Journal of Food Protection, Vol. 70, No. 3, 2007, Pages 706–715

Polychlorinated Biphenyls and Organochlorine Pesticides in Seafood from the Gulf of Naples (Italy)

MARIA CARMELA FERRANTE,[1]* TERESA CIRILLO,[2] BARBARA NASO,[1] MARIA TERESA CLAUSI,[1] ANTONIA LUCISANO,[1] AND RENATA AMODIO COCCHIERI[2]

[1]*Department of Pathology and Animal Health and* [2]*Department of Food Sciences, University of Naples Federico II, Naples, Italy*

MS 06-223: Received 18 April 2006/Accepted 28 September 2006

ABSTRACT

Seven target polychlorinated biphenyls (PCBs; IUPAC nos. 28, 52, 101, 118, 138, 153, and 180) and the organochlorine pesticides (OCPs) hexachlorobenzene (HCB) and dichlorodiphenyltrichloroethane (DDT) and its related metabolites (*p,p′*-DDT, *p,p′*-DDE, and *p,p′*-DDD) were quantified in edible tissues from seven marine species (European hake, red mullet, blue whiting, Atlantic mackerel, blue and red shrimp, European flying squid, and Mediterranean mussel) from the Gulf of Naples in the southern Tyrrhenian Sea (Italy). PCBs 118, 138, and 153 were the dominant congeners in all the species examined. The concentrations of all PCBs (from not detectable to 15,427 ng g^{-1} fat weight) exceeded those of all the DDTs (from not detectable to 1,769 ng g^{-1} fat weight) and HCB (not detectable to 150.60 ng g^{-1} fat weight) in the samples analyzed. The OCP concentrations were below the maximum residue limits established for fish and aquatic products by the Decreto Ministerale 13 May 2005 in all the samples analyzed; therefore the OCPs in the southern Tyrrhenian Sea species are unlikely to be a significant health hazard. Conversely, the mean concentrations of PCBs exceeded (greatly in some cases) the current limits (200 ng^{-1} fat weight) set by the European Union for terrestrial foods. Although the manufacture and use of PCBs are banned or highly restricted, these compounds still are important persistent chemical contaminants in the Gulf of Naples.

Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are chemicals that have been industrially produced and employed on a large scale for many years. Because of high persistence and semivolatility, organochlorine compounds can be transported through the atmosphere or by water across ecosystems. Thus, these compounds enter all the environmental compartments, sometimes contaminating areas far from the original site. They are widely distributed in surface waters, where they tend to accumulate in sediments and aquatic organisms because of their lipid solubility.

To limit their impact on environment, organisms, and humans, PCBs and OCPs have been restricted or banned in many countries, yet various problems related to their high rate of diffusion persist *(18, 37).* Severe injuries to the nervous system and to endocrine reproductive and immune systems have been correlated with chronic exposure to PCBs and OCPs *(17, 21, 31, 33).*

In humans, these pollutants can be absorbed through skin and by respiration. However, more than 90% of daily accumulation comes from diet, which in turn is mainly attributable (90%) to food of animal origin *(15).* In particular, fish consumption is considered a main route of exposure for humans *(1, 19, 35).*

PCB and OCP plasma concentrations in major fish consumers are significantly higher than those detected in people eating less fish. In epidemiological studies, the presence of high concentrations of organochlorines in seafood has been correlated with toxic effects attributable to chronic exposure, such as altered thyroid function and memory and learning impairments associated with PCB exposure during adulthood and fetal development. A correlation has been suggested between the ingestion of PCB-contaminated fatty fish and the increase in the incidence of low birth weight *(27, 29).*

In recent years, public attention has focused on food quality, including organoleptic, nutritional, and dietetic characteristics of foods and the hygienic and healthy factors such as the lack of xenobiotic contamination. Fish quality depends as much on contamination of the aquatic environment as on freshness. Monitoring of food chains to identify and quantify pollutants over time in different environmental compartments is now considered an important factor for increasing food safety *(10).*

Although PCBs and OCPs have been banned in the European Union, contamination can come from developing coastal Mediterranean countries, where these chemicals are still used. The Mediterranean Sea is a closed water body where the dilution, dispersion, and decaying hydrodynamic processes are slower than those in the oceans. This condition favors a longer persistence of pollutants at high concentrations and a great availability for the different levels of aquatic biocenosis. The few studies carried out on the coastal areas of the Mediterranean Sea have revealed high pollution levels in fish, indicating a potential risk for consumers *(3, 13, 14, 28).* These studies have mostly been carried out on the Adriatic Sea; few data are available on PCB and OCP contamination in marine organisms from the Tyrrhenian Sea *(26).*

* Author for correspondence. Tel: 39-81-297820; Fax: 39-81-297122; E-mail: ferrante@unina.it.

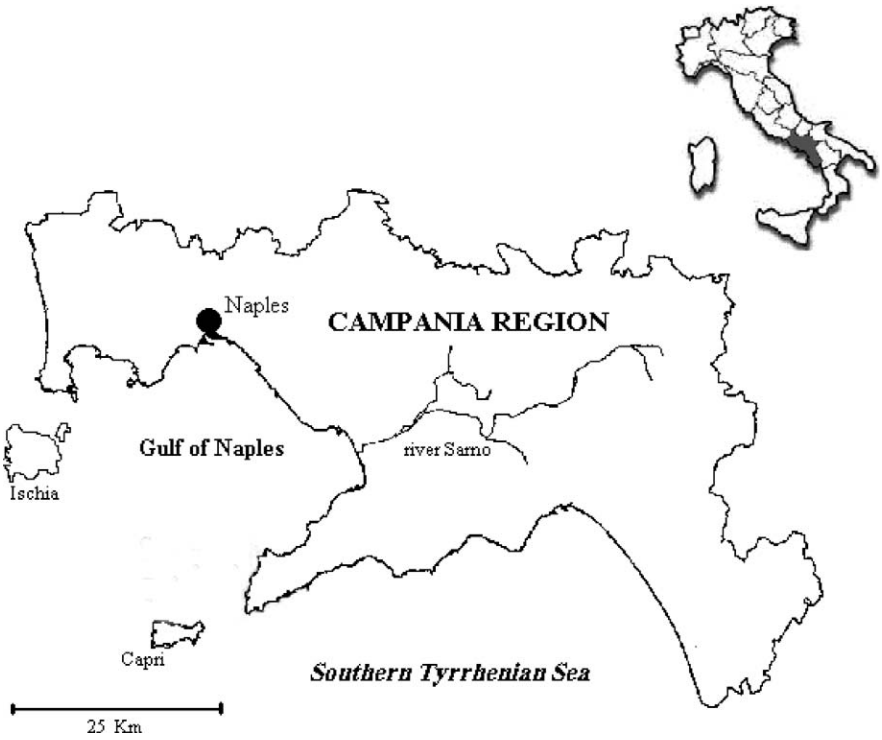

FIGURE 1. *Map of Campania region.*

The aims of this study were to evaluate the organochlorine contamination of seafood caught in the southern Tyrrhenian Sea in the Gulf of Naples (Italy). The distribution profiles of individual pollutants were investigated to assess the potential risks for consumers. A statistical comparison between organochlorine concentrations detected in this study and those previously found by Naso et al. *(26)* was performed to evaluate temporal trends.

