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Mercury speciation in the muscle of two commercially important fish, hake (*Merluccius merluccius*) and striped mullet (*Mullus barbatus*) from the Mediterranean sea: estimated weekly intake

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Abstract

Total mercury and methylmercury concentrations were measured in the muscle tissue of two fish species from the Ionian and Adriatic seas. Higher total mercury and methylmercury concentrations were detected in striped mullet (*Mullus barbatus*), a benthic species (Ionian sea: Hg = 0.40 μg g^{-1} wet wt, MeHg = 0.40 μg g^{-1} wet wt; Adriatic sea: Hg = 0.49 μg g^{-1} wet wt, MeHg = 0.44 μg g^{-1} wet wt), than in hake (*Merluccius merluccius*), a pelagic species (Ionian sea: Hg = 0.09 μg g^{-1} wet wt, MeHg = 0.09 μg g^{-1} wet wt; Adriatic sea: Hg = 0.18 μg g^{-1} wet wt; MeHg = 0.16 μg g^{-1} wet wt). Total mercury residues were determined in all samples of both species from the Adriatic sea, while levels below the limit of detection were registered in 25% and 11%, respectively, of striped mullet and hake samples from the Ionian sea. In 18.8% and 22.2% of striped mullet samples from the Ionian and Adriatic seas, respectively, total mercury concentrations exceeded the maximum level fixed by the European Commission Decision (Hg = 0.5 μg g^{-1} wet wt). In the two different species, mercury was present almost completely in the methylated form with mean percentages between 60% and 100%. The estimated weekly intake for total mercury was below the established the provisional tolerable weekly intake (PTWI) for both species, though their consumption provides a methylmercury intake above the WHO safety limit.

Keywords: Mercury; Methylmercury; Mediterranean sea; Fish

1. Introduction

Mercury may occur naturally in the environment or be released by human activities. In water bodies, inorganic mercury can be converted into highly toxic organomercury compounds. Methylmercury, the most toxic chemical species of the element, is a neurotoxic agent that affects the development of the nervous system, resulting in psychological disturbance, impaired hearing, loss of sight, ataxia, loss of motor control and general debilitation. Poisoning episodes in Japanese people in the 1950s, following the consumption of methylmercury-contaminated seafood, have in fact, demonstrated, irreversible neurological damage and teratogenic effects (De Flora, Bennicelli, & Bagnasco, 1994; Leonard, Jacquet, & Lauwerys, 1983). Seafood consumption appears to constitute a major route of methylmercury exposure for humans, resulting in statistical differences between sub-populations with high and low fish consumption (Holsbeek, Das, & Joiris, 1996; Nakagawa, Yumita, & Hiromoto, 1997). National Agencies have used limits for mercury concentrations in fish to protect against hazardous exposure. The US Food and Drug Administration (FDA) has set an action level of 1 μg g^{-1} wet wt for the concentration of total mercury in fish. In Japan, fish containing total mercury concentrations exceeding the Japanese maximum permitted limit of 0.4 μg g^{-1} wet wt is considered unsuitable for human consumption. In Europe, the limit value for total mercury,

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is set at 0.5 μg g^{-1} wet wt, except for some species, in which it is raised to 1 μg g^{-1}. However, total mercury concentrations above the regulatory limits have been observed many times, particularly in species occupying high trophic levels (Monteiro & Lopes, 1990; Nakagawa et al., 1997; Storelli & Marcotrigiano, 2001b; Storelli, Giacominelli Stuffler, & Marcotrigiano, 2002a) and in species living on or close to the sea bed (Storelli & Marcotrigiano, 2000; Storelli, Giacominelli-Stuffler, & Marcotrigiano, 1998).

In this scenario the importance of monitoring mercury and the methylmercury burden is clear, particularly in the edible tissue of fish important in the diet, in order to safeguard public health. With this objective our study:

(a) measured concentrations of total mercury and methylmercury in the edible fish tissue of hake (*Merluccius merluccius*) and striped mullet (*Mullus barbatus*),
(b) ascertained whether the mercury concentrations were below the maximum level fixed by the European Commission Decision (Official Journal of the European Communities, 1994),
(c) estimated the weekly intake and compared it with the provisional tolerable weekly intake (PTWI) recommended by the Joint FAO/WHO Expert Committee on Food Additives (WHO, 2003).

