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# Maternal and Fetal Mercury and *n*-3 Polyunsaturated Fatty Acids as a Risk and Benefit of Fish Consumption to Fetus

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Maternal fish consumption brings both risks and benefits to the fetus from the standpoint of methylmercury (MeHg) and *n*-3 PUFA (polyunsaturated fatty acids). MeHg is one of the most risky substances to come through fish consumption, and mercury concentrations in red blood cells (RBC-Hg) are the best biomarker of MeHg exposure. Docosahexaenoic acid (DHA, C22:6*n*-3), which is one of the most important fatty acids for normal brain development and function, is also derived from fish consumption. Our objective in this study was to examine the relationships between RBC-Hg and plasma fatty acid composition in mother and fetus at parturition. Venous blood samples were collected from 63 pairs of mothers and fetuses (umbilical cord blood) at delivery. In all cases, fetal RBC-Hg levels were higher than maternal RBC-Hg levels. The geometric mean of fetal RBC-Hg was 13.4 ng/g, which was significantly ($p < 0.01$) higher than that of maternal RBC-Hg (8.41 ng/g). While the average fetal/maternal RBC-Hg ratio was 1.6, the individual ratios varied from 1.08 to 2.19, suggesting considerable individual differences in MeHg concentrations between maternal and fetal circulations at delivery. A significant correlation was observed between maternal and fetal DHA concentrations ($r = 0.37$, $p < 0.01$). Further, a significant correlation was observed between RBC-Hg and plasma DHA in fetus ($r = 0.35$, $p < 0.01$). These results confirm that both MeHg and DHA which originated from fish consumption transferred from maternal to fetal circulation and existed in the fetal circulation 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.

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## 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 (*1*–*3*) since the susceptibility of the developing brain itself is high (*3*–*5*) and higher MeHg accumulates in cord blood than in maternal blood (*6*–*10*). 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 (*3*, *11*–*14*). Serum or plasma is known as a good biomarker of elementary or mercuric Hg exposure (*15*). On the other hand, mercury concentration in red blood cells (RBC-Hg) is the best biomarker of MeHg exposure (*3*, *16*–*18*). Additionally, more than 90% of that in RBC is known to be in the methyl form in high-fish-consuming populations (*19*). Further, hematocrit (Htc) values are quite different between mother and fetus at parturition (Table 1). Therefore, we used total Hg concentrations not in whole blood but in RBCs to reveal the MeHg levels in mothers and fetuses in the present study.

On the other hand, human intake of the *n*-3 longer chain of polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA, C20:5*n*-3) and docosahexaenoic acid (DHA, C22:6*n*-3), is also known to occur through marine products, mainly from fish consumption. Both of these fatty acids are very beneficial for human health (*20*, *21*). Especially, DHA is known to be an important *n*-3 PUFA for normal brain development and function (*22*–*25*). Rapid brain growth occurs primarily during the third trimester in humans (*26*, *27*), and the amount of these fatty acids increases dramatically during the period (*25*). This period corresponds to when the human brain is most susceptible to MeHg (*27*), and also a high accumulation of MeHg in the brain may occur during the period (*5*).

We conducted a study to determine the relationship between RBC-Hg and plasma fatty acid concentrations in fetus to evaluate the risks and benefits of maternal fish consumption by comparing 63 maternal–fetal pairs of blood samples.

## Materials and Methods

Sixty-three healthy Japanese pregnant women, ranging in age from 21 to 41 yr (average 29.6 ± 4.4 yr), planning to deliver in Munakata Suikokai General Hospital, Munakata City, Fukuoka, Japan, gave informed consent to take part in the present trial. Blood samples were collected from the mothers and umbilical cord. The samples included 13 mL of venous umbilical cord blood at birth and 10 mL of venous maternal blood 1 day after parturition before breakfast. Both blood samples were obtained by venipuncture with a small amount of heparin–Na and centrifuged at 3000 rpm for 10 min to separate into RBCs and plasma. Samples were stored at −80 °C until analysis. This study was approved by the Ethics Committee of the National Institute for Minamata Disease (NIMD).

Total Hg in 0.5 g of RBC was determined by cold vapor atomic absorption spectrophotometry (CVAAS) according to the method of Akagi and Nishimura (*29*). The method


