



Total and Methyl Mercury Concentrations in Antarctic Toothfish (*Dissostichus mawsoni*): Health Risk Assessment

Minchul Yoon¹ · Mi Ra Jo¹ · Poong Ho Kim¹ · Woo Seok Choi¹ · Sang In Kang³ · Seok Gwan Choi⁴ · Jong Hee Lee⁴ · Hee Chung Lee¹ · Kwang Tae Son² · Jong Soo Mok¹ 

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Abstract

The concentrations of total mercury (THg) in different organs of the Antarctic toothfish (*Dissostichus mawsoni*) collected from CCAMLR research blocks in Subarea 88.3 and Division 58.4.1 off the coast of Antarctica were determined. The results revealed THg concentrations of 0.165 ± 0.095 mg/kg (0.023–0.454 mg/kg, wet weight) in the Antarctic toothfish. In muscle, methyl mercury (MeHg) accounted for approximately 40% of the THg. In a comparison analysis, muscle and liver tended to bioaccumulate the highest levels of THg, and both THg and MeHg contents showed correlations with fish length and weight. Compared with international guidelines, fish contained 2.5–6.4% and 4.0–10.3% of the provisional tolerable weekly intake for THg recommended by the Joint FAO/WHO Expert Committee on Food Additives and the tolerable weekly intake for MeHg proposed by the European Food Safety Authority, respectively. These results suggest that consumption of the Antarctic toothfish presents no health risk to humans.

Keywords Antarctic toothfish · *Dissostichus mawsoni* · Total mercury · Methylmercury · International guidelines

The Antarctic toothfish (*Dissostichus mawsoni*) is distributed mainly around the mainland of Antarctica south of 60°S and has been caught at depths of 70–2000 m. It is a predator of fishes, squids, and crustaceans (Goldsworthy et al. 2002; Horn 2002). The Antarctic toothfish is considered an economically and ecologically important fishery resource in the Antarctic. However, although it has high ecological and economic values, there is a lack of information on contaminants, such as mercury, in this species. Sea ice and snow in

polar regions have recently been found to contain high concentrations of mercury and could be additional sources of mercury to seawater (Chaulk et al. 2011; Cossa et al. 2011; Gionfriddo et al. 2016). Accordingly, bioaccumulation of the neurotoxicant MeHg in biota distributed in the Arctic region is a major concern for native residents because of their marine-based diet (AMAP 2011). Similarly, Antarctic biota is exposed to large quantities of MeHg. Consequently, the risk of human exposure to mercury is increased by extending fishing activities in this region (Cossa et al. 2011; Soerensen et al. 2014).

MeHg, one of the most toxic forms of mercury, bioaccumulates through increasing trophic levels, for example, via the feeding activity of predator fishes (UNEP 2002). Thus, due to the risk of mercury exposure from fish consumption, the United States has monitored the mercury concentration in fish since the 1990s (US FDA 2010). Australia and New Zealand have set a maximum level of THg of 0.5 mg/kg for fish (FSANZ 2004), while the European Union has set a maximum tolerable level of THg of 0.5 mg/kg for fish and 1 mg/kg for predator fish (EC 2008). Meanwhile, the CODEX Alimentarius Commission suggests a MeHg guideline level of 0.5 mg/kg for fish in general and 1 mg/kg for predatory species (CAC 2009). The joint FAO/WHO Expert

✉ Jong Soo Mok
mjs0620@korea.kr

¹ Food Safety and Processing Research Division, National Institute of Fisheries Science, Busan 46083, Republic of Korea

² South-East Sea Fisheries Research Institute, National Institute of Fisheries Science, Tongyeong 53085, Republic of Korea

³ Department of Seafood and Aquaculture Science/Institute of Marine Industry, Gyeongsang National University, Tongyeong 53063, Republic of Korea

⁴ Distant-Water Fisheries Resources Division, National Institute of Fisheries Science, Busan 46083, Republic of Korea

Committee on Food Additives (JECFA) proposed a provisional tolerable weekly intake (PTWI) for MeHg of 1.6 $\mu\text{g}/\text{kg}$ body weight (bw). However, the European Food Safety Authority (EFSA) more recently proposed a TWI for MeHg of 1.3 $\mu\text{g}/\text{kg}$ bw, lower than the PTWI of the JECFA (EFSA 2012). Moreover, the THg PTWI of 5 $\mu\text{g}/\text{kg}$ bw was withdrawn in 2012 by the JECFA and replaced with a PTWI for inorganic mercury of 4 $\mu\text{g}/\text{kg}$ bw (JECFA 2011). Thus, due to the risk of human exposure to mercury, the standard and intake levels of mercury have been strengthened gradually.

