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Mercury accumulation and speciation in the muscle of red mullet (*Mullus barbatus*) and annular sea bream (*Diplodus annularis*) from Izmir Bay (Eastern Aegean)

L. Tolga Gonul, Filiz Kucuksezgin *

Dokuz Eylul University, Institute of Marine Sciences and Technology, İnciralti, 35340 Izmir, Turkey

Contamination of marine organisms with toxic chemicals such as mercury is of ecological and health concern worldwide (Goldberg, 1995). The presence and behavior of mercury in aquatic systems is of great interest and importance since it is the only heavy metal which consistently biomagnifies through all levels of the aquatic food chain (Lindqvist et al., 1991).

Monomethylmercury (CH_3Hg^+) is the most toxic form and accounts for more than 95% of organic mercury in fish muscle tissues (Bloom, 1992; Porcella, 1994). The lipophilic nature of this organic compound facilitates its penetration across cell membranes. Moreover, methylmercury affinity for the sulphydryl groups of certain proteins and lipid tissues and its long biological halflife leads to its rapid accumulation in living organisms (Storelli et al., 2002). The organic mercury, methylmercury (MeHg), in the marine environment is from biogenic origins, mainly via anaerobic bacteria. This process is chiefly promoted by sulphate-reducing bacteria in the superficial layers of the bottom sediments (Benoit et al., 1998; Gilmour and Henry, 1991; Compeau and Bartha, 1987). When the contaminated sediment is resuspended and transported, the MeHg formed may dissolve in the water column (Covelli et al., 1999; Gill et al., 1999), and then can be taken up by aquatic organisms and accumulated. MeHg readily enters the aquatic food chain and it may be biomagnified as it accumulates in higher trophic levels (Lawrence et al., 1999). It attains the highest concentration in higher consumer fish tissues and marine mammals, at the top of the aquatic food chain (USEPA, 1997). Carnivorous species and marine mammals can be considered good indicators of mercury bioaccumulation (Malm et al., 1995; Di Beneditto et al., 1999).

Izmir Bay is one of the great natural bays of the Mediterranean. The bay can be divided into three zones: the inner, middle and outer bays according to topographical, hydrological and ecological features. Industrial activities cover a large range of industries including food processing, tanneries, paint, chemicals, textile and petroleum refining. During the last ca. 30 years, a large number of studies have been carried out on the physical, chemical and biological oceanography of Izmir Bay.

There are few published data on total mercury concentrations in organisms from Izmir Bay (Parlak and Demirkurt, 1990; Kucuksezgin et al., 2002, 2006), and no data are available on mercury accumulation and speciation in organisms from Izmir Bay. The aim of this study was to comparatively evaluate the total mercury (THg) and MeHg concentrations in two fish species, red mullet (*Mullus barbatus*) and annular sea bream (*Diplodus annularis*), collected from different sites of Izmir Bay. These two species are widely distributed and found all over the bay, are numerically dominant in most catches and the most consumed species.

* Corresponding author. Tel.: +90 232 278 5565; fax: +90 232 278 5082.
E-mail address: filiz.ksezgin@deu.edu.tr (F. Kucuksezgin).

