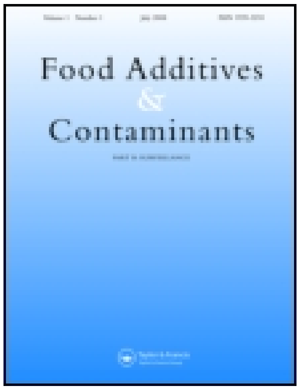

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Organic and total mercury levels in bigeye tuna, *Thunnus obesus*, harvested by Taiwanese fishing vessels in the Atlantic and Indian Oceans

M.H. Chen [a], P.Y. Teng [a], C.Y. Chen [b] & C.C. Hsu [c]
[a] Department of Marine Biotechnology and Resources, National Sun Yat-sen University, 70 Lianhai Rd., Gushan, Kaohsiung 80424, Taiwan
[b] Department of Marine Environmental Engineering, National Kaohsiung Marine University, Kaohsiung 81157, Taiwan
[c] Institute of Oceanography, National Taiwan University, Taipei 10617, Taiwan

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M.H. Chen[a*], P.Y. Teng[a], C.Y. Chen[b] and C.C. Hsu[c]

[a]*Department of Marine Biotechnology and Resources, National Sun Yat-sen University, 70 Lianhai Rd., Gushan, Kaohsiung 80424, Taiwan;* [b]*Department of Marine Environmental Engineering, National Kaohsiung Marine University, Kaohsiung 81157, Taiwan;* [c]*Institute of Oceanography, National Taiwan University, Taipei 10617, Taiwan*

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Muscle samples of 121 and 110 bigeye tuna (*Thunnus obesus*) caught by Taiwanese long-line fishing vessels in the Atlantic and Indian Oceans, respectively, were used to analyze total mercury (THg) and organic mercury (OHg) content. The overall THg and OHg concentrations were 0.786 ± 0.386 (0.214–3.133) and 0.595 ± 0.238 (0.143–2.222) $mg\,kg^{-1}$ wet weight, respectively, similar to the results of previous studies. Our findings, however, reflected the highest THg and OHg concentrations for the species in each ocean among the published data. Mean THg and OHg concentrations in Atlantic tuna were significantly ($p < 0.05$) higher than those in Indian tuna. Two of 121 samples of tuna from the Atlantic Ocean, but no samples from the Indian Ocean, had levels of OHg above $2\,mg\,kg^{-1}$ wet weight set by the Department of Health Taiwan, and 13 of 121 samples of tuna from the Atlantic Ocean and three of 110 samples from the Indian Ocean had levels of OHg above $1\,mg\,kg^{-1}$ wet weight set by US FDA and WHO. Accordingly, for adult Taiwanese men and women with average body weight of 65 and 55 kg, respectively, the maximum allowable weekly intake of bigeye tuna is suggested to be 170 and 145 g, respectively.

Keywords: fish meat; seafood safety; environmental contaminants; oceanic difference; heavy metals; highest record

Introduction

Natural sources of Hg^0 in the atmosphere are from volcanic emissions and forest fire (IOMC 2002). In the atmosphere, the elemental state of Hg^0 is oxidized to the mercuric (+II) state, Hg(II) (Morel et al. 1998). Of this, approximately 40% of the Hg(II) will enter an aquatic environment via rain (Mason et al. 1994). Thereafter, methyl mercury is produced in the environment by biomethylation of the inorganic mercury found in aquatic sediments (Clarkson et al. 2003). A progressive increase in Hg concentrations was found in organisms at different trophic levels of the marine food web, resulting in bioaccumulation of high levels of mercury in the tissues of top predators, such as tuna, shark and swordfish, in marine pelagic ecosystems (Bargagli et al. 1998).

