



NIMD Forum 2009

**Methylmercury and n-3 poly
unsaturated fatty acids (n-3 PUFA)
exposure from fish consumption**

19-20 February 2009

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National Institute for Minamata Disease

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Balancing the risk of methylmercury and benefits of n-3 polyunsaturated fatty acids exposure from fish consumption

L.H.M. Chan*, Choi, K.R. Mahaffey, E. Oken, M. Sakamoto, C.H. Yan

*University of Northern British Columbia

Fish and shellfish are widely available food that provides many nutrients, particularly the n-3 polyunsaturated fatty acids (n-3 PUFAs), to many populations globally. Research conducted over the past several years suggests that there are benefits linked to brain and visual system development in infants and reduced risk for certain forms of heart disease. However, fish and shellfish are also the major source of methylmercury (MeHg), a known neurotoxicant that is particularly harmful to fetal brain development. The objectives of this panel presentation are:

- 1) to review data on the distributions of MeHg and n3-PUFAs across different fish/shellfish species; 2) to estimate dietary intake of both MeHg and n-3 PUFAs in different countries;
- 3) to review the latest biomedical findings on the role and interactions of both n3-PUFAs and of MeHg on prenatal neurodevelopment; and 4) to review the latest epidemiological data on effects of fish consumption on child development. The goal is to develop a consensus to provide dietary advice on how to maximize the dietary intake of n-3 PUFAs and minimize MeHg exposures through optimal choice of fish and shellfish species.

Mercury exposures and health effects of school children living near the mercury emitting facilities in Korea

Guen-Bae Kim^{a*}, Jong-Hwa Lee^a, Hee-Jin Park^a, Seung-Do Yu^a, Dae-Seon Kim^a

^a Department of Environmental Epidemiology, National Institute of Environmental Research,
In-cheon, Korea.

1. Abstract

Coal-fired power plant, incineration has been known as main facilities emitting mercury into atmosphere in Korea. The influences on environment and population in the vicinity of these facilities still have raised large public concerns because most of facilities are situated near the residential area. The exposure and its health effects were examined in several regions where the mercury emitting facilities were in this study. Mercury exposure was evaluated by blood, urinary and hair mercury analysis. Health effect was surveyed with blood and urine test, posturography. The mean blood (B-THg), urinary total mercury (U-THg) for school children living near the coal-fired power plant was 2.69 ppb (geometric avg.), 1.45 $\mu\text{g/g}$ creatinine ($\mu\text{g/g}$ Cr). B-THg, B-MeHg, U-THg, the hair total mercury(H-THg) for school children was 2.45 ppb, 1.95 ppb, 0.68 $\mu\text{g/g}$ Cr, 0.57ppm, respectively in the regions where municipal waste incineration facility was operating. Those (except for B-MeHg) for women were 3.41 ppb, 0.77 $\mu\text{g/g}$ Cr, 0.83 ppm. The relation to health with exposure was not found. The effect of industrial waste incineration on exposure and health was evaluated by comparison of continuous first-morning urine samples for school children with atmospheric total gaseous mercury (TGM) concentration during one month, the same test was done in control region. The mean U-THg and TGM was 1.13 $\mu\text{g/g}$ Cr, 3.37 ng/m^3 in the vicinity of incineration facility. They were 0.70 $\mu\text{g/g}$ Cr, 2.29 ng/m^3 in the control region. Both concentrations were above 40% higher in the region industrial waste incineration was operating. There was distinct relation between the trends of mean U-THg and TGM in both survey regions. Even though some items in health test showed difference between areas, but distinct relation with biological Hg exposure didn't exist.

Keywords: coal-fired power plant, incineration, blood and urinary total mercury, methylmercury, total gaseous mercury

1. Introduction

Although the domestic use of mercury has been decreased significantly during recent 10 years in Korea, it is known that the amounts of mercury emission into atmosphere reached above 50 tons every year which is the eighth in the world (UNEP., 2008). Most of them were emitted from combustion such as power plant. Waste incineration and non-ferrous metal manufacturing was also known to release lots of mercury. Despite of national efforts to reduce mercury emission, increasing demand for power from industry and treatment capacity of waste by incineration has brought about the increase of non-intentional mercury emission inevitably. Furthermore lots of mercury emitting facilities are operating near the large residential area, which raise public and scientific concern about the influence of mercury emission from these facilities on the exposure and health of population. This presentation summarized the results of several related-survey done recent 3 years in Korea.

2. Subjects and Methods

The survey presented here was performed in the areas where there are main Hg-emitting facilities near the large residential complex. One coal-fired power plant (PP) which generating above 1,600 MKW located in western coastal area was selected. One municipal waste (MWI, capacity: above 100 kt) and three industrial waste incineration (IWI, 2 ton/h) facilities in metropolitan area were also investigated. All subjects were consisted of children in elementary school (in all survey) and their mother (only in MWI). Total of 449 school children (average age 12 years) and 41 women (average age 37.5 ± 6.3 years, only in MWI) without any particular exposure to Hg participated with their informed consent. Questionnaire was utilized to collect information on the personal characteristics including dietary habit, history of residence, life style and etc. For investigating mercury exposure, blood and urine, hair (only in MWI) samples were collected. Total mercury in biological samples was measured using Mercury analyzer (Model SP-3D, Nippon Instrument Corporation, Japan). After pre-treatment process, mercury vapor was collected into the tube for gold amalgamation and was measured by atomic adsorption spectrometry. MeHg in the sample was determined by gas chromatography with ECD according to the NIMD method (Akagi et al. 2000). The health effects of mercury exposure were investigated through some blood and test and posturography (CATSYS 2000), oxidative stress biomarker (JAICA, Fukumori., Japan) urinary N-acetyl- β -glucosaminidase . Statistical analysis was done with SPSS version 10.0 for window. One-way analysis of variance and Pearson correlations analysis, multiple linear regression (backward elimination) was used.

3. Results and Discussion

Considering the amount of coal use in power generation, it is estimated that about 9 tons of mercury was emitted into air. So there is large concern about the influence of coal combustion of power plant (PP) on the biological exposure of general population. But it is very difficult to find related survey and data for evaluation of its effect. For this reason, 136 school children living near the power plant (5Km east from PP) and other area (20Km below from pp) were participated. The mean blood (B-THg) and urinary total mercury (U-THg) was 2.69 ppb (geometric avg.), 1.45 $\mu\text{g/g}$ Cr in PP area, which of other area was 2.65, 2.70, respectively. There was not difference between areas in blood Hg level, but urinary mercury level was different from our presumption. The difference in U-THg could be explained from the atmospheric weekly total gaseous mercury concentration (TGM) of two areas. TGM of two area were 3.6, 4.5 ng/m^3 respectively. The Hg emission from PP was estimated to influence on TGM of the area being further below regardless of distance, which was reflected in urine.

There are about 230 municipal waste incineration facilities that treat 590,000 ton/year in Korea. Most of them are situated in the city lots of people live. 69 school children and 41 their mother participated in this investigation for Hg exposure living close to MSI having treatment capacity 100,000 ton/year and control area (10Km away from MSI in upstream of wind direction). In MSI area, B-THg and U-THg, B-THg of children was 2.45 ppb, 0.89 $\mu\text{g/g}$ Cr, 1.95 ppb (80% of B-THg), they were 2.20 ppb, 0.68 $\mu\text{g/g}$ Cr, 1.75 ppb(80%) in control area. Women's B-THg and U-THg was 3.41 ppb, 0.77 $\mu\text{g/g}$ in MWI, 3.03 ppb, 0.87 $\mu\text{g/g}$ in control region. While similar difference was found in B-THg of children and women between two regions, but not in U-THg. The distinct relation between TGM and biological exposure was found in children. TGM was 3.5(MWI) and 2.7(control). The level of urinary NAG, a lysosomal enzyme excreted in condition associated with renal tubular damage (Skalova S., 2002), was showed similar tendency with U-THg level. But the influence of Hg exposure on NAG was estimated to be slight judging from their low correlation. Other test for health effect such as blood biomarker, posturography didn't show distinct relation with biological exposures.

The influence of Hg emission from some industrial waste incineration facilities in the vicinity of residential area on biological exposure and health of the school children (281 subject) was investigated. TGM (daily) and U-THg (every two, first morning urine) was measured during one month. The results were compared with those gained in control area (40Km east from IWI). The mean of TGM and U-THg was 3.37 ng/m^3 , 1.13 $\mu\text{g/g}$ Cr in IWI, they were 2.29, 0.70 in control area. The TGM in IWI was 50% higher than that of control region, such difference was also found in U-THg (40% higher). Urinary MDA and 8-OhdG were analyzed to investigate

the health effects of U-THg. Different from general understandings, their level was higher in subjects living in control area and they didn't show any statistical relations with Hg exposure as like posturography.

In conclusion, we can find that Hg emitted from facilities influenced on the biological exposure, especially in children's U-THg. But the level of Hg exposure so low that distinct relation could not be found with health biomarkers.

Hair mercury: methylmercury exposure in current Japanese

Akira Yasutake and Noriyuki Hachiya

National Institute for Minamata Disease; Email: yasutake@nimd.go.jp

Summary

Methylmercury (MeHg) is an environmental pollutant with neurotoxic effects on the central nervous system. The major exposure route of MeHg to humans is via consumption of fish and shellfish which accumulate the chemical through the food web in an aquatic environment. Hair mercury level is an excellent marker for MeHg exposure, since a portion of MeHg is highly and stably accumulated there. We have been conducting a survey on hair mercury contents among general populations from 14 districts to estimate the current Japanese MeHg exposure level. Total mercury levels of all hair samples collected (12923 in total) were analyzed by the oxygen combustion-gold amalgamation method using an atomic absorption mercury detector. Multiple regression analysis revealed that mercury levels were significantly correlated with several covariates, such as sex, age, the amount of daily intake of total fish/shellfish, a preference for certain fish such as tuna or bonito, and artificial waving. The geometric means for the population without artificial waving were 2.47 and 1.65 ppm for males ($n = 5623$) and females ($n = 3470$), respectively. Hair mercury levels varied with age, and the variations were more significant in males. Since the difference between sexes was not evident at younger ages, some hormonal control might also be involved in the mercury uptake by human hair. The average mercury levels in our hair samples varied among the sampling districts. Tuna is a major carnivorous fish with high mercury accumulations that is often consumed in Japan. The amount of fish consumption and the preference rate for tuna would appear to be responsible for the regional variation in hair mercury levels in Japan.

Recently, a provisional tolerable weekly intake (PTWI) of MeHg was revised considering an effect to fetus by 61st JECFA (FAO/WHO Joint Expert Committee on Food Additives) to 2.0 $\mu\text{g}/\text{kg}/\text{week}$, which was about half that of the Japanese standard, and corresponded to a hair level of 2.2 ppm. Very recently, Japanese Government revised PTWI levels for pregnant women to 2.0 $\mu\text{g}/\text{kg}/\text{week}$, corresponding to a hair level of 2.75 ppm. The distribution of hair mercury levels in Japanese populations in the present study indicated that 25% and 15% of the Japanese females of child-bearing age were estimated to be exposed to MeHg over the PTWI levels of 61st JECFA and Japan, respectively. This would reflect the high Japanese

consumption of marine products. However, not only mercury contamination, but also the nutritional benefit may have to be considered when discussing the risk involved in the current level of fish and shellfish consumption in Japan.

Keywords: hair mercury; Japanese population; methylmercury exposure; PTWI; fish consumption

Methylmercury (MeHg) is formed by saprophyte microorganisms from inorganic mercury compounds in the aquatic environment (ATSDR, 1992). It is accumulated in fish and shellfish through the marine food web. Since the MeHg accumulation increases with the food web, carnivorous fish such as tuna, swordfish and shark often exhibit high levels of mercury. Furthermore, due to the long biological half-life of MeHg, the chemical tends to accumulate throughout the life of fish (Clarkson, 1992). Marine mammals such as whales and dolphins also show high concentrations of mercury. Accordingly, the major route of human exposure to MeHg is the ordinary consumption of fish and shellfish. MeHg is readily absorbed from the gastrointestinal tract and distributed among various tissues including the brain. The permeability of the chemical at the blood-brain barrier is responsible for its hazardous neurotoxic effect.

A WHO report (1990) concluded that the NOAEL (no observed adversary effect level) for adults is 50 ppm of the hair mercury level based on the analytical data of MeHg pollution in the past. Since the developing nervous system of the fetus has been considered highly susceptible to the effect of MeHg (Cox et al., 1989), the report also mentioned a possible association with an increased risk to the neurodevelopment of the fetus when maternal hair levels rise above 10 ppm. Accordingly, recent studies on the health effects of MeHg have focused on the exposure risk to pregnant women and the neuropsychological outcomes in newborns.

In Japan, the provisional regulatory standards of mercury and MeHg in fish and shellfish were determined in 1973 to be 0.4 and 0.3 ppm, respectively, based on the assumption of a safe intake limit of 0.17 mg mercury/person/week (0.48 µg/kg bw/day). In the determination, based on the NOAEL reported by WHO (1990), uncertainty factor 10 was employed. This is almost equal to the former PTWI (3.3 µg mercury/kg bw/week) reaffirmed at the 53rd JECFA meeting (JECFA, 1999). On the other hand, due to high susceptibility of the developing fetus, the exposure limit of MeHg has been suggested for pregnant women. The revised reference dose (RfD) of the US Environmental Protection Agency (EPA, 1997) set the safe exposure limit to

0.1 µg mercury/kg bw/day in 1997. This RfD has been calculated as 1/10 of the benchmark dose obtained in a study of the Iraq incident of 1971-1972 (Cox et al. 1989). However, since the manner of MeHg exposure in that incident was quite different from the ordinary exposure risk incurred through fish consumption, the Committee on Toxicological Effects of Methylmercury convened by the United States National Research Council (NRC, 2000) reevaluated the RfD based on the data obtained in a cohort study conducted in the Faroe Islands (Grandjean et al., 1997). On the other hand, based on the results from cohort studies in the Faroe Islands and the Seychelles, a provisional tolerable weekly intake (PTWI) of MeHg was determined to be 1.6 µg mercury/kg/week using an uncertainty factor 6.4 at the 61st meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2003). Very recently, the PTWI for pregnant women has also revised in Japan to 2.0 µg mercury/kg/week (MHLW 2005). They also used NOEL/BMD of Faroes and Seychelles studies, but employed an uncertainty factor 4. However, a considerable segment of the Japanese population is thought to be exposed to MeHg in excess of the above levels due to their habitually high consumption of marine products (Yasutake et al., 2003; 2004). Here we reported on a survey of the hair mercury levels in a cross section of representative Japanese sub-populations to estimate the current MeHg exposure levels in Japan.

Materials and Methods

Hair sampling

Hair samples were collected during 2000 to 2004 from 12,923 individuals (0 to 95 years old, 6,477 females and 6,446 males) at beauty saloons and barbershops in 14 districts of 12 prefectures: Hokkaido (Abashiri and Tomakomai Cities), Miyagi, Chiba, Niigata, Saitama, Nagano, Wakayama, Tottori, Hiroshima, Fukuoka, Kumamoto (Kumamoto and Minamata Cities), and Okinawa (Fig. 1). Using a questionnaire, we gathered information from each individual on 1) age, 2) sex, 3) frequency of fish and shellfish serving, 4) amount of fish taken per serving, 5) fish species often served, 6) presence of artificial waving, and 7) presence of hair-coloring. The hair samples were also collected at primary schools in each district to supply samples from children. This study has been approved review of the institutional ethical board.

Mercury analysis

0.1 to 0.5 g portion of the hair samples collected were washed well with detergent in an ultrasonic washer, and soaked in acetone twice to remove water, then left to dry on a filter paper. The dried hair was put in a glass vial and cut to small pieces of less than 1-mm length with

scissors. About 20 mg aliquot of the hair sample thus prepared was dissolved in 2N NaOH (0.5 ml) in a 1.5-ml polypropylene tube by heating at 60 °C for 30 min. Ten or twenty μ l of the sample solution was subjected to total mercury analysis according to the oxygen combustion-gold amalgamation method (Ohkawa et al. 1977) using an atomic absorption mercury detector MD-1 (Nippon Instruments, Co., Ltd., Osaka, Japan). Quality of analysis was confirmed by analyzing a reference material of human hair, NIES CRM No.13, with a certified value was 4.42 ± 0.2 ppm. Our data of 5 times analysis was 4.55 ± 0.05 ppm.

Statistical analysis

Because hair mercury concentrations analyzed were distributed in a lognormal profile, Student's t-test was performed on the mercury content data after logarithmic conversion. Multiple regression analysis was conducted by using a SPSS statistical package (SPSS Japan Inc., Tokyo). The amount of daily intake of total fish and shellfish was estimated from serving frequency and the amount of fish and shellfish consumed in each serving.

Results

Distribution of total mercury levels in all the hair samples was shown in Fig. 2. The highest and lowest levels were 40.2 and 0.012 ppm, respectively. Since the data showed a normal distribution after logarithmic conversion, a geometric rather than an arithmetic mean was used as representative of hair mercury levels. Geometric mean values for all males ($n = 6446$) and all females ($n = 6477$) were 2.42 and 1.39 ppm, respectively (Fig. 2A). These levels were somewhat higher than those estimated from mercury concentrations in blood or toenails recently reported in western countries (Sanzo, et al., 2001; Guallar, et al., 2002; CDC, 2003). An artificial waving effectively removed some of the hair mercury (Yamamoto and Suzuki, 1978; Yasutake, et al., 2003), since the waving lotion contained thioglycolate which tightly bound to MeHg. Single treatment with the lotion lowered the hair mercury level by 20 to 30% (Yasutake et al. 2003). Accordingly, the waved hair should showed somewhat lower level than before waving, and lower levels in females obtained here might be, at least partly, due to higher rate of the waving than males. In fact, among the participants in the present study, 8.9% of males and 45.1% of females had the artificial waving. The mean values for subpopulations without artificial waving were 2.47 and 1.65 ppm for males ($n = 5623$) and females ($n = 3470$), respectively (Fig. 2B). Even after excluding the contribution from artificial waving, males still showed a higher level than females, suggesting a presence of other factors such as the amount of fish consumed might be responsible for it.

Geometric means of hair mercury levels at each age were shown in Fig. 3. Hair mercury levels varied with age in both sexes, but such variations were more significant in males. Following a transient decline around the 20s, male levels increased into their 50s and 60s, and declined thereafter. The highest levels in the 50s and 60s were mostly twice those in childhood. The age-dependent variation in male hair mercury well fit to fish consumption feature shown in Fig. 4. On the other hand, the age-dependent variations in females were less significant. Although the difference between sexes was not evident at younger ages, the significant increase with age in male mercury levels accounted for a notable sex difference after the age of puberty. Since the amount of fish consumption shown as per body weight was found to be equal between male and female, it could not account for the sex difference in the hair mercury levels.

The age and sex-dependent variations in the hair mercury levels observed in the whole population were also found in each district (data not shown), but the levels significantly varied among the districts. The geometrical mean values in the hair mercury levels varied among the sampling districts from 1.72 to 3.81 ppm for males and from 1.33 to 2.80 ppm for females (Fig. 5). Such variations seemed to depend on the total amount of the daily intake of fish/shellfish and on the preference for consuming certain fish. Fish species often consumed in Japan found from the questionnaire in the present study were summarized in Table 1 with containing mercury levels reported (MHLW, 2005). In Japan, the provisional regulatory standards of total mercury concentration in fish and shellfish were suggested in 1973 to be 0.4 ppm. It should be noted that tuna fish, 45% of Japanese answered that they often consumed it, showed extremely high mercury content, mostly twice the standard. Since the mercury content of the second highest fish bonito was less than 1/4 of tuna, tuna consumption is supposed to contribute largely to the increase in hair mercury level. Relationships between average hair mercury levels and average amounts of the daily intake of fish/shellfish and the rate of preference for tuna consumption were shown in Fig. 6. The average consumption of fish/shellfish varied from 50.9 to 115.9 g/day among 14 districts, and correlation between the hair mercury level is poor ($r = 0.38$). On the other hand, the preference rate of tuna, a rate of population that answered to eat often, varied from 16.0 to 77.5% among the districts, and significantly correlated with the average hair mercury levels ($r = 0.60$, $p < 0.05$).

Considering all information obtained, multiple regression analysis revealed that mercury levels were significantly correlated with several covariates, such as sex, age, the amount of daily intake of total fish/shellfish, a preference for certain fish such as tuna or bonito, and artificial waving ($p < 0.001$).

Discussion

Human hair is an excellent marker for MeHg exposure, since a part of the chemical absorbed is accumulated there in a constant rate (WHO 1990). Here, we analyzed total mercury levels of general Japanese hair samples collected in 14 districts to estimate the current MeHg exposure level. The hair mercury levels were found to vary depending on age, sex and amount and species of fish/shellfish consumed.

It should be noted that although no difference was observed in the amount fish/shell fish consumption between males and females through ages, males (mean 2.47 ppm) showed higher hair mercury level than females (mean 1.65 ppm). It is documented in experimental animal that significant sex difference was observed in tissue distribution of MeHg, and that tissue uptake and elimination of MeHg could be enhanced by testosterone treatment (Hirayama and Yasutake, 1986; Hirayama et al., 1987). The fact in the present study that the adult males showed higher hair mercury levels than the females of same age, despite the same fish consumption rates, might indicate a positive contribution of male hormone in mercury uptake to human hair.

The exposure level to MeHg can be estimated from hair mercury levels using the following formula (NRC, 2000). Hair level (ppm, $\mu\text{g/g}$) was converted to corresponding blood level ($\mu\text{g/L}$) by multiplying by 1000/250, since an average hair/blood ratio of mercury concentration is 250. The sex difference in the hair mercury levels mentioned above may suggest that it is necessary to employ some different factor(s) for males and females when MeHg exposure level is estimated from hair mercury concentration.

$$d = \frac{C \times b \times V}{A \times f \times bw}$$

where

C = mercury concentration in blood ($\mu\text{g/L}$) = hair level (ppm) x 1000/250

b = elimination rate constant (0.014/day)

V = blood volume (9% of body weight)

A = fraction of the dose absorbed (0.95)

f = absorbed fraction distributed to the blood (0.05)

bw = body weight (kg)

d = dose ($\mu\text{g/kg bw/day}$)

We found through the present study that amount and species of the consumed fish varied among the districts, and that these variations seemed to account for the regional variation in the hair mercury levels. Among fish species often consumed through Japan, tuna is the exclusive carnivorous fish with high mercury accumulations. Since the consumption rate of tuna widely varied among the district, it might contribute the regional variation of the hair mercury. The highest rate of tuna consumption was found in Okinawa and Chiba, while Tottori and Minamata had the lowest rate. The highest hair mercury level found in Chiba among all the districts was probably due to the high consumption of tuna there. Despite Okinawa also showed a marked tendency to consume tuna, their lowest amount of total fish/shellfish consumption among the districts would tend to depress their hair mercury levels. In contrast, the two districts with the lowest hair mercury levels, Fukuoka and Hiroshima, showed both lower amounts of fish consumption and a lower preference for tuna among the districts. Thus, tuna consumption is a critical factor that affects the hair mercury levels in Japanese.

