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Total Mercury and Methylmercury Content in Edible Fish from the Mediterranean Sea

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ABSTRACT

The objective of this study was to determine the current levels of total mercury and methylmercury in the muscle tissues of different fish species caught in the Mediterranean Sea to ascertain whether these concentrations exceed the maximum level stipulated by the European Commission Decision. Total mercury concentrations in the muscles of skates ranged from 0.18 to 1.85 mg/kg (wet weight) (average, 1.00 mg/kg) while levels of 0.11 to 1.92 mg/kg (wet weight) (average, 0.70 mg/kg) and 0.21 to 1.74 mg/kg (wet weight) (average, 0.70 mg/kg) were recorded for blue whiting and red mullet, respectively. For 66.7% of long nose skate samples, 61.4% of thornback ray samples, 42.8% of winter skate samples, and 38% of starry ray samples, the total mercury concentrations exceeded the prescribed legal limit (1.0 mg/kg [wet weight]). Concentrations exceeding the maximum total mercury level stipulated by the European Commission Decision (0.5 mg/kg [wet weight]) were observed in 63.6 and 40% of blue whiting and striped mullet samples, respectively. Mercury was present in the different species almost completely in the methylated form at 55 to 100%. Weekly intakes were estimated and compared with the provisional tolerable weekly intake recommended by the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives.

Mercury is one of the most important pollutants both because of its effect on marine organisms and because it is potentially hazardous to humans. The toxicology and the environmental behavior of mercury are complex, since its toxicity, mobility, and bioaccumulation depend on its chemical form *(8)*.

Methylmercury, which is formed in aquatic sediments through the bacterial methylation of inorganic mercury, is the most toxic chemical species of mercury and therefore of the greatest concern from a human perspective. Owing to its lipophilic nature, this organometallic compound penetrates readily across cell membranes and attaches itself to nucleophilic groups in enzymes concerned with protein synthesis in the central nervous system. The main target organ of mercury toxicity is the central nervous system, and exposure can cause serious brain damage resulting in psychological disturbance, impaired hearing, loss of sight, ataxia, loss of motor control, and general debilitation. Moreover, the absorption of methylmercury during the embryonic phase has led to the retardation of psychomotor development in children.

The human health hazards of environmental mercury were tragically realized during the severe incidents arising from pollution in Minamata and Niigata, Japan, which involved the consumption of fish contaminated with methylmercury by fishers and their families. In fact, nearly all of the mercury in fish muscle occurs as methylmercury *(14, 15, 23)*, and fish consumption is a major route for mercury uptake by humans *(7, 9)*.

To safeguard public health, limits on total mercury levels in seafood have been established in various countries. The U.S. Food and Drug Administration has set a maximum total mercury level of 1 mg/kg (wet weight) in fish. In Japan, fish containing total mercury concentrations exceeding the Japanese maximum permitted limit of 0.4 mg/kg (wet weight) are considered unsuitable for human consumption. In Europe, the total mercury limit, regulated by European Commission Decision 93/351 of 19 May 1993 *(2)*, is 0.5 mg/kg (wet weight), except for some species for which it is 1.0 mg/kg. However, total mercury concentrations above the regulatory limits have been observed on several occasions, particularly for certain species occupying high trophic positions *(16, 17, 19, 22)* and in species that live on or close to the sea bed *(18, 20)*.

Since the toxic effects and metabolic behavior of mercury are largely dependent on its chemical form, it is imperative to differentiate between inorganic and organic mercury when monitoring the quality of fish intended for human consumption. In light of the this concern, this study was initiated to determine the current levels of total mercury and methylmercury in the muscle tissues of different fish species of commercial importance caught in the Adriatic and Ionian Seas. In order to ascertain whether the examined fish could be considered suitable for human consumption, mercury and methylmercury concentrations were checked to ascertain whether they were below the maximum level set by the European Commission Decision *(2)*.