MATERIALS AND METHODS

Sampling. Edible fish, cephalopods, and crustaceans caught in the Tyrrhenian Sea up to 40 miles (64.5 km) off the coast of Italy in the Gulf of Naples were collected from October 2003 to December 2004 by professional fishermen. Bivalves were sampled in sea farms in the Campi Flegrei Bay at about 50 m from the coast (Fig. 1). These marine organisms, selected for their abundance and wide distribution and their availability throughout the year, are a common and inexpensive food for people in southern Italy. The species considered were European hake (*Merluccius merluccius*), red mullet (*Mullus barbatus*), blue whiting (*Micromesistius poutassou*), Atlantic mackerel (*Scomber scombrus*), blue and red shrimp (*Aristeus antennatus*), European flying squid (*Todarodes sagittatus*), and Mediterranean mussel (*Mytilus galloprovincialis*). The specimens, all of commercial size, were wrapped in aluminum foil, immediately refrigerated, and transported to the laboratory.

Analytical sample preparation. Length and the weight for each specimen were measured and recorded. Fish, cephalopods, and crustaceans were dissected, and the edible parts from specimens of almost the same size were pooled and homogenized to obtain about 300 g for a single sample. The lengths of the mussel shells were measured, shells were opened, and the soft tissues were removed, pooled (20 specimens per pool), and homogenized. All the homogenized samples were stored at −20°C until chemical analysis.

Chemical analysis. The concentrations of PCBs (IUPAC nos. 28, 52, 101, 118, 153, 138, and 180), hexachlorobenzene (HCB), and OCPs *p,p′*-DDT, *p,p′*-DDE, and *p,p′*-DDD were determined for each sample by gas chromatography–electron capture detection (GC-ECD) and randomly confirmed by GC–mass spectrometry (MS). These seven PCBs are the target congeners recommended by the European Union as indicators of total PCB contamination. An 10-g aliquot of each sample was extracted with a 70:30 mixture of *n*-hexane and acetone with an accelerated solvent extractor (ASE 100, Dionex Corporation, Sunnyvale, Calif.) under the following conditions: oven temperature of 125°C, static time of 5 min, two static cycles, flush volume of 60% of extraction cell volume, and nitrogen purge at 1 Mpa for 60 s. The extract was passed through a glass tube packed with anhydrous sodium sulfate and evaporated to dryness at room temperature under a flow of N_2. The extracted lipid content was determined gravimetrically.

The separation of the analytes from the lipid fraction and the purification of the extracts were carried out according to the method described by Di Muccio et al. *(14)* and in the *ISTISAN Report (24),* with some modifications. Fifty milligrams of each dried lipid extract was resuspended in *n*-hexane, transferred to Extrelut-3/Extrelut-1 cartridges (Merck Kga A Darmstadt, Germany) with 0.36 g of C-18 Isolute (40 to 60 mesh, Merck), and eluted with acetonitrile. The extracts were concentrated to 1 ml under vacuum at 40°C, cleaned in a glass column containing 2.5 g of Florisil (60 to 100 mesh, Supelco, Bellefonte, Pa.), activated overnight at 130°C, and eluted three times with 10-ml aliquots of *n*-hexane collecting the eluates containing PCBs, HCB, and *p,p′*-DDE. The column was eluted with *n*-hexane-toluene (80:20, vol/vol) to collect the fraction containing *p,p′*-DDD and *p,p′*-DDT. The two fractions were concentrated to about 2 ml at room temperature under a flow of N_2. Internal standards were PCB 209 for the GC-ECD analysis and labeled PCB 180 ($^{13}C_{12}$, Dr. Ehrenstorfer GmbH, Augsburg, Germany) for the GC-MS analysis.

GC analyses of the PCBs and OCPs were carried out with

TABLE 1. *Common and taxonomic names, lipid content, length, and weight of the marine organisms analyzed*

Name			Measurements[a]		
Common	Taxonomic	*n*	Lipid content (%)	Length (cm)	Wt (g)
Red mullet	*Mullus barbatus*	14	1.71 ± 0.65 (0.50–3.00)	13.67 ± 0.62 (12.90–14.63)	38.62 ± 3.33 (35.00–42.40)
European hake	*Merluccius merluccius*	14	0.44 ± 0.19 (0.20–0.90)	21.76 ± 6.49 (10.00–31.50)	99.24 ± 62.86 (30.00–200.00)
Blue whiting	*Micromesistius potassou*	14	0.74 ± 0.23 (0.50–1.50)	18.87 ± 1.68 (17.00–22.50)	56.46 ± 7.36 (50.00–67.50)
Atlantic mackerel	*Scomber scombrus*	14	7.73 ± 7.85 (0.30–19.90)	25.21 ± 2.85 (22.50–27.00)	138.07 ± 43.84 (90.00–260.00)
Blue and red shrimp	*Aristeus antennatus*	14	0.49 ± 0.11 (0.30–0.70)	16.76 ± 1.42 (15.10–19.80)	28.12 ± 4.35 (22.80–34.89)
European flying squid	*Todarodes sagittatus*	14	0.62 ± 0.12 (0.50–0.80)	29.35 ± 5.33 (22.20–39.50)	91.83 ± 35.68 (27.50–127.50)
Mediterranean mussel	*Mytilus galloprovincialis*	14	0.71 ± 0.17 (0.50–0.90)	6.04 ± 0.26 (5.50–6.40)	18.29 ± 1.72 (16.40–21.20)

[a] Values are mean ± standard deviation (range).

an HRGC 5160 Mega Series chromatograph machine equipped with a ^{63}Ni ECD (Carlo Erba, Milan, Italy). The cold on-column mode was used for the injection. Two fused silica capillary columns of different polarities coated with a CP-SIL 5CB (25 m by 0.32 mm inside diameter, 0.25-μm film thickness; Varian Inc., Walton-on-Thames, UK) and Rtx-1701 (30 m by 0.32 mm inside diameter, 0.25-μm film thickness; Restek, Saunderton, UK) were used to separate and quantify the residues. The ECD was kept at 310°C. Hydrogen and nitrogen were used as the carrier gas and makeup gas, respectively. Organochlorines were identified by comparing the retention times on the two columns with those of the corresponding standard compounds and then quantified by comparing the individually resolved peak areas with those of the corresponding standards. The concentrations of resolved peaks from each of the seven individual PCB congeners were summed to obtain the congener sum PCB concentrations (ΣPCB). Dichlorodiphenyltrichloroethane (DDT) concentrations were calculated as the sum of *p,p′*-DDT, *p,p′*-DDE, and *p,p′*-DDD (ΣDDT). Detection limits for PCBs and OCPs ranged from 0.08 to 0.60 ng g^{-1} fat weight. Results were reported as not detectable when the concentrations were lower than the detection limits.