Various levels have been recommended as a safe exposure limit to MeHg in several countries and by international committees as mentioned before. In Japan 0.17 mg mercury/person/week (3.4 μg mercury/kg bw/week) was suggested as a safe exposure limit for adult in 1973. This is almost equal to the former PTWI (3.3 μg mercury/kg bw/week) reaffirmed at the 53rd JECFA meeting (JECFA, 1999), and corresponds to a hair mercury level of about 5 ppm. On the other hand, 0.1 μg mercury/kg bw/day, which was suggested as an RfD by the EPA (1997) and reevaluated by the NRC (2000), is the lowest level, and corresponds to a hair level of 1.0 ppm. The new PTWI 1.6 μg mercury/kg bw/week suggested by 61st JECFA (2003) corresponds to a hair mercury level of 2.2 ppm. Recent PTWI in Japan recommended for pregnant women (MHLW 2005), 2.0 μg mercury/kg bw/week, corresponds to a hair mercury level of 2.75 ppm.

The cumulative frequency of hair mercury levels without artificial waving in our survey was shown in Table 2. The portions that exceeded the 5 ppm which was recommended in Japan (1973) and by the former PTWI (JECFA, 1999) were less than 10% of the total population surveyed. When restricted to females of child-bearing age, 15 to 49 years old, 1.4% of the sub-population had hair mercury concentrations exceeding that level. However, the majority (87% of the total, 80% of females, 74% of females at child-bearing age, and 91% of males) exceeded EPA's 1 ppm. On the other hand, the average hair mercury levels of all Japanese females (1.65 ppm, without waving) and females of child-bearing age (1.43 ppm, without waving) were lower than the both PTWI levels for pregnant women recommended by 61st JECFA (JECFA 2003) and Japan (MHLW 2005). However, considerable population segments (24.7% of

females of child-bearing age) exceeded the PTWI level recommended by the 61st JECFA (2003). Similarly, 14.7% of them exceeded the Japanese new PTWI (MHLW 2005). Although it is difficult to assess the risk level for the Japanese females of child-bearing age, they may not be urgently at risk, since the two PTWIs contain uncertainty factors, 6.4 and 4 for 61st JECFA and Japan, respectively. Furthermore, none of them in our survey exceeded the NOEL/BMD levels obtained in the Faroes and Seychelles studies (JECFA 2003).

For pregnant women and those who may become pregnant, The Ministry of Health, Labor and Welfare, Japan (2005) recently announced a program to regulate the consumption of several kinds of fishes and whales that contained high concentrations of mercury. Such a program may be sufficiently effective to bring about some reduction in fish consumption in Japan. However, not only the risk of mercury contamination, but also food habits and nutritional benefits may have to be considered when determining a regulatory standard of fish and shellfish. Accordingly, sufficient and accurate information for general population must be provided to reach an appropriate decision on fish consumption. Hair analysis may, at least in part, contribute to such decisions by providing information on the MeHg exposure levels of each individual.

References

- ATSDR (1992) Agency for Toxic Substances and Disease Registry, Mercury toxicity. *Am. Fam. Physician*, 46, 1731-1741.
- CDC (2003) Second National Report on Human Exposure to Environmental Chemicals. The Centers for Disease Control and Prevention, United States
- Clarkson, T.W. (1992) Mercury: major issues in environmental health. *Environ. Health Perspect.*, 100, 31-38.
- Cox, C., Clarkson, T.W., Marsh, D.O., Amin-Zaki, L., Tikriti, S. and Myers, G.G. (1989) Dose-response analysis of infants prenatally exposed to methylmercury. An application of a single compartment model to single-strand hair analysis. *Environ. Res.*, 49, 318-332.
- EPA (1997) Mercury Study Report to Congress. United States Environmental Protection Agency, Washington, DC., EPA.
- Grandjean, P., Weihe, P., White, R.F., Debes, F., Araki, S., Yokoyama, K., Murata, K., Sørensen, N., Dahl, R. and Jørgensen, P.J. (1997) Cognitive deficit in 7-year-old children prenatally exposed to methylmercury. *Neurotoxicology*, 19, 417-428.

- Guallar, E., Sanz-Gallardo, M.I., van't Veer, P., Bode, P., Aro, A., Gomez-Aracena, J., Kark, J.D., Riemersma, R.A., Martin-Moreno, J.M. and Kok, F.J. (2002) Mercury, fish oils, and the risk of myocardial infarction. *New Engl. J. Med.*, 347, 1747-1754.
- Hirayama, K., Yasutake, A. and Inoue, M. (1987) Effect of sex hormones on the fate of methylmercury and glutathione metabolism in mice. *Biochem. Pharmacol.*, 36, 1919-1924.
- JECFA (1999) Joint FAO/WHO Expert Committee on Food Additives 53rd Meeting. Rome, 1-10 June 1999. <http://www.who.int/pcs/jecfa/jecfa.htm>
- JECFA (2003) Joint FAO/WHO Expert Committee on Food Additives 61st Meeting. Rome, 10-19 June 2003. <http://www.who.int/pcs/jecfa/jecfa.htm>
- NRC (2000) *Toxicological Effects of Methylmercury*, National Academy Press, National Research Council. Committee on the Toxicology Effects of Methyl-Mercury, Washington DC.
- Sanzo, J.M., Dorronsoro, M., Amiano, P., Aguinagalde, F.X. and Azpiri, M.A. (2001) Estimation and validation of mercury intake associated with fish consumption in an EPIC cohort of Spain. *Public Health Nutr.*, 4, 981-988.
- MHLW (2005) The Ministry of Health, Labor and Welfare, <http://www.mhlw.go.jp/topics/bukyoku/iyaku/syoku-anzen/suigin/index.html>.
- WHO (1990) *IPCS Environmental Health Criteria 101 Methylmercury*. World Health Organization, Geneva.
- Yamamoto, R. and Suzuki, T. (1978) Effects of artificial hair-waving on hair mercury values. *Int. Arch. Occup. Environ. Health*, 42, 1-9.
- Yasutake, A., Matsumoto, M., Yamaguchi, M. and Hachiya, N (2003) Current hair mercury levels in Japanese: survey in five districts. *Tohoku J. Exp. Med.*, 199, 161-169.
- Yasutake, A., Matsumoto, M., Yamaguchi, M. and Hachiya, N (2004) Current hair mercury levels in Japanese for estimation of methylmercury exposure. *J. Health Sci.*, 50, 120-125.

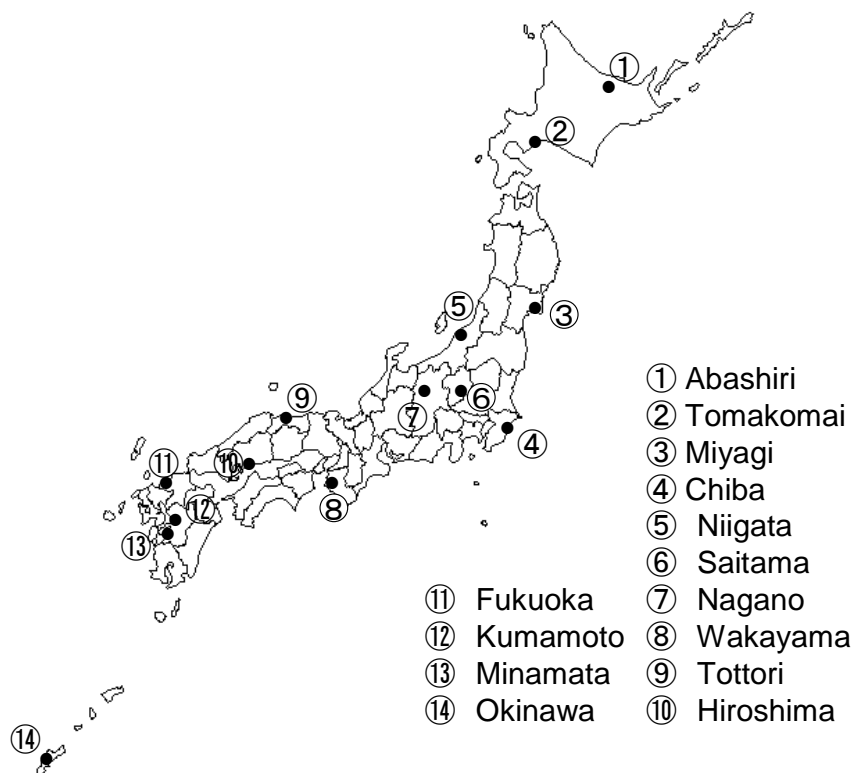


Fig. 1. Hair sampling locations.

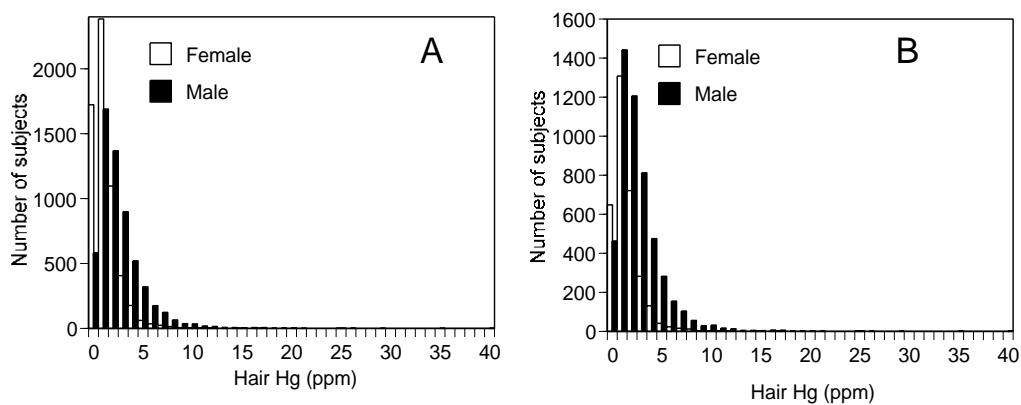


Fig. 2. Distribution of hair mercury content among the total study population (A) and the population without artificial waving (B). Open bar and solid bar indicate female and male populations, respectively.

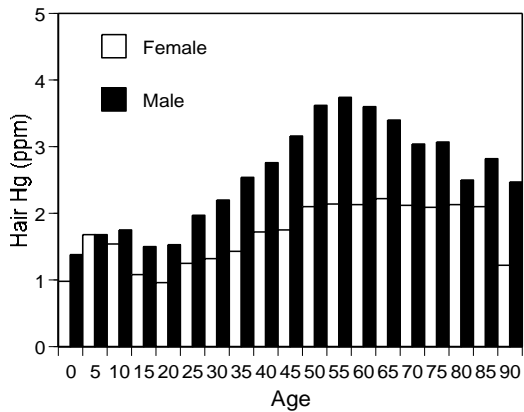


Fig. 3. Age-dependent distribution of the geometric mean of hair mercury content among the population. Open bar and solid bar indicate female and male populations, respectively.

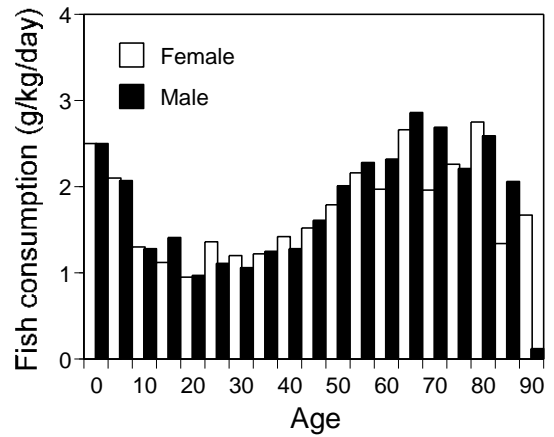


Fig. 4. Age-dependent variation in amount of fish consumption. Open bar and solid bar indicate female and male populations, respectively.

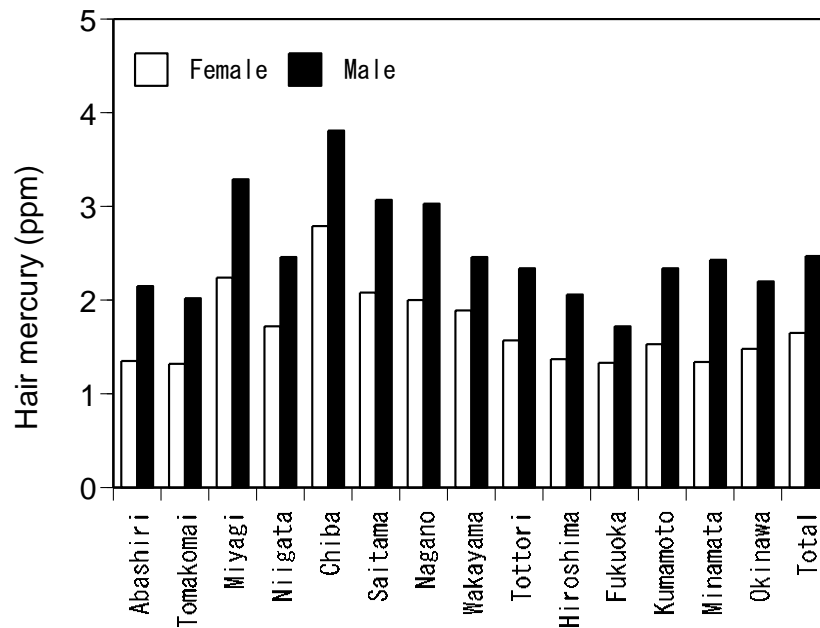


Fig. 5. Geometrical mean of hair mercury levels in 14 districts. Open bar and solid bar indicate female and male populations, respectively.

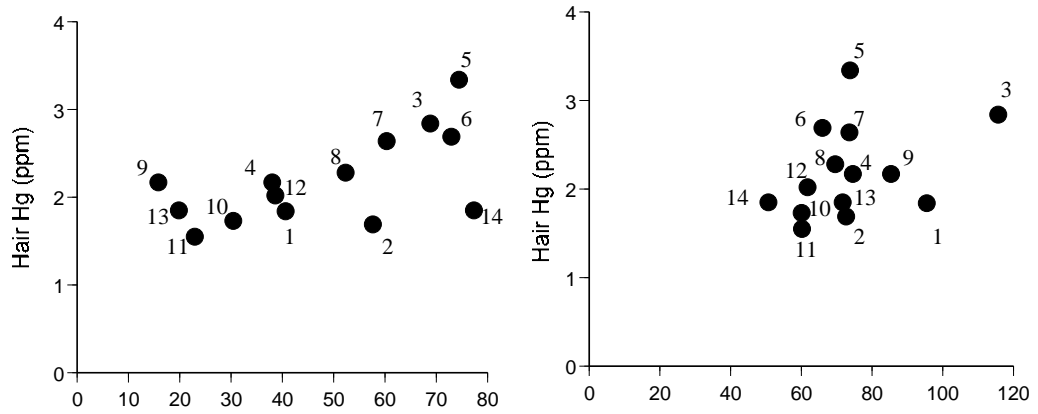


Fig. 6. Relation of hair mercury levels to tuna consumption rates (A) and total amount of fish consumption (B) in 14 districts.

Table 1. Fish and shellfish often consumed in Japan

Ranking	Consumption rate (%)*	Fish	Hg level (ppm)**
1	57.5	Mackerel	0.09
2	54.7	Salmon	0.01
3	49.6	Saurel	0.04
4	49.5	Mackerel pike	0.06
5	46.8	Squid	0.03
6	45.4	Tuna	0.77***
7	45.2	Fish paste	0.01
8	42.3	Shrimp	0.02
9	34.4	Sardine	0.02
10	33.3	Octopus	0.03
11	25.9	Flat fish	0.05
12	24.1	Clam	0.01
13	23.7	Bonito	0.17
14	22.4	Yellowtail	0.13
15	19.3	Sea beam	0.08
16	18.1	Eel	0.04
17	14.8	Crub	0.02

* Rate of population that answered “often consume.”

** Data from The Ministry of Health, Labor and Welfare (MHLW, 2005).

*** Average value of 5 tuna species is shown. An average of higher 3 species is 1.13 ppm.

Table 2. Frequencies (%) of sub-populations exceeding certain levels in current Japanese without artificial waving.

Hair mercury (ppm)	0≤	1<	2<	2.2<	2.75<	5<	10<
		US EPA (1997)		JECFA (2003)	Japan (2005)	Japan (1973)	
Male (total)	100	91.1	62.8	57.6	44.2	13.8	1.9
Female (total)	100	80.1	38.5	32.3	20.6	3.2	0.3
Female (15-49 years)	100	74.1	29.7	24.7	14.7	1.4	0.1
Total	100	86.9	53.6	47.9	35.2	9.8	1.3

Mercury exposure from fish consumption within the Japanese and Korean communities

**Ami Tsuchiya^{a,b}, Thomas A. Hinnert^c, Thomas M. Burbacher^a,
Elaine M. Faustman^{a,b}, Koenraad Mariën^d**

^aDepartment of Environmental and Occupational Health Services, University of Washington, Seattle, WA, USA,

^bInstitute for Risk Analysis and Risk Communication, University of Washington, Seattle, Washington, USA;

^cNational Exposure Research Laboratory, United States Environmental Protection Agency, Las Vegas, Nevada USA;

^dWashington State Department of Health, Olympia, Washington, USA

Abstract

Most fish consumption guidance is based on preventing exposure to contaminants. However, fish may be a culturally important food item and provides nutrients that are essential to optimal growth and health. In this study, we examined high end fish consumers who were women of childbearing age living in the U.S. to determine how intake of n-3 polyunsaturated fatty acids relates to mercury (Hg) exposure and how the relationship can impact fish consumption guidance.

As part of the Arsenic Mercury Intake Biometric Study involving the Japanese and Korean communities, we obtained Hg fish tissue concentrations for species consumed, hair-Hg levels, and fish, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) intake levels. The intake and Hg exposure data were compared with published national data. In total, 214 participants were enrolled into this longitudinal study; 106 Japanese and 108 Koreans. Fish intake (shellfish & finfish combined) for both communities (73 & 82 g/d for Japanese & Koreans, respectively) was close to the 95th percentile for the US general population. Hair Hg levels were also above the national average of 0.2 ppm (1.2 & 0.6 ppm for the Japanese & Koreans, respectively). Although total finfish consumption rates between the two populations were in proximity to each other, Hg intake between the two is significantly different. Consumption patterns suggest that within both populations, there may be a percentage of individuals not obtaining their daily dietary requirement of DHA or DHA+EPA.. Fish consumption guidelines based on contaminant concentrations alone can have the unintended consequence of causing a portion of the population to have an insufficient intake of required nutrients. There are sufficient differences in fish-species consumption behavior and Hg intake levels between these two populations to suggest that Asian populations should not be grouped as a whole, but treated independently.

Keywords: mercury, n-3 fatty acids, fish, nutrition, fish advisories, Japanese, Korean

1. Introduction

Fish contain contaminants such as mercury (Hg), PCBs, dioxin and pesticides that may cause adverse health effects in humans when consumed in large quantities. In particular, health effects of Hg have been well documented beginning with the Minamata and Iraqi incidents (Bakir et al. 1973; Harada 1995; Kondo 2000). As a result of studies on human effects, the US Environmental Protection Agency (USEPA) established a reference dose (RfD) of 0.1 ug/kg/day for methylmercury while the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA) determined the provisional tolerable weekly intake (pTWI) to be 1.6 ug/kg/week (World Health Organization 2003). Because fish is the most prominent source of non-occupational Hg exposure in our diet, regulatory agencies worldwide have developed fish consumption guidelines derived from established reference doses or tolerable intakes. Thus, most public health guidelines are based on preventing overexposure by providing fish consumption advice (Scherer et al. 2008).

Along with concern over contaminant exposure, fish consumption is also associated with improved health (Akabas and Deckelbaum 2006; Cohen et al. 2005). In particular, fish is a major source of n-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). DHA and EPA together are thought to play important roles in neurodevelopment (Akabas and Deckelbaum 2006; Hibbeln et al. 2006). The optimal intake of DHA is thought to be between 100 and 300 mg per day for women (Akabas and Deckelbaum 2006). For DHA and EPA together the optimal intake is considered to be between 400 and 500 mg per day (Gebauer et al. 2006; Kris-Etherton et al. 2002).

Recently, studies have investigated the risk-benefit aspects of seafood consumption; with results being inconclusive (Budtz-Jorgensen et al. 2007; Nesheim et al. 2007; Sakamoto et al. 2004). In this paper, we attempt to better understand this issue by examining high end fish consumers consisting of women of childbearing age living in the U.S. to determine how the intake of PUFAs relates to Hg exposure and how this relationship can impact fish consumption guidance.

2. Subjects and Methods

As part of the Arsenic Mercury Intake Biometric Study, women of childbearing age (18-45 years) who identified themselves as Korean, Japanese or of Japanese or Korean descent participated in the longitudinal study. The recruitment method and sample collection methods have been previously described in detail (Tsuchiya et al. 2008; Tsuchiya et al. 2008). In

summary, participants came to the clinics and participated in a structural interview consisting of a fish consumption survey and a demographic questionnaire while also providing biological samples. The fish consumption survey was developed based on previously used surveys (Mariën and Patrick 2001; Sechena et al. 2003; Toy et al. 1996). Participants were asked to identify the fish species they consumed during the past year and provide amounts and frequency of consumption for each species consumed. Hair from the nape of the neck was collected for Hg analysis. DHA and EPA intakes were estimated based on the consumption of individual species. The amounts of DHA and EPA in fish species were determined by using previously published data (Ackman 2000; Exler and Weihrauch 1976; Kagawa 2005; Mahaffey 2004). The study materials were approved by the State of Washington Department of Social and Health Services Human Research Review Board.

3. Results and Discussion

A total of 214 women of childbearing age (106 Japanese and 108 Koreans) participated in this study. Mean finfish intake for Japanese was 60 g/d and 59 g/d for Koreans with mean total seafood intakes being 73 g/d and 82 g/d, respectively. Intake levels were not significantly different ($p < 0.05$) between communities. These seafood intakes were close to the 95th percentile for the U.S. general population as provided from National Health and Nutrition Examination Survey data (Mahaffey et al. 2004).

The species consumed by the populations were markedly different. The 6 finfish species most consumed by the Japanese were: salmon (29 % of total intake), mackerel (9%), black cod (6%), squid (6%), light tuna (canned) (5%) and halibut (4%). The species most consumed by the Korean population were: squid (23%), mackerel (12%), yellow croaker (11%), salmon (9%), flounder/sole (6%), and light tuna (canned) (6%). In addition to the differences in species consumed, hair-Hg levels were significantly different between the two communities.

The mean hair-Hg level for the Japanese was 1.6 ppm with 55 % of the population exceeding the USEPA's RfD of 1.2 ppm. However, the mean hair-Hg level for the Korean population was 0.75 ppm and only 13% exceeded the RfD. The difference in Hg exposure is likely due to the differences in type of fish species consumed.

Intakes of DHA and EPA were plotted against hair-Hg levels (Figure 1). For the Japanese community, 40 individuals (38%) did not meet the recommended DHA + EPA intake and of those, 12 individuals (11%) exceeded the RfD. Within the Korean community, 62 individuals (57%) did not meet the recommended DHA+EPA intake. However, only 5 (5%) exceeded the RfD. Our results suggest that both communities consumed fish in large quantity, yet a portion

of each population did not meet DHA + EPA recommended intake on a daily bases. Therefore, these two populations could benefit from fish consumption guidance that includes information on minimizing Hg exposure as well as on the benefits of fish consumption so as to reduce both Hg exposure while also reducing the number of individuals not obtaining the recommended levels of n-3 PUFAs. Further, the guidance should be population specific as the Japanese need to reduce their Hg exposure while increasing DHA+EPA intake. In contrast, the Korean population needs to increase their intake of fatty fish species that are low in Hg and have no restrictions placed on fish consumption. The observed differences between the two populations in regards to fish consumption behavior, hair-Hg levels, DHA/EPA intake and recommended public health guidance on fish consumption suggests that Asian populations should not be grouped as a whole, but treated independently.

