Polychlorinated Biphenyls, Organochlorine Pesticides, and Polycyclic Aromatic Hydrocarbons in Wild, Farmed, and Frozen Marine Seafood Marketed in Campania, Italy

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ABSTRACT

Polychlorinated biphenyls (PCBs), organochlorine pesticides, and polycyclic aromatic hydrocarbons (PAHs) were detected in fresh-catch, farmed, and frozen marine fish marketed in Campania, Italy. Additionally, polychlorobiphenyl congeners were found: six were non–dioxin-like (NDL-PCB) (IUPAC no. 28, 52, 101, 138, 153, and 180), and one was dioxin-like (DL-PCB) (IUPAC no. 118). In all, 93% of fresh-catch, 100% of aquaculture, and 74% of the frozen specimens contained PCBs at concentrations varying from 0.12 to 35.11 ng/g, wet weight; NDL-PCBs ranged between 0.12 and 32.44 ng/g. Penta-, hexa-, and heptachlorobiphenyls were predominant. Regarding organochlorine pesticides, hexachlorobenzene was detected in 35% of fresh catch, 36% of farmed, and 46% of the frozen fish specimens, in a range between <0.01 and 3.29 ng/g. Contents of the dichlorodiphenyltrichloroethane isomer amounted to 0.12 to 11.00 ng/g. Finally, PAHs were detected in 100% of the specimens. Benzo[*a*]pyrene was detected in 66% of the aquaculture, 35% of the fresh catch, and 24% of the frozen species, at concentrations varying from 0.03 to 9.18 ng/g. On the basis of annual fish consumption, an average daily intake of NDL-PCBs of 6.02 ng/kg of body weight was estimated. Calculated daily hexachlorobenzene and total dichlorodiphenyltrichloroethane intakes were, respectively, 0.11 and 0.90 ng/kg of body weight per day. The contribution of fish to the daily consumption of the noncarcinogenic PAHs can be considered low; for benzo[*a*]pyrene, the estimated daily intake is considerably lower than the doses considered carcinogenic for experimental animals by the European Union Scientific Committee on Food.

Persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), polychlorodibenzodioxins, polychlorodibenzofurans, organochlorine pesticides (OCPs), and other contaminants, such as polycyclic aromatic hydrocarbons (PAHs), released into the environment represent a significant problem for human health. Although these contaminants can be absorbed through the skin or via respiration, the intake of food, mainly of animal origin, is considered to be the primary source in human exposure *(27)*.

In this regard, seafood can represent a significant source of pollutant intake. However, this observation may well conflict with nutritional recommendations calling for an increase in fish consumption due to the presence of beneficial nutrients, such as lipids. Such nutrients may help reduce the risk of cardiovascular diseases, certain cancers, and type 2 diabetes *(22)*. Official Italian nutritional guidelines, for instance, currently promote fish consumption (two to three times per week) with the aim of reducing saturated fat intake and increasing that of omega-3 fatty acids (Ω_3) *(18)*. Currently in Italy annual fish consumption is estimated to be 22 kg per person; 37.4% of the total Italian fish consumption is accounted for by the regions of southern Campania, Abruzzo, Molise, Puglia, Calabria, Basilicata, and Sicily.

In Italy there are three different marketing categories of seafood products, namely, fresh catch, aquaculture, and

frozen. The fresh-catch category is represented by wild species, which after capture, are stored in ice and marketed within a few (3 to 4) days. The aquaculture sector entails the farming of a wide range of different species and use of varied technologies, reflecting the diversity of available sites. The frozen category, a very important sector for the Italian fish market, consists of seafood frozen after capture and stored at −18°C until marketed. With its guarantee of freshness and lower price, frozen seafood is widely used in the catering sector for hospitals, schools, and factory canteens. For aquaculture products Italy has become the reference market in the Mediterranean for fresh seabass and seabream products. Italy imports huge quantities of seabass and seabream from other Mediterranean countries, mainly Greece, followed by Turkey *(14)*. Production in the seafood sector accounts for 627,000 metric tons, of which 408,880 metric tons are marketed as fresh-catch fish, 129,120 metric tons are from in-shore or off-shore aquaculture plants, and 89,000 metric tons are marketed as frozen *(17)*.

Our study aimed to evaluate (i) the contamination by PCBs, OCPs, and PAHs of the marine seafood sold in Campania, Italy, and (ii) the daily dietary exposure of seafood consumers to the pollutants in question.

MATERIALS AND METHODS

Sampling. From January to May 2007 marine fish and molluscs were randomly purchased in local fish markets, supermarkets, and grocery stores from the main cities of Campania, Italy. Fresh-catch, aquaculture, and frozen fish and molluscs were col-

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lected independently of their geographical origin. Wild Mediterranean mussels were bought from local fishermen. The fresh-catch species were Atlantic mackerel (*Scomber scombrus*), European hake (*Merluccius merluccius*), flathead grey mullet (*Mugil cephalus*), gilthead seabream (*Sparus aurata*), European seabass (*Dicentrarchus labrax*), red mullet (*Mullus barbatus*), common cuttlefish (*Sepia officinalis*), common octopus (*Octopus vulgaris*), European squid (*Loligo vulgaris*), and Mediterranean mussels (*Mitylus galloprovincialis*). The aquaculture species included gilthead seabream, European seabass, and Mediterranean mussels, and the frozen species were European hake, gilthead seabream, European seabass, red mullet, common cuttlefish, common octopus, European squid, and Mediterranean mussels. These species were selected because they are the most commonly consumed in Italy *(17)*.