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TABLE 1. *Numbers of specimens, weight ranges, total mercury and methylmercury concentrations, and percentages of methylmercury with respect to total mercury for different fish species*

Species	No. of pools	Weight range (g)	Total Hg (mg/kg [wet wt])	MeHg (mg/kg [wet wt])	% MeHg
Long nose skate	13	1,847.0 ± 1793	1.67 ± 0.95	1.67 ± 0.95	100
Thornback ray	31	1,522.7 ± 611.7	1.10 ± 0.27	1.10 ± 0.27	100
Winter skate	18	462.1 ± 117.7	0.96 ± 0.51	0.96 ± 0.51	100
Starry ray	27	473.4 ± 280.7	0.81 ± 0.40	0.81 ± 0.36	98.2 ± 3.88
Blue whiting	70	239.6 ± 124.5	0.70 ± 0.48	0.67 ± 0.46	96.4 ± 6.2
Striped mullet	154	47.8 ± 30.6	0.70 ± 0.56	0.68 ± 0.58	94.6 ± 16

People's weekly intakes were also estimated and compared with the provisional tolerable weekly intake (PTWI) recommended by the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives *(25)*.

MATERIALS AND METHODS

During several trawl surveys, specimens of *Micromesistius potassou* (blue whiting), *Mullus barbatus* (striped mullet), and different skate species (*Raja clavata* [thornback ray], *Raja asterias* [starry ray], *Raja miraletus* [winter skate], and *Raja oxyrhynchus* [long nose skate]) were caught in some southern areas of the Adriatic and Ionian Seas of Italy (42°00′N to 36°30′N, 14°25′E to 18°25′E) from June to September 2000. 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 (see Table 1). Muscle tissue was removed from the organisms in each pool and preserved at −25°C until the analysis was carried out. The tissues were dissected with plastic materials that were washed with HNO_3 (5%) and rinsed with distilled and deionized water in order to avoid metal contamination. For analyses of total mercury, homogenized samples of the tissue (1 to 3 g [wet weight]) were digested to a transparent solution with 10 ml of H_2SO_4-HNO_3 concentrated (1:1) under reflux. The resultant solutions were then diluted to a known volume with deionized water *(10)*, and the total Hg concentrations were measured with atomic absorption spectrophotometry (Perkin Elmer 5000) by the cold vapor technique after reduction by $SnCl_2$ (A.V.A. Thermo Jarrel Ash Corp.).

Methylmercury levels were determined according to the method described by Hight and Corcoran *(12)*. Homogenized samples of the tissue (about 1 g [wet weight]) were prewashed three times with 10 ml of acetone and once with 10 ml of benzene. The prewashed tissue was acidified with 5 ml of HCl-H_2O (1 + 1) and extracted three 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 of Na_2SO_4, and analyzed with a gas chromatography system (Carlo Erba model HRGC-5300) equipped with a ^{63}Ni electron capture detector (ECD-400); a splitless injection technique was used. The column consisted of an SPB-5 Supelco fused silica capillary (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 chemical blanks were run; there was no evidence of any contamination in these blanks. TORT-1 lobster hepatopancreas (National Research Council of Canada) was used for analytical quality control. Values obtained in replicate analyses (n = 5) (total Hg, 0.32 ± 0.02 mg/kg [dry weight]; MeHg, 0.123 ± 0.020 mg/kg [dry weight]) were in the range of those for the certified material (TORT-1 lobster hepatopancreas) (total Hg, 0.33 ± 0.06 mg/kg [dry weight]; MeHg, 0.128 ± 0.014 mg/kg [dry weight]).

RESULTS AND DISCUSSION

Total mercury concentrations in the muscle tissue of skates ranged from 0.18 to 1.85 mg/kg (wet weight) (average, 1.00 mg/kg) while levels of 0.11 to 1.92 mg/kg (wet weight) (average, 0.70 mg/kg) and 0.21 to 1.74 mg/kg (wet weight) (average, 0.70 mg/kg) were recorded for blue whiting and red mullet tissues, respectively (Table 1). Among the four species of skates, the highest total mercury concentrations were found for the long nose skate, followed by the thornback ray and the winter skate, while the lowest values were found for the starry ray. The considerable variation in total mercury concentrations among the different skate species could be explained by the different sizes of the samples examined. In fact, animal size is recognized to be of importance in determining the rate of physiological processes influencing the uptake, distribution, and elimination of pollutants *(3, 11)*. This observation is particularly true for mercury because the levels of this element in fish increase with body size, with larger, older fish generally having higher concentrations than smaller, younger fish *(14, 18, 22)*.