Therefore, in the present study, THg concentrations in various organs of the Antarctic toothfish, as a predator, and of grenadiers (*Macrourus* spp.), as prey, collected from the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) research blocks in Subarea 88.3 and Division 58.4.1 off the coast of Antarctica were determined. THg concentrations were compared with biological characteristics (e.g., sex, maturity, total length, and weight) and MeHg concentrations in muscle tissue. Finally, a potential risk assessment of THg and MeHg in Antarctic toothfish was considered, to determine the potential safety of this species as a source of food by comparison with international reference levels.

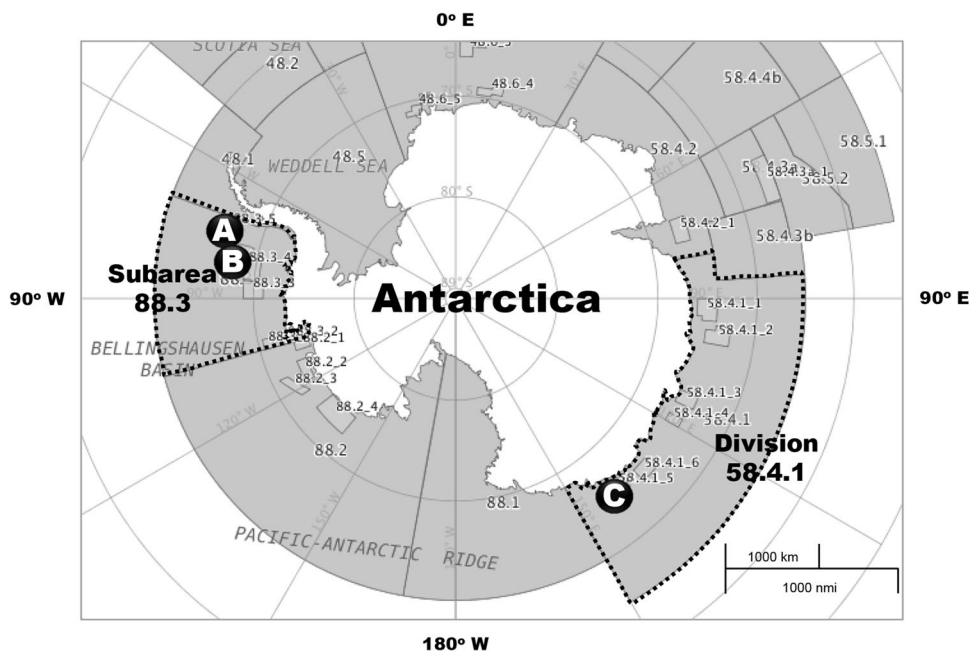
Materials and Methods

Using exploratory longline fishery, field sampling was conducted at two research blocks (88.3_4 and 5) in Subarea 88.3 located from 105°W to 70°W and at one research block (58.4.1_5) in Division 58.4.1 approved by the Commission for the CCAMLR in 2016 (Fig. 1). After random sampling,

the sex, maturity, total length, weight, and fishing depth were measured in 50 Antarctic toothfish and 15 grenadier specimens caught in Subarea 88.3 and in 52 Antarctic toothfish and 15 grenadier specimens caught in Division 58.4.1 in 2016. All biological characteristics were determined using the Scientific Observers Manual produced by CCAMLR (CCAMLR 2011). Various organs (i.e., muscle, gill, and liver) from each fish were separated and homogenized for THg and MeHg analyses. All muscle samples were collected from the head to the first dorsal fin. All samples were kept at -20°C until further analysis.

