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Total Mercury and Methylmercury Accumulation in the Muscle Tissue of Frigate (*Auxis thazard thazard*) and Yellow Fin (*Thunnus albacares*) Tuna from the Gulf of Guinea, Ghana

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The concern for human health and that of the ecosystem has led to increased attention on evaluating mercury (Hg) contamination of the environment. Though the sources of mercury to the atmosphere are both natural and anthropogenic, the emissions of mercury mostly from coal combustion, municipal and medical waste incineration and smelting, exceed the inputs from the natural sources (Mason et al., 1994) with higher levels in developed areas and around point sources (Mason et al., 1997). Present high levels of mercury in the atmosphere are the result of enhanced anthropogenic activities (Meili, 1994) due to population growth and urbanization. Consequent enhanced dry and wet deposition of mercury is often the dominant source of mercury to the aquatic systems (Rolfus and Fitzgerald, 1995) though industrialization and increasing population as a result of rapid urbanization, have also contributed to considerable discharge of domestic wastewater and industrial effluents into the aquatic systems contaminating the waters with mercury. After atmospheric deposition and runoff from surrounding catchments, mercury can be converted to methylmercury (MeHg) from in situ production by natural bacteria in anoxic sediments and soils (WHO, 1990). Methylmercury is a highly toxic, organic form of mercury that is easily absorbed and more readily accumulated by aquatic organisms than inorganic mercury (EPA, 2001). Once methylmercury has been taken up by organisms low in the food chain (such as phytoplankton and zooplankton), it is efficiently accumulated and transferred to organisms higher in the food chain (Watras & Bloom, 1992). Accumulation of methylmercury by fish is of concern since consumption of methylmercury-contaminated fish is the major route for transfer of mercury from the aquatic environment to fish-eating birds and mammals, including humans (Rodgers, 1994). A number of studies have shown that virtually all the mercury in fish is methylmercury (Bloom, 1992; Rodgers, 1994; Lawrence & Mason, 2001). Exposure to high levels of methylmercury has been found to cause neurological damage, as well as fatalities, among adults. Prenatal life and small children are even more susceptible to brain damage due to their enhanced sensitivity to the neurotoxin (Weiss et al., 1999). In view of the changing dietary habits of modern society, which advocates the benefits of fish consumption for maintenance of general health, as well as for prevention of cardiovascular disease (WHO, 1976), the possibility of methylmercury intoxication through ingestion of contaminated fish and fish products is of serious concern. Tuna, which has been recognized as high trophic level fish, are frequently eaten in Ghana, so their methylmercury content should be of concern to human health. The aim of this study is to determine the current levels of total mercury and methylmercury in the muscle tissue of frigate (*Auxis thazard thazard*) and yellow fin (*Thunnus albacares*) tuna from the

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Atlantic Coastal waters of Ghana in the Gulf of Guinea; and to investigate the relationships between tuna fish size (weight and length) and mercury concentration in the muscle tissue.

MATERIALS AND METHODS

All glassware were soaked in detergent solution overnight; rinsed and soaked in 10% v/v HNO_3 solution overnight. They were rinsed with distilled water followed by 0.5% $KMnO_4$ solution and finally rinsed with distilled water before use.

All reagents were of analytical reagent grade (BDH Chemicals Ltd, Poole, England) unless otherwise stated. Double distilled water was used for the preparation of all solutions. Mercury stock standard solution (1000 mg L^{-1}) was prepared by dissolving 0.0677 g of $HgCl_2$ in the acid mixture HNO_3 - H_2SO_4 - $HClO_3$ (2 + 10 + 2) in a 50 ml volumetric flask and diluted to 50 ml with double distilled water. Blank solutions were also prepared alongside and bulked together for use as a diluting solution. The working solutions were freshly prepared by diluting an appropriate aliquot of the stock solution through intermediate solutions using blank solution. $SnCl_2.2H_2O$ solution (10% w/v) was prepared by dissolving 10 g of the salt in 100 ml 1M HCl. The solution was aerated with nitrogen gas at 50 ml min^{-1} for 30 min to expel any elemental mercury from it.