However, mercury accumulates in organisms mainly as methylmercury because of the lipophilic nature of this compound, which facilitates its penetration into the cell. The proportion of total mercury present in the methylated form in marine organisms varies widely among species and with trophic positions. Methylmercury represents 10 to 30% of the total mercury in plants and 20 to 80% of the total mercury in invertebrates *(5)*, while in the muscle tissues of fish and higher predators (e.g., raptorial birds and mammals) methylmercury is the major form of the element. Kamps and Miller *(15)* found that methylmercury accounted for 67 to 100% of the total mercury in the muscle tissue of swordfish and canned tuna. Suzuki et al. *(23)* found organic mercury to account for 95% of the total mercury in the muscle tissue of a variety of fish species. Joiris et al. *(14)* found mercury to be mostly (>85%) in the organic form in the muscle tissue of sardines, while Storelli et al. *(19)* reported methylmercury percentages of 81 to 100% in the muscle tissue of sharks. The data on fish in our paper fit this general picture well; in fact, methylmercury percentages with respect to total mercury were 88 to 100% for skates, 84 to 100% for blue whiting, and 52 to 100% for red mullet.

European Commission Decision 93/351 of 19 May 1993 *(2)* asserts that the mean total mercury content in edible parts of fish products should not exceed 0.5 mg/kg (wet weight). The species listed in annex A of the same European Commission Decision are reputedly risky, because they are able to accumulate large quantities of mercury, the prescribed limit being 1.0 mg/kg (wet weight). Generally, the species listed in annex A are either high-trophic-level predators, such as tuna, swordfish, and sharks, or organisms living in a typically benthic habitat, such as skates, angler fish, and red fish. In this respect, several studies emphasize that predatory and large fish, as well as animals that have close relationships with sediment, accumulate large amounts of mercury. For example, mean total mercury levels of >1.0 mg/kg were detected in skates from the Mediterranean Sea *(18)* and the Atlantic Ocean *(6),* and high levels of mercury were found in other benthic species *(1, 20).* For skates, among the species listed in annex A, total mercury concentrations should not exceed 1.0 mg/kg (wet weight). On this basis, 66.7% of the long nose skate samples, 61.4% of the thornback ray samples, 42.8% of the winter skate samples, and 38% of the starry ray samples had mercury concentrations that exceeded the prescribed legal limit. For the other two species, concentrations exceeding 0.5 mg/kg (wet weight) were detected in 63.6 and in 40% of blue whiting and striped mullet samples, respectively.

The Joint FAO/WHO Expert Committee on Food Additives has established a PTWI of 300 μg of total mercury, of which no more than 200 μg should be present as methylmercury, per person *(25).* These amounts, equivalent to 5 μg of total mercury per kg of body weight and 3.3 μg of methylmercury per kg of body weight, provide guidelines to assess whether consumers are likely to be at risk. Several studies on mercury intake through the diet have shown that with the exception of occupational exposure, seafood consumption is the primary pathway for the exposure of humans to mercury *(7, 9).* In Italy, the per capita weekly average consumption of seafood is 441 g *(13).* On the basis of this weekly average consumption and the mercury levels in the fish in question, the estimated weekly intake of total mercury and methylmercury varied from 5.9 to 12.3 μg/kg of body weight, corresponding to an estimated daily intake of 0.70 to 1.75 μg/kg of body weight. Comparison with the PTWI shows that high exposure is associated with the consumption of the long nose skate (12.3 μg/kg of body weight) and the thornback ray (8.08 μg/kg of body weight), while for the other species (starry ray, 5.90 μg/kg of body weight; winter skate, 7.05 μg/kg of body weight; striped mullet, 5.00 μg/kg of body weight; blue whiting, 4.92 μg/kg of body weight), the estimated weekly intake was slightly above the established PTWI. However, considering that this weekly consumption estimate represents an average across the whole population and that it is unlikely that an individual would consume 441 g of the same fish species per week, the estimated dietary exposure value might be overestimated.