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**TABLE 1. Subject Characteristics ($n$ = 63) and Values, Including Maternal and Fetal Mercury Concentrations in Red Blood Cells and Hematocrit Values**

| category | mean (geomean) | SD | min | max |
|---|---|---|---|---|
| maternal age (yr) | 29.6 | 4.37 | 21 | 41 |
| maternal RBC-Hg level (ng/g) | 9.12 (8.41) | 3.63 | 3.76 | 19.1 |
| maternal Htc value | 31.5 | 3.18 | 23.9 | 38.4 |
| fetal RBC-Hg level (ng/g) | 14.7 (13.4) | 6.37 | 4.92 | 35.4 |
| fetal Htc value | 45.2 | 3.64 | 38.1 | 53.7 |
| fetus/mother RBC-Hg ratio | 1.6 | 0.27 | 1.08 | 2.19 |

involves sample digestion with $HNO_3$, $HClO_4$, and $H_2SO_4$ followed by reduction to $Hg^0$ by $SnCl_2$. The detection limit was 0.1 ng/g. Accuracy was ensured by using certified reference material (DORM-2, dogfish muscle prepared by the National Research Council of Canada) as the quality control material; the Hg concentration found averaged 4.53 $\mu$g/g, as compared to the assigned value of 4.64 ± 0.26 $\mu$g/g. The total analytical precision of this analysis was estimated to be 3.9%. Fatty acid composition analysis in plasma was performed by SRL Inc. (Tokyo, Japan). Lipid was extracted from the sample according to the method of Folch et al. (*30*), and tricosanoic acid (C23:0) was added as an internal standard and then hydrolyzed with 0.5 M HCl. Free fatty acids were extracted with chloroform, and methylated with 0.4 M potassium methoxide–methanol solution and 14 wt % boron trifluoride–methanol. Fatty acid methyl esters were separated by capillary gas chromatography (GC17A, Shimadzu Co., Japan) and identified by comparison with standards (Sigma Chemical Co., Poole, U.K.). Fatty acid compositions were expressed as concentration ($\mu$g/mL of plasma) and percentage by weight of total fatty acids.

**Statistics.** The differences in RBC-Hg concentrations between paired samples were determined by paired $t$-tests. The associations between RBC-Hg and plasma fatty acid concentrations were studied by Pearson and Spearman correlation analysis. Each fetal/mother ratio of fatty acid was analyzed by a one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Significant differences were compared with the sum of the saturated and monounsaturated fatty acids assumed as a reference value. A $p$ value less than or equal to 0.05 was considered to demonstrate statistical significance.

## Results

Table 1 presents the subject characteristics and values, including maternal and fetal mercury concentrations in red blood cells and Htc values. The geometric mean of fetal RBC-Hg at 13.4 ng/g was 1.6 times higher ($p$ < 0.001) than that of maternal RBC-Hg (8.41 ng/g). There was a considerable difference in Htc values between maternal and fetal blood. The mean fetal Htc value (45.2 ± 3.6) was about 1.4 times higher than the maternal level (31.5 ± 3.2). In all 63 cases fetal RBC-Hg levels were higher than maternal RBC-Hg levels. A strong correlation was observed in RBC-Hg between mothers and fetuses ($r$ = 0.92, $p$ < 0.001; Figure 1), the average fetal/maternal RBC ratio was 1.6, and the individual ratios varied from 1.08 to 2.19 (Figure 2).

All of the fatty acid concentrations were lower in the fetuses' plasma than in their mothers' plasma (Table 2). However, the fetal/maternal ratio varied with each fatty acid. The ratio for the sum of saturated and monounsaturated fatty acid concentrations was 0.27. The ratios of $n$-3 and $n$-6 fatty acid concentrations were compared with the value for the sum of saturated and monounsaturated fatty acids

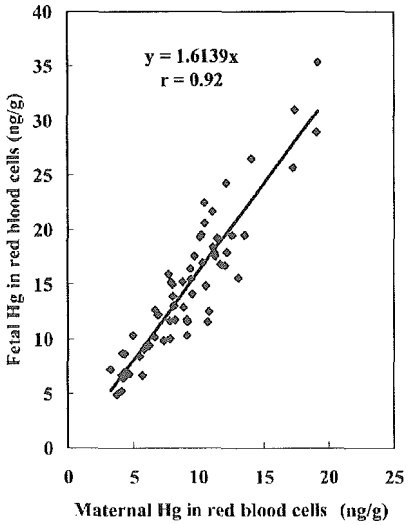


**FIGURE 1. Correlation between maternal and fetal mercury concentrations in red blood cells in 63 maternal–fetal pairs. In all 63 cases fetal RBC-Hg levels were higher than maternal RBC-Hg levels. A strong correlation was observed in RBC-Hg between mothers and fetuses ($r$ = 0.92, $p$ < 0.001).**