For every species of fish and mollusc (cephalopods), nine specimens of about the same size were collected. The specimens were wrapped in aluminum foil, put in ice, and transported to the laboratory in 1 h. Then, muscular tissue (edible part) from fresh-catch and aquaculture seafood was homogenized; for the fresh mussels, the shells were opened and the soft tissues were removed and pooled (20 specimens per pool) and then homogenized; frozen products were defrosted at 4°C overnight and then treated like fresh products. Aliquots of 5 g of the homogenates were lyophilized in glass vessels for about 36 h and then stored in the dark at 4°C for not more than 2 weeks until processed.

PCB and OCP analysis. Seven polychlorobiphenyl congeners, i.e., six non–dioxin-like PCBs (NDL-PCBs) (IUPAC no. 28, 52, 101, 138, 153, and 180) and one dioxin-like PCB (DL-PCB) (IUPAC no. 118), which are generally considered to be PCB contamination markers in biological and environmental matrices, and OCPs hexachlorobenzene (HCB), dichlorodiphenyltrichloroethane (p,p'-DDT), dichlorodiphenyldichloroethylene (p,p'-DDE), and dichlorodiphenyldichloroethane (p,p'-DDD), were analyzed by the Italian Istituto Superiore di Sanità multiresidue analytical method *(19)*.

From lyophilized samples, fat was extracted three times by cold light petroleum with acetone (50:50), and the samples were centrifuged at 200 × g for 15 min, passed through a glass tube packed with anhydrous Na_2SO_4 (Merck KgaA, Darmstadt, Germany), and then dried under vacuum at 40°C. The fat extracted was quantified gravimetrically. The lipidic extracts were dissolved in *n*-hexane, transferred onto Extrelut-3/Extrelut-1 cartridges (Merck KgaA) with the addition of 0.36 g of C-18 Isolute (40-60 mesh; Merck KgaA) and eluted with acetonitrile. The eluates, concentrated to 1 ml under vacuum at 40°C, were poured into a glass column containing 2.5 g of Florisil (60/100 mesh; Supelco, Bellefonte, PA) activated overnight at 130°C. The column was eluted three times with 10-ml aliquots of *n*-hexane collecting the eluates. These eluates contained PCBs, HCB, and a ratio of p,p'-DDE. To obtain the remaining p,p'-DDE and the p,p'-DDD and p,p'-DDT, the column was then eluted with *n*-hexane–toluene (80:20, vol/vol) and the eluate was collected. The eluates gathered together were concentrated to about 2 ml at room temperature under N_2. PCB and OCP analyses were carried out by a Hewlett-Packard 5890-Series III gas chromatograph with an electron capture detector injecting 1 μl of the extracts on an HP-5 (Crosslinked 5%PHME Siloxane, 30-m length, 0.32-mm inside diameter, 0.25-μm film thickness) glass capillary column. Helium and argonmethane were respectively used as carrier and make-up gases. The injector temperature was 300°C, and the temperature of the electron capture detector was 320°C. The temperature program was 60°C for 2 min, increasing by 10°C/min to 170°C, by 2°C/min to

210°C, and by 15°C/min to 260°C, staying at this temperature for 20 min.

PCBs and OCPs were determined by comparing the retention time and the area of each individually resolved peak with that of the corresponding reference standard. PCB 209 (2,2′,3,3′,4,4′,5, 5′,6,6′-decachlorobiphenyl) (Dr. Ehrenstorfer GmbH Labservice Analytica S.r.l. Anzola Emilia, Bologna, Italy) was used as an internal standard.

Detection limits (LODs) and quantification limits (LOQs) for PCBs were (all concentrations are on a wet weight basis), respectively, 0.01 and 0.03 ng/g for PCBs 138, 153, and 180; 0.02 and 0.06 ng/g for PCBs 52, 101, and 118; and 0.04 and 0.12 ng/g for PCB 28. LODs and LOQs for OCPs were, respectively, 0.01 and 0.03 ng/g for HCB and 0.04 and 0.12 ng/g for p,p'-DDT, p,p'-DDE, and p,p'-DDD. Aliquots of commercial homogenized fish muscle used as blanks were extracted and analyzed to test quality control and assurance of the analytical procedure. In addition, aliquots of the homogenized fish muscle fortified with a standard mixtures at three concentrations were analyzed in triplicate. The recoveries for PCBs ranged between 80% ± 3% (PCBs no. 28, 52, and 118) and 90% ± 4% (PCBs no. 101, 138, 153, and 180), and for OCPs they ranged between 90% ± 4% (HCB) and 80% ± 3% (p,p'-DDT, p,p'-DDE, and p,p'-DDD).

The total amount of the PCBs determined was calculated as the sum of the concentrations of the seven congeners (ΣPCBs) and of the six NDL-PCBs (ΣNDL-PCBs) detected, not corrected for recoveries, assuming that the values smaller than the LODs were equal to the LODs. ΣDDTs was calculated as the sum of p,p'-DDT, p,p'-DDE, and p,p'-DDD, assuming that the values smaller than the LODs were equal to the LODs.

