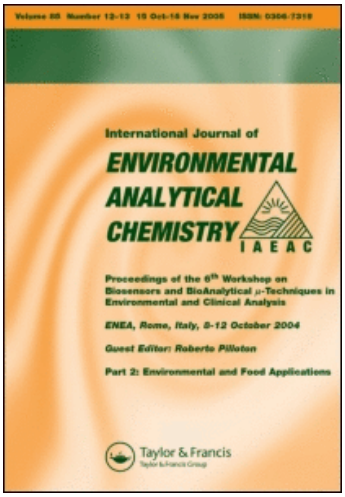

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Methylmercury in marine fish from Malaysian waters and its relationship to total mercury content

Parvaneh Hajeb[a]; Selamat Jinap[a]; Abu B. Fatimah[a]; Bakar Jamilah[a]
[a] Center of Excellence for Food Safety Research (CEFSR), Faculty of Food Science and Technology, Universiti Putra Malaysia, Serdang, Selangor, Malaysia

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Methylmercury in marine fish from Malaysian waters and its relationship to total mercury content

Parvaneh Hajeb, Selamat Jinap*, Abu B. Fatimah and Bakar Jamilah

Center of Excellence for Food Safety Research (CEFSR), Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

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The study evaluated methylmercury concentrations, the methylmercury to total mercury ratio (%MeHg) and their correlations in ten fish species from different trophic levels. Methylmercury levels in fish studied were in the range of 0.007 to 0.914 $\mu g\,g^{-1}$ wet wt. Muscle tissue of predatory fish contained significantly ($p < 0.05$) higher content of methylmercury than non-predatory fish. The methylmercury to total mercury ratio ranged from 49.1% to 87.5%, with the highest ratio in predatory fish. This ratio was always higher in muscle tissue compared to the liver tissues, indicating tissue-specific binding and accumulation of methylmercury in the muscle. All the fish species showed strong positive correlation between methylmercury and total mercury levels ($R^2 > 0.86$). Except for long tail tuna and short-bodied mackerel, all fish species showed lower methylmercury levels and estimated weekly intake as compared to the maximum values established by US FDA (of 0.5 $\mu g\,g^{-1}$) and by FAO/WHO (1.5 $\mu g\,kg^{-1}$ bodyweight), respectively. This study showed that the percentage of methylmercury is rather high in fish and fish represents the major source of this toxic mercury form to the local population.

Keywords: methylmercury; total mercury; correlation; marine fish; exposure assessment

1. Introduction

The presence and behaviour of mercury in aquatic systems is of great interest and importance since it is the only heavy metal which bioaccumulates and biomagnifies through all levels of the aquatic food chain [1] and in higher trophic levels [2]. Amongst organomercurials present in the aquatic environment, methylmercury is the most abundant and also the most toxic form of mercury in the environment, and it represents up to 95% of total mercury in top predators [3]. The relatively high levels of methylmercury found in sediments, biota and water require that other sources of methylmercury be identified. Internal processes, i.e. natural methylation of inorganic mercury in the water column and sediments, represent a significant contribution to the overall burden of methylmercury in the aquatic environment [4].

Because of their risks, mercury and methylmercury are included in the black list of compounds to be monitored in the framework of national and international regulations [5]. Mercury and methylmercury are, therefore, monitored in fish samples by a number of

*Corresponding author. Email: jinap@food.upm.edu.my; sjinap@gmail.com

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organisations. The results are often used to check whether the levels in fish available from markets are below the threshold values and may be consumed safely by humans. In Malaysia, the main mercury sources originate from human activities, such as deforestation and agriculture, electricity-generating power stations, manufacture of cement, pesticides, mirrors and medical equipment, industrial leaks, waste incineration, along with geochemistry, associated with natural mercury release from soil, mercury atmospheric transport and redistribution, as well as mercury methylation potential of ecosystems [6]. Mercury has been reported high in some fish from some parts of Malaysia [7,8].