Consumption of fish is the principal source of human exposure to methylmercury (Reuther and Wheeler 1996). Many countries pay keen attention to the mercury concentration in fish products, in particular imported fish (US FDA 2001; WHO 2008; Department of Health 2009). Despite being a potential Hg accumulator, data on the mercury concentrations in the muscle of bigeye tuna is limited (Sun and Chang 1972; Menasveta and Siriyong 1977; Boush and Thieleke 1983; Kumar et al. 2004; Yamashita et al. 2005; Besada et al. 2006). Information on bigeye tuna in different oceans and various size groups that may contain varying Hg concentrations is even scarcer.

Bigeye tuna, *Thunnus obesus*, is the most important export fish with the highest economic value for Taiwanese far-sea fisheries. The Taiwanese bigeye tuna fishing industry has maintained the highest production in the Indian Ocean since 1989, compared with production in the other two oceans. In the years from 1989 to 2001, production in the Atlantic Ocean ranked second behind the Indian Ocean (Overseas Fisheries Development Council of Republic of China). Therefore, mercury concentrations in tuna are of grave concerns to both fisheries agencies and public consumers in terms of the safety of seafood consumption and sustainable development of marine resources.

Materials and methods

Sampling date, location and pretreatment

A total of 121 samples from the Atlantic Ocean and 110 samples from the Indian Ocean were collected

*Corresponding author. Email: mhchen@mail.nsysu.edu.tw

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between 2005 and 2007 by Taiwanese scientific observers onboard long-line fishing vessels.

The sampling area in the Atlantic Ocean was from 20°E to 45°W and from 40°N to 40°S, whereas in the Indian Ocean it was from 90°E to 35°W and from 15°N to 45°S.

A chunk of caudal peduncle at the base of the fish tail was cut off as a sample onboard. The fork length was recorded in cm and labeled. Samples were kept deep-frozen (−40°C) till the end of the fishing season. After landing in the home port of Chiangcheng, Kaohsiung, the samples were transported to the analytical laboratory. In the laboratory, the white muscles were carefully excavated from the caudal peduncle and sealed in polypropylene bags as analytical material, then frozen at −20°C awaiting chemical analysis.

Methyl mercury extraction (Chen and Chou 2000; Chen et al. 2002)

A 0.2–0.4-g sample of the white muscle tissue was weighed in a 40-ml conical graduated centrifuge tube. Acetone was added to remove lipids covering the surface of the muscle. Then, 5 ml of 3 M potassium bromide (KBr) and 10 ml of 0.1 M copper sulfate ($CuSO_4$) as the extracting agent were added to the 40-ml conical graduated centrifuge tube. This extractant was placed in another conical graduated tube and extracted again with toluene. The upper organic phase was removed and further extracted to 1 ml of 0.01 M sodium persulfate ($Na_2S_3O_3$). This 1 ml $Na_2S_3O_3$ extractant was transferred to a 75-ml graduated test tube for the total mercury digestion procedure and mercury measurement, as follows.

Total mercury digestion (Chen and Chou 2000)

A 0.2–0.4-g sample of white muscle tissue was weighed in a 75-ml graduated test tube. Then, 1 ml of concentrated nitric acid (HNO_3) and 4 ml of concentrated sulfuric acid (H_2SO_4) were added to the 75-ml tubes. The tubes were heated at 75°C for 2 h. After cooling down to room temperature, 11 ml of 5% potassium permanganate ($KMnO_4$) were added for further digestion. Finally, a few ml of 17% H_2O_2 was added to the 75-ml tubes and made up to a final volume of 25 ml by double distilled water.

Mercury determination

Using 5% of tin(II) chloride dehydrate ($SnCl_4$) as the reductant, the mercury concentration was measured on a cold vapor atomic absorption spectrophotomer (CVAAS, Hitachi Z-8200) and hydride formation system (Hitachi, HFS-2) attached to a T-joint device (Chen and Chou 2000). All chemical reagents used in this study were GR-grade from Merck, Germany.

QA/QC

The certified reference materials were DORM-2 (dogfish muscle) and DOLT-2 (dogfish liver), purchased from the National Research Council of Canada, and they were analyzed simultaneously in each batch of the digesting process.