PAH detection. Lyophilized samples were hydrolyzed at 100°C for 150 min with 20 ml of ethanol, 3.5 ml of 50% aqueous KOH (Merck KgaA), and 0.4 ml of 2.5 M aqueous Na_2S (Merck KgaA). The alkaline mixture was cooled and transferred into a separator funnel rinsing the flask with *n*-hexane, ethanol, and distilled water. The funnel was vigorously shaken for 5 min. The organic phase was transferred to a flask, and the aqueous phase was extracted twice more with *n*-hexane. The three *n*-hexane extracts were combined, filtered through anhydrous Na_2SO_4, concentrated to about 3 to 5 ml, and purified on a glass column packed with 8 g of silica gel (70/230 mesh ASTM, 0.063 to 0.200 mm; Merck KgaA) that had been activated at 140°C for 180 min. PAHs were eluted as rapidly as possible with 30 ml of ethyl ether–*n*-hexane (10:90, vol/vol). After elution, the solvent was evaporated to 1 to 2 ml under vacuum at 55°C and transferred to a vial and dried under a mild stream of N_2. The dried extract was reconstituted to 1 ml of acetonitrile. All the glassware used was protected from light by aluminum foil. PAH detection was carried out using a Shimadzu LC10 HPLC chromatograph (Shimadzu ATVP, Tokyo, Japan) with a Restek Pinnacle II PAH steel column 25 cm long with a 4.6-mm inside diameter (Restek Co., Bellefonte, PA), a UV-visible detector (model SPD-10AVP, Shimadzu) set at 254 nm, and a fluorescence detector (model RF-10AVP, Shimadzu) set at 280-nm excitation and at 425-nm emission wavelengths. Elution was carried out by a mobile phase acetonitrile-water at a flow rate of 1 ml/min starting from an acetonitrile-water ratio of 50% for 5 min, changing to 100% acetonitrile in 20 min, and holding at 100% for 15 min. PAHs were identified by comparing the retention time and the area of each individually resolved peak with that of the corresponding reference standard obtained from Restek Co. LODs and LOQs for PAHs (all concentrations are on a wet weight basis) were, respectively, 0.03 and 0.09 ng/g for anthracene (ANT), benzo[*b*]fluoranthene (BBF),

TABLE 1. *Prevalence of PCB contamination and PCB concentrations in the marine fish and molluscs analyzed*

| PCB congener[a] | Fish category: | | | | | |
| | Fresh catch | | Aquaculture | | Frozen | |
	POC (%)[b]	Mean concn ± SD (median) and range[c]	POC (%)	Mean concn ± SD (median) and range	POC (%)	Mean concn ± SD (median) and range
28	25	1.86 ± 1.72 (<0.04) <0.04–14.90	20	1.41 ± 1.52 (<0.04) <0.04–16.31	16	1.53 ± 1.39 (<0.04) <0.04–15.09
52	21	0.76 ± 1.68 (<0.02) <0.02–6.32	37	0.80 ± 1.44 (<0.02) <0.02–6.10	5	0.10 ± 0.40 (<0.02) <0.02–2.35
101	46	1.33 ± 2.15 (<0.02) <0.02–7.29	45	1.39 ± 2.43 (<0.02) <0.02–8.10	27	0.56 ± 1.05 (<0.02) <0.02–4.25
118	25	0.80 ± 1.98 (<0.02) <0.02–9.81	61	0.87 ± 1.02 (0.60) <0.02–3.43	17	0.27 ± 0.72 (<0.02) <0.02–3.05
153	53	1.60 ± 2.67 (0.70) <0.01–6.13	92	1.90 ± 1.27 (1.82) <0.01–5.42	39	0.46 ± 0.90 (<0.01) <0.01–5.00
138	25	1.00 ± 2.94 (<0.01) <0.01–15.00	75	1.15 ± 0.88 (1.15) <0.01–2.64	15	0.13 ± 0.30 (<0.01) <0.01–1.02
180	50	0.98 ± 1.39 (<0.01) <0.01–4.70	58	2.04 ± 3.90 (0.20) <0.01–13.26	15	0.20 ± 0.52 (<0.01) <0.01–2.28
ΣPCBs	93	10.47 ± 8.86 (7.45) 0.15–35.11	100	12.54 ± 8.42 (10.66) 1.52–28.19	74	5.25 ± 5.12 (4.20) 0.12–19.90
ΣNDL-PCBs	93	9.67 ± 7.93 (6.76) 0.13–32.44	100	11.67 ± 8.06 (8.99) 1.50–28.17	80	4.97 ± 4.86 (3.86) 0.12–17.34

[a] ΣPCBs, sum of PCB 28, 52, 101, 118, 153, 138, and 180; ΣNDL-PCBs, sum of PCB 28, 52, 101, 153, 138, and 180.

[b] POC, prevalence of contamination.

[c] Values are in nanograms per gram, wet weight.

benzo[*k*]fluoranthene (BKF), benzo[*a*]pyrene (BaP), benzo[*ghi*]perylene (BPE), and indeno[1,2,3-*cd*]pyrene (IND); 0.05 and 0.15 ng/g for benzo[*a*]anthracene (BA), chrysene (CH), and dibenzo[*a,h*]anthracene (DB); 0.10 and 0.30 ng/g for acenaphthene (AC), fluorine (FLU), phenanthrene (PHE), and fluoranthene (FLUO); 0.20 and 0.60 ng/g for naphthalene (NAP) and pyrene (PY); and 0.40 and 1.20 ng/g for acenaphthylene (ACEN).

The recoveries, tested by analyzing in triplicate samples fortified with a standard mixture at three concentrations, varied from 69% ± 6% for NAP-ACEN-PY-DB, 85% ± 5% for BA-IND-ANT-AC-FLU-PHE-FLUO, and 90% ± 5% for CH-BKF-BaP-BBF-BPE. ΣPAHs was calculated as the sum of the concentrations of the 16 PAHs determined, not corrected for recoveries, assuming that the values smaller than the LODs were equal to the LODs.

Statistical analysis. Statistical data analysis was performed with SPSS 13.0. The comparison was exclusively carried out among specimens of the same species present as fresh catch, aquaculture, and frozen. The differences in mean concentrations of PCBs, OCPs, and PAHs among the same species analyzed were tested, log transforming the values to approximate a normal distribution of the data, assuming that when a result was below the LOD, the value was equal to the LOD. Analysis of variance was carried out. The level of significance was set at P values of <0.05.

