INTAKE OF MERCURY THROUGH FISH CONSUMPTION

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Abstract

Fish has been known as a source of non-occupational mercury exposure to fish consuming population groups, and this is shown by the high hair mercury levels. In this study, hair samples collected from fishermen and their families, and commercial marine fishes were analyzed for mercury and methylmercury by neutron activation and gas chromatography. The results showed a correlation between hair mercury levels and fish consumption patterns. The levels of mercury found in this study were similar to those reported by other workers for fish consuming population groups worldwide.

1. INTRODUCTION

Being a peninsular state, fishing is an important industry in Malaysia, providing people with employment as well as supplying them with protein from the fish. Fish comprises about 70 % of the protein intake of the population, with consumption at about 100 g/d/person. The average mercury levels in commercial marine fishes marketed in Malaysia as reported in our study range from 0.06 - 0.42 mg/kg fresh weight. With such a relatively high intake of fish, it is apparent that fish is a possible source of non-occupational mercury exposure to the general population. The Provisional Tolerance Weekly Intake (PTWI) as set by the WHO and FAO at 0.2 mg Hg may have been achieved by some population groups and this is reflected in the hair mercury concentrations of 5 - 6 mg/kg [1].

In recent years, industrialization and urbanization have encroached onto many coastal fishing villages, which in many cases, cause water pollution. As a result, the livelihood of small inshore fishermen have been threatened or destroyed. At the same time, coastal fishes are contaminated by pollutants from industrial wastes, especially the toxic heavy metals. It is expected that the coastal fishermen and their families are exposed to a higher intake of mercury in the form of methylmercury. In this study, hair samples were collected from fishermen and their families residing in two fishing villages. The aim of this study was to identify certain population groups that may have ingested mercury higher than the PTWI as set by the WHO. We are collaborating with researchers from the Nuclear Energy Unit, the Department of Chemistry and also the Department of Public Health for these studies.

2. METHODS

2.1. Study area

The project is focused on two fishing villages: Kuala Juru in the State of Pulau Pinang and Chendering in Trengganu. Pulau Pinang is one of the most industrialized states in Malaysia. Rapid industrialization of this state has caused many environmental pollution problems and one of the affected areas is Kuala Juru. Chendering in Trengganu serves as a good model for a non-industrialized region in Malaysia.

2.2. Sample preparation

Hair samples were collected from residents of the study areas according to the IAEA protocols. The hair samples are successively washed in acetone, thrice in water, and once more in acetone, as is described in the IAEA protocol. Sufficient amounts of the solvents are added to cover the sample entirely. At each wash, the sample is allowed to stand at room temperature for 10 minutes in contact with constant stirring. After each wash, the liquid is decanted and fresh solvent added. The washing is carried out in a dust-free enclosure.

Fish samples were bought from coastal fishermen at landing jetties. The samples were kept refrigerated and brought to the laboratory. The samples were then cleaned with distilled water. Only body muscle was used in the analysis. The cleaned samples were kept at -50°C and then dried in a freeze dryer. The dried samples were blended and homogenized in a clean blender with stainless steel blades. The homogenized samples were kept in plastics bottles.

2.3. Extraction of methylmercury

The extraction of methylmercury from fish tissue samples was carried according to the methods described in [2]. Two cm^3 of 2M H_2SO_4 saturated with $CuSO_4$, 2 cm^3 of KBr solution and 3 cm^3 toluene were added to 1 g of dried fish tissue sample. The mixture was shaken by vortex for 10 minutes and was centrifuged. The extraction process was repeated with another 3 cm^3 toluene. The toluene layer was then separated quantitatively. Cysteine paper was added to the toluene and shaken for 15 minutes to extract the organic mercury. The paper was then rinsed once with toluene and dried in an acid-free box. The cysteine paper was packed in a polythene vial for neutron activation analysis.

The extraction of methylmercury from hair samples was carried out as in [3]. Each hair sample (approx. 0.1 g) was disintegrated with 2 ml of 7.5M NaOH in a water bath at 90°C for 30 min. Cysteine hydrochloride solution was added to enhance the extraction of methylmercury. The mixture was diluted with 1% NaCl solution to 10 ml. One ml of the solution was then acidified with $CuSO_4$-H_2SO_4, 2 ml 4M KBr was added and was then extracted into 3 ml toluene. Two ml of the toluene was transferred into a test tube and the MeHg was back extracted into thiosulphate solution. The aqueous solution was then acidified and MeHg was extracted into benzene or toluene. The organic solution was then ready for GC analysis.

2.4. Analysis by gas chromatography

A Perkin Elmer Autosystem GC with an Electron Capture Detector (^{63}Ni) and an HP 3390A intergrator was used for the analysis of methylmercury. A Supelco 2-4044 capillary column, 0.32 mm x 30 m with 0.25 micron film packed with SPB-1 (100% dimethyl polysiloxane), was used. The operational temperature was set at 200°C for the injector, 300°C for the detector, and 60 - 300°C for the column. The nitrogen gas flow rates were 30 ml min^{-1} for the detector and 2 ml min^{-1} for the column. The injection volume was 5 μl for standards and samples.