Fish and other seafood form an important part of the Malaysian diet, making up 21% of the per capita consumption of protein intake from all other meats. Statistics have shown that the average Malaysian consumed 58.95–62.58 kg $year^{-1}$ of marine fish annually and the demand for fish consumption is increasing over years [9]. From a human health perspective it is important to assess whether methylmercury poses a risk, therefore estimated weekly intake of methylmercury through fish consumption was also assessed. In a previous survey, we assessed total mercury level in 12 species of common marine fish consumed by Malaysians [9]. The mercury concentrations in muscle and liver samples were determined by cold vapour atomic absorption spectrophotometry. Among the fish analysed, long tail tuna had the highest concentration of mercury followed by short-bodied mackerel. The aim of the present study was to determine methylmercury in the same fish samples and its correlation with total mercury found in the previous study. The correlation will assist to predict methylmercury content in the fish samples, without conducting tedious experiments to measure methylmercury and being exposed to such a highly toxic compound.

2. Experimental

2.1 *Reagents*

All reagents were of analytical reagent grade. Methylmercury chloride (CH_3HgCl) standards was purchased from Fluka (Japan), BCR-463 (total and methylmercury in tuna fish) from Unit for Reference Materials (EC-JRC-IRMM, Belgium), L-cysteine from Fluka (Japan), potassium bromide (KBr, 99.99%), cupric sulfate ($CuSO_4$, 99%) and sulfuric acid (H_2SO_4, 98.08 g mol^{-1}) from Merck (Germany).

All standards and solutions were prepared using deionised water (ELGA LabWater, VWS, UK). Stock standard solution for methylmercury chloride was prepared at 1000 mg L^{-1} (as Hg) in toluene and stored at −20°C. Working solutions were prepared weekly by diluting the stock solutions with toluene to a range of 1–5000 ng mL^{-1} (as Hg) and stored at 4°C.

2.2 *Apparatus*

All glassware used were soaked in detergent solution overnight before being rinsed and soaked in 10% (v/v) HNO_3 overnight. Agilent 6890 gas chromatograph (Wilmington, DE, USA) with split/splitless injection port and micro-electron capture detection (micro-ECD) was utilised for methylmercury analysis. HP-5 column with 30 m × 0.32 mm ID, 0.25 um film was used to determine methylmercury in fish. Splitless injection mode with 280°C was applied. GC oven temperature was set at 150°C during analysis. Helium was used as carrier gas at flow rates of 1 mL min^{-1} and 60 mL min^{-1}, respectively. Methylmercury was

then confirmed by a HP 5890 gas chromatograph coupled to a HP 5973 mass spectrometer (Hewlett-Packard, Avondale, PA). Samples containing methylmercury were injected to GC-MS through a splitless injection port at 220°C. The column used for methylmercury separation was DB-5MS with 30×0.25 mm I.D. Oven temperature was programmed as 50°C to 150°C at first 15°C min^{-1}, then the temperature was increased to 270°C at 30°C min^{-1} and held for 5 min. Helium was used as carrier gas at a flow rate of 0.9 mL min^{-1}. MS quad and MS source temperatures were 150 and 230°C, respectively.

2.3 *Fish samples*

Samples of 55 fresh fish, mainly from the South China Sea (Figure 1), were collected from a major retail outlet in Selangor, Malaysia. The fish samples were delivered in an igloo box filled with ice to the laboratory. Upon arrival, the fish samples were weighted and sizes were measured, and then gutted before the liver tissue was dissected with stainless steel scalpels. A patch of skin was removed from the mid-dorsal area above the lateral line exposing the dorsal skeletal muscle. Samples of muscle tissue were then collected with stainless steel scalpels and they were placed into separate containers. Samples were stored at −18°C (for less than one week) before being used for analysis.

