# Total Mercury in Three Fish Species Sold in a Metro Manila Public Market: Monitoring and Health Risk Assessment

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## ABSTRACT

The total mercury concentrations in *bangus* or milkfish (*Chanos chanos* Forskal), tilapia (*Oreochromis niloticus*) and *galunggong* or round scad (*Decapterus spp.*) purchased from a local market in Metro Manila from 5 August to 20 October 2004 were determined by cold vapor atomic absorption spectrophotometry. The ranges of total mercury concentrations observed from about 30 composite test samples for each fish species were 0.0060 to 0.015 mg $kg^{-1}$ (wet weight) for *bangus*, 0.0041 to 0.017 mg $kg^{-1}$ (wet weight) for tilapia and 0.014 to 0.05 mg $kg^{-1}$ (wet weight) for *galunggong*. Risk assessment for neurological effects associated with the consumption of the fish species with the highest concentration of mercury (0.05 mg $kg^{-1}$ for *galunggong*) was done. The calculated daily dose of total mercury of 0.06 µg $d^{-1}$ $kg^{-1}$ body weight indicates that consumption of any one or any combination of *bangus,* tilapia, *and galunggong* sold in Nepa-Q-Mart from August 5 to October 20 in 2004 does not entail risk of adverse neurological effects.

*Keywords: milkfish*, cold vapor atomic absorption spectrophotometry, *round scad*, health risk assessment, mercury monitoring, *tilapia*

## INTRODUCTION

Among the heavy metals that cause adverse health effects in humans, mercury is one of the most prevalent in the environment. Degassing of the earth's crust is the most important natural source of mercury in the environment; however, human activities result in increasing considerably the presence of mercury in the environment. Burning fossil fuels in incinerators and power plants, mining operations, lead smelting, and pulp and paper processing are some of the activities that release mercury into the air, soil and water. When mercury from the natural and man-made sources finds its way into the water, it can be transformed into its most toxic form, organic methyl mercury, through the action of anaerobic bacteria in the water system. Methyl mercury accumulates in fish and the extent of mercury accumulation in fish depends on the level of mercury in the water and on the place of the fish in the food web (Sloof et al., 1995).

Consumption of methyl mercury-contaminated fish by man poses risks especially to children and childbearing women who are the most vulnerable (Cox et al., 1989, Sloof et al., 1995, FAO/WHO 2003). The adverse health effects of high level mercury contamination that include cardiovascular effects, severe nervous system damage and death have been documented (ATSDR 1992, Goyer 1996).

Health risk through fish consumption can be evaluated by measuring the rate of mercury intake based on the mercury content of the fish (SEG 1971, WHO-IPCS 1991). To minimize the health risk of mercury, government regulatory agencies have issued health advisories on the kinds of fish that consumers should avoid and have provided regulatory limits on mercury in fish. The maximum allowed/recommended levels of methyl mercury in fish are 0.5 mg $kg^{-1}$ in the United States, European Union, Korea, Thailand, Philippines, and the World Health Organization/Food and Agriculture Organization (WHO/FAO), and 0.3 mg $kg^{-1}$ methyl mercury in Japan, China and the United Kingdom **(**UNEP 2003**)**. Other countries have set maximum levels for total mercury in fish at 0.4 mg $kg^{-1}$ in Japan and 0.2 mg $kg^{-1}$ in Australia **(**UNEP 2003**)**.

The Philippines is rich in mineral resources and the exploitation of these mineral resources by big and small scale mining operations in the country is expected to increase the level of mercury in the aquatic environment. The Philippines, being an archipelago, is blessed with long coastal waters as sources of fish, making fish a major source of protein for most Filipinos. In a survey done in 1994-96 (UNEP 2003), the country ranked third among the biggest consumers of fish in Asia (75 g $d^{-1}$ per person) after Japan (107 g $d^{-1}$ per person) and Korea (74 - 94 g $d^{-1}$ per person). In view of the potential enhancement of mercury contamination of the aquatic system by mining operations and industrial wastes and the importance of fish in the Filipino diet, it is important to investigate if the fish that Filipinos consume will not pose adverse health effects due to mercury.

The concentrations of mercury in some commercial fish species from Albay Gulf (Santiago and Africa, 2008), Manila Bay (Prudente et al., 1997) and Laguna Lake (Cuvin-Aralar, 1990) have been reported. This study, however, is the first investigation of the mercury levels of widely consumed fish species sold in a public market in the Greater Manila Area. The health risk associated with the consumption of these fishes based on standard estimation of health risk is also reported.