Pure reference standard solutions were used for instrument calibration, recovery determination, and quantification (Dr. Ehrenstorfer GmbH Labservice Analytica S.r.l. Anzola Emilia, Bologna, Italy). All the solvents were pesticide residue analysis grade (Pestiscan, Labscan, Dublin, Ireland). Prior to analysis of the fish samples, aliquots of commercial homogenized sole, used as blanks, were extracted and analyzed for cross-contamination. Some aliquots of the homogenized sole were spiked with standard mixtures at three concentrations, extracted, and analyzed in triplicate to evaluate the recovery system. The mean recovery for PCB standard spiked samples was 80% ± 7%, the mean recovery the OCPs was 90% ± 10%. The quality of the analytical data also was checked by replicate analysis of Certified Reference Material IAEA-406 (fish homogenate), which indicated that the compounds were quantified to within ±10% of their certified concentrations.

Statistical analysis. Statistical analysis was carried out on the lipid-normalized organochlorine concentrations. PCB and OCP concentrations were log transformed to approximate a normal distribution of the data. Organochlorine concentrations below the detection limits were replaced with half the value of the respective detection limit. Differences in concentrations of organochlorines among the individual marine species were evaluated by one-way analysis of variance (ANOVA). When significant differences were observed among the species, the Tukey-Kramer mul-

TABLE 2. *Percentage of samples with detectable PCB residues and the sum of the PCB concentrations on fat weight and wet weight basis*

Species	% of samples positive for PCBs	PCB (ng g^{-1})				
		Fat wt			Wet wt	
		Median	Mean ± SD (range)		Median	Mean ± SD (range)
Red mullet	100	1,472.70	1,910.20 ± 903.83	(1,105.60–3,750.17)	32.60	30.11 ± 12.01 (4.31–44.77)
European hake	100	3,182.80[a]	4,410.06 ± 3,828.37	(2,112.20–15,427.00)	14.15	16.80 ± 11.06 (4.09–46.28)
Blue whiting	100	1,286.90	1,673.28 ± 1,038.81	(282.42–3,627.09)	9.68	12.42 ± 8.12 (1.55–26.60)
Atlantic mackerel	100	396.93	1,425.99 ± 1,823.13	(64.40–5,648.15)	17.90	23.22 ± 18.25 (1.26–56.38)
Blue and red shrimp	78	207.25	358.22 ± 479.52	(ND[b]–484.93)	0.88	1.49 ± 1.87 (ND–7.07)
European flying squid	100	1,768.27	2,159.75 ± 1,512.01	(275.05–4,952.00)	15.67	15.45 ± 8.36 (1.54–29.79)
Mediterranean mussel	100	1,575.80	2,079.20 ± 2,153.88	(469.52–9,274.04)	12.23	12.33 ± 6.05 (3.38–28.11)

[a] Significantly different at $P < 0.001$ when compared with red mullet, blue whiting, Atlantic mackerel, Mediterranean mussel, and blue and red shrimp and at $P < 0.01$ when compared with European flying squid.

[b] ND, not detectable.

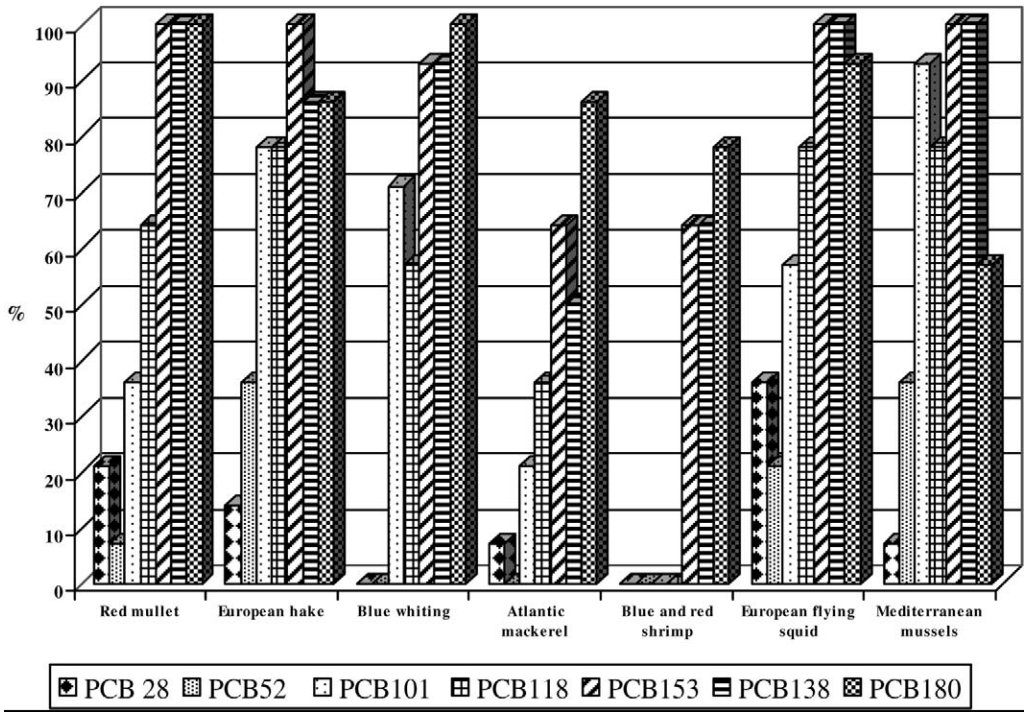

FIGURE 2. *Prevalence of contamination (percentage of samples) by PCB congeners in marine organisms analyzed.*

tiple comparisons test was applied to the ANOVA to determine which means were significantly different.

To compare results of the present study with those reported by Naso et al. *(26),* we performed two tests for differences between means. One test is valid mainly under the assumption that the variances of the two populations compared are equal (Student's *t* test with two tails); the other is based on the assumption of unequal variances (Welch approximate *t* test with two tails). Because we did not raise any valid argument to sustain either the assumption of equal variances or that of unequal variances, we tested for the difference between variances and reported the means difference tests accordingly. For all species compared, however, there is no ambiguity in our results. All analyses and calculations were performed with GraphPad InStat (GraphPad Software, San Diego, Calif.).

RESULTS AND DISCUSSION

The length and the weight of the specimens and their lipid contents are shown in Table 1.