For QA and QC, the analytical results of 11 duplicates of each certified reference material are presented as mean ± standard deviation for DORM-2 (THg = 4.87 ± 0.78 and OHg = 3.83 ± 0.50 mg kg^{-1} dry weight), and for DOLT-2 (THg = 2.40 ± 0.43 and OHg = 0.76 ± 0.05 mg kg^{-1} dry weight). Compared with the certified values of DORM-2 (THg = 4.46 ± 0.26 and OHg = 4.47 ± 0.32 mg kg^{-1} dry weight) and DOLT-2 (THg = 2.14 ± 0.28 and OHg = 0.693 ± 0.053 mg kg^{-1} dry weight), the mean values were all within an 85% confidence interval of the certified values.

In addition, two blanks with only digesting reagents were inserted in each digesting process to detect any alien contaminants. In each measurement, at least six absorbances of the blanks were measured to calculate the instrumental limit of detection (LOD), based on the absorbance of blank plus 3 standard deviations (SDs) and yielded 0.6 μg l^{-1}. Twice the LOD was used as the limit of quantification (LOQ); accordingly, the sample LOD was 0.075 μg g^{-1}.

Statistical analysis

Statistical analyses were performed using SAS software and consisted of a Student's *t*-test to detect the differences in mercury concentrations between the two oceans ($p < 0.05$).

Results and discussion

Total and organic mercury in bigeye tuna

The fork length and body weight of the overall samples were 135.6 ± 31.3 (53–200) cm and 48.6 ± 30.7 (1–137) kg, respectively. The overall total Hg (THg) and organic Hg (OHg) concentrations were 0.786 ± 0.386 (0.217–3.133) and 0.595 ± 0.238 (0.143–2.222) mg kg^{-1} wet weight. THg and OHg levels from the Atlantic Ocean (THg = 0.898 ± 0.466 (0.324–3.133) mg kg^{-1} wet weight, and OHg = 0.656 ± 0.341 (0.220–2.222) mg kg^{-1} wet weight) were significantly higher than those of the Indian Ocean (THg = 0.679 ± 0.231 (0.217–1.880) mg kg^{-1} wet weight, and OHg = 0.529 ± 0.179 (0.143–1.525) mg kg^{-1} wet weight) (*t*-test, $p < 0.0001$). Such oceanic differences were not found in an earlier study on swordfish (Chen et al. 2007).

Table 1. Total mercury concentrations (THg, mg kg^{-1} wet weight) in various size groups by fork length (FL, cm) of bigeye tuna, *Thunnus obesus*, from the Atlantic and Indian Oceans (n = sample size).

	Atlantic Ocean		Indian Ocean		
Size group (FL, cm)	n	Mean ± SD (Range)	n	Mean ± SD (Range)	t-test p value
<90	8	0.410 ± 0.054 (0.341–0.485)	2	0.305 ± 0.125 (0.214–0.393)	0.0807
91–100	7	0.518 ± 0.174 (0.338–0.769)	19	0.664 ± 0.121 (0.444–0.838)	0.0234*
101–110	7	0.600 ± 0.217 (0.324–0.892)	14	0.637 ± 0.152 (0.332–0.895)	0.6561
111–120	15	0.695 ± 0.208 (0.413–1.119)	17	0.635 ± 0.149 (0.414–0.987)	0.3512
121–130	17	0.656 ± 0.190 (0.435–1.102)	8	0.674 ± 0.215 (0.455–1.051)	0.8276
131–140	8	0.803 ± 0.273 (0.378–1.167)	9	0.795 ± 0.165 (0.590–1.110)	0.9472
141–150	9	1.012 ± 0.443 (0.533–1.851)	8	1.118 ± 0.488 (0.532–1.880)	0.6449
151–160	10	0.966 ± 0.261 (0.577–1.380)	11	0.560 ± 0.077 (0.400–0.684)	0.0007*
161–170	11	1.062 ± 0.464 (0.638–2.301)	9	0.661 ± 0.094 (0.493–0.835)	0.0173*
171–180	22	1.359 ± 0.622 (0.495–3.133)	6	0.534 ± 0.122 (0.397–0.719)	<0.0001*
181–190	4	1.266 ± 0.224 (0.934–1.426)	5	0.748 ± 0.170 (0.466–0.900)	0.0054*
191–200	3	1.394 ± 0.437 (0.733–1.608)	2	0.606 ± 0.159 (0.494–0.719)	0.1906
Total	121	0.898 ± 0.466 (0.324–3.133)	110	0.679 ± 0.231 (0.217–1.880)	<0.0001*