## MATERIALS AND METHODS

### Sampling design

Samples of *bangus* (*C. chanos* F.) 19-32 cms, tilapia (*O. nilotica*) 16-24 cms, and *galunggong* (*Decapterus spp.*) 10-30 cms, were purchased in Nepa-Q-Mart, Quezon City in eight batches from 5 August to 20 October 2004. The three species of fish were selected because of the abundance of supply and relatively cheaper price that make them the most affordable among the fishes sold in the market. For each sampling batch, eight fish stalls were chosen at random. Depending on the size of the fish, representative samples (1-5 fishes) from each fish stall were taken at random on each sampling period. For each species, all eight representative fish samples from eight fish stalls were cleaned, cut into pieces, and combined before homogenization.

### Test Procedures

*Preparation of samples.* The procedure for preparation of fresh fish samples before analysis is the AOAC standard method 937.07 (Hollingworth et al., 1990). Briefly, the fresh fish samples were cleaned, scaled and eviscerated, and cut up according to size. Large fishes (≥ 20 cm) were cut into several cross-sectional slices approximately 2.5 cm thick and had their bones removed. For small fishes (≤15 cm) and intermediate-sized fishes, heads, scales, tails, fins, guts, and inedible bones were removed and discarded. All body flesh from head to tail was taken. The fish flesh was homogenized in a Waring blender and subsamples were prepared by quartering technique. The samples were kept in the freezer when analysis could not be done immediately. Before weighing, the sample was thawed to room temperature and rehomogenized. A portion of the subsample (5.0000 ± 0.0001 g) was weighed in a 250 mL Erlenmeyer flask for mercury analysis.

*Digestion of sample and analysis of total mercury.* The procedures for digestion and analysis of the fish sample for mercury were adopted with some modifications from a published method (Bouchard 1973).

Concentrated nitric acid (5 mL) was added to the sample and the flask was covered with polyethylene film to allow digestion of the sample overnight. Five percent chromic acid (10 mL) was added and the digestion was allowed to continue for at least 30 minutes. Ultrapure water (15 mL) was added after digestion was completed. Hydroxylamine crystals (4 g) were added prior to instrumental analysis.

Total mercury was analyzed by cold vapor (flameless) atomic absorption spectrophotometry using Thermo Jarrell Ash Video 11E equipped with a Hamamatsu mercury hollow cathode lamp operated at 3 mA and 1.0 nm spectral bandwidth. Absorption of light was measured at 253.6 nm. The digested sample was transferred quantitatively into a reaction flask which was attached to an aeration apparatus (see Figure 1). Tributylphosphate (8 drops) was added to the sample to minimize foaming. Ten percent stannous chloride solution (10 mL) was immediately added and reaction was allowed to proceed. The absorbance of mercury that was volatilized and carried by air into the absorption cell was measured.

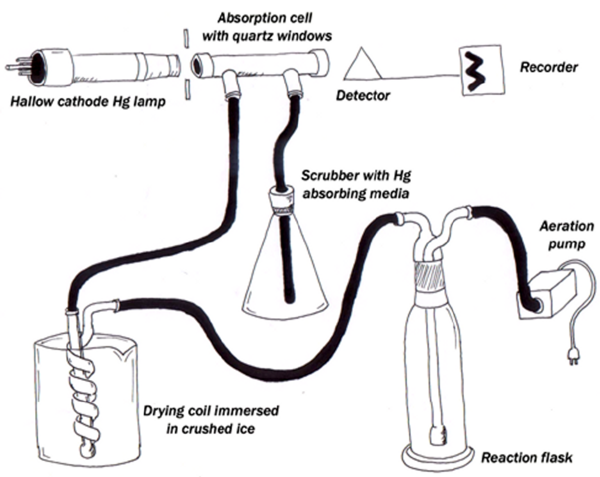


**Figure 1.** Setup for mercury determination by flameless atomic absorption spectrophotometry

The calibration curve was constructed from absorbance data of mercury standards against concentration of standards (0, 0.02, 0.05, 0.10, 0.20, 0.50 and 1.0 µg) using linear regression (Microsoft Excel program). The mercury standards were prepared from mercury standard solution (Titrisol brand) from Merck, USA. The mercury concentration of the samples is expressed in mg $kg^{-1}$ units based on the wet weight of the sample.