References

- Ackman R. 2000. Fatty acids in fish and shellfish. In: *Fatty Acids in Foods and Their Health Implications*, 2nd Edition (Chow C, ed):Marcel Dekker AG, Basel, 153-174.
- Akabas SR, Deckelbaum RJ. 2006. Summary of a workshop on n-3 fatty acids: current status of recommendations and future directions. *The American journal of clinical nutrition* 83(6 Suppl):1536S-1538S.
- Bakir F, Damluji SF, Amin-Zaki L, Murtadha M, Khalidi A, al-Rawi NY, et al. 1973. Methylmercury poisoning in Iraq. *Science* 181(96):230-241.
- Budtz-Jorgensen E, Grandjean P, Weihe P. 2007. Separation of risks and benefits of seafood intake. *Environ Health Perspect* 115(3):323-327.
- Cohen JT, Bellinger DC, Connor WE, Shaywitz BA. 2005. A quantitative analysis of prenatal intake of n-3 polyunsaturated fatty acids and cognitive development. *American journal of preventive medicine* 29(4):366-374.
- Exler J, Weihrauch JL. 1976. Comprehensive evaluation of fatty acids in foods. VIII. Finfish. *J Am Diet Assoc* 69(3):243-248.
- Gebauer SK, Psota TL, Harris WS, Kris-Etherton PM. 2006. n-3 fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. *The American journal of clinical nutrition* 83(6 Suppl):1526S-1535S.
- Harada M. 1995. Minamata disease: methylmercury poisoning in Japan caused by environmental pollution. *Crit Rev Toxicol* 25(1):1-24.

- Hibbeln JR, Ferguson TA, Blasbalg TL. 2006. Omega-3 fatty acid deficiencies in neurodevelopment, aggression and autonomic dysregulation: opportunities for intervention. *Int Rev Psychiatry* 18(2):107-118.
- Kagawa Y. 2005. *Standard Tables of Food Composition in Japan. Fifth Revised and Enlarged Edition*. Tokyo:Kagawa Education Institute of Nutrition.
- Kondo K. 2000. Congenital Minamata disease: warnings from Japan's experience. *J Child Neurol* 15(7):458-464.
- Kris-Etherton PM, Harris WS, Appel LJ. 2002. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation* 106(21):2747-2757.
- Mahaffey KR. 2004. Fish and shellfish as dietary sources of methylmercury and the omega-3 fatty acids, eicosahexaenoic acid and docosahexaenoic acid: risks and benefits. *Environ Res* 95(3):414-428.
- Mahaffey KR, Clickner RP, Bodurow CC. 2004. Blood organic mercury and dietary mercury intake: National Health and Nutrition Examination Survey, 1999 and 2000. *Environ Health Perspect* 112(5):562-570.
- Mariën K, Patrick GM. 2001. Exposure analysis of five fish-consuming populations for overexposure to methylmercury. *J Expo Anal Environ Epidemiol* 11(3):193-206.
- National Research Council. 2000. *Toxicological effects of methylmercury*. Washington, DC:National Academy Press.
- Nesheim MC, Yaktine AL, Institute of Medicine (U.S.). Committee on Nutrient Relationships in Seafood: Selections to Balance Benefits and Risk. 2007. *Seafood choices : balancing benefits and risks*. Washington, D.C.:National Academies Press.
- Sakamoto M, Kubota M, Liu XJ, Murata K, Nakai K, Satoh H. 2004. Maternal and fetal mercury and n-3 polyunsaturated fatty acids as a risk and benefit of fish consumption to fetus. *Environ Sci Technol* 38(14):3860-3863.
- Scherer AC, Tsuchiya A, Younglove LR, Burbacher TM, Faustman EM. 2008. Comparative analysis of state fish consumption advisories targeting sensitive populations. *Environ Health Perspect* 116(12):1598-1606.
- Sechena R, Liao S, Lorenzana R, Nakano C, Polissar N, Fenske R. 2003. Asian American and Pacific Islander seafood consumption -- a community-based study in King County, Washington. *J Expo Anal Environ Epidemiol* 13(4):256-266.
- Toy KA, Polissar NL, Liao S, Mittelstaedt GD. 1996. *A fish consumption study of the Tulalip and Squaxin Island tribes of the Puget Sound region*. Marysville, WA: Tulalip Tribes, Department of Environment.

Tsuchiya A, Hardy J, Burbacher TM, Faustman EM, Mariën K. 2008. Fish intake guidelines: incorporating n-3 fatty acid intake and contaminant exposure in the Korean and Japanese communities. *The American journal of clinical nutrition* 87(6):1867-1875.

Tsuchiya A, Hinners TA, Burbacher TM, Faustman EM, Mariën K. 2008. Mercury exposure from fish consumption within the Japanese and Korean communities. *J Toxicol Environ Health A* 71(15):1019-1031.

World Health Organization. 2003. Joint FAO/WHO Expert Committee on Food Additives. Available: http://www.who.int/ipcs/food/jecfa/summaries/en/summary_61.pdf [accessed June 20 2006].

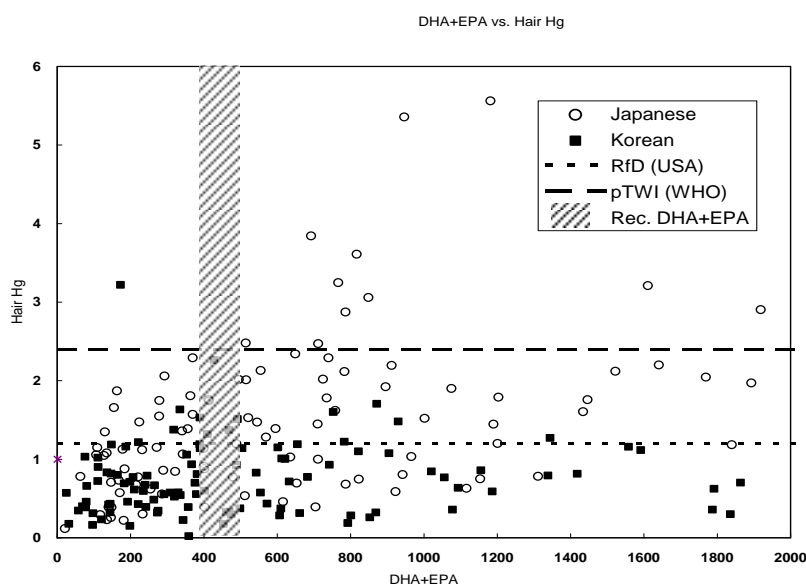


Figure 1. Hair mercury values are plotted against DHA and EPA intake (Tsuchiya et al. 2008). RfD: the biometric equivalent of US EPA's Reference Dose for Hg which is 1.2 ppm in hair (National Research Council 2000). pTWI: biometric equivalent of the provisional Tolerable Weekly Intake for Hg which is 2.4 ppm in hair (World Health Organization 2003). Rec DHA+EPA: recommended DHA and EPA amount of 400-500 mg per day (Akabas and Deckelbaum 2006).

Relationship of methylmercury (MeHg) and docosahexaenoic acid (DHA) in pregnant women and fetuses

Mineshi Sakamoto^{a*}, Shyoichi Kawakami^b, Katsuyuki Murata^c,
Kunihiko Nakai^d, Hiroshi Satoh^d

^aDepartment of Epidemiology, National Institute for Minamata Disease,
Minamata, Kumamoto 867-0008, Japan.

^bGynecology & Obstetrics, Fukuda Hospital, Kumamoto 860-0004, Japan.

^cDepartment of Environmental Health Sciences, Akita University School of Medicine, Akita 010-
8543, Japan

^dEnvironmental Health Sciences, Tohoku University Graduate School of Medicine,
Sendai 980-8575, Japan

Abstract

This study was designed to determine the relationship between methylmercury (MeHg) exposure and docosahexaenoic acid (DHA) concentrations in pregnant women and fetuses to consider the risk and benefit of maternal fish consumption during the gestation period. Venous blood samples were collected from 55 pairs of mothers from early gestation, and mothers and fetuses (cord blood) at parturition. Total mercury (Hg) in blood and fatty acids in plasma were measured. Maternal Hg level showed a tendency to decline from early gestation to parturition. Cord blood Hg level was about 1.8 times higher than mothers. Significant positive correlations with Hg ($r=0.78$) and DHA ($r=0.67$) were observed between mother and fetuses, indicating that both substances in fetuses reflect maternal exposures. Significant positive correlations of Hg and DHA were observed in mothers both at early gestation ($r=0.29$) and at parturition ($r=0.36$). A significant positive correlation with MeHg and DHA was also observed in cord blood ($r=0.36$). These results confirm that both Hg and DHA which originated from fish consumption transferred from mothers to fetus and they existed in the fetal blood with a positive correlation. Pregnant women in particular need not give up eating fish to obtain such benefits. However, they would do well to at least consume smaller fish, which contains less MeHg, thereby balancing the risks and benefits from fish consumption.

Keywords: methylmercury, heavy metals, placental transfer, red blood cells, cord blood

1. Introduction

Methylmercury (MeHg) is a well known and widespread environmental neurotoxicant. In the natural course of events, most human exposure to MeHg is through fish and sea mammal

consumption. Generally, the larger fish and sea mammals at the top of the food chain, such as shark, tuna and whale, contain higher levels of MeHg than the smaller ones. Fetuses are known to be a high-risk group for MeHg exposure since the susceptibility of the developing brain itself is high. Therefore, the effect of MeHg exposure on pregnant women remains an important issue for elucidation, especially in populations which consume much fish and sea mammals.

On the other hand, human intake of the n-3 longer chain of polyunsaturated fatty acids (PUFA), such as eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3), is also known to be produced originally by phytoplankton, mainly from fish consumption. Both of these fatty acids are very beneficial for human health. Especially, DHA is known to be an important n-3 PUFA for normal brain development and function. Rapid brain growth occurs primarily during the third trimester in humans, and the amount of these fatty acids increases dramatically during the period. This period corresponds to when the human brain is most susceptible to MeHg, and also a high accumulation of MeHg in the brain may occur during the period.

We conducted a study mainly to determine the relationship between Hg and plasma fatty acid concentrations in mothers and fetus to evaluate the risks and benefits of maternal fish consumption by comparing 55 maternal-fetal pairs of blood samples.

2. Subjects and Methods

Fifty-five healthy Japanese pregnant women without any particular exposure to Hg provided informed consent to participate in the present trial approximately at early gestation. These women ranged in age from 19 to 40 years (average age 29.3 ± 4.8 years), and resided in Kumamoto City, Kumamoto, Japan. Blood and hair samples were collected at early gestation and at parturition from the mothers. Cord blood samples were collected immediately after birth.

Fatty acid composition analysis in plasma was immediately performed by SRL Inc. (Tokyo, Japan). Blood samples were stored at -80°C until total Hg (THg) analysis. This study was approved by the Ethics Committee of the National Institute for Minamata Disease (NIMD). Total Hg in 0.5 g of blood was determined by cold vapor atomic absorption spectrophotometry (CVAAS) according to the method of Akagi (Akagi et al., 2000).

3. Results and Discussion

MeHg is one of the substances most risky to fetal brain development, and most of the exposure to MeHg is through maternal fish consumption (IPCS, WHO 1990). On the other hand, DHA, which is important for the fetal brain and its growth, is also derived from maternal fish consumption. If

human exposure to MeHg were independent of nutrition from fish, we would aim at zero exposure. However, fish plays an important cultural role among Japanese and contains n-3 polyunsaturated fatty acids, such as DHA and EPA. Therefore, this study was designed to determine the relationship between MeHg exposure and DHA concentrations in pregnant women and fetuses to consider the risk and benefit of maternal fish consumption during the gestation period.

MeHg and DHA levels in cord blood showed the tendency to increase with fish intake (g/day). Significant positive correlations with MeHg ($r=0.78$) and DHA ($r=0.67$) were observed between maternal and cord blood, indicating that both substances in fetuses reflect maternal exposures. Cord blood Hg level was about 1.8 times higher than mothers at parturition. This suggests that MeHg actively transfers to the fetus across the placenta via neutral amino acid carrier (Ashner, M., Clarkson, T.W 1988), when the fetus is most susceptible to MeHg. Therefore, the effect of MeHg exposure remains an important issue for elucidation, especially in populations which consume much fish and sea mammals. Maternal Hg levels at early gestation and at parturition showed a significant positive correlation ($r=0.53$). However, the level showed a tendency to decline from early gestation to parturition, indicating that high amount of MeHg transfers from mothers to fetuses through placenta during gestation.

DHA and arachidonic acid (AA) are abundant in the brain. During rapid brain growth, large amounts of DHA and AA from the maternal circulation must reach the fetus to meet its needs for development. The rapid quantitative accretion of both DHA and AA during the third trimester of pregnancy was noticed in human brain. Breast-milk also contains these fatty acids. Our data showed that the fetal/maternal ratio of DHA and AA are higher than other fatty acids, which are not important for brain, indicating that the fatty acids which are important for the brain and its growth were selectively transferred from maternal blood to cord blood.

Though the origins of the two fatty acids are completely different, DHA showed significant positive correlations with AA, which is also important for fetal brain and its growth. This phenomenon is interesting, and it may suggest that the ratio of these fatty acids is also important.

Hair/blood Hg ratios were 356 at early gestation, 339 at partition, and higher than 250 at the steady state, which indicates that MeHg distribution can not be explained by a single-compartment model. This must be taken into consideration in risk assessment of MeHg during gestation.

Significant positive correlations of MeHg and DHA were observed in mothers both at early gestation ($r=0.29$) and at parturition ($r=0.36$). A significant positive correlation with MeHg and DHA was also observed in fetuses ($r=0.36$). These results suggest that they were derived from fish consumption and were selectively transferred from mothers to fetuses. These results

indicate that both MeHg and DHA, which act contrary to the normal growth and function of the developing brain, were taken into the maternal blood through maternal fish consumption and transfer to fetal blood, and that they showed positive correlations. Therefore, if the ordinary fish consumed are low in MeHg but rich in DHA, children's health will especially benefit from the fish consumption. However, if the fish MeHg concentration is high enough to ruin the effect of DHA, fish consumption will retard children's development. Pregnant women in particular would do well to consume at least smaller fish, thereby reducing the risk from large fish but allowing them to continue to eat them in order to confer the benefits.

References

- Akagi, H., Castillo, E.S., Cortes-Maramba, N. 2000. *Sci Total Environ* 259 (1-3), 31-43.
- IPCS, WHO. *Environmental Health Criteria* 101. Geneva, 1990
- Ashner, M., Clarkson, T.W. 1988. *Teratology*, 19, 145-155

Prenatal low levels mercury exposure on infant development: a prospective study in Zhoushan Islands, China

Chong-Huai Yan Yu Wang*, Yu Gao, Hong Zhang, Xiao-Ming Shen

*XinHua Hospital affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai
Institute for Pediatric Research, Shanghai Key Laboratory of Children's Environmental Health
Shanghai 200092, China

Objective. To explore the relationship between prenatal mercury exposure and infant neurodevelopmental outcomes in Zhoushan cohort.

Material and methods. 408 mother-infant pairs were enrolled in the cohort of Zhoushan Islands. Prenatal mercury exposure was determined by measuring cord blood mercury (CBHg, Range 1.34-18.34 $\mu\text{g/L}$). Based on their CBHg, children were divided into two groups: 80 in low mercury group (LG, CBHg < P20, 3.98 $\mu\text{g/L}$), and 84 in high mercury group (HG, CBHg > P80, 8.09 $\mu\text{g/L}$). Children were followed up at 3 (n=149) and 12 (n=123) months mos. of age with evaluated BSID, hair mercury and questionnaire. The association between CBHg concentrations and neurodevelopmental outcomes at 3 and 12 months of age was examined by multiple regression analysis with adjustment for confounding variables.

Results. The average hair mercury levels were 1058.71 \pm 69.58 (LG), 1903.06 \pm 139.37 $\mu\text{g/kg}$ (HG) of 3-month-infant and 906.05 \pm 110.26 (LG), 1512.41 \pm 93.85 $\mu\text{g/kg}$ (HG) of 12-month-infant. The hair mercury of 12-month were lower than that of 3-month significantly in both group. MDI of 3-month-infant were 109.01 \pm 4.16 of LG, 108.32 \pm 4.23 of HG, PDI were 99.91 \pm 3.28 of LG, and 98.71 \pm 5.21 of HG. MDI of 12-month-infant were 110.60 \pm 4.29 of LG, 109.56 \pm 4.29 of HG; PDI were 104.06 \pm 3.75, and 101.70 \pm 3.59 of HG. There were no significant differences of MDI and PDI between the two groups in both ages. At 3 months, high risk factors for MDI were hair mercury of 3 months and head circumference; and those for PDI were hair mercury of 3 months and gender. At 12 months, the high risk factors for MDI scores were hemoglobin of 3 months, age when children stood up and those for PDI were age when children stood up, average times of fish dinners of mother and hemoglobin of 12 months.

Conclusions. The hair mercury level was significantly related with CBHg. The mercury exposure level of 12-month-infant was lower than that of 3-month-infant. Prenatal low level mercury exposure had no significantly impacts on infants' development in Zhoushan cohort.

**Cohort study of the effects of perinatal exposure to
methylmercury and POPs on development of infants in Japan;
an interim report of Tohoku Study of child development**

Hiroshi Satoh, Kunihiro Nakai, Keita Suzuki*, Naoyuki Kurokawa, Tomoko Oka-Sugawara, Nozomi Tatsuta*, Takashi Ohba and Miyuki Shimada

Environmental Health Sciences, Tohoku University Graduate School of Medicine, Sendai 980-8575,
Japan

*Human Development and Disabilities, Tohoku University Graduate School of Education, Sendai,
980-8576, Japan

Abstract

Possible effects on the development of infants exposed to methylmercury and POPs in utero are of great concern among fish-eating populations, because exposures occur through maternal fish intake. Moreover fish also contains *n*-3 polyunsaturated fatty acids essential for normal brain development in the fetus and infant. Therefore we have been performing a prospective cohort study, the Tohoku Study of Child Development (TSCD), to examine the effects of perinatal exposure to methylmercury and POPs emphasizing PCBs on neurobehavioral development. Healthy pregnant women in Tohoku district, northeastern part of the mainland Japan, were recruited. Biological samples were collected during pregnancy and at delivery. Several types of questionnaire were administered including food-intake frequency questionnaire. The development of infants has been repeatedly assessed by Brazelton Neonatal Behavioral Assessment Scale, Bayley Scales of Infant Development and Kaufman Assessment Battery for Children. Biological samples have been analyzed for total and methylmercury, heavy metals, dioxins, PCBs and several organochlorine pesticides. Since this cohort study is on going and especially chemical analyses have not been completed, in this paper 466 mother-infant pairs who had PCB data were analyzed. The results of multiple regression analyses suggested that perinatal exposures to methylmercury and PCBs have sporadically shown effects on infant development. But the effects seem to be temporary rather than permanent. This is especially true for methylmercury. It is thought we have to continue the follow-up of TSCD infants more and to carry on chemical analyses to increase the numbers of samples and species of substances to be analyzed. We are now carrying out the follow-up examination at 84-month-old including Wechsler Intelligence Scale for Children (3rd edition), Boston Naming Test, Continuous Performance Test and electrophysiological examinations.

Keywords: methylmercury, PCBs, perinatal exposure, postnatal development, Neonatal Behavioral Assessment Scales, Bayley Scales of Infant Development, Kaufman Assessment Battery for Children

1. Introduction

Several epidemiological studies have indicated that perinatal exposure to heavy metals and some persistent organic pollutants (POPs) can cause the developmental deficits such as postnatal growth delay and poor cognitive functions. Especially methylmercury (MeHg) and polychlorinated biphenyls (PCBs) are of great concern among fish-eating populations, because exposures occur through maternal fish intake. Moreover fish also contains *n*-3 polyunsaturated fatty acids essential for normal brain development in the fetus and infant. Therefore we have been performing a prospective cohort study, the Tohoku Study of Child Development (TSCD), to examine the effects of perinatal exposure to methylmercury and POPs emphasizing PCBs on neurobehavioral development in Japanese children (Nakai et al., 2004).

2. Subjects and Methods

Healthy pregnant women were recruited with their informed consents at obstetrical wards of two hospitals in urban area, and one obstetrical ward of a hospital and an obstetric clinic in coastal area in Tohoku district, northeastern part of the mainland Japan. Maternal blood was collected during pregnancy. Cord blood, cord tissue and placenta were collected at delivery. Hair samples were also collected before leaving the hospitals or the clinic. Several types of questionnaire were administered including food-intake frequency questionnaire, educational and occupational backgrounds and life-style before leaving. Breast milk was collected at their own home one month later. The samples were sent to our laboratory under cooling condition by a courier service. Approximately 1,300 mother-infant pairs were obtained after applying exclusion criteria such as congenital anomaly, a severe disease and low-birth weight.

The development of infants have been longitudinally assessed by Brazelton Neonatal Behavioral Assessment Scale (NBAS) at 3-day-old, Bayley Scales of Infant Development (BSID) and Kyoto Scale of Psychological Development at 7- and 18-month-old and Kaufman Assessment Battery for Children (K-ABC) at 42-month-old. Chemical analyses of biological samples are in progress. The followings are the target substances in biological samples: total mercury in hair; total and methylmercury in maternal and cord blood; heavy metals in maternal and cord blood and placenta; dioxins, PCBs and several organochlorine pesticides in maternal and cord blood and breast milk.

Multiple regression analysis was applied to detect the effects of perinatal exposure to chemicals on Birth Weight, NBAS, Mental Development Index (MDI) and Psychomotor Development Index (PDI) of BSID, Mental Processing (MPS) and Achievement Scales (AS) of K-ABC. Since we have been interested in the developmental effects of perinatal chemical exposure through fish consumption, total mercury in the segment of maternal hair 3 cm from the scalp (Hg Hair) (Ohba et al., 2008), PCBs in cord blood (PCB), maternal annual fish consumption (Fish) and cord blood lead (Pb) were set as independent variables and fixed in the model. The other variables including maternal age at delivery (Maternal Age), body weight before pregnancy (BW), alcohol drinking or smoking during pregnancy (yes/no: Drinking or Smoking), Parity (primipara/multipara), Delivery Type (spontaneous/not), years of Education (≤ 12 or $12 <$), Raven's standard progressive matrices (Raven), Sex of babies, Gestational Week, Birth Weight, Apgar score (1 min.) were entered in the regression models and backward stepwise analyses were carried out. Variables were log-transformed when appropriate.

3. Results and Discussion

Since this cohort study is on going and especially chemical analyses have not been completed, the analyses on the association between infant development and perinatal exposures to POPs and heavy metals have been limited. In this paper, 466 mother-infant pairs who had PCB data were analyzed. Chemical analyses on mercury and Pb for these pairs were done. The numbers of female/male babies were 222/244. The mean \pm -SD of Gestational Week was 39.6 \pm 1.25 and Birth Weight 3080 \pm 331g, respectively. The median of Fish was 20.5 kg/year.

As for the exposure measures, medians of total mercury in maternal and cord blood were 5.3, 10.0 ng/ml, and most (>95%) was methylmercury. Hg Hair well correlated with total and methylmercury in maternal and cord blood. The median of Hg Hair was 1.97 ng/mg and within the normal range in Japan. The medians of PCB and Pb were 47.6 ng/g-lipid and 9.7 ng/ml (0.97 micro-g/100mL), respectively. These levels were rather low compared with other studies (Nakamura et al., 2008).

