 50 °C to 150 °C at a rate of 25 °C/min and then to 220 °C (5 min hold) at a rate of 2 °C/min and then to 270 °C (10 min hold) at a rate of 4 °C/min. The electronic impact (EI) source was 70 eV, the acquisition of spectra was performed in SIM analysis.

Retention times and selected ions for organochlorine pesticides and for each PCB are reported in Table 4. The detection limit values were determined according to regulations fixed by the Council of Europe Pharmacopoeia.

Table 7
Concentration of PCBs congeners in fat, liver and muscle samples of *Thunnus thynnus* from the Straits of Messina

Sample	PCBs						
	Congeners	Fat		Liver		Muscle	
		Wet weight (ng/g)	Lipid weight (μg/g)	Wet weight (ng/g)	Lipid weight (μg/g)	Wet weight (ng/g)	Lipid weight (μg/g)
1	138	93.1	0.832	3.5	0.508	nd	nd
	153	43.1	0.385	1.9	0.278		
	180	35.1	0.314	1.0	0.139		
2	138	43.6	0.597	6.9	0.501	nd	nd
	153	39.6	0.542	5.7	0.412		
	180	23.2	0.318	4.3	0.307		
3		nd	nd	nd	nd	nd	nd
4	138	42.3	0.248	2.8	0.264	nd	nd
	153	35.1	0.206	2.3	0.210		
	180	25.8	0.151	1.8	0.169		
5	153	nd	nd	3.5	0.712	nd	nd
	180			2.6	0.519		
6	138	40.6	0.221	3.8	3.792	26.9	0.982
	153	223.0	1.215	3.2	3.206	21.3	0.777
	180	163.00	0.885	2.1	2.142	15.5	0.565
7	138	90.0	0.401	6.5	0.498	1.4	0.224
	153	65.7	0.293	5.2	0.399	2.7	0.436
	180	41.9	0.187	2.9	0.227	1.3	0.208
8	138	54.4	0.129	12.8	0.348	16.5	0.640
	153	41.6	0.0986	11.4	0.309	14.2	0.550
	180	33.2	0.0786	10.4	0.283	11.6	0.451
9	138	78.5	0.201	17.0	0.633	14.6	0.637
	153	48.8	0.125	10.8	0.403	7.7	0.337
	180	38.9	0.0996	10.3	0.384	9.7	0.423
10	138	33.2	0.908	4.9	0.447	nd	nd
	153	16.6	0.455	2.9	0.268	nd	nd
	180	19.1	0.524	2.5	0.227	nd	nd
11	138	257.0	0.408	70.7	1.554	11.9	0.472
	153	206.0	0.327	72.2	1.586	9.9	0.390
	180	200.0	0.318	48.4	1.063	9.7	0.381
12	138	19.0	0.324	29.9	1.213	1.8	0.305
	153	15.1	0.257	17.4	0.706	2.1	0.348
	180	11.6	0.197	14.3	0.580	1.5	0.256
13	138	6.7	0.0399	19.9	0.108	25.1	0.981
	153	48.4	0.289	9.1	0.0494	15.6	0.609
	180	35.9	0.214	nd	nd	11.2	0.437
14	138	91.5	0.268	14.0	0.309	75.9	0.514
	153	62.5	0.183	10.9	0.277	54.1	0.366
	180	47.8	0.140	8.9	0.196	58.5	0.396

nd = non-detectable <= detection limits.

2.6. Accuracy, recovery test and repeatability

The accuracy and repeatability of the method were assessed by performing a spike-and-recovery test on certified standard reference materials of cod-liver oil

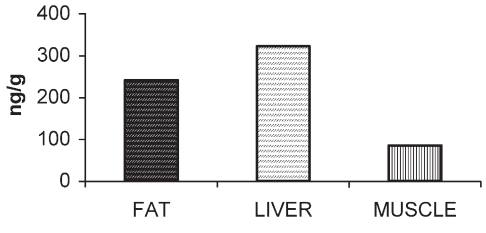

Fig. 2. Mean concentrations of OC pesticides in fat, liver and muscle samples (ng/g wet wt.) of *Thunnus thynnus* from the Straits of Messina.

Table 8
Concentration of OC pesticides in fat, liver and muscle samples of *Thunnus thynnus* from the Straits of Messina

Sample	OCs						
	Metabolites	Fat		Liver		Muscle	
		Wet weight (ng/g)	Lipid weight (μg/g)	Wet weight (ng/g)	Lipid weight (μg/g)	Wet weight (ng/g)	Lipid weight (μg/g)
1	4,4′-DDE	104.0	0.929	2.7	0.388	nd	nd
2	4,4′-DDE	64.1	0.878	10.2	0.733	nd	nd
3	4,4′-DDE	nd	nd	nd	nd	nd	nd
4	4,4′-DDE	58.7	0.344	6.2	0.569	nd	nd
5	4,4′-DDE	nd	nd	3.8	0762	nd	nd
6	4,4′-DDE	399.0	1.446	4.5	4.558	56.9	2.070
7	4,4′-DDE	493.0	2.195	14.1	4.086	5.8	0.952
8	4,4′-DDE	288.0	0.684	57.4	1.559	72.7	3.050
9	4,4′-DDE	148.0	0.381	34.9	1.300	23.9	1.036
10	4,4′-DDE	98.6	2.70	12.4	1.131	5.3	0.106
11	4,4′-DDE	218.0	0.347	11.0	0.242	7.3	0.280
12	4,4′-DDE	31.7	0.540	33.3	1.347	3.5	0.592
13	4,4′-DDE	334.0	1.995	56.2	0.302	72.6	2.830
14	4,4′-DDE	659.0	1.932	3944.0	0.840	515.0	3.480

nd = non-detectable <= detection limits.