2.4 *Chemical analysis*

Samples of liver and muscle tissue were homogenised by repeated chopping, mixing and blending using a commercial blender that had been cleaned and rinsed with dilute nitric acid and deionised water prior to use. A 0.5 g portion of homogenised fish samples was weighed in the extraction tube. Aqueous 2 mL of 14.25M H_2SO_4 (saturated with cupric sulfate), 2 mL of 4 M KBr and 2.5 mL of toluene were added to the sample and the mixture was shaken using an electrical shaking device (Memmert-Schwaback; Germany) for 35 min. After centrifugation using (Vortex, Shanghai, China) at 2200 rpm for 10 min, the

Figure 1. Map of fish sampling in Peninsular Malaysia.

supernatant organic phase was collected. The collected organic extract was subjected to a back-extraction by 1 mL cysteine solution (1.5% w/v). The organic phase was separated from the aqueous phase after shaking for 35 min and centrifugation at 2200 rpm for 10 min, and 1 μL were injected into the gas chromatograph [10].

The homogenised samples of fish muscle tissue (0.5 g wet) were weighted in digestion tubes and 5 mL HNO_3 was added before the mixture was digested in a hot block digestor (Spectron, Ventura, California, USA) at low temperature (40°C) for 1 h and were then fully digested at higher temperature (90°C) for at least 2 h. Digested samples were then cooled to 25°C and subsequently diluted to 40 mL volume with deionised water. Blanks (without samples) were prepared in parallel. Total mercury concentration in the same samples has been measured using cold vapour atomic absorption spectrophotometry CV-AAS [9].

2.5 *Recovery and limit of detection*

The reliability of the analytic method was tested by measuring the element in the reference material (CRM 463-Total and methylmercury in muscle tissue of tuna fish). The concentration of methylmercury in the reference material was reported to be 3.04 $\mu g\,g^{-1}$, where the mean experimental values (seven replications) determined in this study was 2.99 $\mu g\,g^{-1}$ (98.61% recovery). The absolute limit of detection or limit of detection (LOD), and relative limit of detection or limit of quantification (LOQ) for methylmercury, were determined by serial dilutions of the lowest calibrator concentration and established at a ratio of signal-to-noise of 1 : 3 and 1 : 10, respectively. The LOD and LOQ of the method were 2.2 and 7 $ng\,g^{-1}$.

2.6 *Methylmercury exposure*

Methylmercury exposure was assessed using fish consumption data developed by the Ministry of Health Malaysia (MOH) which indicates that average Malaysian adults (≥18 years old) of 50 kg weight are consuming 169 g of fish serving day^{-1} (MOH, personal communication 2006). The consumption data on Malaysia children and child-bearing mothers are not available. The estimated weekly intake (EWI) values of mercury by an adult ($\mu g\,kg^{-1}$ body weight) for each fish species were calculated using the formula below [9]:

$$\text{EWI}\ (\mu g\,kg^{-1}) = \frac{[\text{Mean Hg in fish}\ (\mu g\ g^{-1}\ \text{wet wt}) \times \text{Weekly fish consumption}\ (g)]}{\text{Body weight}\ (50\ kg)}$$

2.7 *Statistical analysis*

The descriptive statistics (mean, standard deviation, range) and one-way analysis of variance (ANOVA) were conducted using SPSS (Version 11.5, SPSS Inc., Chicago, IL, USA). A one-way ANOVA statistical procedure was employed in the assessment of variation in methylmercury concentrations among fish species. A p value of less than 0.05 indicates statistical significance. The strength of the association between total mercury and methylmercury concentrations was measured using Pearson's correlation coefficient.

3. Results and discussions

Fish analysed were divided according to their feeding habit; predatory and non-predatory, including planktivorous and benthophagous (Table 1). The feeding habit classification of the fishes was based on Isa *et al*. [11]. Methylmercury in marine fish in Peninsular Malaysia studied ranged from 0.007 to 0.914 $\mu g\,g^{-1}$ wet weight (Table 1). Significant differences ($p < 0.05$) in methylmercury concentrations occurred among the fish studied. Consistently high levels of methylmercury were found in muscle tissue of two species, i.e. *Thunus Tonggol* (long tail tuna) and *Rastrelliger brachsoma* (short-bodied mackerel). Methylmercury concentration was 0.104 ± 0.013 and $0.131 \pm 0.089\,\mu g\,g^{-1}$ in *Thunus Tonggol* (long tail tuna) and *Rastrelliger brachsoma* (short-bodied mackerel), respectively. These two fish also presented high total mercury levels compared to the other fish studied [9]. Muscle tissue of predatory fish contained significantly ($p < 0.05$) higher content of methylmercury than non-predatory fish. These findings are in agreement with other studies [12,14]. The lowest content of total mercury and methylmercury were found in the muscle tissue of *Decapterus macrosoma* (sardine), whose diet consists mainly of water plants and plankton [7]. No information was found on methylmercury in fish from Malaysian waters and the other neighbouring countries in South East Asia. In comparison with those reported for marine fish from Japan [15], our study showed lower concentrations of methylmercury in marine fish from Malaysia. Yamashita *et al*. [15] reported higher concentrations of total mercury ($3.51 \pm 0.72\,\mu g\,g^{-1}$ wet weight) in different species of tuna fish in Japan.