To reduce interspecies variability and to allow a more accurate comparison of bioaccumulation between marine

TABLE 3. *Prevalence and concentrations of HCB and ΣDDT in edible tissue of some marine organisms*

	HCB (ng g^{-1})					ΣDDT (ng g^{-1})				
		Fat wt		Wet wt			Fat wt		Wet wt	
Species	%[a]	Median	Mean ± SD (range)	Median	Mean ± SD (range)	%[a]	Median	Median ± SD (range)	Median	Mean ± SD (range)
Red mullet	50	9.76	16.23 ± 14.25 (ND[b]–40.00)	ND	0.32 ± 0.41 (ND–1.20)	86	178.52	163.84 ± 115.29 (ND–318.00)	1.14	3.01 ± 2.92 (ND–8.80)
European hake	50	13.96	15.29 ± 12.66 (ND–28.80)	ND	ND (ND–0.12)	93	522.19	533.67 ± 470.02 (ND–1,769.00)	2.28	2.12 ± 1.67 (ND–5.31)
Blue whiting	57	27.10	18.73 ± 14.28 (ND–35.80)	0.19	0.12 ± 0.11 (ND–0.25)	93	167.47	195.79 ± 129.58 (ND–386.20)	1.21	1.38 ± 0.94 (ND–3.02)
Atlantic mackerel	64	18.70	16.20 ± 11.94 (ND–40.49)	1.34	1.66 ± 1.73 (ND–4.30)	21	7.40	34.15 ± 73.06 (ND–278.02)	ND	0.18 ± 0.45 (ND–1.67)
Blue and red shrimp	14	3.13	8.03 ± 7.37 (ND–23.40)	ND	ND (ND–0.12)	61	44.00	66.06 ± 61.14 (ND–201.64)	0.18	0.27 ± 0.29 (ND–1.02)
European flying squid	78	34.50	58.83 ± 51.96 (ND–150.60)	0.23	0.34 ± 0.33 (ND–0.90)	86	117.39	212.40 ± 237.44 (ND–775.40)	0.93	1.29 ± 1.41 (ND–4.65)
Mediterranean mussel	57	14.10	11.71 ± 8.53 (ND–28.13)	0.11	0.10 ± 0.88 (ND–0.20)	71	65.95	113.62 ± 116.91 (ND–342.60)	0.51	0.86 ± 1.01 (ND–2.78)

[a] Percentage of samples positive for HCB or ΣDDT.
[b] ND, not detectable (<0.08 ng g^{-1}).

TABLE 4. *Concentrations of DDT metabolites on fat weight and wet weight bases in edible tissues of the marine organisms*

DDT metabolite	Metabolite (ng g^{-1})[a]							
	European hake		Red mullet		Blue whiting		Atlantic mackerel	
	Fat wt	Wet wt	Fat wt	Wet wt	Fat wt	Wet wt	Fat wt	Wet wt
p,p′-DDT	ND (ND–125.60)	ND (ND–1.62)	104.97 (ND–406.80)	0.52 (ND–1.27)	26.19 (ND–199.40)	0.19 (ND–1.40)	ND (ND–36.23)	ND (ND–0.22)
p,p′-DDE	147.30 (ND–40.00)	1.97 (ND–6.53)	341.30 (ND–1,163.60)	1.42 (ND–3.54)	150.26 (ND–223.20)	1.11 (ND–1.79)	ND (ND–222.85)	ND (ND–1.34)
p,p′-DDD	ND (ND–75.60)	ND (ND–2.27)	ND (ND–198.60)	ND (ND–0.60)	ND (ND–39.78)	ND (ND–0.20)	ND (ND–18.94)	ND (ND–0.11)

[a] Values are median (range). ND, not detectable (<0.08 ng g^{-1} for *p,p′*-DDT and *p,p′*-DDE; <0.09 ng g^{-1} for *p,p′*-DDD).

species, the concentrations of pollutants were normalized to the fat content and were expressed in relation to wet weight.

PCBs were the most abundant pollutants followed by DDTs and HCB. The concentrations of ΣPCB expressed as nanograms per gram of fat weight (f.w.) and wet weight (w.w.) in the muscle of marine species are presented in Table 2. The order of pollution concentrations relative to f.w. was European hake > European flying squid > Mediterranean mussel > red mullet > blue whiting > Atlantic mackerel > blue and red shrimp, whereas on the basis of the w.w. it was red mullet > Atlantic mackerel > European flying squid > European hake > Mediterranean mussel > blue whiting > blue and red shrimp. PCBs were found in all the marine species at concentrations ranging from 64.40 to 15427.00 ng g^{-1} f.w. The only samples without PCBs were three samples of blue and red shrimp.

PCBs were largely made up of hepta-, hexa-, and pentachlorinated PCBs (nos. 118, 138, 153, and 180) in almost all the species analyzed (Fig. 2). These findings are not surprising considering that these congeners are the most abundant in commercial PCB mixtures, such as Aroclor 1254 and 1260, which are the most commonly used in European countries. These compounds have chlorine atoms at the 2, 4, or 5 position in one or both rings.

The concentrations of OCPs in the edible parts of marine species from the Gulf of Naples are shown in Table 3. DDT concentrations on a lipid basis had a trend similar to that of the PCB congeners. The most contaminated species were those at the top of the food chain. The concentration order on the basis of f.w. was European hake > red mullet > blue whiting > European flying squid > Mediterranean mussel > blue and red shrimp > Atlantic mackerel. The order on the basis of w.w. was European hake > blue whiting > red mullet > European flying squid > Mediterranean mussel > blue and red shrimp > Atlantic mackerel.

DDTs were found in 73% of the samples. DDT contamination levels were highly variable, ranging from not detectable to 1769.00 ng g^{-1} f.w. in the European hake. HCB was found in 53% of the samples at low concentrations ranging between not detectable and 150.60 ng g^{-1} f.w.; the European flying squid was the most contaminated marine organism among the other species (median of 34.50 ng g^{-1} f.w.).

p,p′ DDE was the most frequently detected OCP (91%

TABLE 5. *Concentrations of PCB congeners on fat weight and wet weight bases in edible tissues of marine organisms*