(Sigma-Aldrich). Unfortunately, it is not possible to obtain totally residue-free matrix; consequently the blank sample containing *p*,*p*′-DDE 610±40 μg/kg; *o*, *p*′-DDE 30±4 μg/kg, was fortified with researched pesticide solutions at concentrations of 500 μg/kg. The fortified sample was extracted after 30 min as described before. Spike recoveries were repeated three times. Results, expressed as mean±standard deviation, and the total RSD of the spike recoveries of pesticides found are reported in Table 5.

Value of RSD below 3.4% for all pesticides reveal the good repeatability of the method; values of the recoveries always above 94% show the accuracy of the method used.

Spike recoveries were also determined for polychlorinated biphenyl congeners. A certified matrix of cod-liver oil (by Sigma-Aldrich) was utilized at the same way. To confirm the results obtained for PCB the same test was performed for polychlorinated biphenyl congeners. The matrix contained the following congeners: 1) PCB-28 (68±4.0 μg/kg), 2) PCB-52 (149±20 μg/kg), 3) PCB-101 (370±17 μg/kg), 4) PCB-138 (without contaminant), 5) PCB-153 (938±40 μg/kg), 6) PCB-180 (280±22 μg/kg).

The mean values±standard deviation and the total RSD of spike recoveries of PCB are shown in Table 6. The RSD value below 3.63% for all congeners shows the good repeatability of the method used. Recovery value always above 93.4% shows the accuracy of analysis.

3. Results

Levels and congener patterns of polychlorinated biphenyl in various samples (fat, liver and muscle) of bluefin tuna (*T. thynnus*) from the Straits of Messina are shown in Fig. 1 and Table 7. Among the PCB congeners only the 138, 153 and 180 congeners were found. In particular, the ranges of PCB concentrations in different samples were 6.7–257 ng/g w.w. in fat, 1.0–72 ng/g w.w. in liver and 1.27–75 ng/g w.w. in muscle respectively. The determination of organochlorine pesticides in the same samples (fat, liver and muscle) of bluefin tuna show the presence of *p*,*p*′-DDE only in all the samples analyzed (Fig. 2 and Table 8). In particular, the range of *p*,*p*′-DDE concentrations were 31.73–659 ng/g w.w. in fat, 2.68–3944 ng/g w.w. in liver and 3.53–515 ng/g w.w respectively. Moreover, the concentrations of PCBs and organochlorine pesticides showed that the residual levels in muscle samples were always below the MRLs fixed by CE (Decreto del Ministero della Sanità Gazzetta Ufficiale n.115 del 19/05/2000).

4. Discussion

The results obtained show the presence of low concentrations of organochlorine pesticides and PCBs in various organs (fat, liver and muscle) in bluefin tuna (*T. thynnus*) caught in the Straits of Messina.

Regarding the content of the organochlorine pesticides only DDT metabolites (*p*,*p*′-DDE) were found in all samples of liver, fat and muscle respectively. The presence of this metabolite could be correlated to a previous use of DDT in agricultural activity, to high environmental persistence because of their chemical and thermal stability, to different climatic environmental conditions, to marine currents, to different migratory habits of aquatic organisms and to different feeding habits. The low residual levels of metabolites of DDT in muscle at concentrations below CE MRLs (CE n. 97/41, 1999/65 and 1999/71) indicate a situation without toxicological risks for animals and for the consumer of fish. However, the problem of human impact connected to use of these compounds cannot be ignored. The use of OCs in specific areas does not reduce the environmental contamination risks because there is evidence that the climatic and environmental regional conditions can have varying effects on the lifetime and the concentration of these compounds. In fact, DDT use has been prohibited for a very long time, but its low biodegradability and its high liposolubility make it easily accumulated in lipid tissue (WHO, 1993; Gauthier et al., 1997; Nakata et al., 1998; Licata et al., 2004).

Regarding the content of PCBs in all samples of fat, liver and muscle congeners 138, 153 and 180 were also found at concentrations below MRLs (CE n. 97/41, 1999/65 and 1999/71). Specifically, congener 138 had the highest concentrations and congener 180 had the lowest concentrations in fat followed by liver and muscle; congener 153 was present at intermediate levels in fat followed by muscle and liver.

Differences in sample biological parameters such as age, sex and sampling location and the selection of congeners of PCB quantification may account for some of the differences observed.

PCBs 138, 153 and 180 represent prevalent congeners in various marine organisms from the Adriatic Sea (Nakata et al., 1997a,b; Corsolini et al., 1995; Storelli et al., 2004). The accumulation of PCB in different compartments of an aquatic systems is based primarily on physico-chemical mechanisms, but also from biological mechanisms (feeding behaviour, position of the organisms in the food chain, etc.) that may mediate the transfer of these toxic chemicals as well as habitat constitute (Salvo et al., 1998; Capuano et al., 1999; Inagaki et al., 2001). In conclusion, these results suggest that tuna continue to be exposed to OCs and PCBs and show that bluefin tuna is a suitable bioindicator for the monitoring of these contaminants in the open ecosystem of the Mediterranean Sea.

However, particular care should be taken when applying the toxicological profiles obtained from one species to another species, because it is generally known that the degree of tolerance for toxic compounds is strictly species-dependent. This observation implies the need for future studies on bioaccumulation issues of these toxic compounds also in other marine species.

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