*Method Validation.* The method was validated using spiked samples with 0.02 µg (low level) and 0.2 µg (high level) mercury in fish and with reference material DORM-2 (NRC•CNRC Dogfish Muscle Certified Reference Material for Trace Metals). The method detection limit (MDL) is 0.003 mg $kg^{-1}$ total mercury (n=8) calculated based on 5 g of sample. The analysis of DORM-2 showed a bias of + 0.02 mg $kg^{-1}$ and a precision of 3.8 % (n=10). The mean recovery and precision of the 0.2 µg Hg spike samples (n=20) are 137 % and 5 % RSD, respectively. The uncertainty for the measurements was calculated from the uncertainty due to random effects. A summary of the validation and quality control data is presented in Table 1.

*Quality Control.* Duplicate samples of reagent blank and method control sample (sample spiked with 0.4 µg Hg) were included in the analysis of each batch of samples. The absorbance obtained from the reagent blank was subtracted from the absorbance obtained for the sample. Mean recovery and precision of the method control sample (n=8) are 104 % and 13 % RSD, respectively. The fish samples were analyzed in three or four replicates; the mean concentration is reported for the sample from each batch. The concentration of total mercury obtained in the sample was not corrected for recovery.

*Health Risk Assessment.* The allowed concentration of mercury in fish is calculated from the daily reference dose ($Rf_D$) and the daily consumption of fish. The $Rf_D$ for mercury is the daily dose that is considered safe or the dose that does not entail an appreciable risk of adverse effects of mercury (USEPA 2001). The $Rf_D$ is based on the benchmark dose, obtained from the lower 95% confidence limit for a 5% effect in a linear model of the dose-response curve; the response is usually a neurological endpoint **(**USEPA 2001**)**. The USEPA calculated an $Rf_D$ of 0.1 µg $kg^{-1}$ body weight $d^{-1}$ for mercury based on the risk to the adult woman, the population sector which is most vulnerable to the adverse effects of mercury **(**USEPA 2001**)**. Health risk is estimated by comparing the daily dose of mercury from consumption of fish with the reference dose $Rf_D$. Consumption of mercury-contaminated fish will not entail neurological effects if the daily dose of mercury will not exceed the $Rf_D$ of 0.1 µg $kg^{-1}$ body weight $d^{-1}$ **(**USEPA 2001**)**. The daily dose or estimated daily intake (EDI) can be calculated using Equation 1 (Kotsonis et al., 2001):

*EDI =* Hg concentration in fish *(µg/g)* ×
daily consumption of fish *(g $d^{-1}$ per person) /*
weight of person *(kg)* (1)

**Table 1.** Data on validation of method and quality control for analysis of total Hg in fish

| Test Material | n | Mean experimental concentration* | Standard Deviation | Mean theoretical concentration | MDL† | Mean % Recovery‡ | RSD (%)¶ |
|---|---|---|---|---|---|---|---|
| *Unspiked tilapia sample* | 12 | 0.0087 | 0.0003 | | 0.001 | | 3 |
| *0.02mg Hg spiked on tilapia* | 8 | 0.01349 | 0.00009 | 0.0039 | | 123 | 7 |
| *0.2mg Hg spiked on tilapia* | 5 | 0.0622 | 0.003 | 0.039 | | 137 | 5 |
| *Unspiked bangus* | 3 | 0.015 | 0.001 | | | | 7 |
| *0.4mg Hg spiked on bangus* | 8 | 0.097 | 0.013 | 0.079 | | 104 | 13 |
| | | Mean experimental concentration | | Certified concentration | | | |
| *DORM-2(NRC-CNRC) Reference Material* | 10 | 4.66 | 0.18 | 4.64 | | 100 | 3 |

** All concentrations are expressed as mg kg$^{-1}$total Hg, wet weight*
*† Method Detection Limit is calculated as 3×standard deviation*
*‡ Mean %Recovery is calculated as the (difference of the mean experimental concentrations of spiked and unspiked samples divided by the mean theoretical concentration) × 100; mean concentration is the average concentration of the number of samples (n) analyzed*
*¶ RSD (%) is relative standard deviation – calculated as (standard deviation / mean experimental concentration) ×100*