2.5. Neutron activation analysis

2.5.1. Sample irradiation

About 150 mg of each sample (hair or fish tissue) was packed in a pre-cleaned polyethylene vial. Triplicate analysis was carried for each sample. The cysteine paper was also packed in a separate polyethylene vial for activation. Standards were prepared from standard chemical solutions pipetted on ashless filter paper, which were packed in a polyethylene vial after drying. About 50 μL of 10% solution of thioacetamide was pipetted on the filter paper beforehand to preserve mercury. Samples and standards were irradiated in the PUSPATI TRIGA Reactor for 6 hours at a neutron flux of 2.3 x 10^{12} $n \cdot cm^{-2} \cdot s^{-1}$.

2.5.2. Measurement of activities

Mercury was determined by both the ^{197}Hg and^{203} Hg radionuclides. The determination via the 77 keV of ^{197}Hg was carried out by using a low energy photon detector (LEPD). The irradiated samples were cooled for 3 days and counted for 30 minutes. The determination of mercury via the 279.1 keV photopeak of ^{203}Hg is interfered by the 279.6 keV of ^{75} Se, and correction has to be made for the photopeak contribution. The samples were cooled at least for 2 weeks prior to counting. The γ-ray activities of the samples and standards were also measured by a high resolution γ-spectroscopy system comprising of a high purity coaxial N-type Germanium detector with thin Be window (Tennelec), microcomputer based MCA system with 80386 SX data processor and 8192 channel ADC, and TC-244 spectroscopy amplifier (Tennelec).

2.6. Quality control

Accuracy and precision of the analytical technique was evaluated by analyzing IAEA standard reference materials MAA-1 (Copepoda), MAA-2 (Fish Homogenate) and MAB-3 (Fish Tissue) and IAEA-350 (Tuna).

3. RESULTS AND DISCUSSION

Accuracy and precision for the analysis of mercury and organic mercury in the standard reference materials by neutron activation are shown in Table I. The results obtained were in good agreement.

Mercury in fish and hair samples can be analyzed quite easily and conveniently by instrumental neutron activation. Thus, tedious radiochemical separation steps can be

avoided. By employing a LEPD, mercury can be analyzed more quickly through the 69 and 77 keV photopeaks of ^{197}Hg. However, better accuracy and precision can be achieved by analyzing the 279 keV photopeak. The detection limit of the technique used was calculated at 0.01 mg/kg. However, this method is not capable of differentiating the organic mercury into its various species.

The hair mercury levels in the two population groups studied are shown in Table II. The results for the two groups differ significantly. The population in Chendering has lower mercury levels as it is located in a non-industrialized area. About 30% of the Kuala Juru samples contained mercury levels higher than 5 mg/kg. This may be an indication about the percentage of the study population that might have exceeded the PTWI intake of mercury. From the information gathered, some of the donors, especially the adult male population, consumed more than 100 g of fish daily. This is also reflected in the distribution of mercury concentrations in relation to the age of the donors as shown in Table III. Based on the concentrations of mercury in fishes marketed in Malaysia, the PTWI limit may be achieved by consuming about 500 g of fish weekly.

Preliminary results of methylmercury in hair samples are shown in Table IV. The results indicated that the percentage of methylmercury in hair ranged from 14 - 88%. The source of hair methylmercury is the consumption of fish. The concentrations of mercury in the hair samples analyzed showed a positive correlation with the amount of fish consumed. The correlation is within the statistical relationship established by Airey [4] *i.e.*,

$$\text{Hair mercury concentration} = 1.67 + 0.13(\text{Fish consumption}), \quad (1)$$

where the hair mercury concentration is expressed in mg/kg , and annual fish consumption expressed in kg/person/year.

Under this project, we are also trying to establish the levels of mercury and methylmercury in sea fishes marketed in Malaysia. Tuna has been known to contain high levels of mercury, higher than most other fish analyzed. Since tuna is quite popular among certain population groups, tuna samples obtained from local markets were analyzed for mercury. There are three species of tuna available in Malaysia. They are *Euthynnus affinis, Auxis thazarg* and *Thunnus tonggol*. The results of total mercury are shown in Table V. The highest mercury levels were found in the liver.

The total mercury and organic mercury levels of some other popular fish types as analyzed by neutron activation are shown in Table VI. The organic mercury levels were 45 - 95% of the total mercury depending on the species, as well as the location of the samples. Generally, the samples collected along the West Coast contained higher mercury levels, probably due to increased industrialization.

4. PLANS FOR FUTURE WORK

Methylmercury was not analyzed during the previous contract period as we faced difficulty in obtaining a methylmercury standard. Recently, we managed to procure methylmercury from Johnson Matthey in England. However, sharing a GC with other users is not practical and it became important to obtain our own GC. We have now bought a new Perkin Elmer Autosystem GC, which will be dedicated for the analysis of methylmercury.