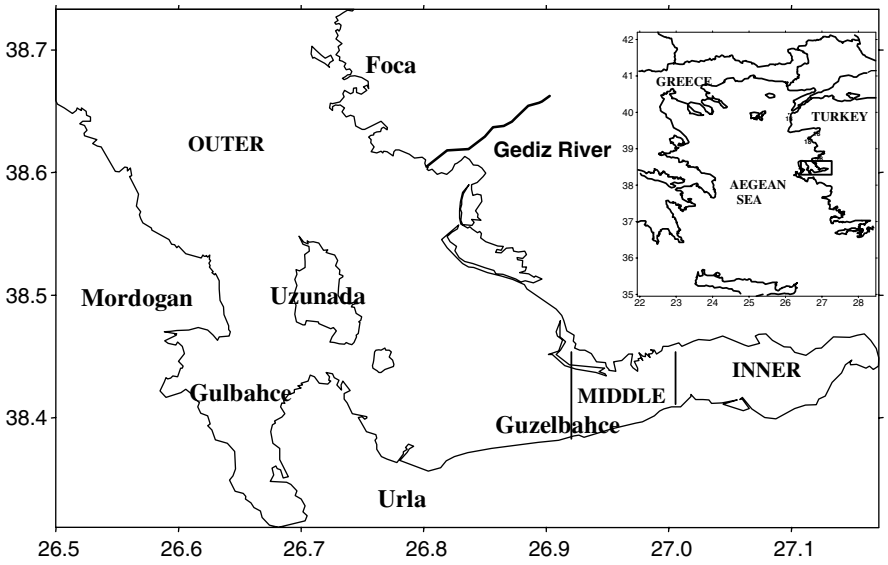

Fig. 1. Sampling areas in the Izmir Bay.

Table 1
Details of individual species in the Izmir Bay

Species	Habitat	Feeding habits	Reproduction	Distribution
Mullus barbatus	Muddy bottom (100–300 m)	Carnivorous (worms, small benthic invertebrates, molluscs, crustaceans)	April–August	Eastern Atlantic, Mediterranean Sea, European and African coasts
Diplodus annularis	Littoral and sandy bottoms (0–90 m)	Carnivorous (crustaceans, molluscs, worms, echinoderms, hydrozoans)	February–April	Mediterranean Sea, Black Sea, Azov Sea, Atlantic from Biscay Bay to Gibraltar

Fish samples (*Mullus barbatus*; $N = 186$, and *Diplodus annularis*, $N = 108$) were collected at four locations (Foca-Gediz, Uzunada, Gulbahce, Guzelbahce) shown in Fig. 1 during the R/V K. Piri Reis Cruise in Izmir Bay in February, April, August and September 2005. The details of individual species are presented in Table 1. The species descriptions are based on UNESCO (1986). Weight and fork length were measured on each fish and the sex was determined. Following this, muscle tissue was removed in the field using surgical sheets, and acid-washed lancets and then preserved at −20 °C until analysis. In the laboratory, approximately 0.5 g of fish muscle was digested with 5:1 HNO_3:$HClO_4$ in a microwave digestion system. The resultant solutions were then diluted to the desired volume with double distilled water and the total Hg concentrations were measured in a VARIAN atomic absorption spectrophotometry (Spectra AA-300 Plus) by cold vapour technique. After reducing the Hg^{2+} to Hg^0 with $SnCl_2$ (stannous chloride), the volatile Hg^0 was bubbled into the closed system of the VGA-76 Vapour Generation Accessory analyser (wavelength: 253.7 nm) (UNEP, 1982).

Measurement of MeHg was conducted using the UNEP/FAO/IAEA/IOC (1992) method. This method is based on liberating the organic form of mercury (R–Hg) from the dried biological matrices by homogenization of the sample in an acid medium. In order to extract all the organic Hg, freeze-dried samples were treated with KBr and $CuSO_4$ in sulphuric acid, which facilitates the liberation of CH_3–Hg bound to thiol groups; the bromine forms a stable compound in a sulphuric medium with CH_3–Hg. The R–Hg–Br was extracted with toluene. This extract was then treated with cysteine solution which selectively complexes the organo-mercury compounds to form water-soluble derivatives. Any co-extracted interfering materials within the toluene phase were thus removed. The complexed organo-mercury compounds within the aqueous phase were liberated in an acid medium and were back extracted into toluene. The resulting solution can then be subjected to gas chromatographic separation for quantification of the individual organo-Hg compounds. Final measurement of the methylmercury content was performed using a Chrompack CP 9000 gas chromatograph, equipped with a splitless capillary injection system and ^{63}Ni electron capture detector (ECD). The capillary column used was a 50 m × 0.25 mm CP-Sil 8CB (0.25 μm film thickness, WCOT fused silica). The injector and detector temperatures were same and maintained at 160 °C. The oven temperature was set to 130 °C. Nitrogen was used both as the carrier (2.50 ml min^{-1}) and make-up (32.5 ml min^{-1}).