Methylmercury stock standard solution (1 μg ml^{-1}) was prepared by dissolving 0.0125 g of methylmercury chloride CH_3HgCl in toluene to make a final volume of 100 ml. Methylmercury-cysteine standard working solution (0.1 μg ml^{-1}) was prepared by mixing 0.5 ml of the methylmercury stock standard solution with 5 ml of 0.1% L-cysteine solution; and the methylmercury extracted into aqueous phase.

Two tuna fish species namely frigate (*Auxis thazard thazard*) and yellow fin (*Thunnus albacares*) tuna were obtained in May 2004 from commercial catches landed at the Tema Fishing Harbour in the Greater Accra Region of Ghana. A total of twenty samples of the two species were obtained. The samples ranged widely in weight (0.62-2.02 kg), and in fork length (35.5-55.2 cm). The samples were placed in clean plastic bags and stored on ice in an ice chest. They were then transported to the laboratory, identified and kept in a freezer at -20° C prior to preparation for chemical analysis. The samples were washed with distilled water, dried in tissue paper and the fork length and body weight of each was taken after defrosting in the laboratory. A portion of the edible muscle tissue was removed from the dorsal part of each fish, homogenized and stored in clean-capped glass vials and kept in a freezer until shipped to Japan for analysis at the National Institute for Minamata Disease (NIMD). The samples were digested for total mercury determination by an open flask procedure developed at the National Institute for Minamata Disease (NIMD) in Japan (Akagi and Nishimura, 1991; Voegborlo et al., 2004). This involved the digestion of about 0.5 g of the samples using 1 ml water, 2 ml mixture of nitric, perchloric (1+1) and 5 ml sulphuric acid added in turns; and heated at a temperature of 200° C in a 50 ml digestion volumetric flask. The digests were diluted to volume with double distilled water and analysed by cold vapour atomic absorption spectrophotometry using an automatic mercury analyzer HG-201(Sano Seisakusho Co., Ltd., Tokyo, Japan). Methylmercury was determined in the fish samples by the dithizone extraction and gas chromatography with electron capture detection method (Akagi and Nishimura, 1991). In the procedure about 0.2 g fish sample was digested with 10 ml of 1 M ethanolic KOH in a 40 ml screw capped conical centrifuge tube at

100° C for 1 h. The sample solution was then washed with 5 ml n-hexane after neutralizing with 10 ml of I M HCl. Methylmercury in the washed sample solution was extracted with 5 ml of 0.01% purified dithizone in toluene. The toluene layer was washed twice with 3 ml of 1 M NaOH and 3 ml was transferred into a 10 ml screw capped conical centrifuge tube and back-extracted with 2 ml of 5 ppm Na_2S in 0.1N $NaOH:CH_3CH_2OH$ (1:1). The Na_2S layer was washed with 2 ml toluene, slightly acidified with 1 M HCl and nitrogen gas bubbled through to expel excess sulphide as H_2S gas. Walpole's buffer at pH 3 (2 ml) was added to the solution followed by 0.5 ml of purified dithizone in toluene to re-extract the methylmercury. The toluene layer was washed with 3 ml of 1 M NaOH and acidified with 2 drops of 1 M HCl. The final extract was centrifuged, the aqueous layer discarded and 5 µl of the toluene layer was injected into Yanaco Gas Chromatograph Model G3800 with column temperature of 150° C; injector and detector temperature of 200° C. Column was 3.0 mm x 1.0 m glass packed with Hg-20A on Uniport HP AW-DMCS, 60-80 mesh (GL Science Co. Ltd., Tokyo, Japan) and carrier gas was nitrogen. In order to validate the methods for accuracy, certified reference material (Dogfish muscle CRM 'National Research Council, Canada' (DORM 2)) was analysed for total mercury and methylmercury. Detection limits and precision of the analyses were determined by repeated analyses of some samples and certified reference material