2. Materials and methods

In June–August 2002, hake (*Merluccius merluccius*) and striped mullet (*Mullus barbatus*) from different seas (Ionian sea and Adriatic sea) were caught during several trawl surveys (Fig. 1). For each species, pools within which individual fish were collected as a function of their similar sizes were formed from the total number of specimens. Muscle tissue was removed from the organisms in each pool and frozen at −25 °C until the analysis was carried out. The tissues were dissected with plastic materials that were washed with HNO_3 and rinsed with distilled and deionized water, in order to avoid metal contamination. For analyses of total Hg, homogenized samples of the tissue (1–3 g wet wt) were digested to a transparent solution with 10 ml of the mixture H_2SO_4–HNO_3 (1:1) under reflux. The resultant solutions were then diluted to a known volume with deionized water (G.U.C.E., 1990) and the total Hg concentrations were measured by atomic absorption spectrophotometry (Perkin Elmer 5000) by the cold vapour technique, after reduction by $SnCl_2$ (A.V.A. Thermo Jarrel Ash Corp.). Methylmercury was determined, following the method described by Hight and Corcoran (1987). Homogenized samples of the tissue (about 1 g wet wt) were pre-washed 3 times with 10 ml of acetone and once with 10 ml of benzene. The pre-washed tissue was acidified with 5 ml

Fig. 1. Sampling sites (bold line).

HCI–H_2O (1 + 1) and extracted 3 times with 10 ml of benzene. After centrifugation, the combined benzene extracts were concentrated in Kuderna–Danish glassware. The extracts were diluted to 25 ml with benzene, mixed with 5 g Na_2SO_4, and analyzed by a gas chromatograph (Carlo Erba model HRGC-5300) equipped with a ^{63}Ni electron capture detector (ECD-400) and splitless injection technique was used. The column consisted of a fused silica capillary SPB-5 Supelco (length = 30 m, inside diameter = 0.50 mm, 5 μm film). Acid-washed glassware, analytical grade reagents and double distilled deionized water were used in the tissue analysis. In order to check the purity of the chemical used, a number of chemicals blanks were run; there was no evidence of any contamination in these blanks. Analytical quality control was achieved using TORT-1 Lobster Hepatopancreas (National Research Council of Canada). Replicate analyses ($n = 5$) (Hg total 0.32 ± 0.02 μg g^{-1} dry wt; MeHg 0.123 ± 0.020 μg g^{-1} dry wt) were in the range of the certified material (Hg total 0.33 ± 0.06 μg g^{-1} dry wt; MeHg 0.128 ± 0.014 μg g^{-1} dry wt). All data were computed on a μg g^{-1} wet wt basis.

3. Results and discussion

Table 1 shows range and mean values of total Hg and MeHg concentrations, together with the percentages of methylmercury to total mercury in muscle tissue of the two fish species. The statistical analysis provided convincing evidence of significant differences in the total

Table 1
Total mercury, methylmercury concentrations (μg g^{-1} wet wt) and percentages of methylmercury with respect to total mercury for the two fish species

	M. merluccius (Ionian sea)	*M. merluccius* (Adriatic sea)	*M. barbatus* (Ionian sea)	*M. barbatus* (Adriatic sea)
Total mercury	ND–0.30	0.04–0.48	ND–1.50	0.08–1.74
	0.09 ± 0.08	0.18 ± 0.12	0.40 ± 0.42	0.49 ± 0.54
Methylmercury	ND–0.30	0.04–0.48	ND–1.50	0.08–1.74
	0.09 ± 0.07	0.16 ± 0.10	0.40 ± 0.42	0.44 ± 0.53
Percentage of methylmercury	73–100	60–100	92–100	68–100
	98.3	90.8	98.9	79.8

mercury and methylmercury loads between the two species analyzed with lower concentrations in hake (Ionian sea: Hg = 0.09 μg g^{-1} wet wt, MeHg = 0.09 μg g^{-1} wet wt; Adriatic sea: Hg = 0.18 μg g^{-1} wet wt, MeHg = 0.16 μg g^{-1} wet wt), than in striped mullet (Ionian sea: Hg = 0.40 μg g^{-1} wet wt, MeHg = 0.40 μg g^{-1} wet wt; Adriatic sea: Hg = 0.49 μg g^{-1} wet wt, MeHg = 0.44 μg g^{-1} wet wt) ($p < 0.05$). Total mercury residues were determined in all samples of both species from the Adriatic sea, while levels below the limit of detection were registered in 25% and 11% of striped mullet and hake samples from the Ionian sea, respectively.