## RESULTS AND DISCUSSION

### Measurement Results for Mercury in *Bangus*, *Tilapia*, and *Galunggong*

Table 2 shows the results of analysis for total mercury in all the samples collected. Total mercury was found in the range of 0.006 to 0.015 mg kg$^{-1}$ (wet weight) for *bangus*, 0.0041 to 0.017 mg kg$^{-1}$ (wet weight) for tilapia and 0.014 to 0.05 mg kg$^{-1}$ (wet weight) for *galunggong*. The average mean of the means of total mercury concentration are 0.010 mg kg$^{-1}$ for *bangus*, 0.009 mg kg$^{-1}$ for *tilapia*, and 0.032mg kg$^{-1}$ for *galunggon*g. However, test of significance using two-tailed tests related to means showed evidence (Table 2) that some random batches among the samples collected for *bangus* and *tilapia* species are not drawn from the population having the measured population average total mercury concentration. Hence, the total mercury concentration for these species is reported as a range of concentration. The average total Hg and expanded uncertainty for *galunggong* is reported as 0.03±0.01 mg kg$^{-1}$ since all the samples have been shown statistically to come from the measured population average. *Galunggong*, which is caught in marine waters, showed the highest contamination with mercury, followed by *bangus*, which is grown in fish cultures in brackish or estuarine waters. Tilapia, which is grown in fresh water fish cultures, showed the least contamination. In Laguna Lake where both tilapia and bangus are grown in aquaculture, the concentrations of mercury were found to be higher in tilapia than in bangus; with the highest concentrations of 0.1 mg/kg dry weight and 0.057 mg/kg dry weight respectively (Cuvin-Aralar, 1990). Since the fish species investigated are all non-predators, the result suggests that the marine water where the *galunggong* were caught is more polluted with mercury than the aquatic environments where *bangus* and tilapia were raised. Fishes, belonging to *Decapterus spp.*, including *galunggong*, are near shore pelagic fishes that feed mostly on zooplanktons such as hyperiid amphipods and crab megalops (Mc Naughton, B., 2008). Unlike in the big pelagic fishes which prey on other fishes, the main pathway of accumulation of mercury in *galunggong* may not be through the food chain. This observation agrees with the result of an assessment of mercury levels in commonly-consumed marine fishes in Malaysia (Hajeb, P. et al., 2009) where the mercury concentration found in scad (0.04 µg/g dry weight) was much lower compared to the concentrations in short=bodied mackerel (0.45 µg/g dry weight) and long-tailed tuna (0.5 µg/g dry weight). It is most likely that the mercury found in *galunggon*g is the result of the exposure of the fish to the marine waters. It is expected that the marine waters would have more methyl mercury in the water column than in freshwater because the sea is a bigger sink for mercury than rivers and lakes. In addition, the water column is deeper and the presence of anaerobic bacteria is greater in marine waters than in the estuarine and fresh waters.

**Table 2.** Result of three-month monitoring of total Hg in fishes sampled from a Metro Manila market

***Bangus (Chanos chanos* Forskal*)***

| Sampling batch | Date of sampling | Mean batch concentration | n | Standard deviation $_{repeatibility}$ | RSD (%) | z |
|---|---|---|---|---|---|---|
| 1 | 5-Aug-04 | 0.0152 | 3 | 0.001 | 7.4 | 7.4 |
| 2 | 24-Aug-04 | 0.0095 | 4 | 0.002 | 19 | -0.7 |
| 3 | 6-Sep-04 | 0.0116 | 3 | 0.0008 | 6.9 | 2.3 |
| 4 | 14-Sep-04 | 0.0104 | 4 | 0.001 | 9.5 | 0.57 |
| 5 | 22-Sep-04 | 0.0089 | 4 | 0.002 | 16 | -1.6 |
| 6 | 29-Sep-04 | 0.0096 | 4 | 0.0006 | 6.6 | -0.57 |
| 7 | 13-Oct-04 | 0.0060 | 4 | 0.0007 | 12 | -5.7 |
| 8 | 20-Oct-04 | 0.0103 | 4 | 0.0009 | 9.1 | 0.42 |
| | *Range of Hg concentration* | 0.0041-0.017 | | | | |
| | *Average of means* | 0.009 | | | | |
| | *Standard deviation$_{reproducibility}$* | 0.004 | | | | |