RESULTS AND DISCUSSION

PCB. One hundred percent of the farmed marine organisms analyzed in this study, 93% of the fresh-catch samples, and 74% of the frozen samples were contaminated by at least one PCB congener at detectable levels (Table 1). In fresh-catch fish, PCB 153 was the main compound, detected in 53% of the samples at up to 6.13 ng/g (median, 0.70

ng/g), followed by PCB 180 (50%) with a maximum value of 4.70 ng/g, and by PCB 101 (46%) with a maximum concentration of 7.29 ng/g. In aquaculture species, PCB 153 was detected in 92% of the samples, with a median concentration of 1.82 ng/g, followed by PCB 138 in 75% with a median concentration of 1.15 ng/g and PCB 118 in 61% with a median value of 0.60 ng/g. In frozen samples, PCB 153 was also prevalent (39% of samples) at concentrations of up to 5.00 ng/g, followed by PCB 101 (27%) at values of up to 4.25 ng/g.

Penta-, hexa-, and heptachlorobiphenyls are reported to be the prevalent compounds in biological samples. Due to their high degree of chlorination and the lack of positions available for enzymatic attack, they remain in the body longer (PCB 153 has an estimated half-life in the human body of 27.5 years) *(1, 2, 4, 15)*. As in other studies, the penta-, hexa-, and heptachlorobiphenyls were the most frequently detected in the present study.

As recently emphasized by Bocio et al. *(4),* comparison of the results of the available studies on PCB contamination in fish is complicated by various factors such as the diversity of their concentrations in the marine waters where fish live, the great variability of the congeners detected, different expressions of the data, use of wet, dry, or lipid weight, and the part of the fish analyzed (e.g., muscle, liver, skin). Selecting the studies that include the same seven PCB congeners in edible tissues of seafood expressed on wet weight, we can observe that the total PCB concentrations found in this study, ranging from 0.12 to 35.11 ng/g, are much lower than those found in fish and shellfish from the Adriatic sea

FIGURE 1. *Median levels of polychlorinated biphenyls (PCBs) (in nanograms per gram, wet weight [ww]) in fresh-catch, aquaculture, and frozen Gilthead Seabream, European Seabass, and Mediterranean mussels marketed in Campania, Italy. Asterisks indicate values that are significantly different among gilthead seabream, European seabass, and Mediterranean mussels in different marketing sectors.*
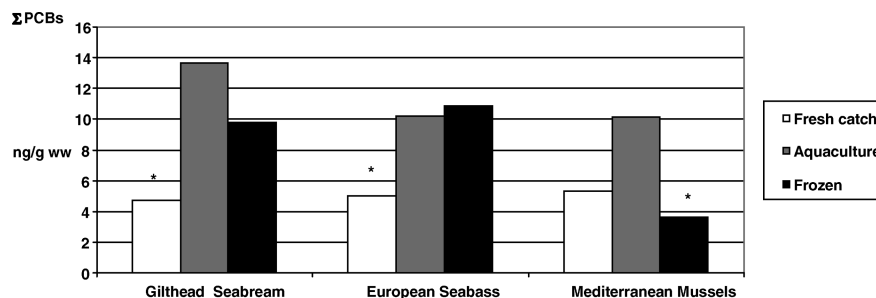
by the Italian Istituto Superiore di Sanità (0.20 to 80.60 ng/g) *(20)* and those referred to by Ferrante et al. *(13)* in fish from the Gulf of Naples (ranging from not detected to 56.38 ng/g).

The levels of contamination by NDL-PCBs varied from 0.13 to 32.44 ng/g (mean, 9.67 ± 7.93 ng/g) in fresh-catch species, from 1.50 to 28.17 ng/g (mean, 11.67 ± 8.06 ng/g) in aquaculture species, and from 0.12 to 17.34 ng/g (mean, 4.97 ± 4.86 ng/g) in frozen ones. These values are lower than those reported by the European Food Safety Authority *(10)* in fish and fishery products, including Baltic fish, from European Union (EU) Member States. The NDL-PCB mean levels of contamination in wild and farmed European seabass found in this study were 4.60 ± 2.80 ng/g and 12.98 ± 9.14 ng/g, respectively (data not shown). These results are in agreement with those reported by Carubelli et al. *(5)* in a study of wild specimens (mean, 3.85 ± 2.35 ng/g) but are higher than those found on the farmed specimens (mean, 7.02 ± 2.79 ng/g) from the Orbetello Lagoon, Tuscany, Italy. NDL-PCBs constitute most of the PCBs found in human tissues and in food *(11)*.

Among the comparable fresh-catch and frozen samples in the study, only the wild European hake was significantly more polluted by PCBs than frozen samples ($P = 0.022$). This finding suggests that the frozen hakes analyzed came

from deep and less polluted fisheries far from the coasts. In wild gilthead seabream and European seabass, PCB levels were significantly lower than those found in frozen and farmed counterparts ($P = 0.02$). Whereas the aquaculture remainder fishes are usually frozen and then purchased, it can be reasonably supposed that the frozen gilthead seabream and European seabass collected in this study came mainly from farming. This hypothesis can be also supported by the lipid content found in these frozen fishes (about 5%), which was very similar to that found in fresh farmed specimens, while in the wild fishes of the same species fat never exceeded a value of 2%. By contrast, the frozen Mediterranean mussels were significantly less polluted than wild and farmed ones ($P = 0.015$ and 0.022, respectively) (Fig. 1).