PCB	PCB (ng g^{-1})[a]							
	Red mullet		European hake		Blue whiting		Atlantic mackerel	
	Fat wt	Wet wt	Fat wt	Wet wt	Fat wt	Wet wt	Fat wt	Wet wt
28	ND (ND–59.00)	ND (ND–1.30)	ND (ND–36.80)	ND (ND–0.13)	ND	ND	ND (ND–24.40)	ND (ND–2.68)
52	ND (ND–33.60)	ND (ND–0.74)	ND (ND–123.00)	ND (ND–0.37)	ND	ND	ND	ND
101	ND (ND–56.00)	ND (ND–4.37)	152.90 (ND–1,629.40)	0.53 (ND–3.23)	74.50 (ND–232.57)	0.50 (ND–1.83)	ND (ND–208.33)	ND (ND–1.24)
118	153.30 (ND–428.56)	3.01 (ND–5.98)	427.90 (ND–3,301.20)	1.55 (1.66–9.90)	145.10 (ND–235.50)	0.99 (ND–1.85)	ND (ND–1,592.60)	ND (ND–7.14)
138	425.50 (210.00–1,744.19)	6.43 (1.36–20.93)	112.00 (6.25–4,397.20)	4.95 (ND–13.19)	322.10 (ND–282.05)	2.39 (ND–3.55)	122.15 (ND–894.88)	2.21 (ND–3.74)
153	339.49 (263.20–1,666.66)	9.05 (1.92–19.99)	962.08 (520.84–4,780.40)	4.16 (1.66–14.34)	294.00 (4.00–1,375.00)	1.88 (ND–9.62)	26.04 (ND–2,812.00)	0.13 (ND–16.87)
180	490.10 (106.67–1,200.00)	10.81 (1.30–20.50)	769.25 (ND–1,213.33)	2.54 (ND–4.85)	482.70 (160.00–990.48)	3.43 (0.95–12.19)	143.74 (ND–955.56)	6.89 (ND–60.41)

[a] Values are median (range). ND, not detectable (<0.60 ng g^{-1} for PCBs 28 and 52; <0.55 ng g^{-1} for PCBs 101 and 118; <0.50 ng g^{-1} for PCBs 138, 153, and 180).

TABLE 4. *Extended*

Metabolite (ng g^{-1})[a]					
Blue and red shrimp		European flying squid		Mediterranean mussel	
Fat wt	Wet wt	Fat wt	Wet wt	Fat wt	Wet wt
ND (ND–28.98)	ND (ND–0.17)	22.36 (ND–170.20)	ND (ND–1.02)	9.38 (ND–204.40)	ND (ND–1.84)
40.57 (ND–169.53)	0.18 (ND–1.02)	107.49 (ND–519.40)	0.76 (ND–3.12)	27.69 (ND–125.60)	0.24 (ND–0.94)
ND (ND–40.60)	ND (ND–0.12)	ND (ND–85.80)	ND (ND–0.51)	ND (ND–56.81)	ND (ND–0.41)

of the samples); *p,p′*-DDD was found in the 55% of the samples analyzed with concentrations ranging from not detectable to 198.60 ng g^{-1} f.w. (red mullet). For all the species but Atlantic mackerel, the median *p,p′*-DDE concentrations were higher than those of the *p,p′* DDT (respectively ranging from not detectable to 1,163.60 ng g^{-1} f.w. and from not detectable to 406.80 ng g^{-1} f.w. in the red mullet) (Table 4). The DDE/DDT ratio is commonly used to assess the chronology of DDT entering the ecosystems *(4).* The DDE/DDT ratio calculated for all the species ranged from 2.9 (Mediterranean mussel) to 9.7 (blue and red shrimp), confirming that these banned pesticides have not been used recently in the zone.

The results indicated a variable PCB congener profile in the different species analyzed, and there were significant differences in the residues of organochlorine pollutants among the species. These differences may be attributable to various abiotic and biotic factors, such as the degree of chlorination and logKow (octanol-water partition coefficient) of the chemicals and the recent habitat and feeding habits of the specimens. Variations in migratory patterns and metabolic detoxification capacity also may have an effect on the magnitude and the pattern of accumulated organochlorines in the investigated species *(2).*

The strong interaction with the sea bed and its sediments may have an important influence on the degree of contamination observed in the benthic and neritic fish (European hake, blue whiting, and red mullet) and cephalopods studied. Organochlorines tend to be adsorbed to the organic fraction of the sediments, which consequently act as a final reservoir and source of these pollutants in the aquatic systems, keeping them available to benthic species.

In this study, the European hake had the highest concentrations of persistent PCBs (31% of the total PCB content; significantly different at $P < 0.001$ compared with red mullet, blue whiting, Atlantic mackerel, Mediterranean mussel, and blue and red shrimp and at $P < 0.01$ compared with European flying squid), although the wide range of the biometric data suggests the inclusion of juveniles with low accumulative capabilities.

Cephalopods, which are known to accumulate numerous contaminants such as OCPs and PCBs *(38, 39, 42),* are an important vector for transferring potentially hazardous contaminants to top marine predators. The European flying

TABLE 5. *Extended*

PCB (ng g^{-1})[a]					
Blue and red shrimp		European flying squid		Mediterranean mussel	
Fat wt	Wet wt	Fat wt	Wet wt	Fat wt	Wet wt
ND (ND–15.00)	ND ND	ND (ND–58.20)	ND (ND–0.35)	ND (ND–18.00)	ND (ND–0.11)
ND	ND	ND (ND–41.80)	ND (ND–0.25)	ND (ND–42.20)	ND (ND–0.33)
ND	ND	68.71 (ND–269.00)	0.55 (ND–1.61)	168.60 (ND–1,098.48)	1.24 (ND–3.33)
ND (ND–30.60)	ND	279.19 (ND–834.80)	1.95 (ND–5.01)	293.43 (ND–2,111.12)	1.97 (ND–6.40)
60.19 (ND–625.00)	0.31 (ND–2.42)	625.30 (108.70–1,354.00)	4.32 (0.65–8.12)	550.50 (152.78–2,138.16)	4.14 (1.12–6.48)
41.10 (ND–175.44)	0.18 (ND–0.68)	539.23 (148.15–1,591.80)	3.57 (0.89–9.55)	545.60 (202.03–2,988.51)	3.72 (ND–9.06)
44.30 (ND–1,026.67)	0.45 (ND–3.97)	611.07 (ND–876.20)	4.28 (ND–5.30)	58.43 (ND–937.77)	0.38 (ND–2.84)

squid had higher median PCB concentrations than did all the other species analyzed except the European hake (1768.27 ng g^{-1} f.w.). This result may be related to the habitat and feeding habits of this squid. The substantial differences in the PCB concentrations might be due to an inability to metabolize these xenobiotics. In support of this hypothesis, Walker *(36)* described in molluscs a reduced synthesis of different forms of cytochrome P-450, which are responsible for the oxidative attack on PCBs.