By the multiple regression analyses, significant variables selected for Birth Weight were Maternal Age, BW, Fish, Sex, Gestational Week and PCB (Kurokawa et al., 2008). Among these variables PCB was negatively associated with Birth Weight. As for the results of NBAS, only motor cluster in the seven clusters in NBAS was negatively associated with Hg Hair, and Maternal Age, Birth Weight and Fish showed positive associations (Suzuki et al., 2006). Perinatal methylmercury exposure showed a negative but slight effect whereas fish intake showed slight positive one. For BSID at 7-month-old, no association with these variables was observed for MDI and Education was only variable to affect PDI. As for BSID at 18-month-old,

sex difference was observed for MDI and Birth Weight negatively associated with PDI. Although perinatal PCB exposure did not show effects on either NBAS, BSID at 7- and 18-month-old, it affected MPS at 42-month-old. Fish also negatively associated. Parity solely affected AS. Pb showed no association with either developmental outcome above.

It has been suggested that perinatal exposures to methylmercury and PCBs have sporadically shown effects on infant development. But the effects seem temporary ones rather than permanent ones so far. This is especially true for methylmercury. PCB showed a negative association with Birth Weight but did not with other outcomes including NBAS and BSIDs. It is interesting that PCB showed a negative association with MPS in K-ABC which was examined later. We do not know reasons at this moment, though it is anticipated neurodevelopmental effects by PCB exposure may be persistent with a long biological half-life of the substances. We do not have data on PCBs in breast milk. It is considered that postnatal exposure to PCBs *via* milk must be evaluated in addition to perinatal exposure.

It is thought we have to continue the follow-up of infant development more and to carry out chemical analyses to increase the numbers of samples and species of substances to be analyzed. We are now carrying out the follow-up examination at 84-month-old including Wechsler Intelligence Scale for Children (3rd edition), Boston Naming Test, Continuous Performance Test and electrophysiological examinations.

References

- Kurokawa, N., K. Nakai, K. Suzuki, T. Nakamura, K. Sakurai, M. Shimada, T. Ohba, C. Satoh,
S. Kameo, K. Okamura and H. Satoh. (2008) *Organohalogen Compounds*. 70: 2256-2259.
- Nakai, K., K. Suzuki, T. Oka, K. Murata, M. Sakamoto, K. Okamura, T. Hosokawa, T. Sakai, T. Nakamura, Y. Saito, N. Kurokawa, S. Kameo and H. Satoh. (2004) *Tohoku Journal of Experimental Medicine* 202: 227-237.
- Nakamura, T., K. Nakai, T. Matsumura, S. Suzuki, Y. Saito and H. Satoh. (2008) *Science of the Total Environment*. 394: 39-51.
- Ohba, T., N. Kurokawa, K. Nakai, M. Shimada, K. Suzuki, N. Sugawara, S. Kameo, C. Satoh and H. Satoh. (2008) *Tohoku Journal of Experimental Medicine*. 214: 69-78.
- Suzuki, K., K. Nakai, T. Nakamura, T. Hosokawa, K. Okamura, T. Sakai, N. Kurokawa, S. Kameo, K. Murata and H. Satoh. (2006) *Organohalogen Compounds*. 68: 1201-1204.

Methylmercury exposure, seafood intake and health risks in Faroese populations

Anna L. Choi¹, Pal Weihe², Esben Budtz-Jørgensen³, Poul J. Jørgensen⁴, Jukka T. Salonen^{5,6}, Tomi-Pekka Tuomainen⁶, Katsuyuki Murata⁷, Hans Petur Nielsen², Maria Skaalum Petersen⁸, Jórún Askham², Philippe Grandjean^{*1,8}

¹Department of Environmental Health, Harvard School of Public Health, Boston, MA

²Faroese Hospital System, Tórshavn, Faroe Islands

³Institute of Public Health, University of Copenhagen, Copenhagen, Denmark

⁴Department of Clinical Biochemistry, Pharmacology, and Genetics, Odense University Hospital, Odense, Denmark

⁵Oy Jurilab Ltd, Kuopio, Finland

⁶Research Institute of Public Health, School of Public Health and Clinical Nutrition, University of Kuopio, Kuopio, Finland

⁷Department of Environmental Health Sciences, Akita University School of Medicine, Akita, Japan

^{*8}Institute of Public Health, University of Southern Denmark, Odense, Denmark

Methylmercury is a worldwide contaminant found in seafood and freshwater fish and is a well-established developmental neurotoxicant. However, essential nutrients in fish and seafood may provide beneficial effects on brain development. The impact on the same health outcomes by two exposures originating from the same food source provides a classic example of negative confounding. We therefore re-examined our previous findings of developmental neurotoxicity in a Faroese birth cohort and included adjustment for maternal fish intake during pregnancy. In this population, most of the methylmercury intake originates from pilot whale meat, which contains only small amounts of n-3 fatty acids. A weak association existed between mercury exposure and fish intake ($r = 0.23$). Both methylmercury exposure and fish nutrition intake are assessed with some degree of imprecision, which needs to be incorporated in the statistical analysis. When taking fish intake into account, the adverse effects of methylmercury increased. Thus, failure to adjust for the benefits of fish intake caused an underestimation of the true extent of methylmercury neurotoxicity.

Recent evidence has suggested that methylmercury may promote or predispose to the development of heart disease, while n-3 fatty acids from seafood also in this case may counteracting the adverse effects. In a pilot study, we examined 42 Faroese whalingmen (aged 30-70 years) to assess possible adverse effects within a wide range of methylmercury exposures from consumption of pilot whale meat. The average number of fish dinners consumed per week in the last year was included as a covariate that was allowed to affect both mercury exposure and outcomes. Exposure levels were assessed from mercury analysis of toenails and whole

blood obtained at the time of clinical examination, and of a hair sample that had been collected seven years previously. Outcome measures include heart rate variability, blood pressure (BP), common carotid intima-media thickness (IMT), and brainstem auditory evoked potentials. Multiple regression and structural equation analyses were carried out to determine the confounder-adjusted effect of mercury exposure. Taking into account correlations among related measures, exposure and outcomes were categorized in groups to derive latent exposure and response variables in structural equation models. Multiple regression analysis was used to compare the predictive validity of individual exposure biomarkers and the latent exposure variable on individual and latent outcomes. The toenail mercury concentrations varied widely and had a geometric mean of 2.0 µg/g; hair concentrations averaged about 3-fold higher. The strongest associations were seen with BP and IMT, with toenail mercury as the strongest predictor. Adjustment for the benefits from fish consumption resulted in strengthened associations between mercury exposure and increased BP and IMT.

The results of both studies suggest that substantial underestimation occurs and that it affects both the extent of mercury toxicity and the fish-associated benefits, when mutual adjustment is not included in the statistical analysis. The underestimation is augmented by imprecise exposure assessment. Regulatory agencies should reconsider current dietary advisories to provide better guidance to consumers in making prudent choices to maintain a nutritious diet with fish and seafood that are low in mercury concentrations.

References:

- Budtz-Jørgensen E, Grandjean P, Weihe P. Separation of risks and benefits of seafood intake. *Environ Health Perspect* 2007; 115: 323-7.
- Choi AL, Cordier S, Weihe P, Grandjean P. Negative confounding in the evaluation of toxicity: The case of methylmercury in fish and seafood. *Crit Rev Toxicol* 2008; 38: 877-93.
- Choi AL, Weihe P, Budtz-Jørgensen E, Jørgensen PJ, Salonen JT, Tuomainen T-P, Murata K, Nielsen HP, Petersen MS, Askham J, Grandjean P. Methylmercury exposure and adverse cardiovascular effects in Faroese whalingmen. *Environ Health Perspect* (in press).

**Maternal fish intake during pregnancy, blood mercury levels,
and child cognition at age 3 years in a US cohort**

**Emily Oken¹, Jenny S. Radesky¹, Robert O. Wright^{*2}, David C. Bellinger³,
Chitra J. Amarasiriwardena⁴, Ken P. Kleinman¹,
Howard Hu⁵ and Matthew W. Gillman^{1,6}**

¹ Department of Ambulatory Care and Prevention, Harvard Medical School and Harvard Pilgrim Health Care, Boston, MA

^{*2} Department of Medicine, Children's Hospital Boston and Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

³ Department of Neurology, Boston Children's Hospital and Harvard Medical School, Boston, MA

⁴ Department of Environmental Health, Harvard School of Public Health, Boston, MA

⁵ Department of Environmental Health, University of Michigan, Ann Arbor, MI

⁶ Department of Nutrition, Harvard School of Public Health, Boston, MA

Correspondence to Dr. Emily Oken, Department of Ambulatory Care and Prevention, 133 Brookline Avenue, Boston, MA 02215 (e-mail: emily_oken@harvardpilgrim.org).

The balance of contaminant risk and nutritional benefit from maternal prenatal fish consumption for child cognitive development is not known. Using data from a prospective cohort study of 341 mother-child pairs in Massachusetts enrolled in 1999–2002, the authors studied associations of maternal second-trimester fish intake and erythrocyte mercury levels with children's scores on the Peabody Picture Vocabulary Test (PPVT) and Wide Range Assessment of Visual Motor Abilities (WRAVMA) at age 3 years. Mean maternal total fish intake was 1.5 (standard deviation, 1.4) servings/week, and 40 (12%) mothers consumed >2 servings/week. Mean maternal mercury level was 3.8 (standard deviation, 3.8) ng/g. After adjustment using multivariable linear regression, higher fish intake was associated with better child cognitive test performance, and higher mercury levels with poorer test scores. Associations strengthened with inclusion of both fish and mercury: effect estimates for fish intake of >2 servings/week versus never were 2.2 (95% confidence interval (CI): –2.6, 7.0) for the PPVT and 6.4 (95% CI: 2.0, 10.8) for the WRAVMA; for mercury in the top decile, they were –4.5 (95% CI: –8.5, –0.4) for the PPVT and –4.6 (95% CI: –8.3, –0.9) for the WRAVMA. Fish consumption of ≤2 servings/week was not associated with a benefit. Dietary recommendations for pregnant women should incorporate the nutritional benefits as well as the risks of fish intake. Abbreviations: CI, confidence interval; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PPVT, Peabody Picture Vocabulary Test; SD, standard deviation; WRAVMA, Wide Range Assessment of Visual Motor Abilities

Omega-3 fatty acids and methylmercury in diet: sources, effects, and public health considerations

Kathryn R. Mahaffey^a

^aProfessorial Lecturer, Department of Occupational and Environmental Health, George Washington University School of Public Health, Washington D.C. USA

Abstract

Fish and shellfish contain both methylmercury, a neurotoxin that at high exposures produces devastating effects on the developing nervous system. Fish and shellfish also contain a group of chemicals, called n-3 polyunsaturated fatty acids (n-3 PUFAs) or omega-3 fatty acids. Specific n-3 PUFAs are important structural components of the developing nervous system. Human can synthesize these from precursors, but it is not clear if the rate of synthesis is rapid enough to meet the needs of the developing fetal nervous system. Compared with males, females (especially during pregnancy), appear to have a higher rate of synthesis of two key omega-3 fatty acids: Eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA). These are synthesized from other dietary fatty acids. It is not clear that eating fish in amounts that provide more omega-3 fatty acids than approximately 2000 milligrams/day of EPA and DHA offers any benefits. The limit of intake providing benefits may be much lower.

Some studies of child development suggest that if methylmercury exposure from fish and shellfish is low enough, EPA and DHA provided in fish and shellfish may be protective against the adverse effects of methylmercury on the developing nervous system. The evidence for this is very limited. Fortunately the quantities of EPA and DHA in specific fish and shellfish species vary a lot. Some kinds of fish have more than 10-times the amount of EPA and DHA than do other kinds of fish. For methylmercury the amount of mercury in specific fish species can differ by 20-to-30 fold. Fortunately the fish that are highest in omega-3 fatty acids are not necessarily high in mercury. Unfortunately some fish that are high in methylmercury do not contain very much of the omega-3 fatty acids. Knowing which fish and shellfish have the most health protective ratios of omega-3 fatty acids and are the lowest in methylmercury will help people chose fish that are the most protective of human health.

Keywords: methylmercury, heavy metals, omega-3 fatty acids, n-3 polyunsaturated fatty acids, eicosapentaenoic acid, EPA, decosapentaenoic acid, DHA, fish, blood mercury, hair mercury, diet.

1. Introduction

The foods that we consume contain some chemicals that are nutrients required by the human body (for example, vitamins, proteins, fats) and other chemicals that are toxic (such as dioxins, heavy metals, polychlorinated biphenyls). Although a nutrient is required in the diet, this same chemical can produce toxicity if consumed in quantities higher than needed for good nutrition. The nutrients if consumed in high doses may be toxic in our diets; examples of nutrients that can also be toxic if consumed at too high a level are vitamin A, vitamin D, and nearly all of the nutritionally required metals such as magnesium and copper. Consequently a public health goal is to identify diets that provide needed nutrients in a balance that is optimal for health, while avoiding chemicals that are known to be toxic (for example, methylmercury and lead).

Exposure to methylmercury through consumption of fish and shellfish varies widely throughout the world (for review articles see Pirrone and Mahaffey, 2005). Based on analyses of blood mercury concentrations or hair mercury levels, populations living in coastal regions and on islands are recognized to have higher exposures to methylmercury than do people living further inland (Mahaffey et al., 2009). These exposures to methylmercury are associated with the type and quantity of fish consumed. A number of reports of mercury exposure in the Pacific region (Chateau-Degat, 2005; Dewailley et al., 2008; Kumar et al., 2006) and Asia have been published in recent years (among others see Fok et al., 2007; Kim et al., 2008; Hsu et al., 2007; Ip et al., 2004; Murata et al., 2007; Sakamoto et al., 2007).

A number of recommendations have been made on how to choose fish and shellfish species that provide higher quantities of omega-3 fatty acids and relatively low quantities of methylmercury (Mahaffey, 2004; Domingo, 2007; Burger, 2008; Burger and Gochfeld, 2009, Ginsberg and Toal, 2009).

2. General Information on Omega-3 Fatty Acids

Fatty acids are chemicals that contain carbon, oxygen, and hydrogen in repeating groups of $-(CH_2)_n-$ that contain a methyl (CH_3) and a carboxyl ($-COOH$) group. *N-3* fatty acids are highly unsaturated (multiple double bonds) with one of the double bonds located three carbon atoms from the methyl end. There are a large number of fatty acids found in nature (Kainz et al., 2006). *N-3* polyunsaturated fatty acids (*n-3* PUFAs) particularly important in human nutrition (NAS/NRC, 2005) include:

- 18:3 α -Linolenic acid
- 20:5 Eicosapentaenoic acid (EPA)
- 22:5 Docosapentaenoic acid (DPA)

22:6 Docosahexaenoic acid (DHA)

Humans can synthesize many fatty acids from other energy sources such as carbohydrates, but do not have the enzymes needed to place a double bond at certain positions in the structure of some fatty acids. Humans cannot synthesize α -linolenic acid and require a dietary source for this chemical. α -linolenic acid is the precursor for the synthesis of EPA and DHA (NAS/NRC, 2005) fatty acids with double bonds at the third (n-3) position. Fatty acids with a double bond between the third and fourth carbons are called n-3 polyunsaturated fatty acids (n-3 PUFAs) or are called omega-3 fatty acids. These are important because they are a part of structural membrane lipids, particularly in nerve tissue and the retina. The two fatty acids considered of especially importance in human neuro-development are EPA (20 carbons in length with five double bonds including one at n-3) abbreviated EPA, and DHA (22 carbons in length with six double bones including one at n-3).

Humans can synthesize omega-3 fatty acids from the chemical precursor, α -linolenic acid, which is required in the diet (Burdge and Calder, 2005). Women appear to be able to convert the dietary component α -linolenic acid (ALA) to the long-chained fatty acids more readily than can men (Burdge and Calder, 2005; Williams and Burdge, 2006; Extier et al., 2009; Bourre, 2007). Too little α -linolenic acid in the diet causes clinical deficiency. The capacity of humans to synthesize EPA and DHA from α -linolenic acid is (1) organ specific (Igarashi et al., 2007), (2) varies substantially from person to person (German et al., 2003), (3) likely affected by conditions of disease (for example, liver toxicity), and (4) is associated with exposure to environmental contaminants, drugs, and other nutrients (Rapoport et al., 2007; Rapoport, 2008). The human body can synthesize EPA and DHA from α -linolenic acid (see review by Brenna, 2002). However, it is not yet known if humans can synthesize DHA at a rate fast enough to provide an optimal amount of this chemical to meet the demands of the developing fetal brain or provide optimal levels of omega-3 fatty acids to protect the heart. Half-lives of DHA in critical tissues, including brain, vary with the adequacy of the dietary supply (DeMar et al., 2004). Wide person-to-person variability exists, especially among term and preterm infants, in the ability to synthesize DHA (Brenna, 2002). This is an area of research at the National Institutes of Health in the United States, as well as other parts of the world.

The fetus may be limited in the ability to form adequate DHA from precursors (Brenna, 2002) and needs to receive pre-formed DHA through placental transfer from the mother (Spector, 2001). This is a complex topic in which research is continuing. There is not a one-for-one increase in fetal DHA as the mother's DHA level is increased (Brenna, 2002; Haggerty, 2004). Long-term DHA status of the mother can be reflected by her body stores of DHA, which may be

mobilized during pregnancy (Haggarty, 2004; Hanebutt et al., 2008). There may be placental regulation of the level of mobilization of maternal adipose tissue (Haggarty, 2002; Stewart et al., 2007).

DHA is important for fetal development because it is incorporated into membranes in the nervous system, especially including the retina (see the review by Innis, 2004). Postnatally, addition of polyunsaturated fatty acids to formula has been shown to improve visual acuity in some, but not all, studies. There are many studies of the effects of post-natal supplementation, but relatively few studies of added DHA during pregnancy. The results of such studies are mixed, with some showing benefits and others showing no benefit on child development.

One of the ways in which conflicting studies are assessed is through reviews with carefully defined procedures for assessing the quality of studies. One of these review systems is the Cochrane Database Systematic Reviews. Two Cochrane reviews (Simmer et al., 2008a, Simmer et al., 2008b) of n-3 PUFAs have looked at the effect of supplementing infant formula with DHA on child development. Simmers et al. (2008a) have concluded on the basis of healthy preterm infants enrolled in eleven randomized trials (Simmer et al., 2008a). When the results were pooled there were no clear long-term benefits for preterm infants receiving formula supplemented with long-chained n-3 PUFAs (Simmer et al., 2008a), as well as no long-term evidence that formula supplemented with n-3 PUFAs and n-6 long-chained PUFAs impaired growth of preterm infants. In an additional review of the data on supplementation in infants born at term (Simmer et al., 2008b), analysis of eleven good quality, randomized studies showed inconsistent results. A meta-analysis of the data showed that the studies did not show beneficial effects of long-chained PUFA supplementation of formula milk on the physical, visual, and neurodevelopmental outcomes of infants born at term (Simmer et al., 2008b).

3. Levels of Pre-formed EPA and DHA in Human Diets

There are many recommendations for inclusion of pre-formed EPA and DHA in the human diet (Bazzard et al., 1994; Simopoulos, 2009). It should be noted that dietary DHA results in a dose-dependent, saturable increase in plasma DHA concentration with modest increases in EPA concentrations. DHA doses of approximately 2,000 milligrams/day result in near maximal increases in plasma DHA (Arterburn et al., 2006).

There is considerable diversity in the amount of pre-formed EPA and/or DHA that is thought to be needed in the diet. Different countries recommend different quantities of the n-3 fatty acids. Expert groups' recommendations range from 100 milligrams/day to 1600 milligrams/day of EPA and DHA (summary statement by Gao et al., *in press*). Typical recommendations either

maintain the status quo, about 100 milligrams to 200 milligrams per day in most western countries, or are intended to actively reduce risk of cardiovascular disease (Harris, 2007). If the recommendations are aimed at prevention of cardiovascular disease, the recommended dietary intake of preformed EPA and DHA is about 400 milligrams to 600 milligrams/day (Harris, 2007). In the United States the usual level of intake is 1600 milligrams/day for men and 1100 milligrams/day for women. In addition, the Food and Nutrition Board of the National Research Council in the United States (NAS/NRC, 2002/2005) has also developed the Acceptable Macronutrient Distribution Range (AMDR) based on evidence from intervention trials with the support of epidemiological evidence suggesting that these levels of intake may have a role in prevention of chronic disease. The AMDR for the omega-3 fatty acids is 0.6 to 1.2 percent of energy intake.

4.Sources of Pre-formed EPA and DHA in Human Diets

Non-fish sources of pre-formed EPA and DHA are becoming more frequent and are important for vegetarians (David and Kris-Etherson, 2003). These include: algae, enriched eggs and milk, additional enriched products not traditionally considered sources of the omega-3 fatty acids, and food supplements. such as fish oil (Barcel-Coblijn et al., 2008; Strijbosch et al., 2008;).

Algae have a lot of capacity for synthesis of the omega-3 fatty acids (Bhaskar et al., 2004) and are responsible for synthesis of more than half the production of omega-3 fatty acids at the base of the food chain (Harwood and Guschina, 2008). Macro algae have the enzyme systems to synthesize EPA and DHA (Polat and Ozogul, 2007). In addition, biotechnology has been used to produce micro algae that are rich in EPA and DHA (see www.Martek.com). Many of the food supplements and foods enriched with omega-3 fatty acids rely on specialized micro-algae as the source of omega-3 fatty acids (Doughman et al., 2007). DHA from algal-oil capsules has been shown to have the same bioavailability as DHA from cooked salmon (Arterburn et al., 2008).

Additional important non-fish food sources of omega-3 fatty acids include eggs (eggs from wild or free-range chickens, and special “omega” eggs) (Butarbutar, 2004), food products enriched with specific omega fatty acids [for example, more than 100 food products in USA have DHA added (see www.Martek.com) including milk (Or-Rashid et al., 2009)], and fish oils (Sargent and Tacon, 1999). Studies with subjects have shown that the omega-3 fatty acids from these sources are absorbed and utilized by humans (Arterburn et al., 2008). Omega-3 fatty acids from hen eggs can be substantially increased by feeding chickens special diets (Herber and Van Elswyk, 1996; Meyer et al., 2003). Hens fed these diets produce eggs containing between 100

milligrams to more than 500 milligrams of the omega-3 fatty acids per 50 gram egg. It is also known that free-range or non-caged chickens often obtain diets that increase the omega-3 content of their eggs. Consequently specialized chicken-raising procedures (e.g., feeding poultry specialized diets) are not necessarily required to increase the omega-3 content of the eggs. Poultry meat may also be a source of omega-3 fatty acids (Simonopoulos, 2000).

5. Fish and Shellfish

Globally, the most commonly consumed sources of omega-3 fatty acids are fish, shellfish, and marine mammals which obtain them from algae that can make these fatty acids. The quantity of omega-3 fatty acids in fish varies greatly between fish species (Mahaffey et al., 2004; NRC-US, 2006, Weaver et al., 2008; Chung et al., 2008; Ozogul et al., 2008; Osman et al., 2007). Estimated intakes of omega-3s (EPA and DHA combined) from individual fish/shellfish species vary greatly in the amounts of both fatty acids (Sidhu, 2003; Mahaffey, 2004; NAS/NRC, 2006; Domingo, 2007). The fat content of specific fish species is moderately predictive of the omega-3 content with certain fatty fish species (especially salmon, mackerel, sardines, herring) being high in omega-3 fatty acids. However, other species that are low in fat (especially shrimp and trout) are also good sources of omega-3 fatty acids. Table 1 provides a summary of the omega-3 levels in various fish species.