OCPs. Fewer than 50% of the marine organisms analyzed in this study were polluted by OCPs at detectable levels (Table 2). The highest prevalence of HCB contamination was found in the frozen fish (46%), followed by the aquaculture and the fresh-catch (36 to 35%) fish. HCB concentrations varied from <0.01 to 3.29 ng/g (aquaculture), with median values of <0.01 ng/g. Among the aquaculture samples, p,p'-DDE was found at detectable amounts in 58% of the samples analyzed at levels of up to 7.16 ng/g

TABLE 2. *Prevalence of OCP contamination and concentrations of OCPs in the marine fish and molluscs analyzed*

	Fish category:					
	Fresh catch		Aquaculture		Frozen	
OCP[a]	POC (%)[b]	Mean concn $\pm$ SD (median) and range[c]	POC (%)	Mean concn $\pm$ SD (median) and range	POC (%)	Mean concn $\pm$ SD (median) and range
HCB	36	0.05 ± 0.06 (<0.01) <0.01–0.24	37	0.30 ± 0.80 (<0.01) <0.01–3.29	46	0.05 ± 0.53 (<0.01) <0.01–0.20
p,p'-DDE	28	0.15 ± 0.29 (<0.04) <0.04–1.42	58	0.72 ± 2.01 (1.55) <0.04–7.16	41	0.14 ± 0.20 (<0.04) <0.04–0.90
p,p'-DDD	14	0.11 ± 0.20 (<0.04) <0.04–0.83	25	0.25 ± 0.75 (<0.04) <0.04–1.70	15	0.08 ± 0.14 (<0.04) <0.04–0.83
p,p'-DDT	18	0.22 ± 0.53 (<0.04) <0.04–2.49	29	0.47 ± 0.92 (<0.04) <0.04–3.80	17	0.06 ± 0.05 (<0.04) <0.04–0.28
ΣDDTs	53	0.48 ± 0.58 (0.19) 0.12–2.57	79	2.45 ± 2.65 (1.63) 0.12–11.00	58	0.28 ± 0.23 (0.15) 0.12–0.97
ΣOCPs	63	0.53 ± 0.59 (0.22) 0.12–2.57	84	2.75 ± 2.65 (1.97) 0.12–11.32	50	0.33 ± 0.26 (0.22) 0.12–1.12

[a] ΣDDTs, sum of p,p'-DDE, p,p'-DDD, and p,p'-DDT; ΣOCPs, sum of HCB, p,p'-DDE, p,p'-DDD, and p,p'-DDT.
[b] POC, prevalence of contamination.
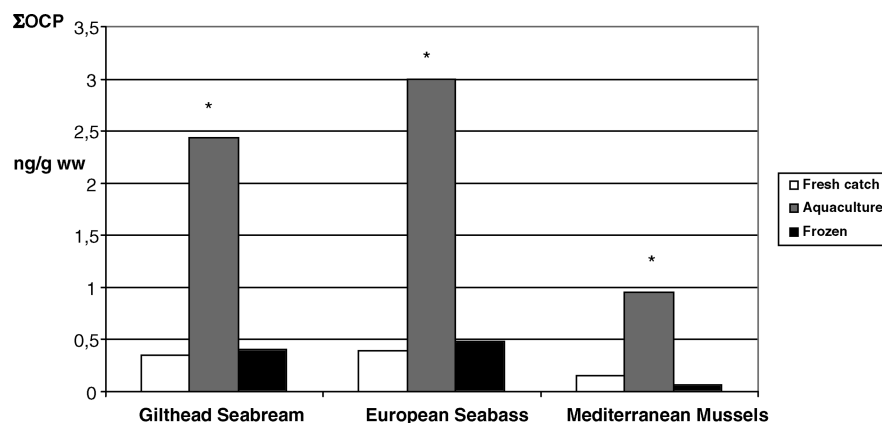[c] Values are in nanograms per gram, wet weight.

FIGURE 2. *Median levels of organochlorines pesticides (OCP) (in nanograms per gram, wet weight [ww]) in fresh-catch, aquaculture, and frozen gilthead seabream, European seabass, and Mediterranean mussels marketed in Campania, Italy. Asterisks indicate values that are significantly different among Gilthead Seabream, European Seabass, and Mediterranean mussels in different marketing sectors.*

(median, 1.55 ng/g), while in frozen and fresh samples the prevalence of p,p'-DDE contamination was 41 and 28%, respectively, with concentrations of up to 0.90 ng/g (median, <0.04 ng/g) and 1.42 ng/g (median, <0.04 ng/g), respectively. The lowest rates of contamination were those of p,p'-DDD and p,p'-DDT. The highest concentration was observed for p,p'-DDT (3.80 ng/g) in aquacultured fish. Consequently, the highest values of the ΣDDTs were obtained in the aquaculture species (0.12 to 11.00 ng/g; median, 1.63 ng/g).

No significant differences were found between OCP concentrations in fresh-catch and frozen species, while the contamination levels found in aquaculture products were significantly higher than in the other two types (Fig. 2). Comparing the OCP concentrations among the three categories in the same species, only wild red mullets were significantly more polluted than their frozen counterparts (P = 0.034).

These HCB and DDT concentrations are lower than those reported by Marcotrigiano and Storelli *(24),* who found, in marine organisms from the Adriatic and Ionian seas (Italy), residues of HCB of up to 4.55 ng/g and residues of p,p'-DDE, p,p'-DDT, and p,p'-DDD of up to 52.24, 6.49, and 9.82 ng/g, respectively. With the exception of the HCB amounts detected in farmed fish, our results are also noticeably lower than those reported in the study by the Italian Istituto Superiore di Sanità cited above (HCB, p,p'-DDE, p,p'-DDD, and p,p'-DDT levels of up to 0.56, 19.88, 3.42, and 4.03 ng/g, respectively) *(20).* By contrast, the total amounts of DDT isomers in this study (0.12 to 11.00 ng/g) were higher than those reported by Binelli and Provini *(3)* in edible clams from Italian and European markets (0.4 to 3.4 ng/g). According to all these authors p,p'-DDE, the most stable among the DDT isomers, was the most prevalent compound. In the samples in which DDE and DDT were found at detectable levels, the p,p'-DDE/p,p'-DDT ratio was calculated. It ranged from 0.01 to 0.2 in 41% of the fresh-catch species, from 0.1 to 0.3 in 14% of the aquaculture species, and from 0.1 to 0.9 in 30% of the frozen ones. The ratio of p,p'-DDE/p,p'-DDT is commonly used to assess the chronology of DDT input into ecosystems *(3).*

This finding could reflect the existence of some differences in the DDT contamination profile of the fisheries. The origin of freshly caught and frozen fish sold in Italy includes many European and non-European coastal countries on the Mediterranean sea and Atlantic, Indian, and Pacific oceans. In some areas, the use of DDT compounds for civil and agricultural purposes cannot be ruled out. With regard to farmed fish and molluscs, the influence of the geographical origin of the feed ingredients could also help to explain the results found.