TABLE 2. *Supposed quantities of fish ingested, mean methylmercury concentrations, and estimated daily intake levels*

MeHg concentration (mg/kg [wet wt])	Fish intake (g)	Estimated daily intake (μg/kg bw)
0.67	100	1.12
	150	1.68
	170	1.90
1.67	100	2.78
	150	4.18
	170	4.73

Italians consume a Mediterranean diet characterized by an abundant intake of fibers, complex carbohydrates, fruits and vegetables, vegetable oils (particularly olive oil), and seafood, including fish. However, people in different geographical areas have different dietary patterns, particularly with regard to seafood consumption. The population of northern Italy consumes less seafood than do the southern and isle populations *(19, 21).* This diversity in seafood consumption gives rise to different mercury intake levels, and therefore health risks may differ considerably for different populations. This observation holds particular importance for certain population sectors, such as fishers and their families, who, having greater access to seafood than other persons, may constitute a group with a high potential health risk.

The earliest clinical signs and symptoms of methylmercury poisoning are diffuse paresthesias in the hands, in the feet, and around the mouth. Increased exposure may result in ataxia, constriction of the visual field, blurred speech, and hearing difficulties. With severe poisoning, patients may develop blindness and general physical and mental debilitation *(24).* According to the WHO *(24),* it is expected that 5% of an adult population will exhibit overt symptoms when their blood concentration of total mercury is between 0.2 and 0.5 mg/liter. This level corresponds to 50 to 125 mg of Hg per kg of hair or to a long-term daily intake of 3 to 7 μg/kg of body weight in the form of methylmercury. Estimated daily intakes of methylmercury calculated on the basis of an average daily seafood consumption of 63 g for the Italian population did not reach the critical level at which toxic effects of mercury could be expected (3 to 7 μg/kg of body weight). However, such a critical threshold may be readily attained or exceeded if the quantities of fish consumed daily increase considerably. High seafood consumption levels are common among some consumer groups, such as professional fishers and their families. For example, Buzina et al. *(4)* found a daily average seafood consumption level of about 150 g for a population living on an Adriatic island made up mainly of the families of professional fishers. For different seafood consumption levels, the corresponding daily intakes were calculated on the basis of the lowest and the highest methylmercury concentrations found. As shown in Table 2, the ingestion of 150 g of fish with a contamination level exceeding the limit prescribed by the European Commission

Decision *(2)* results in a risky daily intake of mercury if exposure is long term.

In conclusion, the results of the present study show that in the muscle tissue of all of the species analyzed, mercury was present mainly in the organic form, while inorganic mercury made a negligible contribution to the total mercury burden. Comparison with the PTWI shows that mercury intake through the consumption of some fish species could constitute cause for concern, because the PTWI was clearly exceeded. This finding may be of significance for sectors of the population who, consuming larger amounts of seafood than other people, can be considered critical groups to which particular attention must be directed.