The accuracy of the Hg analyses was tested by analysis of certified fish muscle homogenate sample, IAEA 407 for total and IAEA 436 for methyl mercury (from the International Laboratory of Marine Radioactivity, IAEA, Monaco). Our measurements (0.214 ± 0.0015 mg kg^{-1} dw for

Table 2
Total Hg (THg) and methyl Hg (MeHg) levels (μg kg^{-1} wet wt.) and the ratios of MeHg to THg in different fish from Izmir Bay

Sampling location	Species	Length (mm)	Weight (g)	Sex	N	THg	MeHg	MeHg %
February 2005								
Foca–Gediz	*Mullus barbatus*	122–136	26–42	F	12	11.0	10.1	92
	Mullus barbatus	156–170	60–74	F	12	23.5	22.3	95
	Diplodus annularis	110–119	24–34	M	11	30.1	26.5	88
Gulbahce	*Mullus barbatus*	121–129	24–32	F	20	14.0	13.0	93
	Mullus barbatus	92–116	12–24	M	20	4.4	3.8	86
Uzunada	*Mullus barbatus*	110–128	24–40	F	17	20.3	17.9	88
	Diplodus annularis	150–168	64–104	F	5	23.1	21.9	95
	Diplodus annularis	153–165	66–88	M	5	31.8	28.6	90
April 2005								
Gulbahce	*Mullus barbatus*	97–115	12–22	M	13	61.1	51.3	84
	Diplodus annularis	110–120	28–42	M	10	92.6	82.4	89
Uzunada	*Mullus barbatus*	149–165	56–84	F	8	77.9	70.9	91
	Diplodus annularis	120–137	38–58	M	10	46.1	42.9	93
Guzelbahce	*Mullus barbatus*	111–120	22–30	F	10	49.5	45.6	92
	Diplodus annularis	116–129	30–42	M	10	45.2	41.2	91
August 2005								
Foca-Gediz	*Mullus barbatus*	140–158	42–72	F	19	103.1	96.9	94
	Diplodus annularis	100–120	20–36	M	13	132.0	112.3	85
Gulbahce	*Mullus barbatus*	142–158	48–66	F	16	82.2	78.5	95
	Diplodus annularis	105–122	24–38	M	18	221.4	194.8	88
Uzunada	*Mullus barbatus*	152–169	54–82	F	7	75.6	71.9	95
September 2005								
Uzunada	*Mullus barbatus*	121–146	28–48	M	17	139.5	121.4	87
	Mullus barbatus	163–185	70–110	F	15	157.9	148.3	94
	Diplodus annularis	125–140	36–58	M	14	127.5	116.0	91
	Diplodus annularis	120–138	36–60	F	12	157.2	154.0	98

Total Hg and 3.57 ± 0.35 mg kg^{-1} dw for Methyl Hg) were in the range of the certified material: 0.222 ± 0.024 mg THg kg^{-1} and 3.68 ± 0.42 mg MeHg kg^{-1} dw.

The concentrations of total mercury and methylmercury for each organism are summarized in Table 2. The concentrations are expressed as μg kg^{-1} wet weight (wet wt.). The results are discussed according to fish type, location and season. Other variables, including the ratio of MeHg to THg (MeHg%), length (mm) and weight (g) are also considered.

Among the two species examined, the highest levels of total mercury, ranging from 23.1 to 221.4 μg/kg wet wt. (mean 90.7 μg/kg), were determined in *Diplodus annularis*. Total mercury concentrations ranged from 4.4 to 157.9 μg/kg wet wt. (mean 63.1 μg/kg) in *Mullus barbatus* (Table 2). The lowest total mercury level (4.4 μg/kg wet wt.) was found in *Mullus barbatus* taken from Gulbahce in February while the highest concentration (221.4 μg/kg wet wt.) was detected in *Diplodus annularis* from Gulbahce in August. According to the results, *Mullus barbatus* accumulated higher levels of total mercury and methylmercury in September in comparison with other months while total mercury and methylmercury levels in August were higher than other months in *Diplodus annularis*.