Weaver et al., (2008) evaluated the species most commonly consumed in North America and grouped species into those providing n-3 fatty acids in amounts of more than 500 milligrams, between 150 and 500 milligrams, or less than 150 milligrams per 100 grams of fish consumed. Fish species that provided the highest levels of n-3 fatty acids were sockeye salmon, farmed trout, farmed salmon, Copper River salmon, Coho salmon, brozini, and toothfish. Species providing the lowest amounts of n-3 fatty acids were mahi-mahi, skate, triggerfish, monkfish, red snapper, wahoo, grouper, corvina, and tuna. Species intermediate in n-3 fatty acids are: haddock, cod, halibut, sole, flounder, crustacea, perch, black bass, tilapia, and swordfish. These groups reflect only their omega-3 fatty acid content, not the mercury concentrations present.

The Japanese diet is relatively low in fat with an average intake of approximately 60 grams/day or 26% of energy (Sugano and Hirahara, 2000). The intake of n-3 polyunsaturated fatty acids (PUFAs) is reported to be approximately 3,000 milligrams /day/capita (Sugano and Hirahara, 2000). In Japan fish, shellfish, and edible fats and oils are the most important source of n-3 PUFAs. The species providing the most PUFAs are horse mackerels, sardines, and tuna (Sugano and Hirahara, 2000). These investigators indicate that fish intake in Japan has declined

especially among younger people. They also indicate that fish from cultured or farmed sources is increasing whereas consumption of wild fish is decreasing.

6. Methylmercury and Child Development

High exposures to methylmercury have produced severe damage to the fetal nervous system (Tsubaki and Irukyama, 1977; Bakir et al., 1973). In addition to the effects of exposures to high-levels of methylmercury, a number of prospective epidemiological studies of lower exposures to methylmercury have been conducted in a number of countries including the Seychelle Islands, the Faroe Islands, New Zealand, the United States, Italy, and Japan.

The studies from the Faroe Islands, the Seychelle Islands and New Zealand were used by the United States National Academy of Sciences (2000) and United States Environmental Protection Agency (Rice et al., 2001) to develop a Reference Dose for methylmercury. This dose is 0.1 microgram per kilogram body weight per day. There have been additional evaluations of the amounts of methylmercury that produce adverse health effects have been developed by various organizations, including the World Health Organization/Food and Agriculture Organization (these were summarized by Mergler et al., 2007).

The major differences between various government assessments are not their estimates of the amount of methylmercury producing adverse effects. The differences are mainly in the amount of exposure considered to be without adverse effects. The magnitude of the factor used to extrapolate from exposures known to produce effects to exposures thought to be safe for a country varies from one assessment of the risks of methylmercury to another. Components of these factors are differences in the distribution of methylmercury within the body and differences in susceptibility of individuals to methylmercury. These factors influencing the distribution of methylmercury within body tissues and susceptibility of various organs to methylmercury include genetic differences and dietary factors.

7. Association between Omega-3 Fatty Acids and Methylmercury Exposure

Among the reports on effects of diet on child development are studies that have used general measures of fish consumption (for example, Daniels et al., 2004; Hibbeln et al., 2007) or include direct measurements of methylmercury exposure, as well as estimates of fish consumption (Daniels et al., 2004; Oken et al., 2008; Jedrychowski et al., 2006 and 2007; Lederman et al., 2008; Davidson et al., 2008). Some of these reports indicate that methylmercury exposure shows adverse effects on child development (e.g., Oken et al., 2008; Lederman et al., 2008) and that increases in fish consumption benefit indices of child development (e.g., Daniels et al.,

2004; Oken et al., 2008; Lederman et al., 2008). Increases in the association between mercury exposure and poorer scores on indices of child development were observed after adjustment for fish intake (e.g., Oken et al., 2008; Lederman et al., 2008). It is important to note that the exposures to mercury studied by Oken et al., (2008) and by Lederman et al. (2008) were at the relatively low end of mercury exposures when compared to populations who routinely consume fish as a major part of their diet.

8. Complexity of the Association between Methylmercury Exposure and Dietary Sources of the Omega-3 Fatty Acids

Both omega-3 fatty acids and methylmercury are contained in fish and shellfish. However, fish species contain widely different quantities of omega-3 fatty acids and methylmercury. Some fish species are high in omega-3 fatty acids and others are high in methylmercury (Mahaffey, 2004; Sakomoto et al., 2004; Philibert et al., 2006; Mahaffey et al., 2008; Domingo, 2007). The amount of methylmercury in fish increases with the trophic level of the fish/shellfish species. By contrast, the pattern of omega-3 fatty acids is not dependent on the trophic level of the fish species. For example, Kainz et al. (2006) demonstrated that although methylmercury concentrations increase steadily with body size in both plankton and fish, the essential fatty acids show irregular retention patterns at different trophic levels. Although both EPA and methylmercury generally increase at higher trophic levels in the planktonic food web, the magnitude of bioaccumulation is much greater for methylmercury compared with EPA. This is because aquatic organisms retain the essential fatty acids at lower rates than they retain methylmercury (Kainz et al., 2006).

The relationship differs for DHA and methylmercury accumulation. Although EPA and methylmercury increase along the planktonic food web, methylmercury bioaccumulates at higher rates than does EPA (Kainz et al., 2006). By contrast, DHA primarily found in phospholipids of cell membranes, is retained differently than EPA with respect to methylmercury (Kainz et al., 2006). Retention of dietary DHA is determined by the taxonomic composition of the planktonic food web and independent of DHA requirements. This variability in comparative rates of bioaccumulation for methylmercury, for EPA, and for DHA, helps to explain divergent patterns of these chemicals in individual fish species.

Data on the association between blood mercury concentration and fish consumption also help to understand food sources of omega-3 fatty acids and methylmercury. As an example, data from the United States evaluating blood mercury concentrations among women of child-bearing age showed that there was a moderate correlation between total fish intake and blood organic

mercury concentration (Mahaffey et al., 2008). There is not a consistent association between the quantity of methylmercury in a particular fish and/or shellfish species and the amount of omega-3 fatty acids present in the fish or shellfish species (Mahaffey, 2004; US NAS/NRC, 2006). The ratio of omega-3 fatty acids to methylmercury intake depended on the species of fish consumed (Mahaffey et al., 2008).

Many of the studies of child-development have relied on frequency of fish consumption to estimate (1) total fish intake or (2) fish intake by category such as fatty fish. One of the limitations of this approach is that the size of the fish serving may be small (e.g., 30 grams) or medium (e.g., 180 to 250 grams) making it difficult to quantitate exposures. Another limitation is that many of these studies have not reported which species of fish are eaten by the people participating in the study. The limited information in such studies of child-development makes it difficult to know the intake of omega-3 fatty acids or methylmercury. Absent biological monitoring of either exposure (e.g., blood or hair mercury concentrations), and blood levels of the omega-3 fatty acids, it is hard to draw conclusions based on estimates of total fish intake rather than intake of specific fish species.

Constituents of fish may attenuate the adverse effects of methylmercury in an exposure range that is yet to be clarified. There is also the issue of negative confounding in the evaluation of methylmercury toxicity (Budtz-Jergensen et al., 2007; Choi et al., 2008). The risks of methylmercury neurotoxicity and the benefits of fish consumption can be underestimated if there are inadequate confounder adjustment and imprecision of exposure estimates (Choi et al., 2008).

9. Conclusions

Much more information is needed on quantities of the omega-3 fatty acids (EPA, and especially DHA) that can be synthesized from α -linolenic acid through women's metabolism. Although there have been several studies of the association between fish intake and child development, a number of the recent studies have been carried out at exposure levels that are relatively low compared with cultures that routinely rely on fish as a major dietary staple. Nonetheless, several of the studies show effects associated with methylmercury exposure as well as benefits of fish consumption.

Fortunately, the ratio of omega-3 fatty acids and methylmercury concentration within individual fish and shellfish species varies considerably (Mahaffey, 2004; NAS/NRC, 2006; Domingo, 2007). It is possible to choose fish species that are both high in omega-3 fatty acids and low in mercury. Although methylmercury exposure is closely linked with the amount and

species of fish eaten, there are non-fish sources of the omega-3 fatty acids. These include: algae, marine oils, products enriched with specific omega-3 fatty acids, and various meat and poultry products from animals fed diets that provide nutrients from diverse sources. None of these sources have high levels of methylmercury.

Fish and shellfish provide most of the preformed omega-3 fatty acids in the diet for most countries. A diet that enough omega-3 fatty acids, but avoids methylmercury from fish remains a goal. The solution requires dietary strategies, public health communication, and pollution control.

References

- Arterburn LM, Hall EB, Oken H. 2006. *Am J Clin Nutr* 83(suppl):1467S-1476S.
- Arterburn LM, Oken HA, Hoffman JP, et al. 2007. *Lipids* 42:1011-24.
- Arterburn LM, Oken HA, Hall EB, et al. 2008. *J Am Diet Assoc* 108:1204-1209.
- Bakir, F., Damluji, SF, Amin-Zaki I, et al. 1973. *Science* 180:230-241.
- Barcel-Coblijn G, Murphy EJ, Othman R, et al. 2008. *Am J Clin Nutr* 88:801-809.
- Béazard J, Blond JP, Bernard A, Clouet P. 1994. *Reprod Nutr Dev* 34:539-68.
- Bhaskar N, Kinami T, Miyashita K, et al. 2004. *Z Naturforsch [C]* 59:310-314.
- Bourre JM. 2007. *Biomedicine Pharmacothera* 61:105-112.
- Brenna JT. *Curr Opin Clin Nutr Metab Care* 5:127-132.
- Budtz-Jergensen E, Grandjean P, Weihe P. 2007. *Environ Health Perspect* 115:323-327.
- Burdge GC, Calder PC. 2005. *Reprod Nutr Dev* 45:581-597.
- Burger J. 2008. *Environ Res* 108:107-116.
- Burger J, Gochfeld M. 2009. *Environ Res*, in press.
- Butarbutar TB. *Southeast Asian J Trop Med Pub Health* 35:1036-1038.
- Chateau-Degat ML. 2005. Neurological signs of ciguatera disease: evidence of their persistency. In: *Portrait Epidémiologique de la Ciguatera dans le Pacifique-sud (PhD Thesis)*. Quebec, Canada: Laval University.
- Choi AL, Cordier S, Weihe P, Grandjean P. 2008. *Crit Rev Toxicol* 38:877-893.
- Chung H, Nettleton JA, Lemaitre RN, et al., 2008. *J Nutr* 138:2422-2427.
- Daniels JL, Longnecker MP, Rowland AS, et al. 2004. *Epidemiology* 15: 3394-402.
- Davidson PW, Strain JJ, Myers GJ et al. 2008. *NeuroToxicology* 29:767-775.
- Davis BC, Kris-Etherton. 2003. *Am J Clin Nutr* 78 (suppl): 640S-646S.

- DeMar JC Jr, Ma K, Bell JM, Rapoport SI. 2004. *J Neurochem* 91:1125-1137.
- Dewailley E, Suhas E, Mou Y, et al. 2008. *Asia Pac J Clin Nutr* 17:461-470.
- Domingo JL. 2007. *Environment Internatl* 33:993-998.
- Doughman SD, Krupanidhi S, Sanjeevi CB. 2007. *Curr Diabetes Rev* 3:198-203.
- Extier A, Langelier B, Perruchot MH, et al. 2009. *J Nutr Biochem* in press.
- Fok TF, Lam HS, Hg PC, et al. 2007. *Environ Int* 33:84-92.
- Gao F, Kiesewetter D, Chang L, et al. In press.
- German JB, Roberts MA, Watkins SM. 2003. *J Nutr* 133:2078S-2083S.
- Ginsberg GL, Toal BF. 2009. *Environ Health Perspect* 117:267-275.
- Haggerty P. 2002. *Placenta* 23 Supp A:528-538.
- Haggerty P. 2004. *Eur J Clin Nutr* 58:1559-1570.
- Hanebutt FL, Demmelair H, Schiessl B, et al. 2008 *Clin Nutr* 27:685-693.
- Harris WS. 2007. *J Cardiovasc Med (Hagerstown)* 8 (suppl 1):550-552.
- Harwood JL, Guschina IA. 2009. *Biochimie*. In press.
- Hibbeln JR, David JM, Steer C, et al. 2007. *Lancet* 369(9561):578-585.
- Hsu CS, Liu PL, Chen LC, et al. 2007. *BJOG* 114: 81-85.
- Igarashi M, Ma K, Chang L, et al. 2007. *J Lipid Res* 48:2463-2470.
- Igarashi M, DeMar JC, Ma Kaizong, et al. 2007. *J Lipid Res* 48:1150-1158.
- Innis SM. 2004. *Adv Exp Med Biol* 554:27-43.
- Ip P, Wong V, Ho M, et al. 2004. *Pediatr Int*. 46:715-721.
- Jedrychowski W, Jankowski J, Flak E, et al. 2006. *Ann Epidemiol* 16:439-447.
- Kainz M, Telmer K, Mazumder A. 2006. *Sci Total Environ* 368:271-282.
- Kim SA, Jeon CK, Paek DM. 2009. *Sci Total Environ* 402:36-42.
- Kumar M, Aalsberg B, Mosley L. 2006. Mercury levels in Fijian seafood and potential health implications. Report for the World Health Organization. Suva, Fiji. Fiji Islands Institute of Applied Sciences.
- Lederman SA, Jones RL, Caldwell KL. et al. 2008. *Environ Health Perspect* 116:1085-1091.
- Mahaffey KR. 2004. *Environ Res* 95:414-428.
- Mahaffey KR, Clickner RP, Jeffries RA. 2008. *Environ Res* 107:20-29.
- Mahaffey KR, Clickner RP, Jeffries RA. 2009. *Environ Health Perspect* 117:47-53.
- Mergler D, Anderson HA, Chan, et al., 2007 *Ambio* 36:3-11.
- Moghadasian MH. 2008. *Crit Rev Food Sci Nutr* 48:402-410.
- Murata K, Dakeishi M, Shimada M Satoh H. 2007. *Tohoku J Exp Med* 213:187-202.
- Nakai K, Suzuki K, Oka T, et al.. 2004. *Tohoku J Exp Med* 202:227-237.

- National Academy of Sciences/National Research Council. Committee on Toxicological Effects of Methylmercury. 2000. *Effects of Methylmercury*. National Academy Press, Washington D.C.
- National Academy of Sciences/National Research Council. 2005. *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients)*. National Academy Press, Washington D.C.
- National Academy of Sciences/National Research Council. 2006. *Seafood Choices: Balancing Benefits and Risks*. National Academy Press. Washington D.C.
- Oken E, Radesky JS, Wright RO, et al., 2008. 167:1171-1181.
- Osman F, Jaswir I, Khaza'ai H, Hashim R. 2007. *J Oleo Sci* 56:107-113.
- Or-Rashid MM, Odongo NE, Wright TC, McBride BW. 2009. *J Agric Food Chem*, in press.
- Ozogul Y, Ozogul F, Cicek E, et al. 2008. *Int J Food Sci Nutr* 29:1-12.
- Philibert A, Vanier C, Abdelouahab, et al. 2006. *Am J Clin Nutr* 84:1299-1307.
- Pirrone N, Mahaffey KR. (Editors) *Dynamics of Mercury Pollution on Regional and Global Scales*. Springer Press. 2005.
- Polat S, Ozogul Y. 2007. *Int J Food Sci Nutr* 12:1-7.
- Raccine R, Deckelbaum. 2007. *Curr Opin Clin Nutr Metab Care* 10:123-128.
- Rapoport SI, Rao JS, Igarashi M. 2007. *Prostaglandins Leukot Essent Fatty Acids*. 77:251-261.
- Rapoport SI. 2008. *Prostaglandins Leukot Essent Fatty Acids* 79:153-156.
- Rapoport SI, Rao J, Igarashi M. 2007. *Prostaglandins Leukotrienes Essent Fatty Acids* 77:251-261.
- Rice DC, Schoeny R, Mahaffey K. 2003. *Risk Anal*. 23: 107- .
- Sargent JR, Tacon AG. 1999. *Proc Nutr Soc* 58:377-383.
- Sidhu KS. 2003. *Reg Toxicol Pharmacol* 38:336-344.
- Simmer K, Schulzke SM, Patoie S. 2008a. *Cochrane Database Syst Rev* 23: CD000375.
- Simmer K, Patole SK, Rao SC. 2008 b. *Cochrane Database Syst Rev* 23:CD000376.
- Simopoulos AP. 2000. *Poultry Sci* 79:961-970.
- Simopoulos AP. 2009. *World Rev Nutr Diet* 99:1-16.
- Spector AA. 2001. *J Mol Neurosci* 16:159-165.
- Stewart F, Rodie VA, Ramsay JE, et al. 2007. *Lipids* 42:335-344.
- Strijbosch RA, Lee S, Arsenault DA, et al. 2008. *Metabolism* 57:698-707.
- Sugano M, Hirahara F. 2000. *Is J Clin Nutr* 71(suppl):189S-196S.
- Wakai K. 2009. *J Epidemiol* 19:1-11.
- Weaver KL, Ivester P, Chilton JA, et al. 2008. *J Am Diet Assoc* 108:1178-1185.
- Tsubaki T, Inkyama K (Eds.), 1977. Minamata Disease. Elsevier Press. Amsterdam.

Table 1.

Concentrations of the Omega-3 Fatty Acids (Eicosapentaenoic and Docosahexaenoic Acid) in Multiple Fish and Shellfish (from Mahaffey, 2004).

Species	EPA + DHA milligrams/100 grams of fish	Species	EPA + DHA milligrams/100 grams of fish
Mackerel	1790	Oysters	350
Salmon	1590	Mussels	350
Sardines	980	Other shellfish	310
Bass	640	Perch	300
Tuna	630	Catfish	280
Trout	580	Scallops	270
Swordfish	580	Pollock	260
Walleye	530	Clams	240
Seabass	490	Cod	240
Shrimp	390	Shark	220
Crayfish	380	Haddock	180
Lobster	360	Pike	140

Potential contamination of mercury from artisanal gold mining in the Talawaan watershed area, north Sulawesi, Indonesia

Markus T. Lasut^{a*} & Yoshiaki Yasuda^b

^a Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado, North Sulawesi, Indonesia

^b Natural Sciences Section, National Institute for Minamata Disease, Minamata City, Kumamoto, Japan

Abstract

Mercury (Hg) discharge through the artisanal gold mining activities is one of the environmental concerns due to its emission that may follow natural methylation of mercury to result in environmental contamination with an impact on human health. To assess the potential impact of Hg emitted from such activities, sampling was carried out in a watershed area of Talawaan, north Sulawesi, Indonesia, where that type of mining is widely occurred. Sediment and biota were collected in the riverine system, while human hairs were collected from the residents living in the remote area. Total mercury (THg) and methyl mercury (MeHg) were measured in each of the samples. The results showed that THg and MeHg were found in all samples. In sediments, the THg concentration mostly decreased with distance from the mining area. The ratio between THg-MeHg in sediment was 0.0035. Accordingly, since there is no anthropogenic source of MeHg in the area, the methylation process of Hg occurred along the riverine system. Methyl mercury was found in the human hair samples in various levels higher than the control value. Since the residents consumed fish from the river daily, mercury impact may potentially occur.

Keywords: methyl mercury, artisanal gold mining, north Sulawesi, Indonesia

1. Introduction

Artisanal gold mining activity in small-scale is mostly conducted by communities with the primitive techniques and equipments to recover gold. It spreads widely in developing countries (Ogola et al., 2002; de Lacerda, 2003), including Indonesia, such as in north Sulawesi (James, 1994; Kambey et al., 2001; Limbong et al., 2003). However, in contradiction, as it practically uses an amalgam technique using Hg to recover gold, it is one of the major environmental concerns of Hg sources (de Lacerda and Salomons, 1998; Kambey et al., 2001; Ogola et al., 2002; de Lacerda, 2003; Limbong et al., 2003). In north Sulawesi, approximately 200 tons of

Hg is used annually in regard to this purpose (Kambey et al., 2001). Forty to 50% of the Hg used in amalgamation is estimated to be lost into rivers during the process as metallic Hg, and other (5 to 10%) Hg is lost into environment during the recovery of gold from Hg amalgam. Further estimation is approximately 1.32 kg loss of Hg for 1 kg gold production (Pfeiffer and Lacerda, 1988 cf de Lacerda and Salomons, 1998).

The objective of the present study is to assess potential contamination of Hg emitted from an artisanal gold mining area to the water-receiving area of Talawaan watershed by analyzing present concentrations and spatial distribution of total Hg (THg) and methyl Hg (MeHg) in sediments and biota, including in human scalp hairs, then discussing potential impacts on human health.

2. Subjects and Methods

Talawaan watershed covers an area of 34,000 ha. In 2004, artisanal gold mining activities cover an area of \pm 822 ha at upper land area of the watershed in 8 villages (Wasian, Tatelu, Tatelu Rondor, Tatelu Warukapas, Talawaan, Kolongan, Tetey, and Mapanget) of north Sulawesi, in which about 2500 to 3000 miners involved (Lingkubi, 2004). The main river of Talawaan (TR), and the other two rivers [Kima (KR) and Bailang (BR)], are flowing through the watershed from the upper land area (artisanal gold mining area) to the beach. The rivers are used by the communities for daily activities (bathing, washing, and fishery).

Sampling was carried out along the three rivers (TR, KR, and BR). Stations were set at each of the rivers based on the distance from the mining area. Sediment and biota (snails, crabs, and fish) were collected at each station, while human hairs were collected from the residents living in an area remote but closely related to the riverine system of TR. Total mercury (THg) and methyl mercury (MeHg) were measured in each of the samples. While THg was measured using mercury analyzer, the MeHg was measured by a gas chromatograph with ECD detector after extracted by dithizone-sodium sulfide extraction method.

3. Results and Discussion

THg was detected in all samples that varied within sampling sites and sample types. The concentrations in the TR sediment varied following the sites from 0.078 to 1.137 ppm. The highest concentration was at the mining area and the lowest was at the river mouth. The mercury concentration decreased with distance from the mining area. The same pattern was also observed in the KR where the highest concentration (0.099 ppm) was found at the sites close to the mining area and the lowest one (0.022 ppm) was at the river mouth. In BR, the situation was somewhat different that the highest THg concentration (0.176 ppm) was found in

the several hundred meters inside area of the river mouth with a thick precipitation of sediment and the lowest (0.009 ppm) was at just the river mouth.

The variation of the THg concentration in sediment showed in the sampling area was due mainly to an introduction of Hg from other source which mainly from anthropogenic one, as introduction of anthropogenic source of Hg may elevate its natural concentration (Yasuda, 2000). Hg discharged from amalgamation in the artisanal gold mining area is in an elemental form through the canals and rivers.

THg was also found in the biota samples in various levels based on the species. Source of the Hg is mostly from sediment where it plays a key role in controlling the metal concentrations in biota (Blanchette., 2001).

MeHg was found in all sediment samples in the ratio of THg-MeHg as 0.0035. It was also found in all samples of the biota. Accordingly, since there is no anthropogenic source of MeHg in the area, methylation process of Hg was occurred in sediment along the riverine system. Moreover, through food webs where bioaccumulation occurred, the concentration of the methylated Hg is increased and magnified (Lasut & Yasuda, 2008).

MeHg was also found in the human hair samples in various levels higher than the control value. Hg methylation generally increases its toxicity as a result of its enhanced penetration through lipid membranes (Bustamante et al., 2006) of organisms and human. Since the residents consumed fish from the river daily, mercury impact may potentially occur.