RESULTS AND DISCUSSION

Twenty samples of tuna comprising two different species namely Frigate (*Auxis thazard thazard*) and Yellow Fin (*Thunnus albacares*) tuna were analysed for total and methylmercury. Total mercury concentration ranged from 0.036 to 0.197 µg g^{-1} and for methylmercury concentration, from 0.035 to 0.188 µg g^{-1} wet weight for all the tuna samples (Table 1). These levels are all below the 0.5 µg g^{-1} wet weight limit recommended by the FAO/WHO (1972) and adopted by many countries (CIFA, 1992). The validity of the method has been proved by the agreement between values obtained for the measured (4.60–4.76 µg g^{-1}) and the certified (4.15–4.79 µg g^{-1}) concentrations in Dogfish muscle (DORM-2) Certified Reference material for total mercury. Detection limit was 0.5 ng Hg g^{-1}. For methylmercury the validity of the method has been proved by the agreement between values obtained for the measured (4.05 – 4.16 µg g^{-1}) and the certified (4.15–4.79 µg g^{-1}) concentrations in Dogfish muscle (DORM-2) Certified Reference material. Detection limit was 5 ng MeHg g^{-1}.

The concentration of mercury in fish has been the subject of intense study in recent years and the mercury content of marine fish has variously been reported (WHO, 1976). Total mercury levels reported for most species of oceanic fish fall in the range of 0 - 0.5 µg g^{-1} wet weight with most values close to 0.15 µg g^{-1} wet weight (WHO, 1976). However mercury levels in tuna fish usually range from 0.2 to 1.5 µg g^{-1} (FAO/WHO, 1972). Holden (1973) also reported mercury content of tuna fish ranging from 0.8 to 1.2 µg g^{-1} with an average content that is between 0.3 and 0.4 µg g^{-1} below which our values fall. Levels in skipjack, white tuna and yellowfin tuna caught in the Atlantic, Pacific and Indian Oceans ranged from 0 to 1.0 µg g^{-1} wet weight with most values ranging from 0.2 to 0.3 µg g^{-1} wet weight (WHO, 1976). The results of this study are either in agreement or lower than the levels reported by the other authors for predatory fish in other areas of the world (WHO, 1976; WHO, 1990: Storelli et al., 2002a; Storelli et al., 2002b; Love et al., 2003; Storelli et al., 2003). Inorganic mercury may be methylated by sulphate-reducing bacteria in an aquatic system.

Table 1. Mean contents of total and methyl mercury ($\mu g\ g^{-1}$) in two tuna fish species samples.

Tuna Specie	Form of Mercury	Range	Mean	Standard Deviation	% Mean (MeHg/THg)
Frigate n=9	Total mercury	0.053-0.197	0.112	0.044	97.32
	Methylmercury	0.055-0.188	0.109	0.042	
Yellow fin n=11	Total mercury	0.036-0.090	0.061	0.021	98.36
	Methylmercury	0.035-0.088	0.060	0.021	

Note: n = no of samples; MeHg = Methylmercury; THg = Total mercury

This microbially-mediated mercury methylation which occurs in the oxygen-minimum layer of the ocean may be the source of methylmercury in the muscle tissues of large pelagic fish such as swordfish and tuna. That mercury in fish appears to be predominantly in the form of methylmercury has been confirmed by many publications (WHO, 1976; WHO, 1990: Bloom, 1992; Larsorsa and Gill, 1995; Andersen and Depledge, 1997; Al-Majeed and Preston, 2000; Storelli et al., 2002a). Swedish measurements of fish, summarized by a Swedish Expert Group (1971), indicated that virtually all of the mercury is present in the form of methylmercury compounds. These findings were confirmed for fish from the North American continent and for swordfish and tuna fish (WHO, 1976).