The THg concentration in homogenized samples was measured directly in triplicate using a combustion gold amalgamation method using a direct mercury analyzer (DMA-80; Milestone, Milano, Italy). Blanks, calibration standards, and certified reference materials (CRMs) were analyzed using the same methods. DORM-4 (dogfish liver; National Research Council, Nova Scotia, Canada) was used as the CRM to verify the accuracy of the results. The THg concentrations were expressed in $\mu\text{g}/\text{g}$ wet weight sample. To analyze MeHg, approximately 2 g homogenized sample were placed in a 50-mL conical tube. After adding 10 mL 25% (v/v) sodium chloride solution (Merck KGaA, Darmstadt, Germany), 4 mL concentrated hydrochloric acid (Merck KGaA, Darmstadt, Germany) and 15 mL toluene (Merck KGaA, Darmstadt, Germany) were added. After centrifuging at 3500 rpm for 15 min, the toluene layer was transferred to a 125-mL separatory funnel, and 10 mL 25% sodium chloride solution and 5 mL L-cysteine solution (Merck KGaA, Darmstadt, Germany) were added. After standing for 10 min, the L-cysteine layer was collected into a 15-mL centrifuge tube. Then, 4 mL hydrochloric acid

Fig. 1 Sampling area of Antarctic toothfish (*Dissostichus mawsoni*) and grenadiers (*Macrourus* spp.) caught at two research blocks (88.3_4; B and 88.3_5; A) in Subarea 88.3 and at one research block (58.4.1_5; C) in Division 58.4.1



solution and 2 mL toluene were added, and the mixture was shaken vigorously for 2 min. The extract was centrifuged (4500 rpm, 30 s), and the upper toluene layer, dehydrated using anhydrous sodium sulfate (Merck KGaA, Darmstadt, Germany), was used as the analyte. Blanks, calibration standards, and reference materials were analyzed using the same methods. For accurate results, MeHg chloride (Sigma-Aldrich, St. Louis, MO, USA) was used as the reference material via spiking. An HR-Thermon-HG (0.53 mm × 15 m; Shinwa Chemical Industries, Ltd., Tokyo, Japan) column was used for the MeHg analysis followed by gas chromatography using an electron capture detector system (Agilent 7890A; Kyoto, Japan).

Statistical data on the mean rates of demersal fish consumption for the global population (55.4 g/week), Americans (80.9 g/week), Asians (43.7 g/week), and Europeans (137.3 g/week), supplied by the Food and Agriculture Organization of the United Nations (FAO 2013), and the average adult body weights of the global population (62.0 kg), Americans (80.7 kg), Asians (57.7 kg), and Europeans (70.8 kg) (Walpole et al. 2012) were applied to perform a health risk assessment.

All experiments were run in triplicate. Data were subjected to analyses of variance (ANOVAs) at $p < 0.05$ and $p < 0.01$ significance levels using SAS software for Windows (ver. 9.2; SAS Institute, Cary, NC, USA). Duncan's multiple range tests and Pearson's correlation coefficients were used to compare differences among the means.

Results and Discussion

The accuracies of the THg and MeHg analyses were assessed using CRMs (DORM-4) and MeHg chloride spiking, respectively. The quantitative recoveries were 91.3% for THg and 92.7% for MeHg, which were within the acceptable values recommended by AOAC International (2002). Table 1 presents the results of the THg and MeHg analysis in different tissues of Antarctic toothfish and grenadiers. The tissue-specific THg concentrations of Antarctic toothfish showed the following trends: muscle > liver > gill in Division 58.4.1 and liver > muscle > gill in Subarea 88.3. No significant differences were observed between male and female specimens, while there were significant differences in muscle THg between two collecting sites ($p < 0.01$) (Fig. 2). The mean concentration of muscle THg in Antarctic toothfish collected from both sampling areas was 0.165 ± 0.095 mg/kg (0.023–0.454 mg/kg), which is similar to previous results (0.14–0.17 mg/kg) for Antarctic toothfish from the CCAMLR Subarea 88.1 (Hanchet et al. 2012), results (0.101–0.139 mg/kg) for same species from two research block 88.1K and 58.4.1C (Son et al. 2014). Conversely, in a closely related and taxonomically similar

Table 1 Tissue-specific THg and MeHg concentrations (mg/kg, wet weight) in fish collected from the Antarctic sea