However, geographic differences between the same fish species has been reported by Kojadinovic et al. (2006), who examined the muscle concentration of THg for swordfish and yellowfin tuna caught at Reunion Islands and found that the fish had higher THg levels than those of the same species from the Mozambique channels in the Indian Ocean.

Fork length (FL) and body weight (BW) of the bigeye tuna from the Atlantic Ocean (FL = 140.6 ± 31.9 (70–200) cm, BW = 55.5 ± 33.2 (6–137) kg) were significantly larger than those from the Indian Ocean (FL = 130.1 ± 30.8 (53–198) cm, BW = 41.1 ± 26.6 (1–110) kg) (t-test, $p < 0.05$). Mercury concentrations in the muscle increased with the size of the fish (Menasveta and Siriyong 1977; Boush and Thieleke 1983; Kumar et al. 2004; Yamashita et al. 2005; Besada et al. 2006). Thus, size is one of the reasons for the higher mercury levels in fish from the Atlantic Ocean.

To exclude the size effect, we compared the mercury concentrations in the different size groups by FL of the tuna (Tables 1 and 2). The THg concentrations in the size groups with FL larger than 151 cm from the Atlantic Ocean were all significantly higher than those from the Indian Ocean (Table 1). In the case of OHg, only two size groups (151–160 and 171–180 cm) showed the same significant oceanic differences (Table 2). There was the opposite trend found in smaller size groups, such as 91–100 cm for THg and 121–130 cm for OHg (Tables 1 and 2). Bigeye tuna from the Atlantic Ocean in the FL range 161–180 cm contained OHg concentrations over 2 mg kg^{-1} wet weight, whereas none of the samples from Indian Ocean tuna contained such high levels of organic mercury. Such variations may be due to dietary differences (Zhu et al. 2007). The main prey species of bigeye tuna in the Atlantic Ocean is the sardine (*Sardina antipa*), while the main prey species of bigeye tuna in the Indian Ocean is squid (*Loligo pealei*) (Zhu et al. 2007). Normally, fish with preferentially piscivorous feeding habits would have higher mercury concentrations than fish that feed mainly on invertebrates (Pinho et al. 2002). Other environmental factors, such as volcanic activity, atmospheric emission and regional industrial developments, may also contribute to Hg bioaccumulation in the long-lived, high trophic predatory fish (Mason et al. 1994; IOMC 2002).

The mercury concentrations detected in bigeye tuna in this study are quite similar to other studies around the world (Menasveta and Siriyong 1977; Boush and

Table 2. Organic mercury concentrations (OHg, $mg\,kg^{-1}$ wet weight) in various size groups by fork length (FL, cm) of bigeye tuna, *Thunnus obesus*, from the Atlantic and Indian Oceans (n = sample size).