REFERENCES

1. Anderson, J. L., and M. H. Depledge. 1997. A survey of total mercury and methylmercury in edible fish and invertebrates from Azorean waters. *Mar. Environ. Res.* 44:331–350.
2. Anonymous. 16 June 1994. European Commission Decision 93/351 of 19 May 1993. *Off. J. Eur. Communities* L. 144.
3. Barron, M. G. 1990. Bioconcentration. *Environ. Sci. Technol.* 24: 1612–1618.
4. Buzina, R., P. Stegnar, K. Buzina-Suboticanec, M. Horvat, I. Petric, and T. M. M. Farley. 1995. Dietary mercury intake and human exposure in an Adriatic population. *Sci. Total Environ.* 170:199–208.
5. Claisse, D., D. Cossa, J. Bretaudeau-Sanjuan, G. Touchard, and B. Bombled. 2001. Methylmercury in molluscs along the French coast. *Mar. Pollut. Bull.* 42:329–332.
6. Collings, S. E., M. S. Johnson, and R. T. Leah. 1996. Metal contamination of angler-caught fish from the Mersey Estuary. *Mar. Environ. Res.* 41:281–297.
7. Cuadrado, C., J. Kumpulainen, and O. Moreiras. 1995. Lead, cadmium and mercury contents in average Spanish market basket diets from Galicia, Valencia, Andalucia and Madrid. *Food Addit. Contam.* 12:107–118.
8. D'Itri, F. M. 1990. The biomethylation and cycling of selected metals and metalloids in aquatic sediments, p. 198–247. *In* R. Baudo, J. Giesy, and H. Muntau (ed.), Sediments: chemistry and toxicity of in-place pollutants. Lewis, Ann Arbor, Mich.
9. Galal-Gorchev, H. 1993. Dietary intake, levels in food and estimated intake of lead, cadmium and mercury. *Food Addit. Contam.* 10:115–128.
10. Gazzetta Ufficiale delle Comunità Europee. 1990. Metodi di riferimento per la ricerca dei residui di metalli pesanti e arsenico no. L 286/33.
11. Gutenmann, W. H., J. G. Ebel, Jr., H. T. Kuntz, K. S. Yourstone, and D. J. Lisk. 1992. Residues of p,p′-DDE and mercury in lake trout as a function of age. *Arch. Environ. Contam. Toxicol.* 22:452–455.
12. Hight, S. C., and M. T. Corcoran. 1987. Rapid determination of methylmercury in fish and shellfish: method development. *J. Assoc. Off. Anal. Chem.* 70:24–30.
13. Istituto Nazionale di Statistica. 2000. Statistica sulla pesca, caccia e zootecnia. Informazione. Alba Grafica S.p.A. Roma no. 94.
14. Joiris, C. R., L. Holsbeek, and N. L. Moatemri. 1999. Total and methylmercury in sardines *Sardinella aurita* and *Sardina pilchardus* from Tunisia. *Mar. Pollut. Bull.* 38:188–192.
15. Kamps, L. R., and H. Miller. 1972. Total mercury-monomethyl-mercury content of several species of fish. *Bull. Environ. Contam. Toxicol.* 8:273.
16. Monteiro, L. R., and H. D. Lopes. 1990. Mercury content of swordfish, *Xiphias glaudius,* in relation to length, weight, age, and sex. *Mar. Pollut. Bull.* 21:293–296.
17. Nakagawa, R., Y. Yumita, and M. Hiromoto. 1997. Total mercury intake from fish and shellfish by Japanese people. *Chemosphere* 35: 2909–2913.
18. Storelli, M. M., R. Giacominelli Stuffler, and G. O. Marcotrigiano. 1998. Total mercury in muscle of benthic and pelagic fish from the South Adriatic Sea (Italy). *Food Addit. Contam.* 15:876–883.
19. Storelli, M. M., R. Giacominelli Stuffler, and G. O. Marcotrigiano. 2001. Total mercury and methylmercury in *Auxis rochei, Prionacee glauca* and *Squalus acanthias* from the South Adriatic Sea. *Ital. J. Food Sci.* 13:103–108.
20. Storelli, M. M., and G. O. Marcotrigiano. 2000. Fish for human consumption: risk of contamination by mercury. *Food Addit. Contam.* 17:1007–1011.
21. Storelli, M. M., and G. O. Marcotrigiano. 2001. Consumption of bivalve molluscs in Italy: estimated intake of cadmium and lead. *Food Addit. Contam.* 18:303–307.
22. Storelli, M. M., and G. O. Marcotrigiano. 2001. Total mercury levels in muscle tissue of swordfish (*Xiphias gladius*) and bluefin tuna (*Thunnus thynnus*) from the Mediterranean Sea. *J. Food Prot.* 64: 1058–1061.
23. Suzuki, T., T. Miyama, and C. Toyama. 1973. The chemical form and bodily distribution of mercury in marine fish. *Bull. Environ. Contam. Toxicol.* 10:347.
24. World Health Organization. 1976. Environmental health criteria. 1. Mercury. World Health Organization, Geneva.
25. World Health Organization. 1993. Evaluation of certain food additives and contaminants. Forty-first report of the joint FAO/WHO Expert Committee on Food Additives. WHO technical report series no. 837. World Health Organization, Geneva.