References

- Blanchette, M. C., Hynes, T. P., Kwong, Y. T. J., Anderson, M. R., Veinott, G., Payne, J. F., Stirling, C., & Sylvester, P. J. (2001). A chemical and ecotoxicological assessment of the impact of marine tailings disposal. *Tailings and Mine Waste '01*. Balkema, Rotterdam, pp. 323-331.
- Bustamante, P., Lahaye, V., Durnez, C., Churlaud, C., & Caurant, F. 2006. Total and organic Hg concentrations in cephalopods from the North Eastern Atlantic waters: Influence of geographical origin and feeding ecology. *Science of the Total Environment*, 368, 585-596.
- de Lacerda, L. D., Salomons, W. 1998. Mercury from gold and silver mining: a chemical time bomb? Springer-Verlag, Berlin, 146 pp.
- de Lacerda, L. D. 2003. Updating global Hg emissions from small-scale gold mining and assessing its environmental impacts. *Environmental Geology* 43, 308-314.

- James, L. P. 1994. The mercury “tromol” mill: an innovative gold recovery technique, and a possible environmental concern. *Journal of Geochemical Exploration* 50, 493-500.
- Kambey, J. L., Farrell, A. P., Bendell-Young, L. I. 2001. Influence of illegal gold mining on mercury levels in fish of North Sulawesi’s Minahasa Peninsula (Indonesia). *Environmental Pollution* 114, 299-302.
- Lasut, M. T., Yasuda, Y. 2008. Accumulation of mercury in marine biota of Buyat Bay, north Sulawesi, Indonesia. *Coastal Marine Science* 32(1), 33-38.
- Limbong, D., Kumampung, J., Rimper, J., Arai, T., Miyazaki, N. 2003. Emission and environmental implications of mercury from artisanal gold mining in north Sulawesi, Indonesia. *Science of the Total Environment* 302, 227-236.
- Lingkubi, O. 2004. Upaya pemerintah dalam mengatasi dampak pencemaran pertambangan di Kecamatan Dimembe [Eng: government actions to overcome pollution impacts from artisanal gold mining activities in Dimembe]. Makalah. Pemerintah Kabupaten Minahasa Utara.
- Ogola, J. S., Mitullah, W. V., Omulo, M. A. 2002. Impact of gold mining on the environment and human health: a case study in the Migori Gold Belt, Kenya. *Environmental Geochemistry & Health* 24, 141-158.
- Yasuda, Y. 2000. Minamata Bay. In: Okada M, Peterson SA, editors. *Water Pollution Control Policy and Management: The Japanese Experience*. Chapter 13. Gyosei Ltd., Tokyo.

Mercury in hair as a diagnostic indicator of exposure to the metal in a coastal population in Venezuela

Rojas Maritza ^(1,3), **Nakamura Kunihiro** ⁽²⁾, **Seijas David** ⁽³⁾, **Squillante G** ⁽³⁾,
Pieters Maria Alejandra ⁽³⁾, **Infante Saba** ⁽⁴⁾.

(1): MRM-CONSULTOX, Valencia, Venezuela;

(2): National Institute for Minamata disease. Minamata, Japan;

(3): Center for Toxicological Investigations of the University of Carabobo (CITUC).

Calle 144 No. Río-211, La Ceiba. Valencia, Venezuela;

(4): Science and Technology School (FACYT). University of Carabobo, Valencia, Venezuela.

(1): Postal address: Calle Urano 91-20 Trigal Norte. Valencia, Venezuela. Tel. 58-241-8430004.

E mail: rojas.m@interlink.net.ve

Abstract

Total and methylmercury in hair (THg-H and MeHg-H) were determined in 160 adults to characterize them in terms of Hg-exposure and potential risk. The study group constituted of 60 individuals living in the Central-North coast of Venezuela. Part of the area was known to be contaminated with mercury from a chlor-alkali plant installed close to one of the tributaries' rivers of the Caribbean Sea. The study group was selected from 4 inclusion criteria points. The control group was composed of 100 individuals selected from Carabobo State with no-known exposure to Hg. A questionnaire was designed to collect demographic, health information, work activities and fishing consumption habits. Hair samples were analyzed for THg. Samples with THg-H > 5 ug/g were also analyzed for MeHg. The mean THg-H was 1.88 ± 1.50 and 0.99 ± 0.87 ug/g for the study and control groups, respectively. The study group was statistically higher than control individuals, however, no statistical differences on THg-H between each of the 4 categories of the study group and control group were found. Mean MeHg-H value was 3.67 ± 1.25 ug/g. Associations were made between Hg-H and several variables. No significant relation was noted between Hg-H levels and clinical symptoms. R analyses and t-tests were used to examine for individual associations between questionnaire variables and THg-H. The main predictors of THg-H levels in the study group were fish consumption and its frequency. As both groups presented relatively low values for THg-H and MeHg-H the results of this study indicate that Hg exposure did not exceed safe levels. However, a more in-depth exposure assessment should be conducted to characterize more accurately this exposure, mainly in terms of water and fish sampling for Hg content.

Key words: Mercury; exposure; mercury in hair

Short Title: Mercury exposure assessment

Resumen

Mercurio total y metil mercurio en cabello (THg-H y MeHg-H) fueron determinados en 160 adultos para caracterizar esta población en términos de exposición a Hg y su potencial riesgo. El grupo Estudio consistió de 60 individuos que viven en la costa Centro-Norte de Venezuela. Parte de esta área se había reportado contaminada, años atrás, con Hg de una planta de clor-soda instalada junto a uno de los ríos tributarios del mar Caribe. El grupo Estudio se seleccionó con base en 4 criterios de inclusión. El grupo Control estuvo compuesto de 100 individuos del Estado Carabobo, sin exposición conocida a Hg. Se administró un cuestionario para obtener información referida a: demografía, salud, actividades laborales y alimentación con pescado. Se analizaron muestras de cabello para determinar THg. Las que resultaron superiores a 5 ug/g, se analizaron también para MeHg. El promedio de THg-H fue de 1.88 ± 1.50 y 0.99 ± 0.87 ug/g para los grupos estudio y control respectivamente. El grupo estudio resultó estadísticamente superior al control, sin embargo, no se hallaron diferencias estadísticamente significantes en el THg-H comparando cada una de las 4 categorías del grupo estudio. La media de MeHg-H fue de 3.67 ± 1.25 ug/g. Se calcularon asociaciones entre Hg-H y algunas variables. No se encontró una relación significativa entre los niveles de de Hg-H y los síntomas reportados. Se usaron R-análisis y t-test y para examinar las asociaciones individuales entre las variables del cuestionario y el THg-H. Los principales predictores de los niveles de THg-H en el grupo estudio fueron el consumo de pescado y su frecuencia. Ya que ambos grupos estudiados presentaron valores relativamente bajos de THg-H y MeHg-H, los resultados de este estudio indican que la exposición a Hg no excedió los niveles permisibles. Sin embargo, se requiere una evaluación más profunda para caracterizar, en forma más exacta esta exposición, principalmente en lo que se refiere a determinar concentraciones de Hg en muestras de agua y de pescado en la zona.

Palabras clave: Mercurio, exposición, mercurio en cabello.

Título corto: Evaluación de exposición a mercurio

Introduction

The major source of environmental mercury is natural degassing of the earth's crust; however, industrial activities can expose individuals and the environment to toxic levels directly or indirectly through the use and misuse of mercurial compounds (1).

Several studies have indicated an association between Hg exposure diet rich in fish (2, 3, 4). This work was done at the Puerto Cabello-Moron coastal area of Venezuela, an ideal location for industries that require large land areas, water and marine transportation. Part of the area was known to be contaminated with mercury because of the installation of a chlor-alkali plant in a petrochemical complex that was close to one of the tributaries' rivers (called "Caño Alpargaton") that empties into the Caribbean Sea (5, 6). Mercury spills from these plants between 1957-1976 produced concern in the 70 km coastal zone from Puerto Cabello to Chichiriviche and in the National Park area. The government of Venezuela requested an evaluation of the impact of the discharges of the major industries in that zone. The evaluation identified some negative impacts such as high Hg levels (> 3 ppm) at the sediments of Caño Alpargaton (6). During the following years, several potentially polluting industries were built in the area where some of the villagers consumed fish as their main source of food intake. While fish is a beneficial food, the primary exposure to MeHg is through consumption of fish, considered the main contamination pathway of MeHg to human beings (7). In some of the villages studied, fish is the primary and frequently, the only source of animal protein. Mercury exposure was evaluated by measuring the Hg content of hair. Total and methylmercury in the hair (THg-H and MeHg-H) are useful bioindicators of long-term exposure to Hg. MeHg-H is specific for MeHg contamination, particularly from ingestion of Hg contaminated fish, where MeHg is easily bio-accumulated and bio-magnified and becomes concentrated in fish, particularly, carnivorous fish (8).

Once incorporated into hair at the root, Hg remains stable while hair continues to grow at approximately 1 cm per month. Hair represents recent and historical exposures while blood Hg content would reflect only recent Hg intake (9). One of the advantages of hair as a biological sample is that the segmental analysis of strands provides the opportunity to reconstitute past exposure history based on their growth rate (9, 10, 11). MeHg builds up in hair during its formation and shows a good correlation with blood Hg levels. There is evidence that THg-H is about 250 to 300 times higher than Hg in blood (7, 8). Therefore a conversion factor of 1:250 has been used to convert THg-H to Hg in whole blood (12).

The authors have updated part of the above mentioned surveillance and have investigated the current potential Hg-contamination in a selected population of villagers in the region of Puerto Cabello-Moron coastal area of Venezuela.

Method

Analytical, case-control study. The Study group included 60 individuals living at the Central-North coast of Venezuela (Falcon-Carabobo States) and was selected according to the following criteria:

- Retired individuals that worked in the petrochemical industry at the time of the contamination (n=20);
- Current petrochemical workers (n=13);
- People living in a fishing village (with high fish intake, locally caught) located at 10 km from the petrochemical industry (n=14).
- People living in Puerto Cabello, a big Carabobo State port, located at 20 km from the petrochemical industry and whose fish intake is also high (n=13).

The Control group consisted of 100 individuals (50 male; 50 female) from Valencia, Carabobo State with no-known Hg-exposure.

A questionnaire including socio-demographic information, health history, Hg-related signs/symptoms if presented during the last 2 years, work activities (potential Hg exposure) and life styles was given by interview to both groups. Respondents were also asked about consumption of fish (yes/no), type of fish and the estimated average number of times per week they ate fish (in the past 6 months). The population was categorized according to frequency of fish intake (1=Daily; 2= 2-5x/week; 3= 1-3x/week; 4= Do not eat fish). We did not ask about daily average quantity of fish consumed (grams) for different meals nor the relative proportion or size (length and weight) of different fish species consumed. The study was carried out during the dry season of the year.

Approximately 1-g hair samples were obtained from the subjects, trimming hair close to the scalp. Hair samples were stored in plastic bags previously identified until analyzed. Samples were sent to Japan and both parameters were analyzed at the Laboratory of the Sciences Institute for Minamata Disease. THg-H was determined in Study and Control groups by a flameless atomic absorption spectrometer with a mercury analysis vaporizer (Rigaku Mercury SP-1; Nippon Instruments Co. Ltd, Tokyo). Samples with THg-H > 5 ug/g were also analyzed for MeHg -methods of Nakamura et al, 1999 (13) and Nakano and Miyamaoto, 2003 (14), by atomic absorption spectrophotometry. Analytical quality control was ensured with standard hair samples provided by the Japanese Institute for Environmental Studies and used as a control.

Volunteers were informed about the objectives of the study and confidentiality issues. Written consent was obtained from the volunteers. They received the results of the analyses.

Statistics:

Descriptive statistical analyses were used to illustrate socio-demographic variables and concentrations of Hg-H and to characterize the fish diet. Mean comparisons were carried out using the Student's t test. R package was used for the one-way analysis of variance (15). This one was the most suitable model for the treatment of our data with independent categorical variables. The proposed model used to estimate the relationship between the dependent variable THg-H and the independent variables sex, group classification, smoking and drinking habits, fish intake and frequency of fish intake, is the following: $THg-H = \mu + \beta_1 \text{sex} + \beta_2 \text{group classif.} + \beta_3 \text{smoking habit} + \beta_4 \text{alcohol intake} + \beta_5 \text{fish intake} + \beta_6 \text{frequency fish intake} + \varepsilon$, where each independent variable is measured in two or more factor levels.

Results

The distribution of the study population according to group, sex, age and Hg-H values is represented in Table 1. The study group had statistically higher THg-H values ($p < 0.01$) than control individuals. Only four individuals (3 male, 1 female) of the study group, had $THg-H > 5$ ug/g. Mean MeHg-H concentration was 3.67 ± 1.25 ug/g. Table 2 shows the distribution of the study group according to classification and mean values of Hg-H.

Percentile distributions with mean THg-H levels for the 4 classifications in the study group are presented in Fig 1. Fig. 2 describes box-plots showing concentrations of THg-H of the study group based on "frequency of fish intake". Each box shows the median, 25th and 75th percentiles. Fifty seven individuals in the study group (95%), referred to eat fish from nearby water bodies. Table 3 shows their frequency of fish intakes according to groups studied and mean values of THg-H (ug/g). Twenty nine fish species were reported. From these, the sea species most frequently reported were: "Lisa" (Mugil sp), followed by "Pargo" (Lutjanus sp), Carite (Scomberomorus sp) and Tuna (Thunnus sp). Twenty five species were sea fish and four were river fish.

Table 4 shows the results obtained from the study group using the routines of the R statistical package for the one-way variance analysis. Only variables that could influence the THg-H values were "fish intake" and "frequency of fish intake". This analysis made for the control group did not reveal any contributing factors to THg-H.

- 1.1 Twenty three Hg-related signs and or symptoms were investigated. In the study group, 28/60 individuals (46.7%) reported some type of sign and/or symptom (26 with $THg-H < 5$ and 2 with ≥ 5 ug/g). The signs/symptoms most reported in that group (all

frequencies) were: sleepiness (13 people; 21.7%), followed by hearing disturbance (10 people, 16.7%), anxiety and sadness (7 people each; 11.7%) and tremor (6 people; 10%). No significant relation was noted between hair mercury levels and clinical symptoms in the current study. The symptom most frequently reported as “very frequently” was headache (8 people; 13.3%), followed by joints pain (6 people; 10%) (Table 5).

Discussion

Since Me-Hg accounts for more than 80% of THg in hair (16), THg could be used to estimate exposure to Me-Hg (17). Mean levels of THg-H were below the safety limit reported by WHO (10 ug/g) and values of MeHg-H were not significantly different from the permissible limit (2 ug/g) (18). None of the women studied had THg-H over 10 ug/g which has been reported to be the upper limit guideline for pregnant women (19). A MeHg-H level around 50 ug/g is associated with a 5% risk of neurological damage to adults (8). Participants' levels were lower than levels associated with such effects among adults (50 ug/g hair) and among children exposed *in utero* (10 ug/g hair) (18). VOY ACA

Our THg-H values are consistent with the ones obtained by Nilson et al, 2001 (20), although those authors obtained MeHg-H levels lower than the present study. Both mean values obtained in our study were lower than the ones reported in studies from other countries such as Brazil (21, 22) and French Guiana (23). However, the statistically higher THg-H values in the study group may indicate that could exist a potential for Hg contamination in the area.

When evaluating the different fish species reported, apart from tuna, we did not find the ones that are more susceptible of Hg uptake as reported by other researchers (7, 22). Due to the great variety of fish species available and reported it is difficult to determine the type of fish predominant as a typical diet in this region, so further epidemiological studies would be recommended.

Levels of THg-H in the Fishing village group were higher than the ones in other groups although not statistically different. This is probably due to the higher fish consumption. THg-H concentrations decreased in the following order: Fishing village > Current workers > Retired workers > Puerto Cabello, which is considered consistent with what was expected.

Table 4 shows that the only variables that could influence the THg-H values in the study group are “fish intake” and “frequency of fish intake”, as the probability of rejection of the F test is 0.035 and 0.034 respectively, which implies that they are significant at 4%. Other factors studied did not contribute significantly to THg-H levels.

As expected, Fig. 2 shows an increase in THg-H concentrations in the study group individuals whose frequency of fish intake was “daily”. The fact that mean THg-H value of the Fishing village residents was the highest in the study group (2.5 ± 1.56 ug/g) and 57% of this population reported a daily frequency of fish intake (mean THg-H 3.55 ± 0.97 ug/g), indicates that diet factors contributed importantly to the Hg-H levels, especially the daily frequency of intake. These findings suggest that the fish from the coast of that area may be contaminated with Hg although with permissible levels. This potential contamination should be further monitored with methods including the Hg determination in fish samples.

Since all Hg-H concentrations in this population were below 10 ug/g (considered as the limit below which clinical signs are not apparent) (24), signs and or symptoms reported could not be considered specific to mercury intoxication so we can not derive definitive conclusions from these results. However, a more in-depth exposure assessment should be conducted to characterize more accurately this exposure mainly in terms of water and fish sampling for Hg content.

References

1. Magos L, Clarkson TW. Overview of the clinical toxicity of mercury. *Ann Clin Biochem.* 2006; 43(Pt 4):257-268
2. Frery N, Maury-Brachet R, Maillot E, Deheeger M, De Merona B, Boudou A. Gold-mining activities and mercury contamination of native Amerindian communities in French Guiana. Key role of fish in dietary uptake. *Environ Health Perspect* 2001; 109 (5): 449-456.
3. Kehrig HA, Malm O, Akagi H. MeHg in air samples from different riverine groups, Amazon, Brazil. *Water, Air and Soil Pollution* 1997; 97:17-29.
4. Boischio AA and Cernichiari E. Longitudinal Hair Mercury Concentration in Riverside Mothers along the Upper Madeira River (Brazil). *Environ Res* 1998; 77(2):79-83.
5. Pérez D. Línea Base de referencia Biológica en el ambiente marino costero del área de Golfo Triste. Informe final del proyecto por contrato Pequiven-Universidad Simón Bolívar. Caracas. 1989.
6. Parra Pardi G. Estudio integral de la cuenca del río Yaracuy. Dirección de Malariología y Saneamiento Ambiental M.S.A.S. Caracas 1974, 341pp.

7. Malm O, Branches F, Akagi H, Castro M, Pfeiffer W, Harada M, Bastos WR, Kato H. Mercury and MeHg in fish and human hair from the Tapajós river basin, Brazil. *Sci Total Environ* 1995; 175(2):141-150.
8. Veiga M and Baker R. *Protocols for Environmental and Health Assessment of Mercury Released by Artisanal and Small-scale Gold Miners*. Published by GEF/UNDP/UNIDO Global Mercury Project. Vienna, ISBN 92-1-106429-5. 2004, 289pp.
9. Canuel R, Boucher de Grosbois S, Atikessé L, Lucotte M, Arp P, Ritchie C, Mergler D, Man Chan HM, Amyot M and Anderson R. New Evidence on Variations of Human Body Burden of Methylmercury from Fish Consumption (Mini monograph). *Environ Health Perspect* 2006; 114 (2):302-306.
10. Lebel J, Mergler D, Branches F, Lucotte M, Amorin M, Larribe F, Dolbec, J. Neurotoxic effects of low-level MeHg contamination in the Amazonian basin. *Env Res Section A* 1998; 79:20-32.
11. Harnly M, Sharon S, Rojas P, Fornes R, Flessel P, Smith D, Kreutzer R, Goldman L. Biological monitoring for mercury within a community with soil and fish contamination. *Environ Health Perspect* 1997; 105(4):424-429.
12. Hightower JM and Moore D. Mercury Levels in High-End Consumers of Fish. *Environ Health Perspect* 2003; 114(4):604-608.
13. Nakamura K, Naruse I, Takizawa Y. A new mass screening method for MeHg poisoning using mercury-volatilizing bacteria from Minamata Bay. *Ecotoxicol and environ saf* 1999; 44(1):100-104.
14. Nakano A and Miyamaoto Y. Development of an analytical method for MeHg determination by atomic absorption photometry. *Proceedings of International Workshop on Health and Environmental Effects of Mercury -Impacts of Mercury in South and Central America*. Belem, Brazil. 2003, 218-238 pp.
15. The R Project for Statistical Computing. Version: 2.2.1. Available from: <http://www.r-project.org>. December 2005.
16. Cernichiari E, Toribara TY, Liang L, Marsh DO, Berlin MW, Myers GJ, Cox C, Shamlaye CF, Choisy O, Davidson P, et al. The biological monitoring of mercury in the Seychelles study. *Neurotoxicology* 1995; 16:613-628
17. NRC (National Research Council). *Toxicological effects of methyl-mercury*. Washington, DC: National Academy Press. 2000.

18. WHO. ENVIRONMENTAL HEALTH CRITERIA 101, Methylmercury. World Health Organization, Geneva, 1990.
19. Skerfving S. Mercury in fish. Some toxicological considerations. *Food Cosmet. Toxicol* 1973; 10:545-556.
20. Nilson SA Jr, Costa M, Akagi H. Total and methylmercury levels of a coastal human population and of fish from the Brazilian northeast. *Environ Sci Pollut Res Int.* 2001; 8(4):280-284.
21. Guimaraes JRD, Fostier AH, Forti MC, Melfi JA, Kehrig H, Mauro JBN et al. Mercury in human and environmental samples from two lakes in Amapa, Brazilian Amazon. *Ambio* 1999; 28:296-301.
22. Leino T and Lodenius M. Human hair mercury levels in Tucuruí area, State of Pará, Brazil. *Sci Total Environ* 1995; 175(2):119-125.
23. Cordier S, Grasmick C, Paquier-Passelaigue M, Mandereau L, Weber JP, Jouan M. Mercury exposure in French Guiana: levels and contaminants. *Arch Environ Health* 1998; 53(4):299-303.
24. Harada M, Nakachi S, Cheu T, Hamada H, Ono Y, Tsuda T, Yanagida K, Kizaki T, Ohno H. Monitoring of mercury pollution in Tanzania: relation between head hair mercury and health. *Sci Total Environ* 1999; 227(2-3):249-256.

**TABLE I. DISTRIBUTION OF THE STUDY POPULATION
ACCORDING TO GROUP, SEX, AGE AND THg-H VALUES.**

Group	AGE (Years)	SEX	THg (ug/g)	THg-H (ug/g)			MeHg-H (ug/g)
				n	% ⁽¹⁾	X ± SD (R)	X ± SD (R)
Study	47.47 ± 14.66 (R= 21-74)	Male	< 5	33	58.92	1.53 ± 1.05 (0.21- 4.38)	-
			≥ 5	3	75	5.78 ± 1.05 (5.16- 7.00)	3.85 ± 1.47 (2.17- 4.90)
			Total male	36	60	1.88 ±1.57 (0.21- 7.00)	-
		Female	< 5	23	41.08	1.72 ±1.21 (0.31- 4.70)	-
			≥ 5	1	25	5.61	3.14
			Total female	24	40	1.88 ±1.42 (0.31- 5.61)	-
		Total Study G.	< 5	56	100	1.61 ±1.11 (0.21- 4.70)	-
			≥ 5	4	100	5.74 ±0.86 (5.16- 7.00)	3.67 ± 1.25 (2.17- 4.90)
			Total	60	100	1.88 ± 1.50(*) (0.21- 7.00)	-
Control	36.86 ± 12.35 (R=18-74)	Male	< 5	50	50	1.08 ±0.84 (0.13- 4.37)	-
		Female	< 5	50	50	0.90 ±0.89 (0.09- 4.31)	-
		Total Control Group	< 5	100	100	0.99 ± 0.87 (0.09- 4.37)	-

R: Range

(1): % based on each total according to THg values: Study: < 5 ug/g (n=56) and ≥ 5 ug/g (n=4); Control: <5 ug/g (n=100).