In this study, mercury was present almost completely in the methylated form in the two species, with percentages between 90 and 106 % (average 97.32 %) in frigate and between 91 and 103 % (average 98.36 %) in yellow fin tuna. The results from this study further corroborate the assertions by the other authors. Therefore, diet consisting particularly of tuna fish, could be the main source of exposure to methylmercury in the general population. Positive relationships between mercury concentrations and fish size have been reported for predatory species (WHO, 1976; (Hueter et al., 1995; Rose et al., 1999; Lacerda et al., 2000). In this study, methyl mercury levels were positively correlated with the weight of the two tuna species namely *Auxis thazard thazard* ($P<0.01$, $r=0.9243$) and *Thunnus albacares* ($P<0.05$, $r=0.4397$). Similarly methyl mercury levels were positively correlated with the fork length of the two species, $r=0.8666$ at $P<0.01$ for *Auxis thazard thazard* and $r = 0.5002$ at $P<0.05$ for *Thunnus albacares*. The relationships are indicated as regression lines in Figures 1 and 2. *Auxis thazard thazard* also showed consistently higher accumulation of mercury than *Thunnus albacares*. This is as a result of consistently higher weight *of Auxis thazard thazard* compared to *Thunnus albacares*. A similar trend is observed with the fork length. These relationships found support the occurrence of biomagnification of mercury in these species. The relatively strong relationship between methylmercury in tuna fish and weight and/or length demonstrates that methylmercury accumulates in tuna fish with increasing size. The concentrations of methylmercury in the tuna fish samples obtained in this study are not high when compared to some other areas of the world and can be said to reflect background methylmercury concentrations that are even much lower than

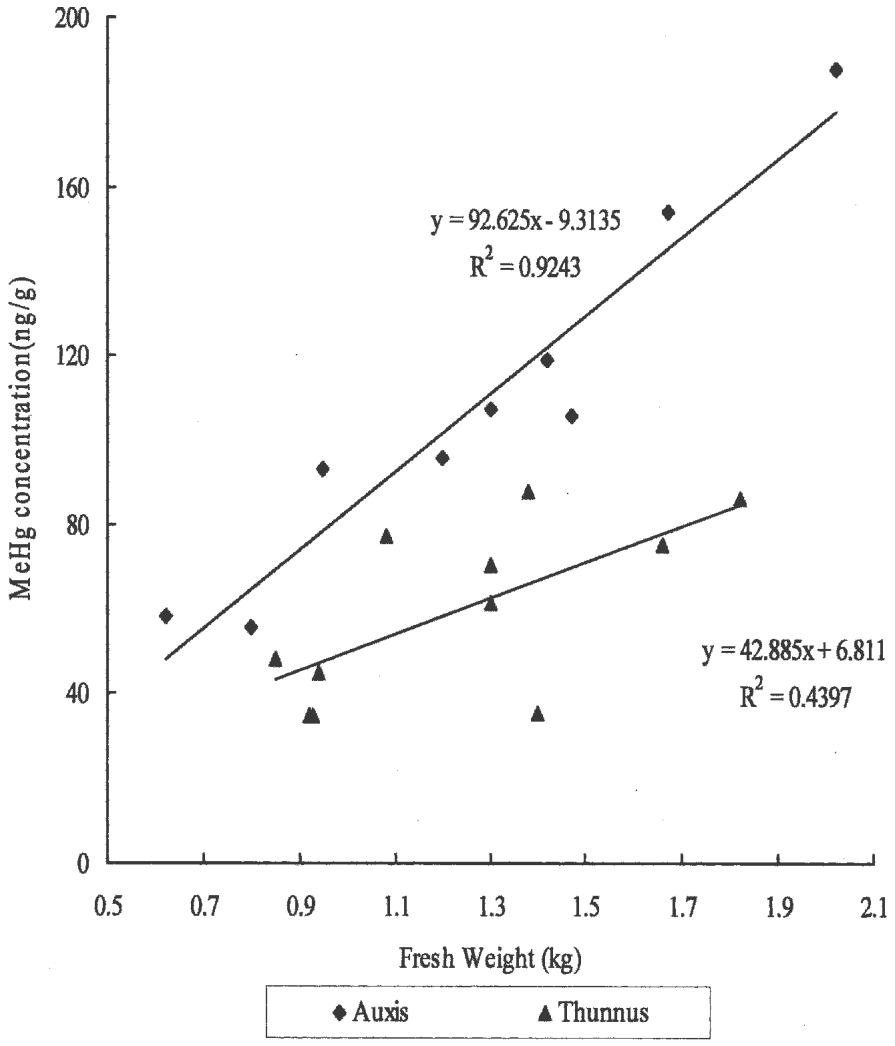

Figure 1. Relationship between MeHg concentrations on weight wet basis and fresh weight of two tuna fish species.

most published methylmercury concentrations in fish from non-polluted areas of the world (WHO, 1990; Storelli et al., 2002; Love et al., 2003).

