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Variation of Methylmercury Concentration in Hair of the Kuala Lumpur Residents in Terms of Race, Gender, Age and Fish Consumption

S. B. Sarmani*, I. Alakili* and El. El. Elkhidir**

*Nuclear Science Programme, School of Applied Physics,Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor Malaysia

**Faculty of Natural Resources and Environmental Studies, University of Kordofan, 160 Elobeid, Sudan

Abstract

This paper reports a piece of research in which the average value of methylmercury in scalp hair of Kuala Lumpur residents is 1.13 $mgkg^{-1}$ in the range of 0.0−4.65 $mgkg^{-1}$ and represents 31.15% of the total mercury. Linear regression approach was used to predict the hair methylmercury concentration in this population. The independent variables included in this model were fish consumption, age, race and gender. In this study, fish consumption, race and age were correlated significantly with hair methylmercury concentration. The interaction between age, gender and fish consumption were also contribute significantly to the level of methylmercury. However, gender had no influence on hair methylmercury level.

Introduction

Mercury is among the most potential hazardous materials and rated in the top category of environmental pollutants. People are exposed to different forms of mercury, which differ with respect to kinetics and toxicology[1]. Methylmercury is the most toxic among the mercury species because of its ability to enter the food chain, accumulating and contaminating humans. One of the main target organs for methylmercury is the central nervous system for adults and foetus. Therefore, it is important to know the background levels of exposure of human population to methylmercury. In this study, in order to assess the level of exposure to methylmercury in Kuala Lumpur residents, simple and efficient extraction technique[2] was used for the separation of methylmercury from hair samples collected from donors living in different area of Kuala Lumpur and analyzed by neutron activation analysis. Linear regression model was used to investigate the influence of the contributory variables such as fish consumption, age, race and gender to the level of methylmercury in this population.

Materials and methods

Study area

Kuala Lumpur, the capital of Malaysia, is the most populous, urbanised and industrialised region in the country. Kuala Lumpur is a plural society made up of three main ethnic groups; Malay, Chinese and Indian.

Sample collection and preparation

The sample of the study was composed solely of randomly picked respondents of Kuala Lumpur residents. The number of the sample was 400 consisting of 46.25% Malay, 31.25% Chinese and 22.5% Indian. Hair samples were collected from donors by single cutting from the occipital region with a pair of clean stainless steel scissors in accordance with the IAEA protocols. During collection of the hair samples, each individual was asked to complete a questionnaire detailing name, sex, age, occupation and dietary habits.

The hair samples were cut to lengths of about 2─5 mm. The hair samples were then washed according to the standard procedure recommended by the IAEA: wash hair in acetone, thrice in water and once more in acetone. The samples were then dried overnight in an oven at 60˚C.

Determination of methylmercury

Sample Digestion: About 0.1g of hair sample was placed in a 15ml test tube with a screw cap and 0.2 ml of 10 M NaOH was added. The test tube was capped tightly and kept in a hot water bath at 90ºC for 15 minutes. Then 2 ml of distilled-deionized water was added to the dissolved sample and the pH was adjusted to 1 using concentrated H_2SO_4. The samples were then cooled to room temperature [3].

Extraction of methylmercury: About 15 mg KBr and 3 ml of toluene were added into the digested samples solution and the mixture was vigorously shaken. The toluene layer was then transferred into a clean test tube. The extraction process was repeated with another 3 ml toluene. The toluene layers were collected together. To back extract methylmercury, cysteine paper was added to the toluene and shaken for 15 minutes. The cysteine paper was washed by shaking for 10 minutes in 10 ml toluene and the paper was then placed in an acid free dry box to dry. After drying, the cysteine paper sheet was encapsulated and sealed in polyethylene envelopes. The cysteine paper is ready for irradiation.

Standard Preparation: The standards were prepared by pipetting about 1µl of known concentration standard chemical solution of methylmercury onto ashless filter paper and packed in polyethylene envelope after careful drying. To prevent mercury losses, about 50 µl of 10% solution of thioacetomide was pipetted onto the filter paper[4]. Two samples along with one standard and blank were packed in clean polyethylene envelope for neutron irradiation.