Organic Hg (mainly methylmercury) varied between 21.9 and 194.8 μg/kg wet wt. (mean 82.1 μg/kg) in *Diplodus annularis*, and between 3.8 and 148.3 μg/kg wet wt. (mean 57.8 μg/kg) in *Mullus barbatus* (Table 2). The average of methylmercury for all fish ($N = 294$) was 68.4 ± 10.96 μg kg^{-1}. Methylmercury concentrations varied from 3.8 to 194.8 μg kg^{-1} wet wt. in all species. The highest (194.8 μg kg^{-1}) and the lowest (3.8 μg kg^{-1}) methylmercury concentration were found at Gulbahce in *Diplodus annularis* and *Mullus barbatus*, respectively.

Table 3
Range of Total Hg (THg), methyl Hg (MeHg), inorganic Hg levels (μg kg^{-1} wet wt.) and length (mm) in biota in different sampling areas

Sampling area	THg	MeHg	Inorg Hg	Length
Foca-Gediz				
Mullus barbatus	11.0–103	10.1–96.9	0.88–6.2	122–170
Diplodus annularis	30.1–132	26.4–112	3.7–19.6	100–120
Gulbahce				
Mullus barbatus	4.4–82.2	3.8–78.5	0.63–9.8	92–158
Diplodus annularis	92.6–221	82.4–195	10.2–26.6	105–122
Uzunada				
Mullus barbatus	20.3–158	17.8–148	2.5–18.1	110–185
Diplodus annularis	23.1–157	21.9–154	1.2–11.5	120–168
Guzelbahce				
Mullus barbatus	49.5	45.6	3.9	111–120
Diplodus annularis	45.2	41.2	4.0	116–129

The percentage of MeHg was high and constant in muscle. The mean percentages of methylmercury to total mercury for all fish samples were $91.0 \pm 0.77\%$ (Mean $\pm$ SE) with a range of 84–98%, indicating that organic mercury was the predominant form of mercury in the muscle tissue of fish (Table 2).

A general examination of the statistical summary shows the range of total, organic and inorganic mercury levels and length in *Mullus barbatus* and *Diplodus annularis* within different regions (Table 3). Hg and MeHg concentrations in *Mullus barbatus* increased at Uzunada while Hg levels decreased at Gulbahce. On the other hand, *Diplodus annularis* demonstrated maximum levels at Gulbahce and minimum levels at Foca–Gediz. Mercury concentrations at Uzunada were similar to the Foca–Gediz sampling area. The Hg levels were lower in *Mullus barbatus* than in*Diplodus annularis*.

It is important to have an overall picture of the obtained THg in the analysed species from a public health perspective. So as to achieve this, it was first necessary to convert concentrations to wet weight values using dry weight/wet weight ratio (0.36 ± 0.03 for *Mullus barbatus*, 0.26 ± 0.02 for *Diplodus annularis*). It is then possible to apply the categorization of Chvojka et al. (1990) who described THg in the wet weight of fish from 0.05 to 0.15 $\mu g\ g^{-1}$ as very low, from 0.15 to 0.25 $\mu g\ g^{-1}$ as low, from 0.25 to 0.35 $\mu g\ g^{-1}$ as medium, from 0.35 to 0.45 $\mu g\ g^{-1}$ as high and total Hg (THg) above 0.45 $\mu g\ g^{-1}$ as very high. The results from Izmir Bay are shown in Fig. 2. It is clear that there are no fish in the "medium" category and that *Mullusbarbatus* and *Diplodus annularis* represent significantly lower health threats.