Bivalves are suitable bioindicators for monitoring toxic contaminants in coastal waters because of their wide distribution, tolerance to a wide range in salinity, resistance to stress and high concentrations of a wide range of chemicals, and ease of sampling *(22).* Mussels have a high protein content, averaging 67% of body weight *(7, 34)* which is comparable to that of other food items at higher trophic levels. This fact underscores the importance of mussels as a source of animal protein and explains the high level of consumption. Although mussels occupy the lowest position on the food chain among the species investigated, these bivalves often had higher concentrations of organochlorines than those found in the other species. Neither the nature of the habitat nor the feeding habits offer a satisfactory explanation for these results. The main explanation might be that bivalves, similar to cephalopods, have a very limited capacity to metabolize PCBs through the cytochrome P-450 system *(5)*; hence, the concentrations of the single PCB congeners and the total congener pattern are not much modified by biotransformation and thus closely reflect the state of pollution of the coastal waters. Storelli et al. *(32)* reported that mussels are high in the lower chlorinated species. In contrast, in our study the PCB congeners 118, 138, and 153 were predominant (only the PCB 180 was present at lower concentrations than the other highly chlorinated congeners) (Table 5).

PCB concentrations in blue and red shrimp were remarkably low in comparison to those found in the other marine species and also in the mussels (the same trophic level) (Table 2). Blue and red shrimp live in deep water and feed on decomposing organisms. In this case the feeding habits might explain the low PCB accumulation by biomagnification. As already observed in mussels, more chlorinated congeners were found in higher concentrations than were the lower chlorinated congeners (90% for PCBs 138, 153, and 180) (Table 5), and this profile is unlike previous data reported in crustaceans, in which remarkably low concentrations of some penta- and hexachlorinated congeners have been found in brown shrimp *(16)* and lobsters and crabs *(18).*

Mackerel generally had lower organochlorine concentrations than did the other fish species, molluscs, and crustaceans (Tables 2 and 3). These results may be ascribed to the pelagic habitat and long-range horizontal and vertical migration of mackerel *(8).* The time spent in less contaminated open seas has a continuous washout effect on organochlorine compounds.

During the study period, we observed a seasonal trend in contamination levels of the species considered. The unclear influence of many factors such as habitat, physiology, lipid content, geographical origin, and feeding behavior could not account for pollutant deposit and elimination. However, the differences between seasons were not significant.

The mean concentrations of ΣPCB, ΣDDT, and HCB in the edible parts of red mullet, Atlantic mackerel, European hake, and Mediterranean mussel are compared in Table 6 with concentrations found in the same species in a study conducted in 2003 *(26).* The mean concentrations of ΣPCB in red mullet, Mediterranean mussel, and Atlantic mackerel found in the present study are higher than those reported by Naso et al. *(26).* For red mullet, the difference was significant ($P < 0.01$). In agreement with data reported by Naso et al. *(26),* PCBs 118, 138, 153, and 180 were the congeners most frequently detected and at the highest concentrations.

Our results are consistent with the high concentrations of other organochlorine compounds (dioxins) found in dairy products from the Campania region during the monitoring program carried out by the Italian Ministry of Health in 2004. Such high concentrations may be indicative of persistent point sources of PCBs in the Campania region despite the reduced production and use of these compounds.

The mean concentrations of both ΣDDT and HCB found in this study were generally lower than those reported by Naso et al. *(26)* (Table 6). For ΣDDT in Atlantic mackerel and HCB in red mullet and European hake, differences in mean concentrations previously reported were significant ($P < 0.05$). This temporal trend of decreasing OCP concentrations can be ascribed to the length of time since legal restrictions were introduced.

For the OCPs, the maximum residue limits were established for fish and aquatic products by the Decreto Ministeriale of 27 August 2004 *(12).* Almost all the samples analyzed had a <5% lipid percentage and are classified in group 1, with maximum residue limits of 0.050 and 0.010 μg g^{-1} w.w. for DDTs and HCB, respectively. Only some specimens of Atlantic mackerel had a >5% lipid percentage (mean, 7.73%) and were included in group 2 with a maximum residue limit of 0.100 μg g^{-1} w.w. for DDTs and 0.020 for HCB. All samples analyzed were below these limits, as stipulated by the ban on DDT for agricultural use. These data suggest that the OCPs in marine species caught in the Gulf of Naples are unlikely to cause a significant health hazard.

The European Union legislation has not fixed a maximum residue limit for the seven PCB congeners in fish, shellfish, or crustaceans. The current limits (200 ng g^{-1} f.w.) were established by the European Union for terrestrial edible classes of food *(9).* In all the marine species analyzed, the median PCB concentrations exceeded (by a large amount in some cases) this residue limit (Table 2). Although the manufacture and use of PCBs are banned or highly restricted, these compounds still are significant persistent chemical contaminants in the Gulf of Naples.

Because PCBs accumulate in organisms through the food chain, the diet is considered the main source of human exposure (>90%) *(15)*; animal fats and fish are the main sources of PCBs in an average western European diet *(3,*

TABLE 6. *Comparison of concentrations of ΣPCB, ΣDDT, and HCB: present study versus Naso et al. (26)*

Compound	Pollutant (ng g^{-1})[a]							
	Red mullet		European hake		Atlantic mackerel		Mediterranean mussel	
	This study	Naso et al. *(26)*	This study	Naso et al. *(26)*	This study	Naso et al. *(26)*	This study	Naso et al. *(26)*
ΣPCB	1,903.82 ± 909.96[b]	1,120.06 ± 208.26	4,421.45 ± 3,831.46	4,478.94 ± 4,183.52	1,425.99 ± 1,823.13	1,006.93 ± 1,221.16	2,070.61 ± 2,158.37	1,519.48 ± 417.22
ΣDDT	158.71 ± 120.52	220.93 ± 65.37	532.46 ± 471.02	646.35 ± 414.56	34.15 ± 73.06[c]	180.91 ± 180.93	113.62 ± 116.91	177.81 ± 118.99
HCB	16.23 ± 14.656[c]	28.05 ± 16.67	15.29 ± 12.66[c]	30.23 ± 20.96	16.20 ± 11.94	15.77 ± 21.49	11.71 ± 8.43	12.45 ± 10.32

[a] Values are mean ± SD.
[b] Mean in the present study is significantly different from that reported by Naso et al. *(26)* ($P < 0.01$).
[c] Mean in the present study is significantly different from that reported by Naso et al. *(26)* ($P < 0.05$).

25, 44). However, because dietary habits vary greatly among populations and because PCB concentrations vary across geographical areas, the main sources of PCBs and the extent of exposure may differ across populations. Dietary intakes of 0.5 to 48 μg/day have been reported in different countries. The data available in the 1980s indicated that an average intake of 5 to 15 μg/day for the general population in industrialized countries was a reasonable estimate *(40).* Individual intakes of specific foods vary greatly within a single population, and PCB concentrations also are affected by food processing, i.e., washing and cooking *(43).* To correctly estimate risks associated with PCBs, we must measure exposure to specific PCB congeners because each of these compounds has particular properties and characteristic patterns of persistence in the human body and toxicity potential *(30)*. The cytochrome P-450 monooxygenase system and conjugating enzymes are the two most important multienzyme systems involved in the metabolism and detoxification of xenobiotic compounds *(20).* PCBs 118, 138, 153, and 180 tend to be refractory to metabolic degradation by monooxygenases and, consequently, tend to be eliminated more slowly because of their high degree of chlorination and the lack of unsubstituted positions on their biphenyl rings that could be available for enzymatic attack *(36).* The higher congeners (hexa and hepta forms) have a lower clearance rate than do the lower forms, so they remain in the body longer (PCB 153 has an estimated half-life in the human body of 27.5 years) *(6, 41).* Corsolini et al. *(11)* reported that the most abundant PCB congeners found in human adipose tissue from Italian surgical patients were 138, 153, and 180. In our study, these PCBs were the predominant congeners in all the marine species analyzed.