Literature has shown that the methylmercury concentration in liver is higher than in muscle tissue [13–16]. In this study, apart from *Rastrelliger brachsoma* (short-bodied mackerel), no significant difference ($p > 0.05$) in methylmercury concentration was found between liver and muscle in the other fish species. There was a significant difference ($p < 0.05$) in methylmercury concentrations of the liver tissues among different fish species.

The percentages of methylmercury in muscle and liver tissues of the marine fish found during this study are shown in Table 1. Methylmercury level as a percentage of total mercury in the muscle comprised $84.6 \pm 6.5\%$ in *Rastrelliger brachsoma* (short-bodied mackerel), $74.2 \pm 5.3\%$ in *Scomberomorous commerson* (narrow-barred Spanish mackerel), $55.3 \pm 9.1\%$ in *Parastromateus niger* (black pomfret), $88.5 \pm 6.6\%$ in *Thunus Tonggol* (long tail tuna), $75.0 \pm 2.9\%$ in *Epinephelus tauvina* (greasy grouper), $49.8 \pm 6.8\%$ in *Anodontostoma chacunda* (chacunda gizzard shad), $56.1 \pm 5.5\%$ in *Selaroides leptolepis* (yellow-banded scad), $75.2 \pm 7.3\%$ in *Euthynnus afinis* (Eastern little tuna), $59.3 \pm 4.2\%$ in *Nemipterus delagone* (delagoa threadfish bream), and $81.2 \pm 8.6\%$ in *Lates calcarifer* (giant perch). Results of the methylmercury analysis showed that most of the mercury is in the methylmercury form; methylmercury represented an average of $75.6 \pm 9\%$ of the total mercury in all the analysed specimens. Table 1 also shows the percentage of methylmercury in muscle and liver tissues of the Malaysian marine fish as compared to the mercury level obtained in a previous study [9]. This ratio also was always higher in the muscle tissue compared to the liver tissues, indicating tissue-specific binding and accumulation of methylmercury in the muscle. The current findings on methylmercury levels in fish are comparable to those observed for marine fishes in other studies [15–25]. The sole study on methylmercury in seafood in Malaysia by Rahman *et al*. [25] revealed that in a variety of samples studied, methylmercury was in the range of 45%–94% of the total mercury. Spanish mackerel and Indian mackerel were reported to have higher

Table 1. Total mercury (Hajeb *et al.* [10]) and methylmercury concentration (μg g^{-1} wet wt.) in liver and muscle of fish species.