Sample irradiation

Samples, standard and blank were irradiated in the TRIGA reactor of the Malaysian Institute for Nuclear Technology and Research for 6 hours in a neutron flux of $2.3.10^{12}$ $n.cm^{-2}.s^{-1}$. The irradiated samples were then cooled for three days prior to counting.

Measurement of activities

After cooling, the samples, standards and blanks were counted for 1800s using HPGe detector. For calculation of mercury concentrations, the 77 kev peak of ^{197}Hg ($t_{1/2}$= 64.1h) and the 279.1 kev peak of ^{203}Hg ($t_{1/2}$=46 day) were used. Gamma ray energy calibration was made with standard sources prior to every set of experiments. The nuclides were quantified by comparing net photopeak area with those of standards.

Linear regression approach

To investigate the influence of some independents variables (fish diet, age, race and gender) that we assumed to have an impact on the level of methylmercury in hair of Kuala Lumpur residents, a multiple linear regression approach was carried out. In an attempt to estimate the contribution of these factors the following predicted regression model was fitted:

$$Y_i = \beta_0 + \beta_1 D_1 + \beta_2 D_2 + \beta_3 D_3 + \beta_4 X_1 + \beta_5 X_2 + \beta_6 X_1 X_2 + \beta_7 D_3 X_2 + \mu_I \quad (1)$$

Where,

Y = denotes the level of methylmercury concentration in hair measured in $mgkg^{-1}$,

D_1= dummy variable representing Chinese race,

D_2= dummy variable representing Indian race. Malay race is control group,

D_3= dummy variable for gender (1 = female, 0 = male),

X_1= An age of the respondent measured in years,
X_2 = Refers to the amount of fish diet consumed per respondent per week measured in kg,
X_1X_2 = Interaction between age and amount of fish diet consumed,
D_3X_2 = Interaction between gender and amount of fish diet consumed,
μ_i = An error term assumed to capture the contribution of other variables.
β_0 is a constant, and $\beta_1, \beta_2, \beta_3, \ldots \beta_7$ are the coefficients of the independent variables that to be estimated, and *i* = Subscript of respondent (*i* = 1, 2,…, 400).

Results and discussion

Running ten replicates of the two certified reference materials IAEA-085 and IAEA-086 checked the accuracy of the analytical method. The results are given in Table 1, which show no significant difference (P > 0.05) between our results and the certified values. The precision of the method was tested by ten replicates analysis of the certified reference materials IAEA-085. The precision of this method for the methylmercury was 9.93% "expressed in terms of the coefficient of variation for the replicate analysis".

Table 1 Results of Methylmercury Determination in IAEA Reference Materials (mg kg^{-1})

SRM	This Work	Certified Value	F-test	t-test	P-Value
IAEA-085	23.46 ± 2.33	25.06 ± 1.55	0.44	1.81	0.09
IAEA-086	0.32 ± 0.05	0.29 ± 0.02	3.10	1.76	0.09

Methylmercury levels in 400 hair samples obtained from donors living in different area of Kuala Lumpur were determined using instrument neutron activation analysis. The sample population consisted of 47 % males and 53 % females. The Malay, Chines and Indian form 46 %, 31 % and 23 % of the sample population, respectively. The age of the donors varied between 2 to 80 years old with an average (± standard division) of 32.80±15.95 years old. Detailed breakdown on ethnography, sex and age are given in Table 2.