The wide variability of mercury levels among the different species is in accordance with the process of uptake of this metal in fish and the interaction of numerous parameters, either abiotic (water and sediments) or biotic (size, sex, longevity, growth rate, feeding habits, trophic position, habitat). It has been clearly documented that benthic fish show higher total mercury concentrations in their muscles than pelagic organisms, confirming the significant process of sedimentation and persistence of this metal in sea depths. In this context, striped mullet, a benthic species, that lives in deep water in close contact with the upper layer of sediment, the site of mercury methylation, showed higher levels than hake, a pelagic fish. Moreover, the importance of size to body mercury loading is widely recognized in marine organisms. Usually, older individuals show higher mercury levels than younger ones as a consequence of a longer exposure time (Dixon & Jones, 1994; Lansen, Leermakers, & Baeyens, 1991; Pellegrini & Barghigiani, 1989; Storelli & Marcotrigiano, 2000; Storelli et al., 1998). The positive relationship between mercury and methylmercury concentration and size of fish presented in our paper fits this general picture well. In fact, Fig. 2 shows that, in both the organisms studied, total mercury and methylmercury concentrations increased with the increase in weight (*M. merluccius* Ionian sea: mercury $r = 0.59$ $P < 0.001$, methylmercury $r = 0.53$ $P < 0.001$;

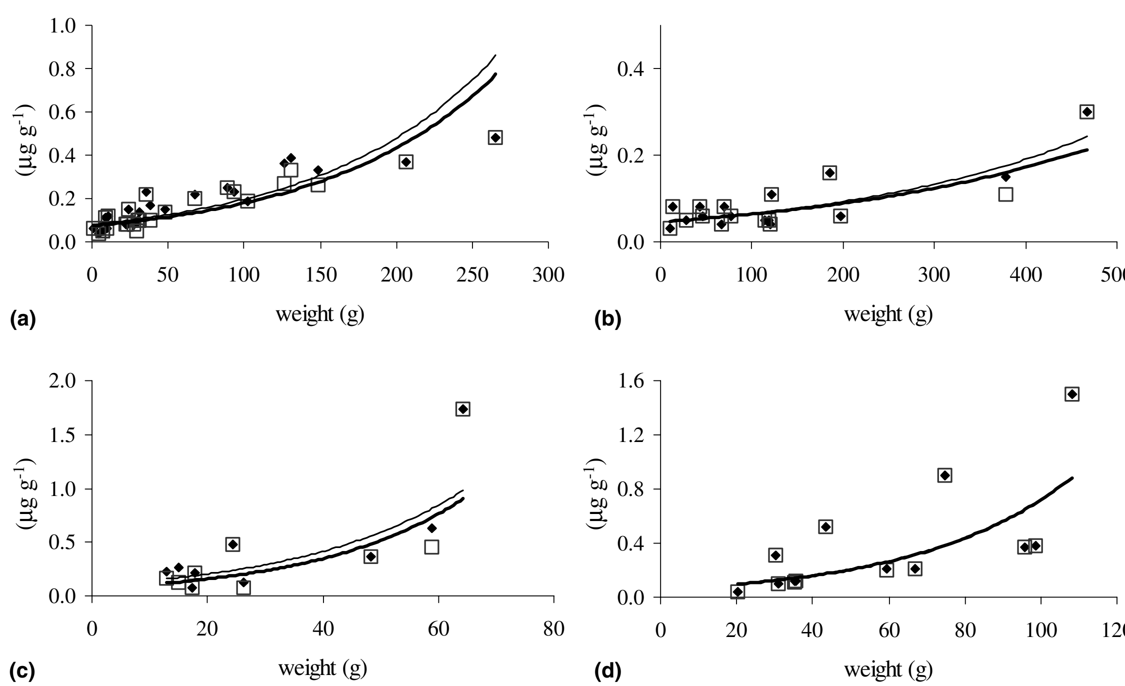

Fig. 2. Correlation between total mercury (◆) and methylmercury (□) concentrations and weight in muscle tissue of *M. merluccius* (Adriatic sea: a; Ionian sea: b) and *M. barbatus* (Adriatic sea: c; Ionian sea: d).