Common name (Scientific name)	*N*	Body weight (g) Total length (cm) Mean (range)	Food habit (Based on Isa *et al.* [11])	Portion	Total mercury (μg g^{-1} wet wt.) Mean ± SD	Total mercury (μg g^{-1} wet wt.) Maximum	Methylmercury (μg g^{-1} wet wt.) Mean ± SD	Methylmercury (μg g^{-1} wet wt.) Maximum	Methylmercury/ total mercury %
Short-bodied mackerel	6	155.5 (126.2–183.0)	Predatory	Muscle	0.149 ± 0.376	1.013	0.131 ± 0.089	0.914	84.6 ± 6.5
(*Rastrelliger brachsoma*)		21.2 (20.3–22.5)		Liver	0.023 ± 0.01	0.032	0.011 ± 0.002	0.023	53.3 ± 3.5
Narrow-barred Spanish mackerel	5	900.1 (235.5–1950.0)	Predatory	Muscle	0.014 ± 0.023	0.043	0.008 ± 0.045	0.021	74.2 ± 5.3
(*Scomberomorous commerson*)		40.8 (32.0–61.7)		Liver	0.028 ± 0.042	0.089	0.013 ± 0.003	0.040	36.0 ± 5.1
Eastern little tuna	5	696.0 (283.5–1560.0)	Predatory	Muscle	0.014 ± 0.009	0.024	0.007 ± 0.001	0.014	75.2 ± 7.3
(*Euthynnus afinis*)		26.9 (26.9–44.2)		Liver	0.005 ± 0.002	0.008	ND	ND	–
Long tail tuna	4	1016.0 (379.4–1650.0)	Predatory	Muscle	0.122 ± 0.381	0.961	0.104 ± 0.013	0.888	88.5 ± 6.6
(*Thunus Tonggol*)		34.7 (28.3–44.2)		Liver	0.124 ± 0.033	0.144	0.056 ± 0.007	0.074	49.1 ± 2.7
Greasy grouper	6	398.5 (276.1–614.6)	Predatory	Muscle	0.012 ± 0.002	0.013	0.007 ± 0.001	0.008	75.0 ± 2.9
(*Epinephelus tauvina*)		31.2 (27.8–36.5)		Liver	0.005 ± 0.001	0.009	ND	ND	–
Giant perch	3	402.3 (177.5–818.2)	Predatory	Muscle	0.024 ± 0.002	0.054	0.016 ± 0.002	0.044	81.2 ± 8.6
(*Lates calcarifer*)		31.5 (25.7–42.1)		Liver	0.006 ± 0.002	0.009	ND	ND	–
Chacunda gizzard shad	7	68.8 (50.9–91.0)	Planctivorous	Muscle	0.040 ± 0.063	0.163	0.022 ± 0.005	0.089	49.8 ± 6.8
(*Anodontostoma chacunda*)		12.9 (12.8–15.5)		Liver	0.006 ± 0.001	0.022	ND	ND	–
Yellow-banded scad	6	69.4 (19.2–158.1)	Planctivorous	Muscle	0.032 ± 0.043	0.090	0.024 ± 0.005	0.046	56.1 ± 5.5
(*Selaroides leptolepis*)		15.6 (10.5–22.0)		Liver	0.029 ± 0.038	0.064	0.011 ± 0.004	0.024	37.7 ± 6.1
Black pomfret	6	220.1 (105.9–448.6)	Zoobenthic	Muscle	0.044 ± 0.081	0.188	0.021 ± 0.009	0.123	55.3 ± 9.1
(*Parastromateus niger*)		19.9 (16.6–24.8)		Liver	0.063 ± 0.049	0.123	0.022 ± 0.001	0.044	31.6 ± 3.9
Delagoa threadfish bream	7	161.2 (67.4–316.1)	Benthophagous	Muscle	0.029 ± 0.018	0.077	0.024 ± 0.004	0.052	59.3 ± 4.2
		20.7 (16.0–26.8)		Liver	0.031 ± 0.014	0.043	0.013 ± 0.001	0.023	40.1 ± 3.5

ND: not detected; N: number of samples; SD: standard deviation.

concentration of methylmercury. The highest methylmercury concentrtation (94% of the total mercury) was found in Spanish mackerel collected from Kuala Perlis. In comparison with those reported for marine fishes from Malaysia [25] and Japan [15], our study showed lower levels of methylmercury in marine fishes in Malaysia. Lower levels of mercury from Malaysian oceans compared to Japanese waters may be due to less sources of pollution in this region. Table 1 shows the increase in the ratio of methylmercury to total mercury from non-predatory and predatory fish. This clearly confirms the well-known fact that methylmercury is accumulated and biomagnified in trophic food chains in the aquatic environments.