(*): Significant difference with respect to Control group (t-Student for independent samples)

**TABLE II. “STUDY” GROUP ACCORDING TO CLASSIFICATION
AND MEAN VALUES OF Hg-H**

Classification	THg-H (ug/g)	THg-H (ug/g)			MeHg-H (ug/g)
		n	% ⁽¹⁾	X±SD(*) (R)	X ± SD (R)
Retired workers	< 5	18	32.1	1.07±0.75 (0.21-2.97)	-
	≥ 5	2	50	6.09±1.27 (5.19-7.00)	3.33±1.64 (2.17-4.49)
	Total	20	33.3	1.57±1.72 (0.21-7.00)	-
Current workers	< 5	13	23.2	2.04±1.07 (0.60-4.70)	-
	≥ 5	0	-	-	-
Fishing Village	< 5	13	23.2	2.29±1.42 (0.31-4.38)	-
	≥ 5	1	25	5.16	4.90
	Total	14	23.3	2.50±1.56 (0.31-5.16)	-
Puerto Cabello	< 5	12	21.4	1.20±0.63 (0.40-2.70)	-
	≥ 5	1	25	5.61	3.14
	Total	13	21.6	1.54±1.36 (0.40-5.61)	-
TOTAL	< 5	56	100	1.61±1.11 (0.21-4.70)	-
	≥ 5	4	100	5.74±0.86 (5.16-7.00)	3.67±1.25 (2.17-4.90)
	Total	60	100	1.88±1.50 (0.21-7.00)	-

R: Range

(1): % based on each frequency according to THg values: < 5 ug/g (n=56) and ≥ 5 ug/g (n=4);

(*): No statistical differences between studied groups (one-way ANOVA)

Fig. 1

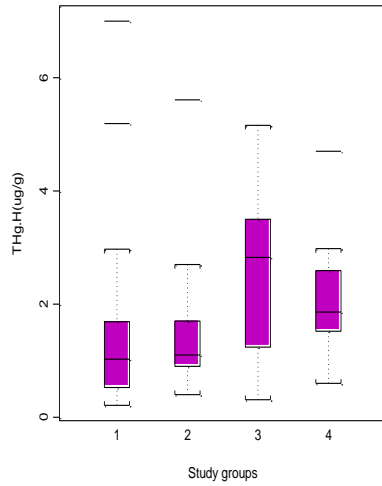


Fig. 2

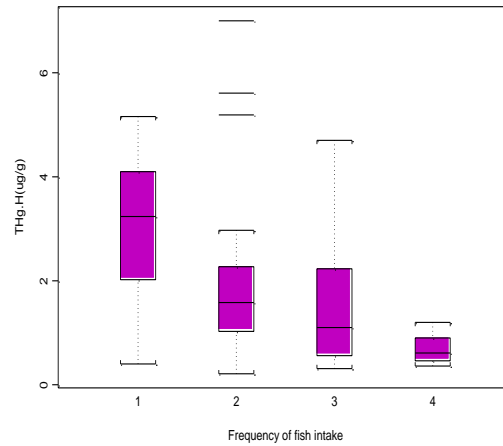


Fig. 1: Percentile distributions of mean THg-H levels

(1= retired workers; 2= current workers; 3= fishing villagers; 4= Puerto Cabello individuals).

Fig. 2: Concentrations of THg-H of the Study Group based on “frequency of fish intake”

(1=daily; 2= 2-5x/week; 3= 1-3x/week; 4= Do not eat fish).

1.2 TABLE III . FREQUENCY OF FISH INTAKE IN THE STUDY AND CONTROL GROUPS ACCORDING TO GROUP CLASSIFICATION AND MEAN VALUES OF THg-H (ug/g)

STUDY GROUP	Frequency	n	% (1)	THg-H X ± SD	1.3 Range
Retired workers	Daily	2	10	0.6 ± 0.3	0.4 – 0.8
	2-5 times/week	16	80	1.8 ± 1.8	0.21 - 7
	1-3 times/week	2	10	0.4 ± 0.2	0.3 – 0.6
	Total	20	100	1.5 ± 1.7 (*)	0.2 – 7
Fishing Village	Daily	8	57	3.5 ± 0.9	2.2 – 5.16
	2-5 times/week	3	21	1.8 ± 0.9	1.2 – 2.8
	1-3 times/week	2	15	0.4 ± 0.1	0.3 – 0.5
	Don't eat fish	1	7	0.3	-
	Total	14	100	2.5 ± 1.5 (*)	0.3 – 5.1
1.4 Current workers	2-5 times/week	6	46	1.8 ± 0.4	1.2 – 2.5
	1-3 times/week	6	46	2.4 ± 1.3	0.6 – 4.7
	Don't eat fish	1	8	0.6	-
	Total	13	100	2 ± 1 (*)	0.6 – 4.7
Puerto Cabello	2-5 times/week	8	57	1.8 ± 1.6	0.4 – 5.6
	1-3 times/week	4	28.5	1.0 ± 0.4	0.4 – 1.4
	Don't eat fish	1	7	0.9	-
	Total	13	100	1.5 ± 1.3 (*)	0.4 – 5.6
CONTROL GROUP	Daily	2	2	0.79 ± 0.57	0.38 – 1.20
	2-5 times/week	9	9	1.02 ± 0.98	0.13 – 3.38
	1-3 times/week	25	25	1.20 ± 1.09	0.27 – 4.37
	Don't eat fish	64	64	0.91 ± 0.76	0.09– 4.31
	Total	100	100	0.99± 0.87^(*)	0.09 – 4.37

(*): No statistical differences on THg-H between groups studied.

(1): % based on total individuals in each group

TABLE IV. ANALYSES OF VARIANCE OF THE USED MODE

Variables	Df	Sum of Sq	Mean Sq	F	P _r (F)
Sex	1	0.118	0.118	0.058	0.811
Group classification	1	5.402	5.402	2.657	0.109
Smoking habit	1	1.662	1.662	0.818	0.370
Alcohol intake	1	0.038	0.038	0.018	0.892
Fish intake	1	9.506	9.506	4.676	0.035

TABLE V. DISTRIBUTION OF THE SYMPTOMS OF THE STUDY GROUP
ACCORDING TO CLASSIFICATION AND WHOSE PRESENCE
WAS “VERY FREQUENTLY”, IN THE LAST 2 YEARS.

Symptoms	Retired	Fishing Village	Current Workers	Pto. Cabello	Total	%/60
Gums Inflammation	0	1	0	0	1	1.7
Writing impairment	1	0	0	0	1	1.7
Anxiety	1	0	0	0	1	1.7
Somnolence	0	0	0	1	1	1.7
Fear	0	1	0	0	1	1.7
Appetite decrease	1	1	0	0	2	3.3
Bronchitis	0	0	1	1	2	3.3
Paresthesia	0	1	0	1	2	3.3
Hearing impairment	1	0	1	0	2	3.3
Doubtfulness	1	1	0	0	2	3.3
Sweating	2	1	0	0	3	5.0
Sadness	1	2	0	0	3	5.0
Tremor	2	1	0	0	3	5.0
Nasal irritation	0	1	1	2	4	6.7
Sleepiness	1	2	1	0	4	6.7
Muscular pain	2	2	0	1	5	8.3
Joints pain	2	3	0	1	6	10.0
Headache	0	5	2	1	8	13.3

Hair mercury investigation of mercury-polluted areas around the world

**Masatake Fujimura¹, Akito Matsuyama², Masaaki Nakamura³,
Akira Yasutake¹, Mineshi Sakamoto², Kunihiro Nakamura¹.**

1: Basic Medical Science, National Institute for Minamata Disease

2: Epidemiology, National Institute for Minamata Disease

3: Clinical Medicine, National Institute for Minamata Disease

The most effective method to understand the human health risk of harmful substances such as methylmercury at the early stage is assessment of the exposure level.

"How much of the harmful substance is taken into the body?"

It is clear that methylmercury uptake from food etc. into the body is eliminated by urine etc., and accumulates in hair and fingernail at a constant rate. The concentration of methylmercury in hair is an effective indicator to understand the level of human exposure to methylmercury. The purpose of this investigation is to assess the methylmercury exposure level around the world by measuring the hair mercury level of the inhabitants in regions where methylmercury pollution is possible by gold mining, factory pollution related and fish dining habits etc., and to contribute to prevention of related health hazard.

These results were obtained from a 6-year investigation (2003-2008). Hair samples were collected from 2,054 inhabitants (males: 881, females: 1,173) in 8 countries (Kazakhstan, China, South Korea, Colombia, Benin, Indonesia, Venezuela and French Guiana) around the world. The concentrations of total mercury in the hair were determined according to the oxygen combustion-gold amalgamation method using a mercury analyzer MA 2000 (Nippon Instruments). Methylmercury analysis was involved immersion of the samples in 2N HCL, heating at 100 for 5 minutes to leach out methylmercury from the sample, methylmercury extraction into toluene, and determination by gas-liquid chromatography with electron capture determination method using a gas chromatograph G 3800 (Yanaco).

Some hair samples from Benin included very high concentration of mercury (>1000 ppm). The ratio of methylmercury to the total mercury, which was an indicator of the human body internal exposure, ranged from 0.6 to 1.0%. Since no miner's hair included in these hair samples, it was suggested that cosmetics such as hair conditioner involved in mercury caused high value of mercury. In the case of Indonesia, the ratio of methylmercury to the total mercury ranged from 1 to 30% in a lot of samples. Samples included miners' hair who worked in the gold mine. The high concentration of mercury was ascribed to external exposure while gold

mining. The average concentration of hair total mercury in French Guiana was remarkably high at 7.6 ppm (male) and 9.3 ppm (female), respectively. The ratio of methylmercury to the total mercury, ranged from 80 to nearly 100%. It was collected from inhabitants who have a habit of eating fish in the mercury-polluted area near a gold mine mountain. The high concentration of methylmercury was ascribed to consumption of mercury-polluted fish.

Methyl mercury exposure from fish consumption among communities near Ethiopian Rift Valley Lakes (ERVLs)

***Solomon Aragie**

PhD candidate, Hawassa University, Ethiopia solo2429@yahoo.com

Abstract: Fish is an important component of the diet in Ethiopia and it is an excellent source of important nutrients. Yet, such benefits may be offset by the presence of contaminants such as, methylmercury (MeHg). Our objective is to investigate MeHg exposure levels and nutritional benefits from fish consumption in communities near ERVLs. This research involves three phases: Phase I was focused on the assessment of fish consumption pattern and major health problems of the population, which was conducted using a structured food frequency questionnaire. Phase II will focus on MeHg and n-3 polyunsaturated fatty acids (n-3PUFAs) exposure assessments and Phase III is planned to involve a cohort study of child development. The diets of the study population are dominated by a single staple food and the source of animal proteins for most of the study population, for instance, for 100 % of Arbamich and 82.1 % of Zeway fishermen was fish only and they never ate other animal proteins. There are six important fish species landing in the study areas. (*Tilapia*) *O. niloticus* consumption rate in Zeway, Awassa and Arbaminch is 81.1 %, 78 % and 79.3%, respectively. Catfish and *Barbus* species are the second and the third most frequently consumed species next to tilapia. Mean fish meal consumption rate in the study population per week was 8.5 (range 1- 14 meals). Half (47.9%) of the population had 10 to 14 meals per week. The average weekly fish meal consumption was significantly different among fishermen in Arbaminch, Zeway and Awassa (13.5, 10.2 and 9.5 respectively $P < 0.01$). Mean amount of fish consumption per week per individual was 1309g (range 125g - 3500g per week). Highest amount of fish was consumed in Arbaminch than Awassa and Zeway (3062.5g, 1046.8g and 1343.7 g respectively) and the result was statistically significantly different ($P < 0.05$). In the health status survey, Mental illness was reported as their major family health problem by 24.5 % of households from Zeway islands. Fishermen and their assistants in all study areas and Zeway fishermen families have been found to consume grater quantities of fish per week. Such frequent fish consumptions, may pose a health risk if consumed fish is contaminated with MeHg. Therefore, the second phase will focus on the assessment of MeHg and n-3PUFA exposure among fishermen and their families living around these lakes.

Key words: Ethiopian Rift Valley Lakes; Fish consumption; Methylmercury; Polyunsaturated fatty acid.

Introduction: Fish is an important component of the diet in Ethiopia where about 49% of the total population is considered ‘under-nourished’ (FAO, 1995). Estimated annual fish yield potential of the country is about 51,000 tonnes (Mebrat, 1993). The ERVLs are being used for commercial fisheries, irrigation, power generation, recreation and for some industrial purposes. The sources of major pollutants affecting these lakes are factories, agriculture and sewage. Mineral extraction from Lake Abijata, effluents from the tannery at Koka reservoir and the textile industries in Awassa and Arba Minch have affected the concerned fisheries. Expanding horticultural production is making increasing use of chemical inputs. Aquatic food chains are capable of accumulating certain environmental contaminants up to toxic concentrations. MeHg is the most dramatic and best documented example of high bioaccumulation (Clarkson, 1995). Mercury concentration was reported from one of the rift valley lakes, Lake Awassa. The Mercury concentration in the effluent discharged to and the concentration in the water from Tikur Wuha is 5.1µg/l and 2.8µg/l (Zinabu and Pearce, 2003). Desta et al (2006) reported the high mercury concentration in some fish species such as, piscivorous Big Barbs (*Barbus intermedius*) in Lake Awassa. The level of mercury was beyond the marketing limit of the European Union (0.5 mg/kg) and FAO/WHO’s guideline for safe fish consumption (0.3 mg/kg). Communities living around these lakes have a long history of relying upon fish as a major part of their diet and using fish for commercial fisheries. Compared to other group of populations, fishermen and their families may eat more and variety of fish than the general population. Yet, nothing is known about the exposure level as well as health effects caused by contaminants through fish consumption. The purpose of this study is therefore to investigate MeHg exposure levels and nutritional benefits from fish consumption in communities near ERVLs. This study involves three phases: Phase I was focused on the assessment of fish consumption pattern and major health problems of the population, Phase II will focus on MeHg and n-3 PUFAs exposure assessments and Phase III is planned to involve a cohort study of child development.

Methods: Phase I: The study area includes fish landing sites and surroundings at lakes situated in the southern part of the Ethiopian rift valley. More than 70% of the total populations were surveyed. People fishing at the time of survey and head of households available at home were included. This cross-sectional study involved questionnaire administration to be completed by each participant. The questionnaire was designed using (UNEP DTIE, 2008) guideline. The study was approved by ethics committee of Addis Ababa University. Fish weight estimation and

species identification were performed with the help of staffs from Ethiopian Fish Production and Marketing Corporation (EFMPC).

Phase II: To assess exposure to mercury, hair and blood samples will be collected from fishermen families and children who are working as assistants to the fishermen. We will also determine plasma polyunsaturated fatty acid concentrations of the study population. In addition, Fish samples from different species will be used for chemical analysis including mercury and fatty acid concentrations using standard procedures. Phase III: Since fetus is a highest risk group for MeHg exposure. At the third stage, we are also going to collect the samples from pregnant women and fetuses (cord blood) for the future cohort study of child development.

Results and Discussion: The total number of households surveyed was 307, consisting of 184 in Zeway Islands 69 in Arbaminch and 54 in Awassa. In addition, 133 fishermen and 21 children working as assistants were surveyed at fishing sites. The primary source of income for all participants is fishing. All respondents have permanent residence in the area.

The diets of the study population are dominated by a single staple food such as cereals with some vegetables such as cabbage or tomato. Overall, the mean number of fish meals eaten per week by the whole population was 8.5. The survey also gathered information on consumption of other major sources of animal proteins and fruits. The source of animal proteins for most of the study population, for instance, for 100 % of Arbaminch fishermen and 82.1 % of Zeway fishermen was fish only and they never ate other animal proteins. Therefore, fish can be considered as the only source of animal proteins for our study population.

There are six important fish species landing in the study areas. The dominant species was (Tilapia) *Oreochromis niloticus*. Tilapia was the most frequently consumed fish in Zeway, Awassa and Arbaminch by 81.1 %, 78 % and 79.3% of the study population, respectively. Catfish and Barbus species are the second and the third most frequently consumed species next to tilapia. The mercury concentrations of piscivorous big barbs, *O. niloticus* and *C. garipinus* from lake Awassa were 0.01 to 0.94 mg/kg; 0.0028 and 0.082 mg/kg and 0.002–0.154 mg/kg respectively (Desta et al., 2006). Since the mercury concentration of the big Barbu species was beyond FAO/WHO's guideline for safe fish consumption, it may pose a health risk to those people who frequently consumed this species. However, mercury concentration of these species from the other lakes such as, lake Zeway and Chamo were not determined so far. According to recent observation in lake Zeway and estimation of annual commercial catch in lake Awassa, the landings of Catfish and big Barb become increasing (Bjokli, 2004). Given the high fishing

pressure on tilapia and its overexploitation, the share of big Barb and Catfish in the commercial catch from these lakes is expected to rise in the future.

Our study showed that all fish sources are nearby lakes and the study populations consume locally self-coughed fish. Mean fish meal consumption rate in the study population per week was 8.5 (range 1- 14 meals). Half (47.9%) of the study population had 10 to 14 meals per week. These indicate that, on average, people ate fish everyday and twice a day on some days. The average weekly fish meal consumption was significantly different among fishermen in Arbaminch, Zeway and Awassa (13.5, 10.2 and 9.5 respectively $P < 0.01$). In general, large population of Arbaminch fishermen (100%) and Zeway fishermen and their families (61.4%) had the highest number of meals (10 to 14 meals) consumed per week compared to the other study groups.

The amount of fish consumed per week by the study population ranges from 125g to 3500g. The mean fish consumption rate by the whole population was 1309g/week. Arbaminch fishermen consume significantly higher amount of Fish per week than Awassa and Zeway fishermen (mean: 3062.5g Vs 1046.8g, 1343.7 g respectively, $P < 0.005$). Arbaminch and Awassa fishermen families had the lowest amount of fish per week (mean: 527.1g and 564.8g respectively). Large proportion (63.5%) of Arbaminch fishermen consume the highest amount of fish (3000g to 3500g) per week compared to the other study groups ($P < 0.01$). Decreased fishing opportunity by family members, the long distance between fishing sites and their residence and access to fish markets to sell fish in a better price may be considered as possible reasons for fish consumption differences between fishermen and their respective family members. Easy access to lakes may account for the higher consumption rates among the Zeway fishermen families compared to Awassa and Arbaminch fishermen families.

The estimated fish consumption rate by the study population (63kg/year) was more than 6 times higher than the amount of fish consumed by the local people living in the areas such as, Arbaminch, Sodo and Awassa (8.5kg/year) and in Gambella, close to River Baro (10kg/year) (FAO, 1995). It also exceeds the estimated total per capita fish consumption of Sub-Saharan Africa (6.7kg/year) as well as per capita consumption of most developing countries (14kg/year) (Delgado, et al., 2003). In Ethiopia, extreme regional variation in fish consumption was reported (René, 1991). Seasonal fish production, troughs in demand, fasting habit and the repeated changes in the prices of animal products are major factors associated with fish consumption rate in Ethiopian (René, 1991).

In general most (87.9%) of the study population exceeds the weekly recommended amount of fish to be eaten (US EPA, 2004). However, since there is no fish consumption advisory in

Ethiopia and / or in the absence of data regarding the levels of toxic pollutants in various fish species, it is difficult to judge whether their fish consumption pattern exposed them to toxic pollutants such as MeHg or not.

The study populations in all study areas had complaint for infectious diseases that are very common in Sub Saharan African countries. But unique to population from Zeway islands, Mental illness was reported as their major family health problem by 24.5 % of the households. In the previous study, a relatively high prevalence of mental illness (1.8% of Bipolar disorders and 0.06% schizophrenia) among Zay people was reported (Abebaw *et al.*, 2004). Sixty-six percent of them were from Zeway islands. In contrast with several studies that showed the association of greater seafood consumption with lower prevalence of bipolar disorders (Hibbeln 1998; Noaghiul and Hibbeln 2003), a relatively high prevalence of bipolar disorder was observed in our population who consume more than 10 fish meals and on the average 1344g of fish per week. Even this prevalence of mental illness was significantly higher compared to the prevalence observed among non fish eating communities of Ethiopia (Kebede and Alem, 1999). It is also known that insufficient dietary intake of omega-3 essential fatty acids increases the risk of affective disorders (Hibbeln and Salem, 1995) and this is also supported by the results of a double-blind, placebo-controlled trial that reported a reduction in the number of severe affective episodes and a reduction in depression scores in bipolar patients receiving 9.6 g/day of EPA and DHA (Stoll *et al.*, 1999). Therefore, the present unexpected rate of mental illness among fish eating community on Zeway islands can be explained by the fact that: either 1) the most frequently consumed fish species in the present study may have very low omega 3 PUFAs or 2) the association of omega 3 PUFAs with bipolar disorders may vary from population to population or 3) the prevalence of the disorder would be much higher than the present findings, if the diets of this population were not dominated by fish.

In conclusion, Phase I of the present research showed Fishermen and their families living around ERVLs solely rely on fish to cover their daily proteins. The most frequently consumed fish species was Tilapia followed by Catfish and Barbus species. Mental illness was identified as a serious health problem by many people interviewed on the two Zeway islands. Fishermen and their assistants in all study areas and Zeway fishermen families including women and children have been found to consume grater quantities of fish per week. These frequent fish consumptions, while having significant general health benefits, it may pose a health risk if consumed fish is contaminated with toxic chemicals such as MeHg. Therefore, the Second phase of the research will focus on the: 1) Determination of the levels of MeHg and n- 3PUFAs contents of frequently consumed fish species.2) Assessment of both MeHg and n-3PUFAs

exposure level of the study population. 3) Association of frequent fish consumption and prevalence of mental illness among Zeway fishermen.

References:

- Abebaw et al., (2004) *J. Affective disorder*, 80: 1-10
- Bjørkli S. (2004). MSc thesis, Agricultural University of Norway
- Clarkson, T.W., (1995). *Am. J. Clin. Nutr.*, 61 682-86
- Delgado et al., (2003) IFPRI Washington, D.C., U.S.A
- Desta et al. (2006) *Ecology of Freshwater Fish*, 15: 532-43
- FAO (1995). *Fisheries and Aquaculture*, Rome, FAO
- Hibbeln JR (1998) *Lancet*; 351:1213
- Kebede and Alem (1999) *Acta Psychiatr. Scand*, 100: 11-17
- Mebrat, A. (1993). pp 45-53. Rome, FAO
- Noaghiul, S. and. Hibbeln, J.R (2003) *Am. J. Psychiatry*,
160:2222–2227
- René, F., (1991). JEFAD/FMRS/91/20. UNECA.
- Stoll A ,et al (1999). *Arch Gen Psychiatry*; 56:407–412
- UNEP DTIE (2008) *Guidance for identifying populations at risk*, Geneva, Switzerland
- US EPA (2004) fact sheet. EPA-823-F-04-016.
- Zinabu, and Pearce (2003) *Hydrobiologia* 492:171-178.

Clinical evaluation of mercury exposure in riverine from Tapajós Basin, in the Amazon.

**Maria da Conceição Nascimento Pinheiro¹; Teiichi Oikawa¹;
Carlos Araújo da Costa¹; José Luiz Fernandes Vieira¹;
Geraldo de Assis Guimarães¹; Amélia A. de Araújo¹;
Luiz Carlos de Lima Silveira^{1,2}; Manoel Villarroel¹.**

1-Núcleo de Medicina Tropical/UFPA. 2. Instituto de Ciências Biológicas/UFPA.