For the OCPs, the maximum residue limits were established for fish and aquatic products by the Ministerial Decree of 27 Agosto 2004 *(6)* on the basis of the lipid percentage of food. In all the species analyzed in this study, in which the lipid contents varied from 0.2 to 14.6 g%, OCP contamination never exceeded the EU legal limits for fish or other aquatic products.

PAHs. In all, 16 PAHs were found: AC, ACEN, ANT, BA, BaP, BBF, BKF, BPE, CH, DB, FLU, FLUO, IND, NAP, PHE, and PY. All the marine organisms analyzed in this study were polluted by PAHs at detectable levels (Table 3). The total PAH contents ranged between 0.82 and 134.28 ng/g (median, 17.59 ng/g). The highest prevalence of the contamination, except for BKF, was found in the aquaculture species. The most prevalent compounds were PHE and BA, which were found at detectable levels in 96% of the analyzed samples and at concentrations ranging, respectively, from <0.09 to 2.33 ng/g (median, 0.32 ng/g) and from <0.05 to 3.47 ng/g (median, 0.14 ng/g). In fresh-catch fish BA was the most widely detected compound (89%) followed by PHE, FLU, PY (86%), and CH (71%). In frozen fish FLUO contamination prevailed (88%), followed by BA (85%) and PHE (83%). BaP, recommended as a carcinogenic PAH marker by the EU Scientific Committee on Food due to its genotoxic and carcinogenic properties, was detected in 66% of the aquaculture, 35% of the fresh-catch, and 24% of the frozen samples analyzed (Table 4). The aquacultured species showed the highest levels of BaP contamination (range, <0.03 to 9.18 ng/g; median, 0.42 ng/g), while the frozen species (range, <0.03 to 4.22 ng/g; median, <0.03 ng/g) and the fresh-catch species (range, <0.03 to 3.55 ng/g; median, <0.03 ng/g) showed lower and similar levels of BaP contamination.

Farmed gilthead seabream and Mediterranean mussels were significantly more polluted than wild and frozen mem-

TABLE 3. *Prevalence of PAH contamination and concentrations of PAHs in the marine fish and molluscs analyzed*

	Fish category:					
	Fresh catch		Aquaculture		Frozen	
PAH	POC (%)[a]	Mean concn ± SD (median) and range[b]	POC (%)	Mean concn ± SD (median) and range[c]	POC (%)	Mean concn ± SD (median) and range[c]
NAP	68	9.65 ± 14.62 (2.70) <0.20–54.25	79	5.20 ± 4.80 (4.76) <0.20-15.24	51	4.02 ± 8.70 (0.89) <0.20–40.90
ACEN	39	8.33 ± 18.87 (<0.40) <0.40–79.04	75	9.41 ± 13.44 (5.00) <0.40–62.00	66	7.84 ± 14.80 (3.25) <0.40–83.78
AC	64	15.50 ± 20.89 (3.84) <0.10–62.76	75	6.70 ± 7.19 (3.52) <0.10–25.47	41	4.17 ± 7.80 (<0.10) <0.10–29.23
FLU	25	0.17 ± 0.55 (<0.10) <0.10–2.83	58	0.70 ± 1.02 (<0.10) <0.10–4.54	61	0.17 ± 0.29 (<0.52) <0.10–1.73
PHE	86	0.23 ± 0.23 (0.15) <0.10–1.00	96	0.47 ± 0.53 (0.32) <0.10–2.33	83	0.28 ± 0.24 (0.28) <0.10–1.08
ANT	64	0.09 ± 0.19 (0.06) <0.03–0.80	88	0.15 ± 0.10 (0.14) <0.03–0.37	73	0.06 ± 0.07 (0.04) <0.03–0.34
FLUO	86	1.32 ± 2.12 (0.90) <0.10–9.56	88	2.50 ± 3.31 (1.19) <0.10–13.43	88	0.97 ± 1.72 (0.63) <0.10–1.10
PY	86	0.56 ± 0.55 (0.33) <0.20–1.95	88	0.56 ± 0.53 (0.49) <0.20–2.20	73	0.47 ± 0.77 (0.26) <0.20–4.45
BA	89	1.92 ± 1.80 (1.67) <0.05–6.00	96	0.36 ± 0.70 (0.14) <0.05–3.47	85	0.50 ± 0.69 (0.26) <0.05–3.48
CH	71	0.23 ± 0.48 (0.09) <0.05–2.50	88	0.40 ± 0.36 (0.42) <0.05–1.56	78	0.23 ± 0.34 (0.11) <0.05–1.31
BBF	50	0.36 ± 0.63 (0.06) <0.03–2.76	88	0.24 ± 0.17 (0.21) <0.03–0.66	41	0.21 ± 0.53 (<0.03) <0.03–3.05
BKF	68	0.43 ± 0.57 (0.19) <0.03–1.85	46	1.00 ± 1.81 (<0.03) <0.03–6.52	41	0.20 ± 0.43 (<0.03) <0.03–2.45
BaP	35	0.40 ± 0.86 (<0.03) <0.03–3.55	66	0.85 ± 1.84 (0.42) <0.03–9.18	24	0.18 ± 0.67 (<0.03) <0.03–4.22
BPE	14	0.11 ± 0.25 (<0.03) <0.03–1.21	25	0.85.± 1.84 (<0.03) <0.03–5.63	10	0.11 ± 0.27 (<0.03) <0.03–1.64
DB	14	0.22 ± 0.71 (<0.05) <0.05–3.69	25	1.03 ± 2.00 (<0.05) <0.05–6.66	2	0.11 ± 0.45 (<0.05) <0.05–2.91
IND	7	0.03 ± 0.07 (<0.03) <0.03–0.33	25	0.07 ± 0.11 (<0.03) <0.03–0.41	2	0.02 ± 0.6 (<0.03) <0.03–0.38
ΣPAHs	100	43.47 ± 38.77 (26.33) 1.18–117.73	100	33.44 ± 24.44 (28.44) 1.29–105.94	100	21.50 ± 27.76 (10.11) 0.82–134.28