***Tilapia (Oreochromis nilotica)***

| Sampling batch | Date of sampling | Mean batch concentration | n | Standard deviation $_{repeatibility}$ | RSD (%) | z |
|---|---|---|---|---|---|---|
| 1 | 5-Aug-04 | 0.0066 | 3 | 0.00069 | 10 | -1.7 |
| 2 | 24-Aug-04 | 0.011 | 4 | 0.00066 | 6 | 1.4 |
| 3 | 6-Sep-04 | 0.0088 | 3 | 0.0004 | 4.1 | -0.14 |
| 4 | 14-Sep-04 | 0.0044 | 4 | 0.0004 | 10 | -3.2 |
| 5 | 22-Sep-04 | 0.0166 | 4 | 0.0018 | 11 | 5.4 |
| 6 | 29-Sep-04 | 0.0093 | 4 | 0.0004 | 4.6 | 0.21 |
| 7 | 13-Oct-04 | 0.0041 | 4 | 0.0004 | 10 | -3.5 |
| 8 | 20-Oct-04 | 0.0091 | 4 | 0.0004 | 4.5 | 0.07 |
| | *Range of Hg concentration* | 0.0041-0.017 | | | | |
| | *Average of means* | 0.009 | | | | |
| | *Standard deviation$_{reproducibility}$* | 0.004 | | | | |

Galunggong (Decapterus spp)

| Sampling batch | Date of sampling | Mean batch concentration | n | Standard deviation $_{repeatibility}$ | RSD (%) | z |
|---|---|---|---|---|---|---|
| 1 | 5-Aug-04 | 0.014 | 3 | 0.0013 | 9.4 | -1.1 |
| 2 | 24-Aug-04 | 0.0426 | 4 | 0.0022 | 5.2 | 0.90 |
| 3 | 6-Sep-04 | 0.0167 | 4 | 0.0025 | 15 | -0.95 |
| 4 | 14-Sep-04 | 0.0184 | 3 | 0.0026 | 14 | -0.83 |
| 5 | 22-Sep-04 | 0.0357 | 3 | 0.0054 | 15 | 0.41 |
| 6 | 29-Sep-04 | 0.0463 | 4 | 0.0023 | 5 | 1.16 |
| 7 | 13-Oct-04 | 0.0367 | 4 | 0.0022 | 6 | 0..47 |
| 8 | 20-Oct-04 | 0.0503 | 4 | 0.0027 | 5.3 | 0.74 |
| | *Range of Hg concentration* | | | 0.014-0.050 | | |
| | *Concentration of average of means* | | | 0.03 | | |
| | *Standard deviation of the average of means* | | | 0.014 | | |
| | *Pooled standard deviation for repeatability* | | | 0.00231 | | |
| | *Combined standard deviation for random effects* | | | 0.014468 | | |
| | *Combined standard uncertainty for random effects* | | | 0.005 | | |
| | *U, expanded uncertainty* | | | 0.01 | | |

**all concentrations are expressed as mg kg$^{-1}$ total Hg, wet weight*

*†z value from two tailed test of means, calculated as* $[(\text{ave of means} - \text{batch mean})/(\text{std of the average of means}/\sqrt{8})]$ *where std is standard deviation; a z value of more than ± 2.58 indicates evidence at 95% CI that the batch does not belong to the population with total mercury concentration equal to the average of means.*

*‡Combined standard uncertainty for random effects is combined uncertainty due to repeatability and reproducibility, calculated as* $\sqrt{(\text{combined std due to random effects})^2/8}$

*where combined std due to random effects is calculated as* $\sqrt{\text{std}^2_{reproducibility} + \text{pooled std}^2_{repeatability}}$

*¶U is calculated as 2× standard uncertainty for random effects*

## Health Risk Assessment

The per capita fish consumption for the Filipino adult was reported as 75 g $d^{-1}$ in 1998 (UNEP 2003) and 69 g $d^{-1}$ in 2003 (FNRI 2003a). The published average weight for an adult Filipino woman is 54 kg and for an adult Filipino male, 60 kg (FNRI 2003b). The health risk assessment was calculated for the adult Filipino woman to give bias to the sector of the population most vulnerable to the effects of mercury. Based on the 2003 data on fish consumption and average weight of a Filipino adult woman, a daily dose of total mercury of 0.06 µg $kg^{-1}$ body weight $d^{-1}$ was estimated for the consumption of fish with the maximum total mercury contamination (0.05 µg $g^{-1}$). The calculated daily dose or the estimated daily exposure due to consumption of fish is less than the $Rf_D = 0.1$ µg $kg^{-1}$ body weight.$d^{-1}$. To exceed the $Rf_D$ , the same fish consumption rate would require a maximum concentration of 0.08 mg $kg^{-1}$ of fish. The risk assessment indicates that the consumption of any one or any combination of *bangus,* tilapia, *and galunggong* bought from Nepa-Q-Mart within August 5 to October 20, 2004 will not entail risk of adverse neurological effects for an average adult Filipino consumer.

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