Table 2 The Population Characterises

Ethnic Group	**Ethnicity**		**Age (years)**	**Male**		**Female**	
	N	**%**		**N**	**%**	**N**	**%**
Malay	185	46.25	2 ─ 80	85	45.90	100	54.1
Chinese	125	31.25	4 ─ 75	62	49.60	63	50.40
Indian	90	22.50	3 ─ 70	42	53.33	48	46.67
Total sample	400	100%	2 ─ 80	189	47.25	211	52.75

The average level of methylmercury concentration in this population hair was 1.13 mg kg^{-1} in range of 0−4.65 mg kg^{-1}. The percentage of methylmercury to total mercury was 31.15 on average and ranged from 0.0−75.81. By ethnic group, the level of methylmercury concentration were 0.75mg kg^{-1} (ranged from 0−4.0 $mgkg^{-1}$), 1.52$mgkg^{-1}$ (ranged from 0−4.65$mgkg^{-1}$), 1.31$mgkg^{-1}$ (ranged from 0−4.02mg kg^{-1}) for Malay, Chinese and Indian, respectively. For male was 1.03$mgkg^{-1}$ (in range of 0−3.87mg kg^{-1}) and for female was 1.25 $mgkg^{-1}$ (in the range of 0−4.65$mgkg^{-1}$). The frequency

distribution of methylmercury in hair samples of Kuala Lumpur residents is shown in Figure 1.

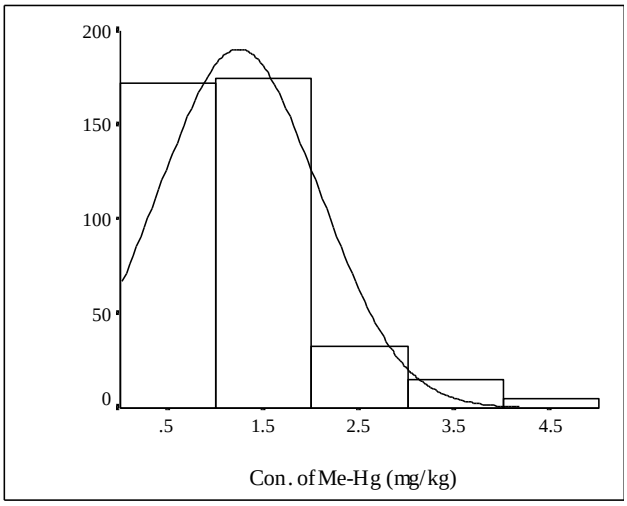

Figure 1 The frequency distribution of methylmercury concentration in the hair samples of Kuala Lumpur population

Figure 1 show that even among individuals with level of methylmercury considerably high, the hair methylmercury contents in Kuala Lumpur risdents do not reach the levels of toxicity (50 mg kg^{-1}) set by WHO[5].

The results of the correlation of the independent variables with the measured methylmercury content in hair was considered helpful for finding out to what extent these variables influence the accumulation of methylmercury in the hair of the inhabitants. Results of the analysis of multiple regression between the levels of methlmercury in hair and the various examined contributing factors are presented in the Table 3. Nevertheless, the result of the diagnostic test of multicollinearity between the independent variables is satisfactory. The presence of heteroscedasticity and autocorrelation problems was indicated. In such situations of these problems and as a remedial technique we estimated the model by using Newey-West procedure to correct OLS standard errors.

The model has a high explanatory power with coefficient of determination $R^2 = 0.87$. This indicates that 87% of the methylmercury concentration levels among the individuals of Kuala Lumpur community are explained by the contributing factors under considerations. A high value of multiple R (93%), which measures the correlation between the observed and predicted values of the methylmercury concentration levels; indicates that there is obviously linear relationship between the methylmercury concentration levels and the contribution of the causative: race, gender, age and fish consumption factors. Further, the F-value of 373.97 indicates the overall significance of the model at 1%. This means the rejection of null hypothesis that the regression coefficients $\beta_1 = \beta_2 = \beta_3 = ... = \beta_7 = 0$ and the acceptance of the alternative hypothesis that at least one variable coefficient is not equal to zero. Moreover, it indicates that such association between the methylmercury concentration levels and the contributing factors

under considerations could not be of random origin. The estimated regression model is as follow:

$$Y = -0.38 + 0.36\ D_1 + 0.28\ D_2 - 0.07\ D_3 - 0.01\ X_1 + 1.94\ X_2 + 0.42\ X_1X_2 + 0.02\ D_3X_2 \quad (2)$$

The regression coefficient of a variable measures the change in the methylmercury concentration levels associated with a unit change in the explanatory variable given that, all other explanatory variables are held constant.