A simpler grouping, utilizing the WHO permissible limits (WHO, 1990), can be also used where 0.5 $\mu g\ g^{-1}$ is the limit for THg and 0.3 $\mu g\ g^{-1}$ for MeHg. The results indicate that none of the fish analyzed was $\geqslant 0.500\ \mu g\ g^{-1}$ for THg and $\geqslant 0.300\ \mu g\ g^{-1}$ for MeHg and nor did they exceed the WHO limit.

To assess the potential health impact, the 43 $\mu g\ day^{-1}$ maximum intake limit for THg (WHO, 1989) and the 30 $\mu g\ day^{-1}$ maximum intake limit for MeHg of a 70 kg person was used as a guideline (USEPA, 1984). Using the

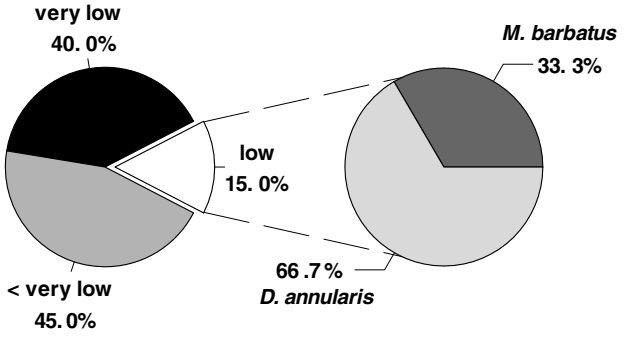

Fig. 2. The distribution of THg in fishes from Izmir Bay according to concentration categories (Chvojka et al., 1990).

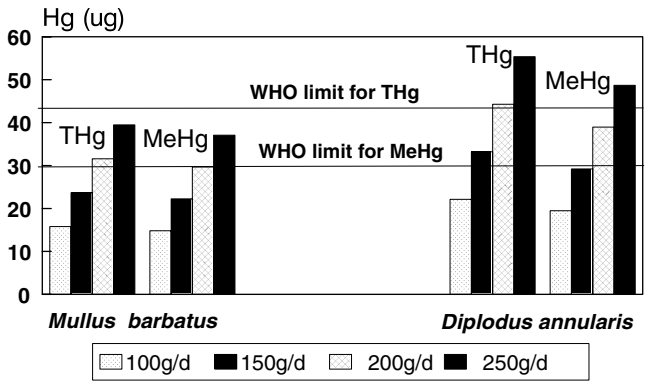

Fig. 3. The estimated human intake of THg and MeHg (μg) from *Mullus barbatus* and *Diplodus annularis* according to quantities of fish consumed.

observed concentrations in the fish and a range of fish consumption (100, 150, 200 and 250 $g\ d^{-1}$) the results shown in Fig. 3 were obtained (Note: Among the species and samples that showed maximum concentrations were considered). From this it can be seen that at a consumption rate of 250 $g\ d^{-1}$ or more, the WHO limit was exceeded for THg in *Diplodus annularis* while it was not exceeded for THg in *Mullus barbatus*. For MeHg, at a consumption rate of 200 $g\ d^{-1}$ the EPA limit was approached and exceeded at a rate of 250 $g\ d^{-1}$ for *Mullus barbatus* while at a consumption rate of 150 $g\ d^{-1}$ it was approached and exceeded at a consumption rate of 200 $g\ d^{-1}$ or more for *Diplodus annularis*.

There was a strong and statistically significant ($p < 0.01$) correlation between T–Hg and MeHg concentrations ($R = 0.99725$). The relationships between fork lengths and mercury concentrations were significant ($R = 0.57$, $n = 186$, $a = -114.4$, $b = 1.29$, $p < 0.05$ for THg, $R = 0.61$, $n = 186$, $a = -111.7$, $b = 1.25$, $p < 0.05$ for MeHg) in *Mullus barbatus*. There were no significant correlations between fork lengths and mercury concentrations in *Diplodus annularis*. There were significant correlations between both THg and MeHg concentrations and length, reflecting an accumulation with age as described commonly in the literature (Lange et al., 1994; Joiris et al., 1995a; Holsbeek et al., 1997) for *Mullus barbatus*. This result represents the fact that weight is in effect a proxy for age and hence exposure. The levels of mercury in fish increase with body size,