English name (CCAMLR/FAO code)	Scientific name	N (sex ratio)	Length (cm)	Weight (g)	Muscle		Gill		Liver	
					THg (mg/kg)	MeHg (mg/kg)	% of MeHg	THg (mg/kg)	THg (mg/kg)	THg (mg/kg)
Division 58.4.1										
Antarctic toothfish (TOA)	<i>D. mawsoni</i>	50 (1)	143.7 ± 17.6 (87.0–180.0)	40.8 ± 15.3 (7.5–80.0)	0.221 ± 0.096a* (0.066–0.454)	0.106 ± 0.064** (0.025–0.343)	51.3 ± 26.7 (15.6–111.6)	0.085 ± 0.054c (0.029–0.319)	0.128 ± 0.078b (0.022–0.319)	
Grenadiers (GRV)	<i>Macrourus</i> spp.	15	–	–	0.336 ± 0.118a (0.124–0.536)	0.145 ± 0.069 (0.028–0.260)	40.9 ± 10.9 (21.3–56.6)	0.100 ± 0.051b (0.037–0.226)	0.101 ± 0.063b (0.013–0.237)	
Subarea 88.3										
Antarctic toothfish (TOA)	<i>D. mawsoni</i>	52 (1.08)	115.4 ± 33.0 (57.0–167.0)	24.6 ± 18.9 (1.6–75.0)	0.110 ± 0.054a* (0.023–0.315)	0.035 ± 0.029** (0.004–0.179)	29.8 ± 10.6 (10.5–56.9)	0.094 ± 0.039a (0.022–0.188)	0.156 ± 0.112b (0.017–0.583)	
Grenadiers (GRV)	<i>Macrourus</i> spp.	15	–	–	0.365 ± 0.157a (0.080–0.608)	0.184 ± 0.109 (0.029–0.407)	47.8 ± 12.9 (28.2–67.4)	0.150 ± 0.079b (0.073–0.389)	0.158 ± 0.063b (0.058–0.279)	

With the same column (a horizontal column), different letters indicate significant difference ($p < 0.05$)

*Level of significance ($p < 0.05$)

**Level of significance ($p < 0.01$)

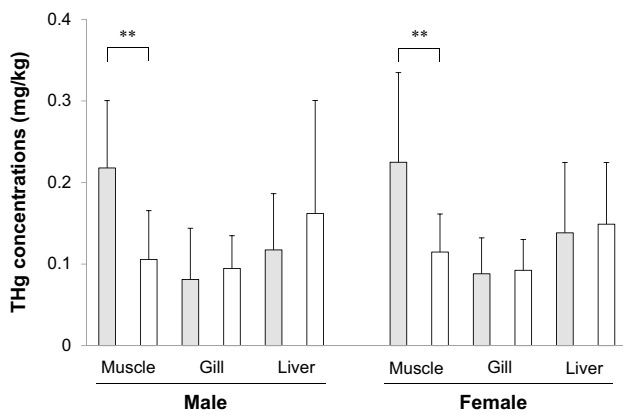


Fig. 2 Sex-specific THg concentrations in Antarctic toothfish caught in CCAMLR Division 58.4.1 (gray box) and Subarea 88.3 (white box). There were significant differences in muscle THg between two collecting sites ($p < 0.01$). The asterisks mean level of significance

species, the Patagonian toothfish (*Dissostichus eleginoides*), the mean concentrations of THg were found to be markedly higher than those in Antarctic toothfish in this study. Méndez et al. (2001) reported THg concentrations of 0.12–1.16 mg/kg in Patagonian toothfish collected from the southwestern Atlantic Ocean. The THg concentrations in Patagonian toothfish collected from the Ross Sea and Macquarie Island were 0.43 and 0.33 mg/kg, respectively (McArthur et al. 2003; Hanchet et al. 2012).

To determine potential dietary exposure for toothfish, THg and MeHg concentrations were quantified in grenadiers, an important prey species in this region. The THg concentrations in muscle were 0.336 ± 0.118 mg/kg (0.124–0.536 mg/kg) and 0.365 ± 0.157 mg/kg (0.080–0.608 mg/kg), respectively (Table 1). Moreover, high concentrations were observed in muscle compared with other organs ($p < 0.05$). The mean THg concentrations in muscle were considerably lower in Antarctic toothfish, as a predator species, than in grenadier, as a prey species, while the mean concentrations in other tissues between these two species were similar. In addition, blue antimora (*Antimora rostrata*) and rattail (*Macrourus whitsoni*), as a prey species of the Antarctic toothfish, from the Antarctic sea had THg concentrations of 0.10–0.25 and 0.36–0.42 mg/kg, respectively (Hanchet et al. 2012). Moreover, the mean concentration of THg in Antarctic Notothenioid fishes collected from collected in 2011 from McMurdo Sound and Ross Sea was 0.219–0.713 mg/kg (Wintle et al. 2015). These results showed that the mean THg concentrations in the predator species Antarctic toothfish were considerably lower than those in prey species and taxonomically similar species. Although the Antarctic toothfish is a predatory fish with a higher trophic level similar to the Patagonian toothfish, it has low THg concentrations. To explain this discrepancy, Hanchet et al. (2012) suggested