Abstract

For many years the riverside from Tapajós basin has been showed mercury levels that exceed those safe to health. To investigate the toxic effects associated to mercury exposure the authors propose to valuable the occurrence of neurological effects in the inhabitants from a small community Tapajós basin in the Amazon. A group of 26 subjects, 4 men and 22 women, $39,7 \pm 15,8$ years old (range: 14-66 years old) were clinically examined and subjected to electromyography (ENMG). Exclusion criteria comprised early exposure to mercury vapor, leprosy, diabetes and other systemic diseases that could potentially affect the nervous system. Symptoms were presented in the 15(55,5%) subjects, such as anxiety (19,2%), headache(7,7%), lumbar pain (7,7%), cervical pain (3,8%), general fatigue(3,8%) insomnia(7,7%), tremor of hands(3,8%), numbness of hands(11,3%), psychic irritability(3,8%) and memory disturbance(3,8%).. Three women with paresthesias also had Phalen's(hands) and Tinel's positives (wrist) and electromyography(ENMG) with prolongation of the median distal latency (Carpal Tunnel Syndrome). In a case was observed sensory disturbances of the extremities the glove-stocking type associated with tremor, vibration sound disturbance, Romberg's sign, hyporreflexia, ataxia(finger-nose-test), inability to walk straight and disturbance of equilibrium (Romberg's test). He also had slowed sensory and motor nerve conduction velocities in the upper and lower extremities. That case showed the similar symptoms to methylmercury intoxication. However, the hair mercury level from 3,2 to 11ppm. We need the further study to reveal the cause of that syndrome.

Key words: Mercury exposure. Mercury toxicity . ENMG. Riverine. Amazon.

Support: CNPq; FAPESPA; IBNnet; JICA.

Introduction

Methyl mercury is an environmental toxic compound that biomagnifies through the aquatic food web, placing at risk humans who consume significant quantities of predatory fish from upper trophic levels or who rely heavily on fish as a food source (Clarkson, 2002).

Human exposure to methyl mercury at levels exceeding those considered safe and with risk of adverse effect has been registered across the world, including levels lower than those that produced the historic epidemics of methyl mercury poisoning in Japan and Iraq. In some populations, however, there is growing evidence that current exposures are sufficient to alter normal function of several physiological and developmental systems, indicating that methyl mercury exposure still constitutes an important public health problem.

The neurotoxic effects of excessive exposure to methyl mercury have been repeatedly verified and include somatosensory disturbance (peripheral nerves of limbs – glove and stocking type), auditory disturbance, constriction of visual field, ataxia, tremor, dysarthria, abnormal reflexes and psychiatric signs (Harada, 1995; Fugino 1994)

For more than two decades, riverside communities from Tapajós basin located in the west region of Brazil's Pará State have been exposed to mercury levels that do not save to health through diet ((Akagi et al, 1995; Barbosa et al, 1995; Malm et al. 1997; Lebel, et al. 1997; Pinheiro et al, 2007). Studies on the mercury content of fish in the Amazon river system have shown that levels often surpass 0.5ppm fresh weight (Barbosa et al. 1995; Lacerda and Solomons, 1991; Malm et al, 1995; Brabo et al, 2000).

In this work, we have performed a neurological examination of inhabitants of a small community of the Tapajós basin to evaluate the degree of nervous system impairment in this population that could be related to the mercury exposure.

Methods

Population

The study targeted people living in a small village situated on the bank of a tributary of the Amazon River. This village is approximately 120 Km from the most extensive gold-mining fields in Tapajós River. A house-to-house survey revealed that this village had a total population of 780 people. Part of these inhabitants, about 120 persons (≥ 14 years old) who had some complaints, or wished to undergo a medical examination were underwent general medical

examination performed from September, 2008. Twenty six (26) subjects were selected to symptoms and signs neurological and electromyographic evaluation. The participation criteria included older than 14 years old, long-term local residents (for more ten years), mercury levels higher than 6ppm. They were given health evaluation by physician staff of Tropical Medicine Institute of Federal University Pará. The neurologic examination was conducted by a physician expert in neurology and neurophysiology. We excluded any exposed subjects with diagnosis of leprosy, diabetes mellitus, cerebrovascular diseases, and early exposure to mercury vapor.

Epidemiological information

Information concerning demographics, medical and work history, dietary habits, including fish consumption, with an emphasis on number fish meals were collected by a questionnaire, administered during interview by a physician.

Hair sample and Analysis

Hair strands from root taken from the occipital region were analyzed to total mercury. Mercury concentration was determined by atomic absorption spectrophotometry. In 1996 and 1998 hair sample were analyzed in Environmental Science Center of Tokyo/Institute of Environmental Science Technology, Yokohama, Japão, in 2000 were performed in the Laboratório de Caracterização Química of the Instituto de Pesquisas Energéticas e Nucleares (IPEN), University of São Paulo, Brazil, in 2003, 2005, 2006 in the Laboratory of the Tropical Medicine Institute of Pará Federal University. All the results were in $\mu\text{g/g}$ (ppm).

Clinical evaluation

Examination of the peripheral nervous system was carried out by a neurologist physician (MV) in all patients and performed according to standardized protocol. Sensory function was examined in all extremities and included pain and light touch perception, vibration, joint position sense according to a distal to proximal distribution. Equilibrium, Romberg's test, coordination, muscle strength, reflex.

Neurophysiological test.

For neurophysiologic evaluation was used a Medelec Synergy EMG (Oxford Instruments, Surrey, England). Right and left median, ulnar, deep peroneal and tibial motor nerves, right and left radial, median, ulnar and sural sensory nerves and tibial F-waves were studied. Recording were performed on Medelec Synergy EMG machine. Median and ulnar nerve compound muscle action potentials (CMAPs) were recorded from the abductor pollicis brevis (APB) and the abductor digiti minimi (ADM) muscles and stimulation was delivered at the wrist and the elbow. Peritoneal and tibial CMAPs were recorded from the extensor digitorum brevis (EDB) and the abductor hallucis (AH) muscles and stimulation was delivered at the ankle and the knee. Median, ulnar and radial sensory nerves action potentials (SNAPs) were recorded with ring electrodes from digit II, digit I and digit V and stimulation was applied at the wrist, 14cm from the recording electrodes. Sural SNAPs were recording posterior to the malleolus and stimulation 14cm proximally in the midcalf. The skin temperature was above 31°C in all subjects and was usually between 31 and 34°C.

In the sensory NCS low filter were set at 10Hz and high filters at 5 kHz. Sweep speed was 1ms per division, sensitivity was adjusted between 10 and 20µv per division, and stimulus duration was 0,1ms.

In the motor NCS low filter were set at 3Hz and high filters at 10kHz. Sweep speed was 2 ms per division, sensitivity was adjusted between 1 and 2 mv per division, and stimulus duration was 0,2.

Ethical evaluation

The study protocol was approved by the ethics communities of the Tropical Medicine Institute of the Federal University of Pará, Brazil. All participants gave their informed consent prior to study.

Results and Discussion

In this study 26 subjects had history of residence at Tapajós river for more 10 years and intake fish meals frequently and actually eat more than six fish meals for week. Some studies

showed that the fish is important source of protein the dietary in riverine communities from Amazonian (Lebel et al,1997; Eve et al, 1996) and mercury levels were related to the frequency of fish consumption (Grandjean et al,1993; Eve et al, 1996). In polluted area as Minamata in Japan the history of residence time, the occupational activities and food qualities was suggestive of exposure to a high degree of pollution(Tsubaki and Takahashi,1986). In this study, there was an epidemiological information suggesting low exposure to mercury for long time.

Reference values for mercury levels in hair from non-exposed individuals based on a review of existing data from other countries is 2ppm (WHO,1990). WHO advisory maximum tolerable levels for hair is 6ppm. In present study the maximum total mercury concentration found was 71,5ppm, and the means $26,5 \pm 29,6$ ppm for males and $15,54 \pm 5,9$ ppm for females. It is quite clear that contamination with methylmercury is present.. Hair mercury levels measured in our study were similar to those reported in other studies along the Tapajós River (Akagi et al,1995; Barbosa et al, 1995; Malm et al. 1997; Lebel, et al. 1997; Pinheiro et al, 2007) and in other regions of the Brazilian Amazon (Barbosa et al. 1998; Boischio and Cernichiari,1998).

Combinations of epidemiological information, mercury level in hair sample and neurological signs observed in residents from polluted area are very important for the mercury poisoning 's diagnosis. Sensory disturbance is one of the cardinal symptoms of methylmercury intoxication. Sensory impairment of the extremities is of the glove-stocking type, sometimes with perioral dysesthesia were observed in cases acute and chronic of Minamata disease which is the neurological disorder induced by oral ingestion of organic mercury accumulated in fishes and seafood (Harada,1995)

In this study, 26 subjects of total 120 inhabitants who wished to undergo a medical examination or had in this time some symptom or sign was examined for a physician to neurological symptoms and signs. Peripheral nerve involvement was observed in four cases. Three women with more than forty years old had paresthesias, positive phalen's sign (hand) and tinell's sign (wrist), and electroneuromyography(ENMG) with prolongation of the median distal latency that suggested Carpal Tunnel Syndrome. The mean of mercury levels in hair sample in each woman were 8.1ppm, 8.5ppm and 22ppm however there was not evidence of other neurological abnormalities.

In a case was observed sensory disturbances of the extremities the glove-stocking type associated with tremor, vibration sound disturbance, Romberg's sign, hyporreflexia, ataxia(finger-nose-test), inability to walk straight and disturbance of equilibrium (Romberg's test). Although he had living for a long time mercury exposure area their mercury levels were from 3,2 to 11ppm. According OMS/1990 the highest level without neuronal symptoms was

50ppm. He also had slowed sensory and motor nerve conduction velocities in the upper and lower extremities. In high pollution area as Minamata-Japan, the motor and sensory conduction velocities were usually delayed in accordance with the severity of disease, the sensory one being more severely affected, although there are some in whom conduction velocities are normal even when there is numbness (Tsubaki and Takahashi, 1986). Our patients had present a slowed sensory and motor nerve conduction velocities in the upper and lower extremities as observed in some cases of mercury poisoning but, others cause can determine similar electrophysiologic abnormalities as in the Spinocerebellar Ataxias (Van de Warrenberg, 2009).

That case showed the similar symptoms to methylmercury intoxication. However, the hair mercury level from 3,2 to 11ppm. We need the further study to reveal the cause of that syndrome.

Table 1- Levels mercury in hair sample and fish meals in inhabitants from Tapajós river, 2008

Sex	n cases	Fish meals/week mean±DP	Hgtotal (µg/g) mean±DP
Male	4	6,75±3,09(4-11)	20,28 ± 12,9 (6,53-71,5)
Female	22	6,60± 2,57(4-11)	15,06 ± 5,9 (6,16 – 24,2)
P value		>0,05	<0,05

Levels mercury in hair sample and fish meals in inhabitants from Tapajós river are showed table 1. Male and female have more six fish meals for week and mercury concentration in men are higher than women ($p < 0,05$).

Table 2: Symptoms and signs in 26 subjects from Tapajós Village , 2008.

Subjective	Number of cases(%)
Anxiety	5 (19,2)
Headache	2 (7,7)
lumbar pain	2 (7,7)
cervical pain	1 (3,8)
general fatigue	1 (3,8)
Insomnia	2 (7,7)
Tremor of hands	1 (3,8)
numbness of hands	3 (11,3)
numbness of hands and feet	1 (3,8)
psychic irritability	1 (3,8)
memory disturbance	1 (3,8)
Objective	
Somatosensory disturbances	4(15,4)
Vibration sound disturbance	1 (3,8)
Tremor of hands	1 (3,8)
Hyporreflexia	1 (3,8)
Ataxia(finger-nose-test)	1 (3,8)
Inability to walk straight	1 (3,8)
Disturbance of equilibrium (Romberg's test)	1 (3,8)
Phalen's sign (hand)	3 (11,3)
Tinel's (wrist)	3 (11,3)
Electrophysiologic findings	
Axonal polineuropathy	1(3,8)
Prolongation of the median distal latency (Carpal Tunnel Syndrome)	3(11,3)

References

- AKAGI, H.; MALM, O.; KINJO, Y.; HARADA, M.; BRANCHES, F.J.P.; PFEIFFER, W.C.; KATO, H. Methylmercury pollution in the Amazon, Brazil. *The Science of the Total Environment*, v. 5, p. 85-95, 1995.
- AKAGI, H.; MALM, O.; BRANCHES, F.J.P. Human exposure to mercury due to mining in the Amazon, Brazil. A review. *Environmental Sciences*, v. 3, p.199-211, 1996.
- BARBOSA, A.C.; SILVA, S.R.L.; DÓREA, J.G. Concentration of mercury in hair of indigenous mothers and infants from the Amazon Basin. *Archives of environmental contamination and toxicology*, v. 34, p. 100-105, 1998.
- BOISCHIO, A.A.P.; CERNICHIARI, E. Longitudinal hair mercury concentration in riverside mothers along the upper Madeira River (Brazil). *Environmental Research*, v. 77, p. 79-83, 1998.

- BRABO, E.S.; SANTOS, E.O.; JESUS, I.M.; MASCARENHAS, A.F.S.; FAIAL, K.F. Mercury contamination of fish and exposures of on indigenous community in Para State, Brazil. *Environmental Research*, v. 84, p. 197-203, 2000.
- CLARKSON, TW. The Three Modern Faces of Mercury. *Environment Health Perspect*, 110: 11-23, 2002.
- EVE, E.; OLIVEIRA, E.F.; EVE, C. The mercury problem and diets in the Brazilian Amazon: planning a solution. *Environmental Conservation*, v. 23, p. 133-139, 1996.
- GRANDJEAN, P.; CARDOSO. B.; GUIMARÃES, G. Mercury poisoning. *Lancet*, v. 342, p. 991, 1993.
- FUGINO, T. Clinical and Epidemiological Studies on Chronic Minamata Disease Part I: Study on Katsurajima Island. *Kumamoto Medical Journal. Kumamoto Japan*. v.44, n. 4, 1994.
- HARADA M. Minamata disease: methylmercury poisoning in Japan caused by environmental pollution. *Critical Reviews in Toxicology*, v. 25, p. 1-24, 1995.
- LACERDA, L.D.; SALOMONS, W. Mercúrio na Amazônia: uma bomba química? Rio de Janeiro: CETEM/CNPq, 1992, 78 p. Série Tecnologia Ambiental.
- LEBEL, J.; ROULET, M.; MERGLER, D.; LUCOTTE, M.; LARRIBE, F. Fish diet and mercury exposure in a riparian Amazonian population. *Water, Air and Soil Pollution*, v. 97, p. 31-44, 1997.
- LEBEL, J.; MERGLER, D.; BRANCHES, F.; LUCOTTE, M.; AMORIM, M.; LARRIBE, F.; DOLBEC, J. Neurotoxic effects of low-level methylmercury contamination in the Amazonian Basin. *Environmental Research*, v. 79, p. 20-32, 1998.
- HARADA M. Neurotoxicity of methylmercury, Minamata and the Amazon. In: Yasui, M., Strong, M.J., Ota, K.K., Verity, M.A. (eds.) *Mineral and Metal Neurotoxicology*, p. 177-187. New York, U.S.A.: CRC Press, 1997.
- MALM, O.; BRANCHES, F.J.P.; AKAGI, H.; CASTRO, M.B.; PFEIFFER, W.C.; HARADA, M.; BASTOS, W.R.; KATO, H. Mercury and methylmercury in fish and human hair from the Tapajós River Basin, Brazil. *The Science of the Total Environment*, v. 175, p. 141-150, 1995.
- SHIN J. O. Nerve conduction techniques. In: SHIN J. O *Clinical Electromyography. Nerve Conduction Studies*. 2a ed. Williams&Wilkins. 1993, p.39-45.
- TSUBAKI, T. TAKAHASHI, H. Clinical aspects of methylmercury poisoning in Minamata. In: Tsubaki, T., Takahashi, H. (eds.) *Recent advances in Minamata Diseases Studies*. Tokyo, Japan: Kodansha, 1986, p. 41-57.
- WORLD HEALTH ORGANIZATION (WHO). International Program in Chemical Safety (IPCS). *Environmental Health Criteria 101: Methylmercury*. Geneva, Switzerland: WHO, 1990.

Mercury levels in household members hair and in fish from fishing villages in Zhoushan, China (Poster Session)

Jinping Cheng*1, Lili Gao1, Xiaojie Liu2, Mineshi Sakamoto2, Wenhua Wang1

1 School of Environmental Science and Engineering, Shanghai Jiao Tong University, Shanghai 200240, China

2 Department of Epidemiology, National Institute for Minamata Disease, Minamata, Kumamoto 867-0008, Japan

Zhoushan Island is situated on the coast of the East China Sea, near Hangzhou and Shanghai. The offshore area is an important fishing ground, where the total length of the continental coastline is 1500 km and the total sea area of the fishing ground is about 100 000 km². The annual fish catch is about 800 000 t, representing one third of the Chinese national total. Seafood dominates the diet of the Zhoushan coastal populations, and includes many species of fish and shellfish, as well as a variety of other invertebrates. According to the dietary survey of Jiang et al (2005), A healthy adult in the coastal city of Zhoushan consumed 105±182 g fish meat each day which is greater than the average rate of consumption of marine products in China as a whole (23 g/person per day). Our previous studies showed that hair Hg levels in subjects from Zhoushan City were higher (mean 2.44 µg/g for males and 1.94 µg/g for females) than the levels found in hair samples from subjects in Shanghai, Ningbo, Dalian and Xiamen districts. This study aims to assess Hg exposure by measuring total mercury (T-Hg) and methyl mercury (MeHg) in the hair of fishermen and their household members in Zhoushan fishing village. Factors such as the quantity of fish consumed, age, gender and Hg levels in fish were investigated.

Health benefits and chemical risks associated to dietary habits: fish consumption, mercury and omega-3 fatty acids (Poster Session)

Domingo JL,¹ Bocio A,¹ Martí-Cid R,¹ Llobet JM,² Castell V,³ Mata E³

¹Laboratory of Toxicology & Environmental Health, School of Medicine, "Rovira i Virgili" University, Reus; ²Toxicology Unit, School of Pharmacy, University of Barcelona, Barcelona; ³ACSA, Department of Health, Generalitat de Catalunya, Barcelona, Spain

In recent years, and based on the importance of fish as a part of a healthy diet, there has been a notable promotion of fish and seafood consumption. For cardiovascular risk reduction in the general population, prestigious institutions such as the American Heart Association have suggested, among other dietary recommendations, to consume fish, especially oily fish, at least twice a week. Oily fish are rich in omega-3 polyunsaturated acids (PUFAs) such as EPA (eicosapentaenoic) and DHA (docosahexanoic), which have shown to have protective effects in preventing coronary heart disease among other important health benefits. However, recent studies have also shown that fish consumption might also mean a potential source of exposure to various chemical pollutants. In our laboratory, we determined in 14 edible marine species the concentrations of EPA and DHA, as well as those of a number of chemical contaminants, including mercury (Hg). To quantitatively establish the intake of these pollutants (risks) versus that of EPA + DHA (benefits), we designed a simple software, RIBEPEIX (<http://www.fmcs.urv.cat/portada/ribepeix/>). The concentrations of EPA, DHA, and chemical pollutants were used as database of the program. Although it seems quite evident that fish must be an important part of a balanced diet, to choose the most suitable species in terms of levels of PUFAs and pollutants, with special attention to Hg, the frequency of consumption, and the meal size are essential aspects to balance benefits and risks of a regular consumption of fish and seafood. Recently, we extended that information to a long series of macro- and micronutrients contained in widely consumed foodstuffs (other than fish and seafood), and with an important nutritional value. A new web, RIBEFood (<http://www.fmcs.urv.cat/ribefood/>) was designed. RIBEPEIX and RIBEFood may be used as easy tools to optimize the dietary habits of any subject by increasing the intake of beneficial nutrients and by reducing those of toxic pollutants, including Hg. They can be useful not only for professionals (cardiologists, general physicians, nutritionists, endocrinologists, toxicologists, etc.), but also for the general population, as both are available (free) in Internet.

Estimated daily intake of mercury by general population in Korea (Poster Session)

**Kwangsik Park¹, Eun Jung Park¹, Young Hee Chung², Sang Hoon Nam²,
Dae-Seon Kim³**

¹College of Pharmacy, Dongduk Women's University Seoul Korea

²National Institute of Environmental Research, Incheon, Korea

³School of Environmental Health, University of British Columbia, Canada

Introduction

Even if only a small amount is released to the environment, mercury causes bio-accumulation in the ecosystem. Mercury accumulation into human body is through not only digestive organs but also skin or respiration system. According to National Survey for Hazardous Materials in General Population, Korea, blood mercury concentrations were 4.34ug/L in 2005, 3.80ug/L in 2007.

This study was accomplished to estimate the daily intake of total mercury through food intake and environmental media by general population in Korea.

Methods

In order to estimate the amount of mercury intake, we used data from reported scientific papers and governmental statistics. Pollution degrees in environmental media were analyzed from recent official data under the consideration of area and season. Even if data of the amount of food intake and pollution were not enough, we tried to use the representative value of them.

Data of human exposure were calculated and combined by environmental media. Exposure amount through food intake was estimated by foodstuffs under the consideration of intake amount and mercury content in that food. Then, daily exposure amount was calculated finally.

. Intake through air =

$$\frac{\text{pollution}(\mu\text{g}/\text{m}^3) \times \text{daily respiration}(\text{m}^3/\text{day}) \times \text{unit converting factor}(10^{-3}\text{mg}/\mu\text{g})}{\text{body weight}(\text{kg})}$$

. Intake through water was not considered by the reason no mercury was detected in drinking and raw water in whole country.

. Daily soil intake was used 0.05g/day, the suggestion by US EPA IRIS

$$\text{inhale soil} = \frac{\text{pollution}(\mu\text{g}/\text{m}^3) \times \text{soil intake}(\text{g}/\text{day}) \times \text{unit factor}(10^{-3}\text{mg}/\mu\text{g})}{\text{body weight}(\text{kg})}$$

. Estimation of mercury intake through foodstuff ($\mu\text{g}/\text{kg}$) = Hg in foodstuff ($\mu\text{g}/\text{kg}$) x consumption(kg/day)

Result & Discussion

Mercury in ambient air was used the highest value between 200 ~ 2004, $5.32 \pm 3.53\text{ng}/\text{m}^3$, reported by Ministry of Environment (MOE), Korea. Mercury intake through water was disregarded by not detection in any water monitoring station in Korea. Mercury concentration in soil was $0.043 \pm 0.233\text{mg}/\text{kg}$ (range: ND ~ 12.060) in 6,988 samples from soil monitoring net work by MOE.

For mercury concentration in foodstuffs, data were available from Ministry of Health and Food & Drug Agency.

In these context, estimated daily intake of mercury is $18.8\mu\text{g}/\text{day}$ totally; $0.106\mu\text{g}/\text{day}$ through air, $0.002\mu\text{g}/\text{day}$ through soil, $18.719\mu\text{g}/\text{day}$ through foodstuffs.

If daily intake of mercury is compared with other countries, it is higher than England and the Netherlands. But it showed similar amount with Japan which has similar dietary habits.

On the other hand, daily intake of mercury by Korean is about 7.8% of daily maximum tolerable amount by UNEP, $240\mu\text{g}/\text{day}$.

Conclusion

Daily intake of mercury by general population in Korea was $18.8\mu\text{g}/\text{day}$, totally- $0.106\mu\text{g}/\text{day}$ through air(0.5%), $0.002\mu\text{g}/\text{day}$ through soil(0.01%), $18.719\mu\text{g}/\text{day}$ through foodstuffs(99.4%). Mercury intake through fish and shellfish intake occupied about 90% in total foodstuffs, and 89% in total samples. National level projects for mercury monitoring in fish and shellfish are going on in Korea from this result and suggestion.