[a] POC, prevalence of contamination.

[b] Values are in nanograms per gram, wet weight.

bers of the same species ($P = 0.01$), while frozen European seabass was significantly more contaminated than wild and farmed members of the same species ($P = 0.01$) (Fig. 3). The different distributions of the pollutants evaluated between farmed and frozen gilthead seabream and European seabass are not in agreement with what was noted concerning PCB contamination levels in the same species. As PAH distribution in the aquatic environment depends on many factors and PAHs are metabolized differently in the various marine organisms, their absorption patterns can vary considerably. As observed in European hake concerning PCB pollution, PAH levels were also significantly higher in wild specimens than in frozen ($P = 0.04$).

EU Regulation 1881/2006 set the maximum admissible concentrations for BaP in many foods *(7)*. In fish products, the limits vary from 2 μg/kg (fish muscular tissue) to 5 μg/

TABLE 4. *Benzo(a)pyrene concentrations in marine fish and molluscs analyzed*

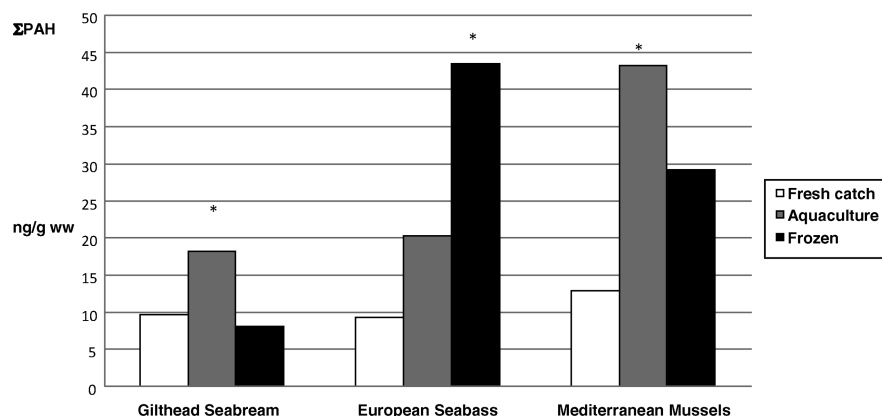|	Mean ± SD BaP concn, median, and range (ng/g, wet wt)		
Fish category	Muscle fish	Cephalopods and crustaceans	Bivalve molluscs
Fresh catch	0.27 ± 0.62, <0.03, <0.03–1.98	0.71 ± 1.23, <0.03, <0.03–3.55	<0.03, <0.03, <0.03
Aquaculture	0.27 ± 0.33, 0.07, <0.03–0.97		2.02 ± 2.92, 1.00, 0.42–9.18
Frozen	0.06 ± 0.09, <0.03, <0.03–0.26	0.33 ± 0.99, <0.03, <0.03–4.22	<0.03, <0.03, <0.03

FIGURE 3. *Median levels of polycyclic aromatic hydrocarbons (PAHs) (in nanograms per gram, wet weight [ww]) in fresh-catch, aquaculture, and frozen gilthead seabream, European seabass, and Mediterranean mussels marketed in Campania, Italy. Asterisks indicate values that are significantly different among gilthead seabream, European seabass, and Mediterranean mussels in different marketing sectors.*

kg (cephalopods-crustaceans) and 10 μg/kg (bivalve molluscs). All the samples analyzed in this study were under those limits (Table 4).

The PAH composition pattern was dominated by PAHs with three rings (FLUO, ANT, and PHE) and four rings (PY, BA, CH, BKF, and BBF) followed by those with five rings (BaP, BPE, DB, and IND) (Table 3). A similar trend was also reported by Binelli and Provini *(3)* in clams marketed in Italy and in Europe and by Perugini et al. *(25)* in fish from the Adriatic (Italy). The prevalence of medium-low condensate ring structures suggests that both oil pollution and combustion emissions in the atmosphere contribute to marine pollution.

The results of this study demonstrated a diffuse contamination by PCBs, OCPs, and PAHs in the fish products marketed in Campania, Italy, but the levels of the pollutants never exceeded the published EU legal limits. PCB, OCP, and PAH concentrations were similar to or lower than those reported elsewhere for Italy or other European countries.

Farmed gilthead seabream and mussels analyzed in this study showed the highest levels of concentration for all the chemicals considered. The lipidic content of farmed fish, on average about threefold higher than that of wild fish (data not shown), may well affect the absorption of lipophilic chemical pollutants. Furthermore, feeds for fish farming could reasonably be considered a major source of organic pollutants in fish contamination. Since 2002, the maximum permitted levels for a range of contaminants including dioxins, OCPs, heavy metals, etc., in animal feedstuff have been set by EU Directives 2002/32/EC and 2006/77/EC *(8, 9),* while no specific limits are currently available for NDL-PCBs and PAHs in feedstuff.