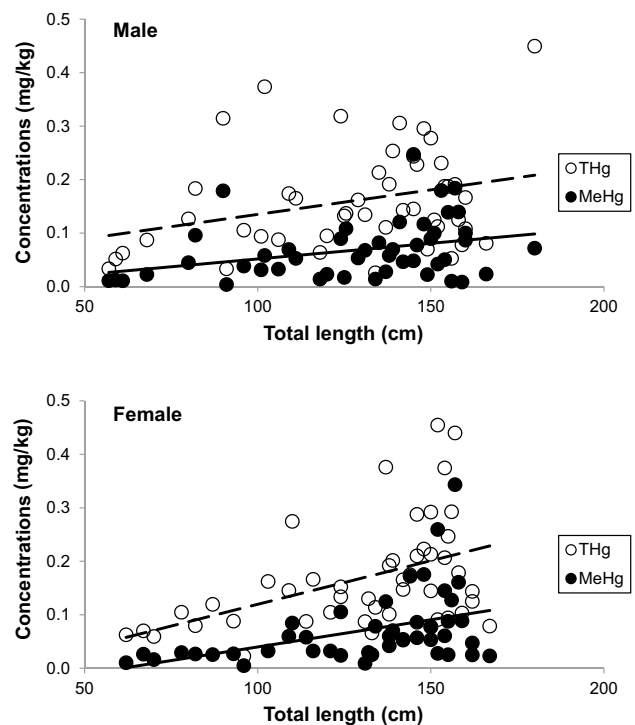


Fig. 3 Relationships between muscle mercury concentrations and total length in Antarctic toothfish. Regression lines were fitted for the THg (dashed line) and MeHg (solid line)

that the Antarctic toothfish either has a low rate of mercury assimilation from prey or a high rate of mercury elimination. More recently, Peng et al. (2016) reported that the low mercury concentrations in herbivorous fish are attributed to high physiological losses of both MeHg and inorganic mercury, as well as a relatively low dietary MeHg assimilation efficiency. Moreover, the transcription of heavy-metal binding protein, hepatic metallothionein, is induced in fish by heavy-metal exposure (Hogstrand and Haux 1990; Tom et al. 2004), and may provide an evidence for explanation of low levels of THg and MeHg in this species. Therefore, in comparison of mercury contents in these two species, the significant difference may be due to these unique physiological features in Antarctic toothfish. In addition, based on length-specific mercury concentrations, smaller fish had lower THg and MeHg concentrations likely reflects the condition that smaller toothfish are likely the younger individuals within the population and have experienced shorter lifetime exposure to dietary mercury via prey fish consumption. (Fig. 3). Grenadiers account for only 14% of the diet of sub-adult Antarctic toothfish but the majority of the diet of adult Antarctic toothfish (> 100 cm) (Stevens 2006). Moreover, among prey fish, grenadiers have relatively high mercury concentrations (Hanchet et al. 2012). Therefore, mercury bioaccumulation in Antarctic toothfish may be strongly affected by their feeding habits.

As an organic form of mercury, MeHg shows strong biomagnification up the food chain, and dietary transfer of MeHg is considered the main risk for mercury in higher trophic level animals, including humans (Fisher and Hook 2002; Bravo et al. 2014). In particular, fish muscle is the primary MeHg storage organ (Bloom 1992; Peng et al. 2016). In general, the proportion of MeHg to THg in fish is approximately 83% (45%–124%) (Kannan et al. 1998). In the present study, this proportion was only 29.8%–51.3% in Antarctic toothfish. The reasons for this lower proportion of MeHg in Antarctic toothfish are unclear. However, the biological factors, ecological variations, and unique biokinetics of this species may influence this difference (Kannan et al. 1998; Peng et al. 2016). In addition, regarding tissue-specific mercury concentrations, muscle and liver tended to have relatively high THg levels and gills lower levels in the two studied species. These results were similar to reports of freshwater fishes from Lake Mead, marine fish from Izmir Bay, and other Antarctic fish (Honda et al. 1983; Kucuksezgin et al. 2002; Cizdziel et al. 2003; Agusa et al. 2007). This suggests that liver has an important role in the metabolic processes of contamination and detoxification in fish. According to Peng et al. (2016), muscle is a primary tissue for metal storage, and the high storage of MeHg is explained by its negligible elimination.