Table 4
Values of one-way analysis of variance for all sampling periods

Variable	Season			Sampling area		
	Df	*F*	*p* level	*df*	*F*	*p* level
Mullus barbatus						
THg	3	69.2899	***	3	1.1219	ns
MeHg	3	56.38798	***	3	1.0826	ns
Diplodus annularis						
THg	3	11.53691	**	3	0.8440	ns
MeHg	3	10.49812	**	3	0.7384	ns

$^{*}p < 0.05$;$^{**}p < 0.01$; *** < 0.001, ns: non-significant.

so larger, older fish have generally higher concentrations than smaller, younger fish.

In *Mullus barbatus,* accumulation of organic Hg concentrations increased with length, while inorganic Hg concentrations remained at a constant low level (i.e., the relative methyl Hg concentrations increased with length). This pattern is generally regarded as normal in those fish species exhibiting a high relative organic Hg concentration. Some fish species may be capable of the demethylation of MeHg, albeit over long time periods. The same positive correlations have been found earlier by several authors.

The results of one-way ANOVA between stations and seasons during sampling periods are given in Table 4. Concentrations in muscle tissue of *Mullus barbatus* differed significantly among seasons for THg ($p < 0.05$, $df = 3$, $F = 69.2899$) and MeHg ($p < 0.05$, $df = 3$, $F = 56.38798$). In muscle tissues of *Mullus barbatus,* THg and MeHg levels in April differed significantly from February and September, as shown by a Post hoc Tukey HSD analyses.

Concentrations in muscle tissue of *Diplodus annularis* differed significantly among season for THg ($p < 0.05$, $df = 3$, $F = 11.53691$) and MeHg ($p < 0.05$, $df = 3$, $F = 10.49812$). THg and MeHg levels in September differed significantly from February (Post hoc Tukey HSD analyses). August was different from February and April. Lastly, April was different from August in muscle tissue of *Diplodus annularis*.

The significant differences between sampling areas for THg ($p < 0.05$, $df = 3$, $F = 1.1219$) and MeHg ($p < 0.05$, $df = 3$, $F = 1.0826$) were not found in muscle tissue of *Mullus barbatus*. In the same way concentrations in muscle tissue of *Diplodus annularis* did not differ among sampling areas for THg ($p < 0.05$, $df = 3$, $F = 0.8440$) and MeHg ($p < 0.05$, $df = 3$, $F = 0.7384$). Hg, as shown with a Post hoc Tukey HSD analysis, displayed no difference between sampling areas.

A one-way ANOVA test showed non-significant differences for THg ($p < 0.05$, $df = 1$, $F = 1.299$) and MeHg ($p < 0.05$, $df = 1$, $F = 1.211$) in *Mullus barbatus* and *Diplodus annularis*. Such variability is in accordance with the process of mercury uptake in fish and the interaction of biotic parameters such as size, sex, longevity, growth rate, feeding habits and trophic position. Therefore, a knowledge of the physiology and ecology of the organisms is necessary in order to understand the meaning of the data concerning pollutant concentrations.

Various workers have addressed measurements of mercury in fish from different regions of the world and some of these data are summarized in Table 5. The findings of this study are not similar to other studies, especially for the difference of the mean Hg content between carnivorous and small benthic invertebrates (Nakagawa et al., 1997; Joiris et al., 1997). Carnivorous fish can be used as a good indicators for the monitoring of mercury pollution (Vigh et al., 1996) while other species with other feeding habits should also be monitored for human health aspects. A geographic comparison of total Hg and methylmercury in the examined species is difficult because to our knowledge there are no studies on mercury and its speciation in *Mullus barbatus* and *Diplodus annularis* from the Aegean Sea.

This study was the first to document the speciation and distribution of mercury in organisms from Izmir Bay. Results obtained therefore provide useful information in order to further assess mercury accumulation and speciation in the Bay.