Strong positive correlation ($R^2 > 0.86$) was found between methylmercury and total mercury levels in the fish species studied (Table 2). These data together with the fact that methylmercury makes up more than 75% of total mercury in most of the marine fish suggests that the mercury present in marine fish will be efficiently accumulated in fish consumers. However, there was no significant correlation found between methylmercury concentration and the length or weight of all the fish species analysed. This may be due to the scarce number of fish samples analysed.

Estimated weekly intake (EWI) of total mercury and methylmercury were compared for all the fish species (Figure 2). While the estimated weekly intake of total mercury in all fish species were well below the provisional tolerable weekly intakes (PTWI) of 5 μg per kg body weight recommended by the Joint FAO/WHO Expert Committee on Food Additives [29], the estimated weekly intake of methylmercury in *Rastrelliger brachsoma* (short-bodied mackerel) and *Thunus Tonggol* (long tail tuna) were higher than PTWI of 1.5 μg per kg body weight. However, people are consuming a variety of fish. The estimated daily intake for the adult population in the areas studied revealed interesting conclusions. It was assumed that the major route of exposure is consumption of contaminated fish.

This study improves the baseline data and information on methylmercury concentration in marine fish commonly marketed in Malaysia. An interesting outcome of this study is the observation that the percentage of methylmercury is rather high in fish, and fish represents the major source of this toxic mercury form to the local population. In general, it can be concluded that the most at risk population are the coastal inhabitants with higher fish consumption. An increase in the ratio of methylmercury to total mercury from non-

Table 2. Correlation (R^2) between total mercury and methylmercury in muscle tissue of different fish species.

Common name	Correlation between Total Hg and MeHg (R^2)
Short-bodied mackerel	0.9982
Narrow-barred Spanish mackerel	0.9956
Black pomfret	0.9996
Long tail tuna	0.9998
Greasy grouper	0.8648
Chacunda gizzard shad	0.9972
Yellow-banded scad	0.9906
Eastern little tuna	0.9858
Delagoa threadfish bream	0.9994
Giant perch	0.9999

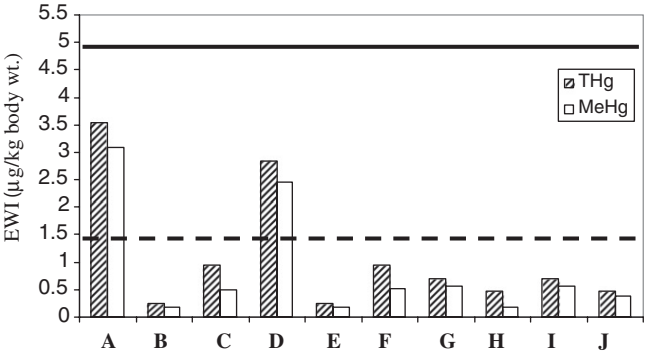

Figure 2. Estimated weekly intake (EWI) of total mercury (Hajeb *et al.* [10]) and methylmercury ($\mu g\,kg^{-1}$ body wt.) of different fish species.
1: Short-bodied mackerel; 2: Narrow-barred Spanish mackerel; 3: Black pomfret; 4: Long tail tuna; 5: Greasy grouper; 6: Chacunda gizzard shad; 7: Yellow-banded scad; 8: Eastern little tuna; 9: Delagoa threadfish bream; 10: Giant perch.
PTWI (Provisional tolerable weekly intakes) of 5 and 1.5 µg of total mercury and methylmercury per kg body weight recommended by Joint FAO/WHO Expert Committee on Food Additives (2005).
A: Shirt bodied Mackerel; B: Narrow barred Spanish mackerel; C: Black pomfret; D: Long tail tuna; E: Greasy grouper; F: Chacunda gizzard shad; G: Yellow banded scad; H: Easter little tuna; I: Delagoa threadfish bream; J: Giant perch.

predatory and predatory fish clearly confirms the well-known fact that methylmercury is accumulated and biomagnified in trophic food chains in the aquatic environments. Finally, from the estimated weekly intakes of total mercury and methylmercury studied for the adult population in Malaysia, it can be concluded that the major route of exposure is consumption of contaminated fish.

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