	Atlantic Ocean		Indian Ocean		
Size group (FL, cm)	*n*	Mean ± SD (Range)	*n*	Mean ± SD (Range)	*t*-test *p* value
<90	8	0.308 ± 0.041 (0.228–0.349)	2	0.268 ± 0.176 (0.143–0.393)	0.7996
91–100	7	0.385 ± 0.101 (0.262–0.539)	19	0.427 ± 0.047 (0.355–0.509)	0.3245
101–110	7	0.366 ± 0.014 (0.220–0.572)	14	0.425 ± 0.083 (0.311–0.612)	0.2405
111–120	15	0.560 ± 0.234 (0.259–0.999)	17	0.521 ± 0.103 (0.357–0.802)	0.5596
121–130	17	0.472 ± 0.128 (0.271–0.756)	8	0.608 ± 0.188 (0.387–0.939)	0.0439*
131–140	8	0.658 ± 0.185 (0.378–0.847)	9	0.694 ± 0.182 (0.501–1.043)	0.6945
141–150	9	0.818 ± 0.347 (0.460–1.577)	8	0.770 ± 0.365 (0.331–1.525)	0.7873
151–160	10	0.706 ± 0.196 (0.476–1.046)	11	0.477 ± 0.081 (0.346–0.632)	0.0051*
161–170	11	0.870 ± 0.486 (0.495–2.185)	9	0.574 ± 0.130 (0.343–0.702)	0.0776
171–180	22	0.880 ± 0.400 (0.370–2.222)	6	0.507 ± 0.119 (0.352–0.676)	0.0008*
181–190	4	0.883 ± 0.299 (0.568–1.288)	5	0.625 ± 0.136 (0.451–0.807)	0.1258
191–200	3	1.111 ± 0.232 (0.715–1.151)	2	0.451 ± 0.047 (0.417–0.484)	0.0567
Total	121	0.656 ± 0.341 (0.220–2.222)	110	0.529 ± 0.179 (0.143–1.525)	<0.0001*

Thieleke 1983; Kumar et al. 2004; Yamashita et al. 2005; Besada et al. 2006). However, we recorded the highest THg and OHg concentrations in bigeye tuna from the two oceans among the published data: THg = 3.133 and OHg = 2.222 $mg\,kg^{-1}$ wet weight for a male sample with FL = 177 cm for the Atlantic Ocean, and THg = 1.880 and OHg = 1.525 $mg\,kg^{-1}$ wet weight for a female sample with FL = 144 cm from the Indian Ocean. In comparison with other high trophic predatory fish, bigeye tuna does not hold the record Hg concentration. Swordfish (*Xiphis gladrius*) caught in the two oceans both had record Hg concentrations (THg = 3.97 and OHg = 3.92 $mg\,kg^{-1}$ wet weight for the Atlantic Ocean, and THg = 2.54 and OHg = 1.93 $mg\,kg^{-1}$ wet weight for the Indian Ocean) (Sun and Chang 1972; Kureishy et al. 1979; Andersen and Depledge 1997; Adams 2004; Yamashita et al. 2005; Besada et al. 2006; Kojadinovic et al. 2006; Chen et al. 2007). Swordfish also contained the highest mean Hg concentrations in the two oceans (THg = 1.20 and OHg = 0.96 $mg\,kg^{-1}$ wet weight for the Atlantic Ocean, and THg = 1.47 and OHg = 1.10 $mg\,kg^{-1}$ wet weight for the Indian Ocean) (Sun and Chang 1972; Kureishy et al. 1979; Andersen and Depledge 1997; Adams 2004; Yamashita et al. 2005; Besada et al. 2006; Kojadinovic et al. 2006; Chen et al. 2007). In addition, the mean THg concentrations of blackfin tuna, *Thunnus atlanticus,* and little tunny, *Euthynnus alletteratus,* caught in the Florida Atlantic Ocean also contained slightly higher Hg concentrations than the Hg levels of the bigeye tuna found in this study in the same ocean (Adams, 2004).

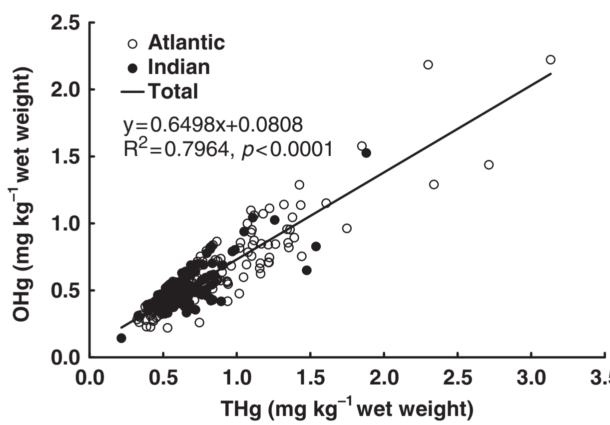

Figure 1. Linear relationship between organic mercury (OHg, $mg\,kg^{-1}$ wet weight) and total mercury concentration (THg, $mg\,kg^{-1}$ wet weight) of bigeye tuna, *Thunnus obesus*, from the Atlantic and Indian Oceans.

