

**TOXIC ORGANIC MICROCONTAMINANTS IN EDIBLE MARINE  
SPECIES FROM THE ADRIATIC SEA**

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**Introduction**

The ubiquitous polychlorinated biphenyls (PCBs), dibenzodioxins (PCDDs) and dibenzofurans (PCDFs) are known to occur in aquatic environment and accumulate in aquatic organisms, so that consumption of fish and shellfish from polluted areas may be a significant human dietary source for these highly toxic compounds eventually posing a real health risk. It is important to carry out studies to determine the levels of these microcontaminants in food in order to assess human exposure. In this context, a number of studies have been recently published regarding dioxin-related contamination of fish products. The present work, complementing the past and ongoing assessments of Venice lagoon biota [1], was carried out to estimate the extent of PCB and PCDD/F contamination in selected fish products from fishing harbors representing three areas of the Adriatic sea (see map). This research is part of a large monitoring program focused on a number of toxicologically important microcontaminants and involving several edible marine species from the Adriatic area.

**Materials and Methods**

Marine species with high commercial value were obtained in two sampling campaigns (April–June 1997, and November 1997–January 1998) from the northern (nine sites), central (six sites), and southern (four sites) areas of the Adriatic sea. As this research is part of a large monitoring program and in order to obtain preliminary data on PCB and PCDD/F contamination, some species were selected for analysis from all of the species sampled. High, medium, and low priority was assigned on the basis of sampling frequency (i.e., number of sites) and more than one trophic level was required to be represented. Only species with high and medium priority ranking were chosen for this study (see Table 1).

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Species <sup>b</sup>	North <sup>c</sup>					Centre <sup>c</sup>					South <sup>c</sup>					Priority <sup>d</sup>			
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
Anchovy	3	1	3	3	2					3	3	3	2			3	3	3	H
Squid	3	3	3	3						3	3	3	2			3	3	3	H
Hake	2	1	3	3	2					3	3	3	2			3	3	3	L
Mussel	2	1	1	3					2			1	2			3	1		2 M
Spottail mantis shrimp	1	3	3	3						3	3	3	2			3	2	2	L
Anglerfish		2	3							1	3	3	2			3	3	3	L
Norway lobster		1	3	3						3	3	3	2			3	3	3	H
Cuttlefish	3	3	3	3	2					3	3	3	2			3	3	3	L
Mackerel	1	1	3	1	2					3	3	3	2			3	3	3	H
Sole	1	3	3	3	2					3	2	3	2			1	1	3	L
Red mullet	1		3		3					3	3	3	2			3	3	3	H
Clam		2	3	3	2	1	1	1	2	2	3	1	2	1	1	3	2		H

**Table 1.** Sampling scheme.<sup>a</sup> Priority of the diverse marine species for a preliminary microcontaminant assessment.

(a) 1, specimens collected during the first sampling campaign; 2, specimens collected during the second sampling campaign; 3, specimens collected during the first and the second sampling campaigns.

(b) Anchovy, *Engraulis encrasicolus*; squid, *Loligo vulgaris*; hake, *Merluccius merluccius*; mussel, *Mytilus galloprovincialis*; spottail mantis shrimp, *Squilla mantis*; anglerfish, *Lophius piscatorius*; Norway lobster, *Nephrops norvegicus*; cuttlefish, *Sepia officinalis*; mackerel, *Scomber scombrus*; sole, *Solea vulgaris*; red mullet, *Mullus barbatus*; clam, *Chamelea gallina*.

(c) A, Caorle; B, Cesenatico; C, Fano; D, Goro; E, Rimini; F, Rosolina Mare (Goro); G, Cavallino (Caorle); H, Cattolica (Fano); I, Marano Lagunare, Laguna di Marano (Caorle); J, Civitanova Marche; K, San Benedetto; L, Pescara; M, Termoli; N, Porto S. Giorgio (Civitanova Marche); O, Roseto degli Abruzzi (S. Benedetto); P, Manfredonia; Q, Mola di Bari; R, Molfetta; S, Zapponeta (Manfredonia).

(d) H, high; M, medium; L, low.



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Edible parts of individual specimens of the same sample were suitably washed and pooled together. The final sample matrix was made by properly mixing pooled samples from both sampling campaigns. Each matrix was combined with fully  $^{13}\text{C}$ -labelled standards, homogenized, and lyophilized [1]. A 200- to 500-mg aliquot of freeze-dried material was subjected to  $\text{CO}_2$  Supercritical Fluid Extraction (SFE) in a Hewlett-Packard 7680T extractor for the analysis of PCBs [2]; clean-up required a chromatographic filtration on activated alumina. A 10-g portion of freeze-dried material was extracted with a pressurized 1:1 (v/v) mixture of *n*-hexane-acetone, using a Dionex ASE 200 instrument for PCDD and PCDF determination; cleanup required a sequence of canonical steps, ending with chromatographic filtration on activated alumina. Determination of organic analytes was carried out by HRGC-LRMS(SIM) or -HRMS(SIM), the latter being used to measure PCDDs and PCDFs or else, as a confirmatory technique. About 60 PCB congeners of the tri- to octachlorosubstituted homologous groups, and seven PCDD and ten PCDF congeners were determined based on their toxicological importance. **Layout of sampling sites.** Marine species were collected at 19 sites within the northern, central, and southern areas of the Adriatic sea (only main fishing harbors shown).