Table 3 Regression results of mercury level in Kuala Lumpur predicted model

Independent variable	Regression coefficient	Standard Error	t-value	P-value	Partial correlation
Constant	-0.38	0.11	-3.57	0.0004	
Chinese race	0.36	0.04	9.06**	0.0000	0.44
Indian race	0.28	0.05	6.04**	0.0000	0.34
Gender	-0.07	0.10	-0.74	0.4580	-0.05
Age	-0.01	0.003	-1.99*	0.0473	-0.11
Fish diet	1.94	0.19	10.35**	0.0000	0.52
Age*Fish diet	0.42	0.162	2.58*	0.0103	0.17
Gender*Fish diet	0.02	0.005	3.02**	0.0027	0.18

$R^2 = 0.87$ Multiple R = 0.93
F-ratio = 373.97** P-value = 0.0000
N = 400; * Significant at 5%; ** Significant at 1%.

Fish is widely recognised as the major source of methylmercury record[5]. Being fish constitutes the main source of protein for the great majority of Kuala Lumpur residents, it is important to study the influence of fish consumption on methylmercury accumulation by this population. As expected, The amount of fish diet taken per week per resident of this community was highly significant contributed to the methylmercury concentration levels (1.94). Therefore, variations in methylmercury levels in this population are to be attributed to different fish feeding habits. This result confirms that, fish consumption is the main route of methylmercury exposure in the Kuala Lumpur residents. This finding confirms and extends previously findings that reported in different parts of the world[6-14].

The contribution of Chinese and Indian races to the methylmercury concentration levels among residents of Kuala Lumpur community were found to be significant and resulted in 0.36 and 0.28, respectively. These high levels of Chinese and Indian races contribution could be attributed to the difference in fish consumption frequency between

the three races. Our results revealed that Chinese and Indian groups consume more fish than Malay group. A total of 79% of Chinese group and 63% of the Indian group consume fish in range of 6 − 14 times/week, while 51% of the Malay group consume fish in the same range.

Age variable is negatively and significantly contributes to the level of methylmercury in this population. This result revealed that, as donor's age increase the level of methylmercury in hair decrease significantly. This finding is inconsistent with the Barbosa[15] et al. 2001 who found no correlation between age and the level of methylmercury in hair. However, the positive and significant interaction between age and fish diet show that the older people tended to consume more fish than the younger.

Gender factor was found to be negatively and insignificantly contribute to the level of methylmercury in hair of this population. This finding accords with Kyle & Ghani[16] 1982 and Barbosa 2001. However, the interaction term between gender and fish diet indicated positive and significant contribution to methylmercury concentration level among these residents, and showed that female consumes more fish than male. On the other hand, Akagi[17] et al. 1995 and Malam[18] et al. 1995 found that males consume more fish than females and as a consequence males presented higher level of methylmercury than females.

The estimated results of partial correlation showed that the contribution of the amounts of fish diet intake to the mercury concentration levels in hair of Kuala Lumpur residents (other variables held constant) ranked first with 52%. This indicating that, fish consumption is the dominant factor, which contribute highly to the level of total mercury in the hair samples of the Kuala Lumpur population. The remains factors according to their contribution ranked as: Chinese race (44%), Indian race (34%), gender and fish diet interaction (18%), and interaction of age and fish diet (17%).

Conclusion

The present work shows a marked impact of fish consumption on hair methylmercury levels in Kuala Lumpur residents confirming that, fish diet is an important source of methylmercury exposure in this population. Indian and Chinese race and age also contributed significantly to the overall methylmercury level. On the other hand, gender negatively and insignificantly contributed to the hair methylmercury level. However, significant interaction between age, gender and fish diet also contributed to the methylmercury level. According to this study, at present, methylmercury pollution does not appear to be a serious problem in Kuala Lumpur. However, due to the large quantities of fish and other seafood consumed by the Malaysian people in their daily diet, the methylmercury pollution in Kuala Lumpur and other Malaysian cities should be continuously and crucially observed for head hair methylmercury level and health.

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