The OHg% (OHg/THg) in bigeye tuna, *Thunnus obesus*, were 34.71–100%, with a mean of 77.59 ± 15.0%. The results are consistent with earlier

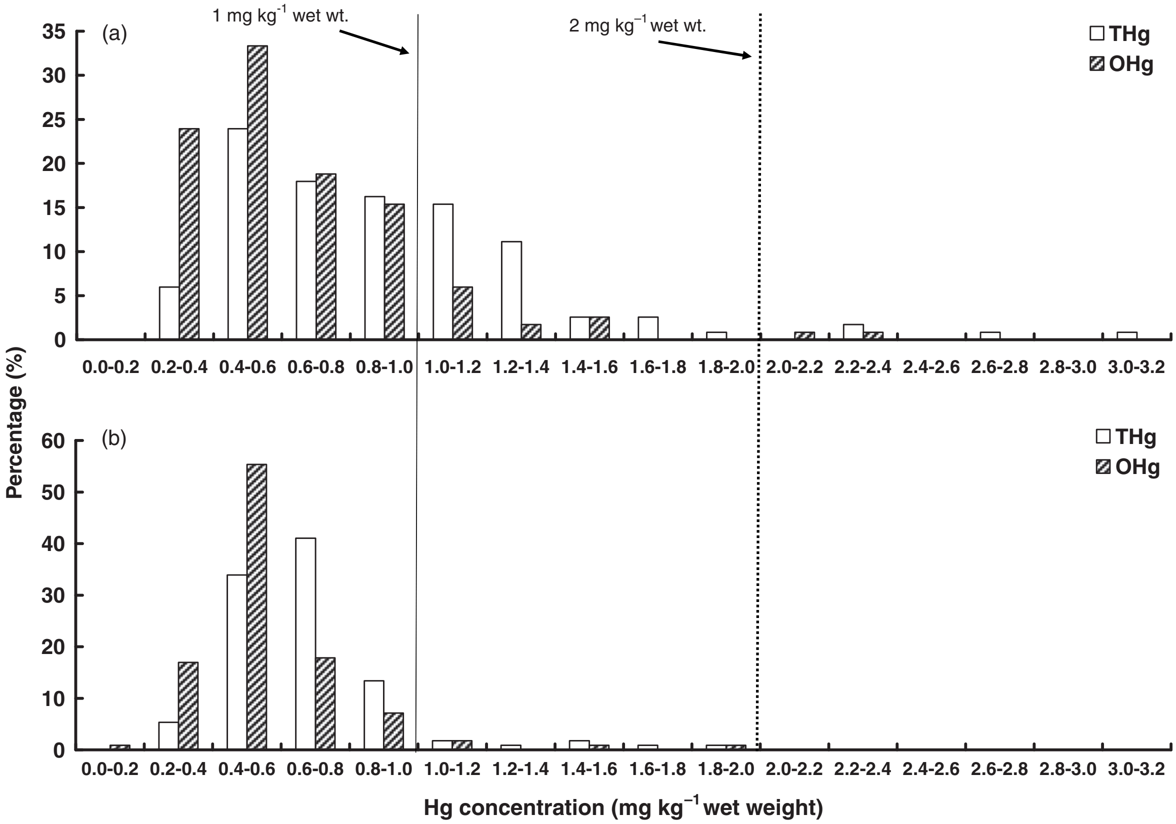

Figure 2. Frequency of total and organic mercury concentrations in muscle of bigeye tuna, *Thunnus obesus*, from the (a) Atlantic and (b) Indian Oceans.
Notes: [a]The seafood safety limit for total mercury concentration in the EU is $1\,\mathrm{mg\,kg^{-1}}$ wet weight. [b]The WHO and FDA seafood safety limit for methylmercury concentration is $1\,\mathrm{mg\,kg^{-1}}$ wet weight. [c]The seafood safety limit for methylmercury concentration of the Department of Health Taiwan is $2\,\mathrm{mg\,kg^{-1}}$ wet weight.