Until now, no systematic analysis has been performed to evaluate the risk assessment for local populations or for Italians as a whole associated with consumption of PCBs in contaminated marine organisms. The consumption of seafood products in southern Italy, including Sicilia but not Sardinia, was 157,026 tons in 2003, which corresponds to 38.3% of Italian seafood consumption *(23),* the highest in Italy.

Our findings suggest that the European Union should set limits for PCBs (the seven targets congeners) in seafood products, mainly for the most commonly consumed species of fish, crustaceans, and molluscs based on the increase in PCB contamination over time, which is related to high local levels of environmental pollution. A more complex analysis of seafood preferences, an evaluation of the mean daily intake of seafood, and the exploitation of the results of monitoring studies are recommended for a more accurate assessment of the consumer health risk associated with these foods.

ACKNOWLEDGMENTS

This work has been supported by Ministry of University and Scientific and Technological Research grants (MPI 40% 2002). The authors are grateful to Dr. Ciro Sbarra for his important help in collecting specimens.

REFERENCES

1. Alcock, R. E., P. A. Behnisch, K. C. Jones, and H. Hagenmaier. 1998. Dioxin-like PCBs in the environment—human exposure and the significance of sources. *Chemosphere* 37:1457–1472.
2. Ashley, J. T., R. Horwitz, J. C. Steinbacher, and B. Ruppel. 2003. A comparison of congeneric PCB patterns in American eels and striped bass from the Hudson and Delaware River Estuaries. *Mar. Pollut. Bull.* 46:1294–1308.
3. Bayarri, S., L. T. Baldassarri, N. Iacovella, F. Ferrara, and A. di Domenico. 2001. PCDDs, PCDFs, PCBs and DDE in edible marine species from the Adriatic Sea. *Chemosphere* 43:601–610.
4. Bordajandi, L. R., G. Gòmez, M. A. Fernàndez, E. Abad, J. Rivera, and M. J. Gonzàlez. 2003. Study on PCBs, PCDD/Fs, organochlorine pesticides, heavy metals and arsenic content in freshwater fish species from the River Turia (Spain). *Chemosphere* 53:163–171.
5. Cajaraville, M. P., M. J. Bebianno, J. Blasco, C. Porte, C. Sarasquete, and A. Viarengo. 2000. The use of biomarkers to assess the impact of pollution in coastal environments of the Iberian Peninsula: a practical approach. *Sci. Total Environ.* 247:295–311.
6. Chen, P. H., M. L. Luo, C. K. Wong, and C. J. Chen. 1982. Comparative rates of elimination of some individual polychlorinated biphenyls from the blood of PCB-poisoned patients in Taiwan. *Food Chem. Toxicol.* 20:417–425.
7. Cheong, L. 1982. Country report: Singapore, p. 69–71. *In* F. B. Davy and M. Graham (ed.), Bivalve culture in Asia and the Pacific. Proceedings of a workshop held in Singapore 16 to 19 February 1982. International Development Research Centre, Ottawa, Ontario, Canada.
8. Collette, B. B., and C. E. Nauen. 1983. Scombrids of the world. An annotated and illustrated catalogue of tunas, mackerels, bonitos and related species known to date. FAO fisheries synopsis 125. FAO species catalog, vol. 2. Food and Agricluture Organization of the United Nations, Rome.
9. Commission of the European Communities. 1999. EU Commission decision 1999/788/EC of 3 December 1999 on protective measures with regard to contamination by dioxins of certain products of porcine and poultry origin intended for human or animal consumption. G.U. EU-L 310/62. Commission of the European Communities, Brussels.
10. Commission of the European Communities. 2004. Communication from the Commission to the Council, the European Parliament and the European Economic and Social Committee on implementation of the Community Strategy for dioxins, furans and polychlorinated biphenyls. COM (2001) 593. 240. Commission of the European Communities, Brussels.
11. Corsolini, S., S. Focardi, K. Kannan, S. Tanabe, and R. Tatsukawa. 1995. Isomer-specific analysis of polychlorinated biphenyls and 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalents (TEDs) in red fox and human adipose tissue from central Italy. *Arch. Environ. Contam. Toxicol.* 29:61–68.
12. Decreto Ministeriale. 2004. Prodotti fitosanitari: limiti massimi di residui delle sostanze attive nei prodotti destinati all'alimentazione. (Recepimento delle direttive n. 76/895/CE, n. 86/362/CE, n. 86/363/CE, n. 90/642/CE.) G.U. 292 del 14/12/2004.
13. di Domenico, A., L. Turrito-Baldassarri, G. Ziemacki, E. De Felip, G. Ferrari, C. La Rocca, M. Cardelli, G. Cedolini, M. Dalla Palma, M. Grassi, M. Roccabella, F. Volpi, F. Ferri, N. Iacovella, F. Rodriguez, O. D'Agostino, R. Sansoni, and G. Settimo. 1998. Priority microcontaminants in biota samples from the Venice Lagoon: a selection of concentration data and elements of risk analysis. *Organohalogen Comp.* 39:199–204.
14. Di Muccio, A., P. Stefanelli, E. Funari, D. A. Barbini, T. Generali, P. Pelosi, S. Girolimetti, G. Amendola, F. Vanni, and S. Di Muccio. 2002. Organochlorine pesticides and polychlorinated biphenyls in 12 edible marine organism from the Adriatic Sea, Italy, spring 1997. *Food Addit. Contam.* 19:1148–1161.
15. Duarte-Davidson, R., and K. C. Jones. 1994. Polychlorinated biphenyls (PCBs) in the UK population: estimated intake, exposure and body burden. *Sci. Total Environ.* 151:131–152.
16. Duinker, J. C., M. T. J. Hillebrand, and J. P. Boon. 1983. Organochlorines in benthic invertebrates and sediments from the Dutch Wadden Sea. *Neth. J. Sea Res.* 17:19–38.
17. Faroon, O., M. S. Keith, D. Jones, and C. de Rosa. 2001. Effects of polychlorinated biphenyls on development and reproduction. *Toxicol. Ind. Health* 17(3):63–93.
18. Farrington, J. W., and J. Westall. 1986. Organic chemical pollutants in the oceans and groundwater: a review of fundamental properties and bio-geochemistry, p. 361–425. *In* G. Kullenberg (ed.), The role of the oceans as a waste disposal option. NATO ASI series. D. Reidel, Boston.
19. Fernandez, M. A., B. Gomara, L. R. Bordajandi, L. Herrero, E. Abad, M. Abalos, J. Rivera, and M. J. Gonzàlez. 2004. Dietary intakes of polychlorinated dibenzo-*p*-dioxins, dibenzofurans and dioxin-like polychlorinated biphenyls in Spain. *Food Addit. Contam.* 21:983–991.
20. Garcia, L. M., C. Porte, and J. Albaigés. 2000. Organochlorinated pollutants and xenobiotic metabolizing enzymes in W. Mediterranean mesopelagic fish. *Mar. Pollut. Bull.* 40:764–768.
21. Garner, C. E., W. N. Jefferson, L. T. Burka, H. B. Matthews, and R. R. Newbold. 1999. In vitro estrogenicity of the catechol metabolites of selected polychlorinated biphenyls. *Toxicol. Appl. Pharmacol.* 154:188–197.
22. Goldberg, E. D., V. T. Bowen, J. W. Farrington, G. Harvey, J. H. Martin, P. L. Parker, R. W. Risebrough, W. Robertson, E. Schneider, and E. Gamble. 1978. The mussel watch. *Environ. Conserv.* 5:101–125.
23. Instituto di Servizi per Il Mercato Agricola Alimentare–AcNielsen. 2003. Osservatorio consumi ittici, report. I consumi domestici e il comportamento di acquisto delle famiglie italiane. Instituto di Servizi per Il Mercato Agricola Alimentare, Rome.
24. Istituto Superiore di Sanità. 2002. ISTISAN report 02/38 no.1123-3117. Istituto Superiore di Sanità, Rome.
25. Liem, A. K., P. Furst, and C. Rappe. 2000. Exposure of population to dioxins and related compounds. *Food Addit. Contam.* 17:241–259.
26. Naso, B., D. Perrone, M. C. Ferrante, M. Bilancione, and A. Lucisano. 2005. Persistent organic pollutants in edible marine species from the Gulf of Naples, Southern Italy. *Sci Total Environ.* 344:83–95.
27. Patandin, S., C. Koopman-Esseboom, M. A. de Ridder, N. Weisglas-Kuperus, and P. J. Sauer. 1998. Effects of environmental exposure to polychlorinated biphenyls and dioxin on birth size and growth in Dutch children. *Pediatr. Res.* 44:538–545.
28. Perugini, M., M. Cavaliere, A. Giammarino, P. Mazzone, V. Olivieri, and M. Amorena. 2004. Levels of polychlorinated biphenyls and organochlorine pesticides in some edible marine organisms from the central Adriatic Sea. *Chemosphere* 57:391–400.
29. Rylander, L., U. Stromberg, and L. Hagmar. 1995. Decreased birthweight among infants born to women with a high dietary intake of fish contaminated with persistent organochlorine compounds. *Scand. J. Work Environ. Health* 21:368–375.
30. Safe, S. H. 1998. Development validation and problems with the toxic equivalency factor approach for risk assessment of dioxin and related compounds. *J. Anim. Sci.* 76:134–141.
31. Salama, J., T. R. Chakraborty, L. Ng, and A. C. Gore. 2003. Effects of polychlorinated biphenyls on estrogen receptor-beta expression in the anteroventral periventricular nucleus. *Environ. Health Perspect.* 111:1278–1282.
32. Storelli, M. M., A. Storelli, G. Barone, and G. O. Marcotrigiano. 2004. Polychlorinated biphenyl and organochlorine pesticide residues in *Lophius budegassa* from the Mediterranean Sea (Italy). *Mar. Pollut. Bull.* 48:743–748.
33. Svensson, B. G., T. Hallberg, A. Nilsson, A. Schultz, and L. Hagmar. 1994. Parameters of immunological competence in subjects with high consumption of fish contaminated with persistent organochlorine compounds. *Int. Arch. Occup. Environ. Health* 65:351–358.
34. Tanabe, S., M. S. Prudente, S. Kan-atireklap, and A. Subramanian. 2000. Mussel watch: marine pollution monitoring of butyltins and organochlorines in coastal waters of Thailand, Philippines and India. *Ocean Coast. Manage.* 43:819–839.
35. U.S. Environmental Protection Agency. 2004. Update: national list-