M. merluccius-Adriatic sea: mercury $r = 0.71$ $P < 0.001$, methylmercury $r = 0.72$ $P < 0.001$; *M. barbatus*-Ionian sea: mercury $r = 0.57$ $P < 0.001$, methylmercury $r = 0.57$ $P < 0.001$; *M. barbatus*-Adriatic sea: mercury $r = 0.61$ $P < 0.001$, methylmercury $r = 0.62$ $P < 0.001$) illustrating the greater accumulation in larger size fish than smaller. However, if mercury accumulates with age, the observed variability would also reflect different spectra of size between specimens from different localities. To test this hypothesis, a comparison of total mercury contents was carried out between organisms of the same species and of equal size from different localities. The results of this investigation indicated, however, markedly higher values for species from the Adriatic sea than in those from the Ionian sea (Fig. 3), suggesting that the variability observed might reflect the variability of mercury levels encountered in the environment. On the other hand it has been put in evidence that, in the eastern Mediterranean sea, the areas subjected to a higher contamination are the Aegean sea and the Adriatic sea (Catsiki, Papathanassiou, & Bei, 1991; Fytianos, Evgenidou, & Zachariadis, 1999; Storelli, Giacominelli Stuffler, & Marcotrigiano, 2002b).

Numerous recent studies have demonstrated that most, if not all, of the mercury that is bioaccumulated in fish muscle tissue is as methylmercury. Anderson and Depledge (1997) reported percentages between 63% and 86% of organic mercury in the muscle tissue of different fish species. Joiris, Holsbeek, and Moatemri (1999) found that mercury is mostly in the organic form (>85%) in the muscle of sardines, while Storelli et al. (2002a) and Storelli, Giacominelli Stuffler, Storelli, and Marcotrigiano (2003) in the muscle tissue of tuna fish and of other fish species, reported organic mercury percentages between 75–100% and 94–100%, respectively. Methylmercury percentages to total mercury, recorded in this study, varying from 60% to 100%, generally fall in the range of the values in the above mentioned literature.

The presence of such large proportions of this toxic metallo-organic compound of mercury in fish muscle tissue, in combination with the fact that seafood consumption is the main source of mercury intake in people not occupationally exposed (Cuadrado, Kumpulainen, & Moreiras, 1995; Galal-Gorchev, 1993), amply underlines the need for preventive measures to safeguard public health.

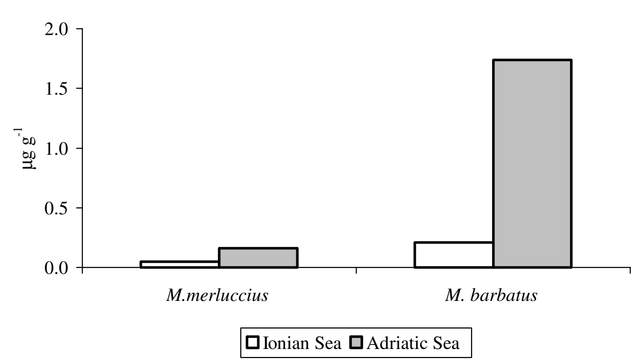

Fig. 3. Comparison of total mercury levels in similar sized specimens from different marine areas.

It has already been mentioned that governmental agencies have set limits for mercury concentrations above which the fish is considered unsuitable for human consumption. European Commission Decision 93/351 of 19 May 1993 (Official Journal of the European Communities, 1994) sets the maximum limit for mercury in seafood at 0.5 $\mu g\,g^{-1}$ wet wt, increasing to 1 $\mu g\,g^{-1}$ wet wt for the edible parts of some species in Annex A of the same ECD, which for physiological and ecological reasons concentrate mercury more easily in their tissues. For fish analyzed in the present study total mercury concentrations should not exceed 0.5 $\mu g\,g^{-1}$. Considering this standard, concentrations exceeding it were observed solely in 18.8% and in 22.2% of striped mullet samples from the Ionian and Adriatic seas, respectively.

Also, the Joint Food and Agriculture Organisation/World Health Organisation (FAO/WHO) Expert Committee on Food Additives has established regulatory guidelines regarding dietary mercury intake. It recommends a provisional tolerable weekly intake (PTWI) of 300 μg of total mercury per person, of which no more than 100 μg should be present as methylmercury, amounts equivalent to 5 μg/kg body wt of total mercury and 1.6 μg/kg body wt of methylmercury (WHO, 2003).

To estimate the "degree" of mercury and methylmercury intake through seafood, our results were interpreted in terms of the FAO/WHO provisional tolerable weekly intake. By using the means of fish weekly consumption of the Italian population of 441 g (ISTAT, 2000), mean mercury and methylmercury concentrations in fish, and human body weight (60 kg), weekly intake calculated ranged from 0.66 to 3.60 μg/kg body wt for total mercury and from 0.66 to 3.23 μg/kg body wt for methylmercury (Table 2). As can be seen in Fig. 4, the estimated weekly intake of total mercury was below the established PTWI for both species, though their consumption determined a methylmercury intake higher than the WHO safety limit.