studies in many kinds of fish (May et al. 1987; Andersen and Depledge 1997; Wagemann et al. 1997; Storelli et al. 2002; Yamashita et al. 2005). OHg and THg concentrations in the muscle of bigeye tuna were linearly correlated according to the equation: $\mathrm{OHg} = 0.6498\ \mathrm{THg} + 0.0808$ ($r^2 = 0.9041$, $p < 0.0001$; Figure 1), whereas the relationship in swordfish was $\mathrm{OHg} = 0.8100\ \mathrm{THg}–0.0296$ (Chen et al. 2007).

Evaluation of food safety

In the Atlantic and Indian Oceans, 1.7% and 0% of the bigeye tuna, respectively, contained OHg levels above the food safety limit set by the Department of Health Taiwan, as 2 mg methylmercury $\mathrm{kg^{-1}}$ wet weight (Department of Health 2009) (Figure 2). Furthermore, 13 and 2.7% of the bigeye tuna from the Atlantic and Indian Oceans, respectively, failed the food standards set by the US FDA (The Food and Drug Administration in the United States) (US FDA 2001) and World Health Organization (WHO 2008), establishing an action level of $1\,\mathrm{mg\,kg^{-1}}$ wet weight to regulate methylmercury concentrations in predatory migratory and commercial fish. Moreover, a maximum level of total mercury was set by the European Union for tuna (*Thunnus* species) as $1\,\mathrm{mg\,kg^{-1}}$ wet weight (Commission Regulation 2006). In the Atlantic and Indian Oceans, 36 and 6% of bigeye tuna samples analyzed exceeded this limit (Figure 2).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) set the provisional tolerable weekly intake (PTWI) level for methylmercury at $1.6\,\mu\mathrm{g\,kg^{-1}}$ body weight $\mathrm{week^{-1}}$ (FAO/WHO 2003). Accordingly, considering an average body weight of 65 and 55 kg for adult males and females, respectively, in Taiwan (Kao et al. 1999) and the mean organic Hg concentration ($0.595\,\mathrm{mg\,kg^{-1}}$ wet weight) in the muscle of bigeye tuna, the maximum allowable weekly intake of bigeye tuna would be 170 and 145 g for an adult man and woman, respectively. This intake is slightly less than 6 ounces (one average meal) and 5 ounces tuna steaks, respectively, or one and half servings of "Sashimi". However, for pregnant women and children who need to limit their consumption of tuna due to the possible risks to their developing nervous systems, Health Canada set the PTWI level of organic mercury for women of child-bearing age (18–34 years old, 50 kg) and young children (20 kg) to $1.4\,\mu\mathrm{g\,kg^{-1}}$ body weight $\mathrm{week^{-1}}$ (Health Canada 2004). Therefore, the maximum allowable weekly intake of bigeye tuna would be 115 or 45 g for women of child-bearing age and children, respectively. Thus, the suggested weekly consumption is one 4 ounces serving of tuna steak or "Sashimi" for young women, and only one and half ounces of tuna meat for a child of 20 kg.

Conclusions

In terms of mean value and highest level, the THg and OHg concentrations in muscle of bigeye tuna from the Atlantic Ocean were higher than those from the Indian Ocean. Such oceanic differences may be due to differences in dietary composition and, possibly, global mercury emissions. A total of 3–13% of the fish products may exceed the US FDA and WHO food safety standard (methylmercury $< 1.0\,\mathrm{mg\,kg^{-1}}$ wet weight). Accordingly, to reduce the health risk of Hg poisoning, weekly consumption of less than 140 g is suggested.

Acknowledgments

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