In the analysis of the relationships between biological characteristics and mercury concentrations, both THg and MeHg concentrations in muscle were significantly and positively correlated with total length and weight ($p < 0.01$) (Table 2). Similar relationships have been found previously in Patagonian toothfish and Antarctic toothfish (Hanchet et al. 2012). In the present study, the THg concentrations differed between the collection sites (0.221 versus 0.110 mg/kg). Based on the length–mercury content relationship in the

Table 2 Pearson correlation coefficients for the relationships between the tissue-specific mercury concentrations and their biological characteristics and fishing depth

Variable	Element	Pearson correlation coefficients		
		Muscle	Gill	Liver
Total length	THg	0.397**	0.102	0.165
	MeHg	0.383**	–	–
Weight	THg	0.369**	0.032	0.118
	MeHg	0.356**	–	–
Maturity	THg	–0.065	0.010	0.079
	MeHg	–0.023	–	–
Fishing depth	THg	–0.088	0.023	0.203*
	MeHg	–0.152	–	–

*Level of significance ($p < 0.05$)

**Level of significance ($p < 0.01$)

Antarctic toothfish, this suggests that this difference may be associated with length variation in this species.

In the present study, approximately 13% of grenadiers exceeded the safety mercury levels set by Australia and New Zealand (0.5 mg/kg) and the European Union (0.5 mg/kg), whereas the THg levels in even the largest Antarctic toothfish (180 cm) at both collection areas were below this level, with the exception of one liver sample (0.583 mg/kg) collected from Subarea 88.3. The reason for this unusually high THg concentration is unclear; however, biological and ecological variations in fish may have been influencing factors. As a predator species, the THg levels in the Antarctic toothfish were within the safety level set by the European Union for predators (1 mg/kg). In addition, the MeHg levels in both species were below the safe levels suggested by the CODEX Alimentarius Commission (0.5 mg/kg for fish in general, 1 mg/kg for predatory species). Since the 1990s, toothfish consumption has increased markedly in the United States and Asian countries, including Japan (Cascorbi 2002). Compared with international guidelines, the percentages of the PTWI of THg recommended by the JECFA on Food Additives and the TWI of MeHg proposed by the EFSA are 2.5–6.4% and 4.0–10.3%, respectively (Table 3). Europeans had the highest exposure rates (0.320 µg/kg bw/week for THg and 0.134 µg/kg bw/week for MeHg), explained by their higher consumption (137.3 g/week) of demersal fish (FAO 2013). Moreover, the percentages of PTWI and TWI of relatively highly contaminated fish muscle were within

Table 3 Estimate weekly intake of THg and MeHg in Antarctic toothfish, in comparison with the reference dose by JECFA

Country	Element	EWI ^a (µg/kg bw/week)		% of PTWI, TWI ^b	
		Mean ^c	90th ^d	Mean	90th
World	THg	0.147	0.264	2.9	5.3
	MeHg	0.062	0.129	4.7	9.9
America, USA	THg	0.165	0.296	3.3	5.9
	MeHg	0.069	0.144	5.3	11.1
Asia	THg	0.125	0.223	2.5	4.5
	MeHg	0.052	0.109	4.0	8.4
Europe	THg	0.320	0.572	6.4	11.4
	MeHg	0.134	0.279	10.3	21.5

^aEWI (estimate weekly intake)=the measured metal concentration in fish muscle tissue × the fish consumption rate (g/week)/the average adult body

^bThe percentage of PTWI (provisional tolerable weekly intake) for THg (5 µg/kg bw/week) recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA 2003) and TWI (tolerable weekly intake) for MeHg (1.3 µg/kg bw/week) proposed by the European Food Safety Authority (EFSA 2012)

^cThe measured mean concentration in fish muscle tissue

^dThe measured 90th percentile concentration in fish muscle tissue

the guideline levels. These results suggest that consumption of Antarctic toothfish presents no health risk to various global populations.

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