The lowest total mercury level was found in *Mullus barbatus* while the highest concentration was detected in *Diplodus annularis*. This distribution pattern reflects the fact that *Diplodus annularis* can be used as a good indicator for monitoring of mercury pollution while other species with other feeding habits should also be monitored for human health aspects. Moreover, mercury levels in Febru-

Table 5
Levels of total Hg and MeHg in fish from the different regions of the world (as μg/kg wet wt)

Area	∑Hg (mean, min–max)	MeHg (mean, min–max)	MeHg%	References
Black Sea				
Phocoena phocoena	332 (90–720)	216 (60–540)	65	Joiris et al. (2001)
Brazilian estuary				
Micropogonias furnieri	199 (63.0–556.1)	195 (65.0–561)	98	Kehrig et al. (2002)
Bangladesh				
Rastrelliger kanagurta	64.0 (24–124)	48.0 (17–120)	77	Joiris et al. (2000)
Tachysurus thalassinus	120 (57–370)	101 (34–340)	83	
Ionian Sea				
Sphyrna zygaena	12150 (8550–21070)	14000 (7450–19570)		Storelli et al. (2003)
Mediterranean Sea				
Chimaera monstrosa	3104 (1300–5160)	2670 (1140–4560)	83.6	Storelli et al. (2002)
Torpedo nobiliana	2420 (1650–3590)	1900 (1150–2760)	81.0	
Myliobatis aquila	830 (670–1010)	630 (400–840)	71.6	
Izmir Bay (Aegean)				
Mullus barbatus	63.1 (4.4–157.9)	57.8 (3.8–148.3)	91.0	This study
Diplodus annularis	90.7 (23.1–221.4)	82.1 (21.9–194.8)	90.8	

ary for *Diplodus annularis* and *Mullus barbatus* were three to seven times lower in comparison with the other seasons.

Of the total 294 individuals analyzed, mercury was mostly in the organic form in all species (>84% MeHg). The result of this research demonstrated that the percentage of MeHg was high and constant in muscle tissue of *Mullus barbatus* and *Diplodus annularis*.

Total mercury and methylmercury concentrations in *Mullus barbatus* were positively correlated with length, reflecting an accumulation of Hg with time (considering length as directly depending on age). However, there were no significant correlations between fork lengths and mercury concentrations in muscle tissue of *Diplodus annularis*.

The maximum Hg content was 221.4 μg $\sum$Hg kg^{-1} wet wt., corresponding to 194.82 μg MeHg kg^{-1} wet wt. For a person eating 200–250 g of *Diplodus annularis* daily, the maximum amount of MeHg ingested is of 39–49 μg daily (273–343 μg weekly), a value of the same order of magnitude as the 210 μg permissible tolerable weekly intake (PTWI) for methylmercury proposed by United States Environmental Protection Agency (USEPA, 1984). Our results indicated that *Diplodus annularis* exceeded this limit.

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Organochlorine contamination (PCBs and DDTs) in deep-sea fish from the Mediterranean sea

Maria M. Storelli, Veronica G. Perrone, Giuseppe O. Marcotrigiano *

Pharmacological–Biological Department, Chemistry and Biochemistry Section, Medicine Veterinary Faculty, University of Bari, Strada Provinciale per Casamassima, Km 3, 70010 Valenzano, Bari, Italy

Organic contaminants, such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (e.g., DDTs), are a group of persistent compounds whose presence in the environment is a clear indication of anthropogenic pollution. Several studies have shown that these compounds exhibit a great variety of sex-, strain-, and species-specific toxic effects, including immuno, hepato and neurotoxicity, as well as reproductive alterations (Ahlborg et al., 1994). Several of these adverse effects are caused by the most toxic members of the PCB family, the so-called "dioxin-like", 3,3′,4,4′-T_4CB, (IUPAC 77), 3,3′,4,4′,5-P_5CB (IUPAC 126), and 3,3′,4,4′,5,5′-H_6CB (IUPAC 169), whose properties are similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD).