### Results and Discussion

PCB, PCDD and PCDF cumulative fresh-weight-based (fw) findings in the selected marine species are summarized in Table 2. PCDD/F results are also presented after conversion to 2,3,7,8-T<sub>4</sub>CDD toxicity equivalents (I-TE) through the current I-TEF system [3]. Figures below determination limits were entered as half their nominal value to calculate the sum of congener concentrations. Analyses of PCBs revealed that a remarkable difference of contamination levels seems to characterize species obtained from the northern, central, and southern sampling sites respectively, between 7.62 and 177, 2.25 and 157, and 4.47 and 94.1 ng/g fw. Furthermore, as might be expected, contamination levels appear to differ among species. The greatest PCB concentrations were found in mackerel (94.1-177 ng/g fw), that is the species with the highest fat content (11.1% [4]). The industrial use of PCBs is severely restricted in the European Union from 1976, but maximum residue levels for PCBs in foodstuffs are not yet established. Hence, the tolerance limits for total PCBs set or recommended by different countries such as the USA (2000 ng/g fw [5]), Canada (2000 ng/g fw [5, 6]) or France (2000 ng/g [7]), were used for comparison. Total PCB concentrations found in fish products from this study are far below these limits, and are comparable with PCB contamination levels detected in specimens from marine background zones [8]. A preliminary application of multivariate analysis to the available data highlights [or confirms] the existence of a recurring PCB pattern made up by the majority of congeners detected and independent of both the species and the fishing area. Of the species assayed, however, the anchovy appears to be the most sensitive one in that it exhibits the most appreciable variation of analyte concentration levels with the fishing area.

On the whole, PCDD and PCDF contamination levels appear to be low. However, although several congeners were below quantification limit, analytical results and I-TE data were representative in almost all cases, according to the criteria set forth by our laboratory [9, 10]. In general, I-TE findings are greater for those species at higher levels in the trophic web (mackerel > red mullet > anchovy), although the higher fat content of the first two species (11.1 and 6.2 %, respectively [4]) also accounts for part of the greater fw contamination measured. Contamination levels fall within 0.23 and 1.07 pgTE/g fw in the aforesaid species, while all remaining species exhibit contamination levels ranging from approximately 0.07 to 0.25 pgTE/g fw. With the exception of few species (northern and central Norway lobster, northern mussel, and southern clam), PCDF analytical contributions are greater than those of PCDDs. Lastly, I-TE cumulative findings in species from the northern area are in general greater than those from the central and southern areas (with the questionable exception of mackerel). The low contamination levels found in the species investigated are comparable with those detected in specimens from marine background zones [8, and references therein]. Higher contamination levels have been found in sea fish from other countries such as Germany and Sweden [11–13], but similar I-TE contributions are reported in sea fish from Japan [14], The Netherlands [15, 16], or United Kingdom [17].

In conclusion, marine species appear to show, as expected, a trend towards higher contamination levels with increasing anthropic impact. However, contamination levels determined give no indication of particular health risks associated with consumption of these products.

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**Table 2.** Total PCB, PCDD and PCDF concentration levels (fresh weight based) in samples of selected marine species from the Adriatic sea northern, central, and southern areas.

Species/Area	PCBs ng/g	PCDDs pg/g	PCDFs pg/g	Total PCI pg/g	pgTE/g
Anchovy					
<i>North</i>	132	0.50	1.53	2.03	0.47
<i>Centre</i>	43.2	0.34	0.90	1.24	0.34
<i>South</i>	33.6	0.33	0.71	1.04	0.23
Squid					
<i>North</i>	81.5	0.30	0.93	1.23	0.25
<i>Centre</i>	41.1	0.20	0.81	1.01	0.17
<i>South</i>	22.0	0.25	0.48	0.73	0.12
Mussel					
<i>North</i>	45.0	1.54	1.52	3.07	0.24
<i>Centre</i>	6.65	0.58	0.98	1.56	0.16
<i>South</i>	13.5	0.49	0.89	1.38	0.11
Norway lobster					
<i>North</i>	7.62	0.69	0.62	1.31	0.14
<i>Centre</i>	2.25	0.68	0.65	1.33	0.085
<i>South</i>	4.47	0.46	0.77	1.23	0.12
Mackerel					
<i>North</i>	177	0.34	2.49	2.83	0.59
<i>Centre</i>	157	0.53	2.38	2.90	0.94
<i>South</i>	94.1	0.32	3.05	3.37	1.07
Red mullet					
<i>North</i>	90.2	0.60	1.49	2.09	0.56
<i>Centre</i>	42.9	0.29	1.09	1.39	0.37
<i>South</i>	41.1	0.53	0.99	1.52	0.37
Clam					
<i>North</i>	10.3	0.53	1.38	1.91	0.13
<i>Centre</i>	5.55	0.50	0.55	1.05	0.10
<i>South</i>	3.72	0.38	0.38	0.76	0.066

TE: 2,3,7,8-T<sub>4</sub>CDD toxicity equivalents.

Rounding off of the data reported merely reflects the outcome of a mathematical approximation and does not imply analytical significance.

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