Although the results found in this work are reassuring, one should not forget that the estimated intake does not take account of intake from food other than fish. It also is important to note that a higher fish consumption would be of concern in terms of the risk of detrimental health effects. Italians consume a Mediterranean diet, characterized by high consumption of carbohydrates, fruits, vegetables, olive oil, and fish. Although the dietary pattern of Italians is the same among people living in the different regions the quantities of food consumed vary greatly. This is especially true of seafood consumption, it being higher in the population of southern

Table 2
Mean total mercury and methylmercury concentrations (μg g^{-1} wet wt) and estimated weekly intake and estimated daily intake (μg/kg body wt)

Species	Total Hg	Estimated weekly intake	MeHg	Estimated weekly intake
M. merluccius (Ionian sea)	0.09	0.66	0.09	0.66
M. merluccius (Adriatic sea)	0.18	1.32	0.16	1.17
M. barbatus (Ionian sea)	0.40	2.94	0.40	2.94
M. barbatus (Adriatic sea)	0.49	3.60	0.44	3.23

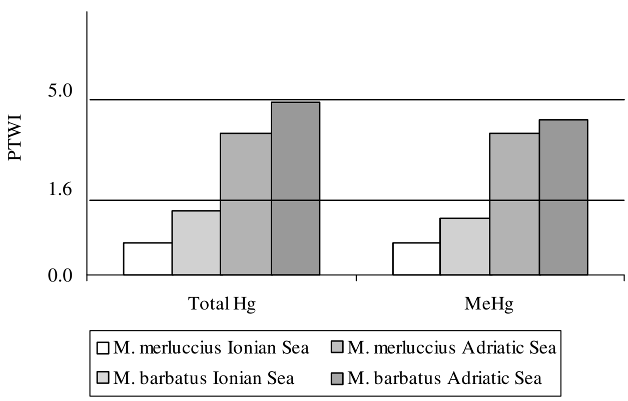

Fig. 4. The estimated weekly intake (μg kg^{-1} body wt) of mercury and methylmercury from *M. merluccius* and *M. barbatus* according to the WHO limits (total mercury = 5 μg kg^{-1} body wt; methylmercury = 1.6 μg kg^{-1} body wt).

Italy and in Italian isles than in the northern Italian population (Storelli & Marcotrigiano, 2001a). In particular there are certain population segments, such as fishermen and their families who eat greater amounts of seafood and are exposed to a higher risk than the general fish-eating population. For example, the daily consumption of 100 g of striped mullet, a quantity habitual for some populations (Buzina et al., 1995; Valentino, Torregrossa, & Saliba, 1995) of the Mediterranean basin, would result in a mercury and methylmercury intake (total mercury: 4.60–5.72 μg/kg body wt, methylmercury: 4.60–5.13 μg/kg body wt) surpassing the safety limit.

Concluding, the results of this work demonstrate that mercury intake, through the consumption of certain fish, particularly of benthic species, is a problem which deserves more attention.

References

Anderson, J. L., & Depledge, M. H. (1997). A survey of total mercury and methylmercury in edible fish and invertebrates from Azorean waters. *Marine Environmental Research, 44*, 331–350.

Buzina, R., Stegnar, P., Buzina-Suboticanec, K., Horvat, M., Petric, I., & Farley, T. M. M. (1995). Dietary mercury intake and human exposure in an Adriatic population. *The Science of the Total Environment, 170*, 199–208.

Catsiki, V., Papathanassiou, E., & Bei, F. (1991). Heavy metals levels in characteristic benthic flora and fauna in the central Aegean sea. *Marine Pollution Bulletin, 22*, 566–569.

Cuadrado, C., Kumpulainen, J., & Moreiras, O. (1995). Lead, and cadmium and mercury contents in average Spanish market basket diets from Galicia, Valencia, Andalucia and Madrid. *Food Additives and Contaminants, 12*, 107–118.

De Flora, S., Bennicelli, C., & Bagnasco, M. (1994). Genotoxicity of mercury compounds: A review. *Mutation Research, 317*, 57–79.

Dixon, R., & Jones, B. (1994). Mercury concentrations in stomach contents and muscle of five fish species from the north east coast of England. *Marine Pollution Bulletin, 28*, 741–745.