The results of this study provide a basis for assessment of human exposure of the coastal population to methylmercury from consumption of tuna fish. The generally low levels of mercury found in tuna fish muscle from the coastal waters of Ghana in this study suggest that there is very little input or production of methylmercury in the marine environment. Since fish accumulate more methylmercury than inorganic mercury, the low mercury levels in fish from this marine environment seems to indicate low concentrations of methylmercury in the Gulf of Guinea.

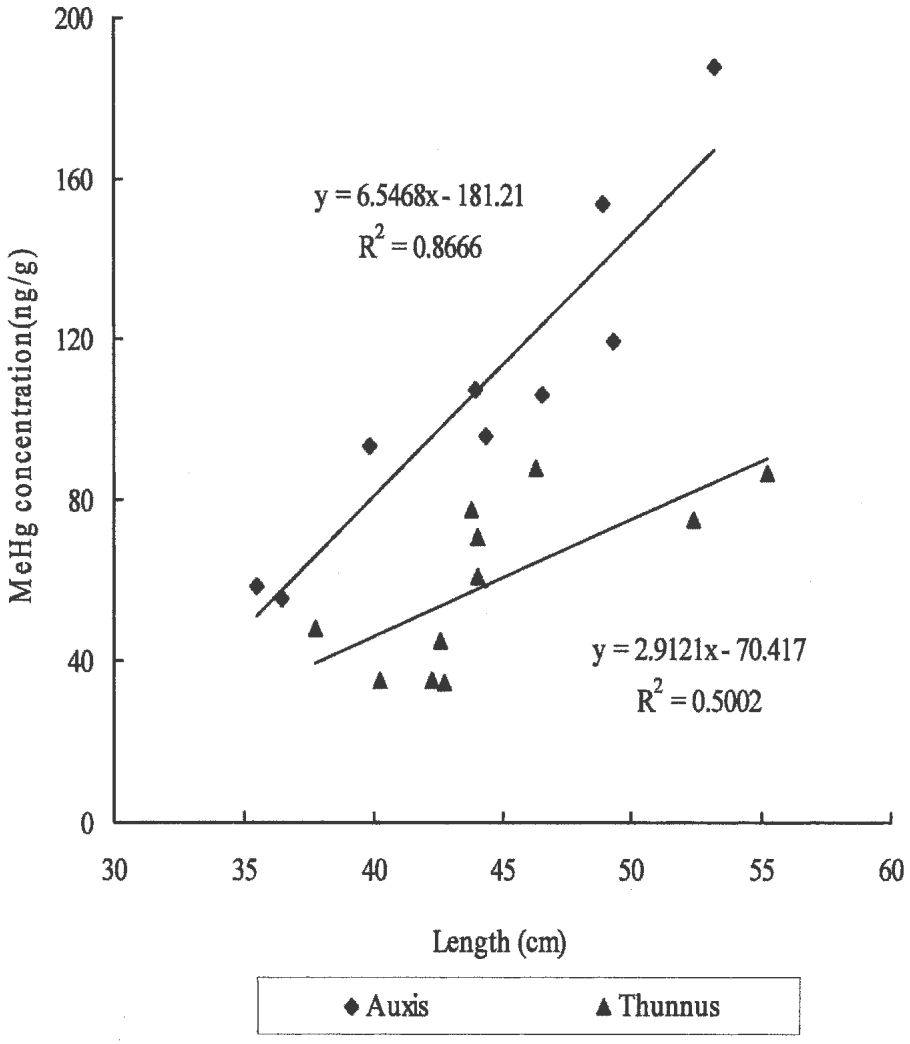

Figure 2. Relationship between MeHg concentrations on weight wet basis and fork length of two tuna fish species.

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