In marine environments, the final fate of these contaminants is to be collected in the sediments of the abyss (Harvey and Steinhauer, 1976; Tatsukawa and Tanabe, 1984). Transported by living or dead biota, or absorbed to the detritus, these hydrophobic pollutants can reach the sea floor. From this final resting ground, these pollutants can be resuspended by physical mixing or by the activity of bottom-dwelling organisms and bioaccumulate the food chain. Consequently, fish living on or near the sea floor might be highly prone to accumulation. It has been, in fact, reported that the deep-sea fauna shows higher loads of organochlorine compounds as compared to surface living species (Froescheis et al., 2000; de Brito et al., 2002a). Despite this, only a few studies have determined the presence of PCBs and DDTs in deep-sea biota (Berg et al., 1997, 1998; Froescheis et al., 2000; de Brito et al., 2002a), especially in the Mediterranean sea (Solé et al., 2001; Storelli et al., 2004), a marine area at risk by these substances (Meadows, 1992; Kuetting, 1994; Borrell et al., 1997).

In consideration of the above, the present study measures the hepatic levels of polychlorinated biphenyls (PCBs) and organochlorine pesticides (DDTs) in two different species of deep-sea fish *Nezumia sclerorhynchus* and *Coelorhynchus coelorhynchus* from the Mediterranean sea (Adriatic sea) in order to ascertain their contamination status. In addition, a comparison of our results with those from other deep-sea environments is included to evaluate the relative significance of the contamination.

Specimens of hollowsnout grenadier (*C. coelorhynchus*) (specimen number: 352; weight: 2.2–82.3 g, average 33.0 ± 22.9; length: 4.2–8.7 cm, average 6.0 ± 1.3) and roughtip grenadier (*N. sclerorhynchus*) (specimen number: 1054; weight: 10.0–34.1 g, average 21.8 ± 7.7; length: 3.3–4.4 cm, average 3.9 ± 0.4) were caught in the Mediterranean sea (Adriatic sea) between May and June 2003. From the total number of specimens, pools were formed (roughtip grenadier no. 12; roughtip grenadier no. 8) within which individual fish were gathered as a function of their similar size. From the fish of each pool, liver was taken and deep frozen at −20 °C until chemical analysis. The extractive analytical procedure and the instrumental conditions for determining polychlorinated biphenyls (PCBs = 8, 20, 28, 35, 52, 60, 77, 101, 105, 118, 126, 138, 153, 156, 169, 180 and 209) and DDT compounds (DDTs = *p*,*p*′-DDT, *p*,*p*′-DDE, *o*,*p*′-DDT, *p*,*p*′-DDD, *o*,*p*′-DDD) concentrations have been described in detail elsewhere (Storelli et al., 2004, 2006). Briefly, aliquots (1–2 g) of the homogenised samples were ground with anhydrous sodium sulphate in a mortar. The mixture was extracted with petroleum ether according to Erney's procedure (Erney, 1983). The extracts were then concentrated and subsamples were taken in order to determine the tissue fat content by gravimetry. An aliquot of the remaining extract was dissolved in hexane and mixed with conc. H_2SO_4 for the clean up, following the procedure described by Murphy (1972). For the separation of non-*ortho* PCB congeners, 3,3′,4,4′-T_4CB, (IUPAC 77), 3,3′,4,4′,5-P_5CB (IUPAC 126), and 3,3′,4,4′,5,5′-H_6CB (IUPAC 169) from other PCBs the method reported by Tanabe et al. (1987) was used. Analyses were made on a Carlo Erba HR gas chromatograph 8000 Top with an automatic injection system and an electron capture detector ECD-800, Ni^{63} (temperature:

* Corresponding author. Tel.: +39 80 5443866; fax: +39 80 5443863.
E-mail address: g.o.marcotrigiano@veterinaria.uniba.it (G.O. Marcotrigiano).