While there are extensive toxicological data on DL-PCBs that can be used to assess health risks, in contrast very little toxicological information is available on NDL-PCBs and little is known about their toxicity profiles. The European Commission has expressed concern that NDL-PCBs can cause neurobehavioral, reproductive, and perinatal development problems, as well as promote tumors. The adverse effects reported in laboratory animals following exposure to individual NDL-PCBs were effects on the thyroid, liver, and brain biochemistry, as well as immunotoxicity, estrogenicity, and reproductive and neurodevelopmental effects. However, such effects are not all specific

to NDL-PCBs but can also follow exposure to polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, and DL-PCBs; moreover, epidemiological studies do not allow an estimation of the toxicity that may differentiate the effects that can be specifically attributed to the NDL-PCBs since human exposure usually involves environmental mixes containing both NDL-PCBs and DL-PCBs.

The average daily intake of total NDL-PCBs for adults in Europe is estimated to be between 10 and 45 ng/kg of body weight (bw) with higher intakes observed among young children. In certain groups exposed to highly contaminated marine food, such as Baltic Sea fishermen, higher exposures may occur. For major consumers of fish and fish products, an average exposure of 35 ng/kg/day is estimated *(23)*. Unfortunately, data on per capita fish consumption in Campania are not available. However, given an annual average consumption of fish by Italians (22 kg) and the values obtained in this study for NDL-PCBs, ranging from 0.12 to 32.44 ng/g, with a median of 6.01 ng/g, we estimated that the average daily dietary intake of NDL-PCB for adults (assuming a 60-kg bw) can vary between 0.12 and 32.45 ng/kg of bw, with an average of 6.02 ng/kg of bw. These values are consistent with those reported by the European Food Safety Authority but show that, in some cases, the consumption of fish, in particular from aquacultured species, can significantly contribute to the average daily exposure to NDL-PCB estimated in Europe.

Regarding OCP, for HCB we estimated intake levels ranging from 0.013 to 3.29 ng/kg of bw per day with a median value of 0.11 ng/kg/day (assuming a 60-kg bw), which represents a rather low contribution to the established admissible daily intake (0.16 μg/kg/day) *(28)*. Regarding DDT isomers, on the basis of the residue levels found in this study, the daily levels of intake via consumption of fish can be estimated to range from 0.12 to 11.05 ng/kg/day (median value, 0.90 ng/kg/day). For the sum of *p,p′*-DDE, *p,p′*-DDD, *p,p′*-DDT, *o,p′*-DDE, *o,p′*-DDD, and *o,p′*-DDT the established admissible daily intake is 10 μg/kg of bw *(21)*. Our values, albeit related only to the *p,p′* DDT isomers, show a low contribution of fish to the admissible daily intake.

Regarding exposure to PAHs, the EU estimated that the dietary exposure to each of the most abundant PAHs, such as ANT, PHE, FLUO, and PY, may range from 60 to

80 ng/kg/day, for a person weighing 70 kg. The EU Scientific Committee on Food *(26)* concluded that at these levels of intake the risk of heritable effects from dietary exposure to PAHs is low. Considering the concentrations of these PAHs in our samples and the Italian annual average fish consumption, the contribution of fish to daily PAH intake from fish can vary between 0.025 and 11.54 ng/kg of bw (assuming a 70-kg bw).

In assessing the risk of long-term adverse health effects following dietary intake of PAHs, 15 compounds have shown clear carcinogenic effects in various types of bioassays in experimental animals. Since characterization of the risk is very difficult, the EU Scientific Committee on Food has generally recommended that exposures should be as low as reasonably achievable. The EU estimated (data obtained from 1983 to 2002 by European national surveys) a maximum daily intake of BaP from food of approximately 420 ng per person, equivalent to approximately 6 ng/kg/day for a person weighing 70 kg; this value is about 5 or 6 orders of magnitude lower than the daily doses observed to induce tumors in experimental animals *(26)*. Considering the BaP concentrations in our samples and the Italian annual average fish consumption, the contribution of fish to BaP daily intake can vary between 0.025 and 7.89 ng/kg, with a median value of 0.025 ng/kg (assuming a 70-kg bw). These data are consistent with those reported by the EU Scientific Committee on Food.

As for the consumption of wild and farmed fish, the European Food Safety Authority *(10)* maintains that the current levels of contaminants would not justify a general recommendation against fish consumption but also recommends that research should be carried out on novel and "cleaner" feeds for fish farming. The measures carried out by governments in many countries worldwide in banning, or severely restricting, the use of PCBs and OCPs have resulted in a progressive decrease in marine water pollution. To reduce chemical environmental pollution worldwide, international action plans have been implemented since 1994 to provide advice on the environmentally sound management of risks from chemicals *(16)*. In 2001, the Stockholm Convention on persistent organic pollutants formed a framework, based on the precautionary principle, with the priority aim of the reduction in production and use and the safe elimination of a group of persistent organic pollutants harmful to human health and the environment, informally called the "dirty dozen" (aldrin, chlordane, DDT, dieldrin, dioxins, endrin, furans, heptachlor, HCB, Mirex, PCBs, Toxaphene). The United States was a signatory to the Stockholm Convention but has yet to ratify it, unlike other countries including the EU, which implemented it via Council Decision 2006/507/EC *(12)*. Nevertheless, the globalization of trade makes active surveillance of the quality of marine products ever more necessary, particularly when coming from countries not included in the international plans for the protection of aquatic environments from chemical pollution. The control of feedstuff contamination and the introduction of farming techniques in order to decrease the lipidic content of fish should achieve lower bioconcentrations of organic contaminants in farmed species.

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