Currently, we are developing our capability in analyzing methylmercury by both neutron activation method and by gas chromatography. The detection limit for GC analysis is better than the NAA method that we used. In addition, smaller sample sizes can be used in the determination of methylmercury by the GC method. For NAA, we normally used >1 g samples for the extraction of organic mercury, as compared to the 0.1 g samples used for the GC analysis. Another important factor is the analysis time. The determination of methylmercury by NAA requires about 3 weeks of analysis time. However, we are still keen to develop and apply the NAA method.

When we have developed the capability in the routine analysis of methylmercury, our major work in the future is in the application of the methods. We will take an active part in the interlaboratory analysis, as well as larger scale screening work for identifying critical population groups. In this regard, we have included in our group researchers from the Department of Public Health, Faculty of Medicine who are interested in the heavy metal poisoning. There has been some isolated cases of arsenic poisoning reported by the department in Malaysia. We have plan to screen the hair mercury levels of pregnant women among the general population groups.

PUBLICATIONS

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TABLE I. MERCURY IN IAEA STANDARD REFERENCE MATERIALS (mg/kg)

	This work	Certified value
MAA - 1	0.33 ± 0.05	0.28 ± 0.01
MAA - 2	0.49 ± 0.14	0.47 ± 0.02
MAB - 3	0.54 ± 0.19	0.510 ± 0.070
IAEA - 350	3.88 ± 0.41	4.99 ± 0.26
IAEA - 350	3.31 ± 0.09[a]	3.96 ± 0.27[b]

[a] - total organic mercury
[b] - Analysis by M. Horvat in Ref. [1]

TABLE II. TOTAL MERCURY IN HAIR (mg/kg)

	Kuala Juru	Chendering
Sample	106	98
Range	0.45 - 16.87	0.34 - 9.07
Arith. mean	3.61	2.20
Median	2.96	1.77
Geom. mean	3.49	1.87

TABLE III. DISTRIBUTION OF MERCURY CONCENTRATIONS

Age (y)	Range (mg/kg)	Mean (mg/kg)
1 - 9	0.45 - 8.47	3.27
10 - 19	0.58 - 10.43	3.16
20 - 29	0.87 - 16.87	3.83
30 - 39	1.04 - 6.75	3.09
40 - 49	1.91 - 12.55	5.22
50 - 59	1.94 - 8.33	4.05
60 above	1.29 - 16.65	6.25

TABLE IV. TOTAL MERCURY AND METHYLMERCURY IN HAIR (mg/kg)

Sample	Age	Sex	Hg-T	MeHg	%MeHg
T05	69	M	2.16	1.34	62
T07	12	F	1.68	0.24	14
T22	8	F	1.84	0.73	40
T23	6	M	1.69	1.05	62
T29	46	F	1.56	0.68	43
T49	34	F	1.09	0.96	88
T64	29	F	0.52	0.44	83
T71	63	F	2.13	1.24	58
T77	12	M	2.89	2.54	88
T81	9	M	1.76	1.04	59

TABLE V. MERCURY IN MALAYSIAN TUNA (mg/kg) FRESH WEIGHT

Organ	Hg	Se	Zn
White muscle	0.81 ± 0.18	1.64 ± 0.51	11.47 ± 1.04
Red muscle	1.20 ± 0.08	1.98 ± 0.07	9.78 ± 0.58
Liver	3.35 ± 0.27	8.02 ± 0.64	50.79 ± 1.60
Roe	1.29 ± 0.79	4.55 ± 0.21	151.72 ± 2.96
Spleen	1.96 ± 0.62	7.83 ± 0.55	115.59 ± 7.22
Intestine	1.39 ± 0.21	4.37 ± 0.19	13.51 ± 0.92

TABLE VI. TOTAL MERCURY AND ORGANIC MERCURY IN FISH (mg/kg) FRESH WEIGHT

Sample	Location	Hg-T	Hg-O	Hg-O/Hg-T (%)
Indian mackerel	Mersing	0.126	0.084	67
	Bachok	0.059	0.048	81
	K. Trengganu	0.059	0.053	90
	Paka	0.092	0.062	67
	Marang	0.064	0.048	74
Spanish mackerel	Mersing	0.076	0.064	85
	K. Perlis	0.076	0.071	94
	Batang Tiga	0.074	0.044	59
	Benut	0.069	0.044	63
	Kuala Kedah	0.140	0.113	80
Squid	Kuala Perlis	0.163	0.144	84
	Mersing	0.086	0.058	67
	Kuantan	0.138	0.096	70
	Pekan	0.067	0.038	57
	Kuala Selangor	0.093	0.053	79
Prawn	Kuantan	0.273	0.145	53
	Kuala Kedah	0.255	0.115	45
	Rompin	0.158	0.123	78
	Mersing	0.255	0.140	55
	Sg. Buloh	0.215	0.175	81
Cockle	Morib	0.056	0.040	71
	Juru	0.110	0.088	80