ing of fish and wildlife advisories. Environmental Protection Agency, Washington, D.C.
36. Walker, C. H. 2001. Organic pollutants—an ecotoxicological perspective. Taylor and Francis, London.
37. Wania, F., and D. Mackay. 1996. Tracking the distribution of persistent organic pollutants. *Environ. Sci. Technol.* 30:390A–396A.
38. Weisbrod, A. V., D. Shea, G. LeBlanc, M. J. Moore, and J. J. Stegeman. 2000. Organochlorine bioaccumulation and risk for whales in a northwest Atlantic food web. *Mar. Environ. Res.* 50:431–441.
39. Weisbrod, A. V., D. Shea, M. J. Moore, and J. J. Stegeman. 2001. Species, tissue and gender-related organochlorine bioaccumulation in white-sided dolphins, pilot whales and their common prey in the northwest Atlantic. *Mar. Environ. Res.* 51:29–50.
40. World Health Organization. 1993. Polychlorinated biphenyls and terphenyls. Environmental health criterion 140, 2nd ed. International Programme on Chemical Safety, World Health Organization, Geneva.
41. Yakushiji, T., I. Watanabe, K. Kuwabara, R. Tanaka, T. Kashimoto, N. Kunita, and I. Hara. 1984. Rate of decrease and half life of polychlorinated biphenyls (PCBs) in the blood of mothers and their children exposed to PCBs. *Arch. Environ. Contam. Toxicol.* 13:341–345.
42. Yamada, H., K. Takayanagi, M. Tateishi, H. Tagata, and K. Ikeda. 1997. Organotin compounds and polychlorinated biphenyls of livers in squid collected from coastal waters and open oceans. *Environ. Pollut.* 96:217–226.
43. Zabik, M. E., J. B. Harte, M. J. Zabik, and G. Dickmann. 1992. Effect of preparation and cooking on contaminants distribution in crustaceans: PCB in blue crab. *J. Agric. Food Chem.* 40:1197–1203.
44. Zuccato, E., S. Calvarese, G. Mariani, S. Mangiapan, P. Grasso, A. Guzzi, and R. Fanelli. 1999. Level, sources and toxicity of polychlorinated biphenyls in the Italian diet. *Chemosphere* 38:2753–2765.