Fytianos, K., Evgenidou, E., & Zachariadis, G. (1999). Use of macroalgae as biological indicators of heavy metal pollution in Thermaikos gulf, Greece. *Bulletin of Environmental Contamination and Toxicology, 62*, 630–637.

Galal-Gorchev, H. (1993). Dietary intake, levels in food and estimated intake of lead, cadmium and mercury. *Food Additives and Contaminants, 10*, 115–128.

G.U.C.E. (1990). Gazzetta Ufficiale delle Comunità Europee. L 286/33 del 1990. Metodi di riferimento per la ricerca dei residui di metalli pesanti e arsenico.

Hight, S. C., & Corcoran, M. T. (1987). Rapid determination of methylmercury in fish and shellfish: Method development. *Journal of Association of Official. Analytical. Chemists, 70*, 24–30.

Holsbeek, L., Das, H. K., & Joiris, C. R. (1996). Mercury in human hair and relation to fish consumption in Bangladesh. *The Science of the Total Environment, 186*, 181–188.

ISTAT (2000) (Istituto Nazionale di Statistica) Statistica sulla pesca, caccia e zootecnia. Informazione No. 94-2000 Alba Grafica S.p.A., Roma.

Joiris, C. R., Holsbeek, L., & Moatemri, N. L. (1999). Total and methylmercury in sardines *Sardinella aurita* and *Sardina pilchardus* from Tunisia. *Marine Pollution Bulletin, 38*, 188–192.

Lansen, P., Leermakers, M., & Baeyens, W. (1991). Determination of methylmercury in fish by headspace-gas chromatography with microwave-induced-plasma detection. *Water Air Soil Pollution, 56*, 103–115.

Leonard, A., Jacquet, P., & Lauwerys, R. R. (1983). Mutagenicity and teratogenity of mercury compounds. *Mutation Research, 114*, 1–18.

Monteiro, L. R., & Lopes, H. D. (1990). Mercury content of swordfish, *Xiphias glaudius*, in relation to length, weight, age, and sex. *Marine Pollution Bulletin, 21*, 293–296.

Nakagawa, R., Yumita, Y., & Hiromoto, M. (1997). Total mercury intake from fish and shellfish by Japanese people. *Chemosphere, 35*, 2909–2913.

Official Journal of the European Communities, (1994). L. 144 of 16 June 1994.

Pellegrini, D., & Barghigiani, C. (1989). Feeding behaviour and mercury content in two flat fish in the northern Tyrrhenian sea. *Marine Pollution Bulletin, 20*, 443–447.

Storelli, M. M., & Marcotrigiano, G. O. (2000). Fish for human consumption: Risk of contamination by mercury. *Food Additives and Contaminants, 17*, 1007–1011.

Storelli, M. M., & Marcotrigiano, G. O. (2001a). Consumption of bivalve molluscs in Italy: Estimated intake of cadmium and lead. *Food Additives and Contaminants, 18*, 303–307.

Storelli, M. M., & Marcotrigiano, G. O. (2001b). Total mercury levels in muscle tissue of swordfish (*Xiphias glaudius*) and bluefin tuna

(*Thunnus thynnus*) from the Mediterranean sea. *Journal of Food Protection, 64*, 1058–1061.

Storelli, M. M., Giacominelli-Stuffler, R., & Marcotrigiano, G. O. (1998). Total mercury in muscle of benthic and pelagic fish from the south Adriatic sea (Italy). *Food Additives and Contaminants, 15*, 876–883.

Storelli, M. M., Giacominelli Stuffler, R., & Marcotrigiano, G. O. (2002a). Total and methylmercury residues in tuna-fish from Mediterranean sea. *Food Additives and Contaminants, 19*, 715–720.

Storelli, M. M., Giacominelli Stuffler, R., & Marcotrigiano, G. O. (2002b). Mercury accumulation and speciation in muscle tissue of different species of sharks from Mediterranean sea, Italy. *Bulletin of Environmental Contamination and Toxicology, 68*, 201–210.

Storelli, M. M., Giacominelli Stuffler, R., Storelli, A., & Marcotrigiano, G. O. (2003). Total and methylmercury content in edible fish from the Mediterranean sea. *Journal of Food Protection, 66*, 300–303.

Valentino, L., Torregrossa, M. V., & Saliba, L. J. (1995). Health effects of mercury ingested through consumption of seafood. *Water Science Technology, 32*, 41–47.

WHO (2003). Summary and conclusions of the sixty-first meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), JECFA/61/SC, Rome, 10–19 June 2003.