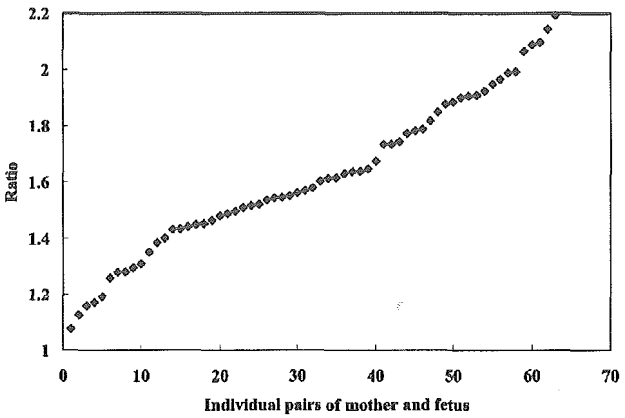


**FIGURE 2. Individual fetus/mother ratios of Hg concentrations in red blood cells in 63 maternal–fetal pairs. The average fetal/maternal RBC ratio was 1.6, and the individual ratios varied from 1.08 to 2.19.**

assumed as a reference value. The ratios for linoleic acid (LN, C18:2$n$-6)) and linolenic acid (LnN, C18:3$n$-3) were significantly ($p$ < 0.01) lower than the value for the sum of saturated and monounsaturated fatty acids. On the other hand, those for arachidonic acid (AA, C20:4$n$-6), DHA, and dihomo-$\gamma$-linolenic acid (DGLA, C20:3$n$-6) were significantly ($p$ < 0.01) higher than the reference value. Further, there were significant correlations in LN ($r$ = 0.31, $p$ < 0.05), DGLA ($r$ = 0.34, $p$ < 0.01), AA ($r$ = 0.39, $p$ < 0.01), EPA ($r$ = 0.39, $p$ < 0.01), and DHA ($r$ = 0.37, $p$ < 0.01) concentrations between maternal and fetal plasma (Table 2).

Maternal RBC-Hg concentrations showed significant correlation coefficients with maternal plasma EPA ($r$ = 0.36, $p$ < 0.01) and DHA ($r$ = 0.33, $p$ < 0.05) concentrations (Table 3). Further, fetal RBC-Hg concentrations showed a significant positive correlation with fetal plasma EPA ($r$ = 0.32, $p$ < 0.05) and DHA ($r$ = 0.35, $p$ < 0.01) (Table 3 and Figure 3).

## Discussion

MeHg is one of the most risky substances to fetus brain, and most of the human exposure to MeHg is through maternal fish consumption. On the other hand, DHA, which is important for the fetus brain and its growth, is derived also from maternal fish consumption. If human exposure to MeHg were independent of nutrition from fish, we would aim at

**TABLE 2. Comparison of Maternal and Plasma Fatty Acid Concentrations and Correlation Coefficient between Maternal and Fetal Plasma Fatty Acid Concentrations in 63 Maternal–Fetal Pairs[a]**

| fatty acid | mother ($n$ = 63) μg/mL | mother ($n$ = 63) % | fetus ($n$ = 63) μg/mL | fetus ($n$ = 63) % | fetus/mother | correlation coeff |
|---|---|---|---|---|---|---|
| saturated and monounsaturated | 2361 (476) | 61.6 | 619 (156) | 65.0 | 0.273 (0.08) | |
| linoleic (C18:2$n$-6) | 1013 (177) | 26.4 | 118 (34) | 12.4 | 0.118 (0.03)[b] | 0.31[c] |
| linolenic (C18:3$n$-3) | 30 (9) | 0.8 | 1.1 (1.3) | 0. 1 | 0.070 (0.04)[b] | 0.23 |
| dihomo-γ-linolenic (C20:3$n$-6) | 55 (17) | 1.4 | 33 (9.2) | 3.5 | 0.628 (0.20)[b] | 0.34[b] |
| arachidonic (C20:4$n$-6) | 187 (45) | 4.9 | 115 (29) | 12.0 | 0.637 (0.16)[b] | 0.39[b] |
| eicosapentaenoic (C20:5$n$-3) | 35 (19) | 0.9 | 7.1 (3.7) | 0.7 | 0.308 (0.13) | 0.39[b] |
| docosahexaenoic (C22:6$n$-3) | 149 (41) | 3.9 | 59 (16) | 6.2 | 0.417 (0.12)[b] | 0.37[b] |

[a] Saturated = palmitic (C16:0) and stearic (C18:0); monounsaturated = palmitoleic (C16:1$n$-7) and oleic (C18:1$n$-9). Values are mean (SD). Fetal/mother plasma fatty acid concentration ratio for $n$-3 and $n$-6 series against the sum of saturated and monounsaturated fatty acids as a reference value. Data were analyzed by a one-way ANOVA followed by Dunnett's multiple comparison test. The associations between maternal and fetal fatty acids were studied by correlation analysis. [b] $p < 0.01$. [c] $p < 0.05$.

**TABLE 3. Correlation Coefficient between Mercury Concentrations in Red Blood Cells and Fatty Acids in Both Mothers and Fetuses[a]**

| | | maternal mercury | fetal mercury |
|---|---|---|---|
| maternal ($n$ = 63) | linoleic (C18:2$n$-6) | −0.09 | −0.10 |
| | linolenic (C18:3$n$-3) | 0.23 | 0.19 |
| | dihomo-γ-linolenic (C20:3$n$-6) | −0.20 | −0.01 |
| | arachidonic (C20:4$n$-6) | −0.05 | −0.14 |
| | eicosapentaenoic (C20:5$n$-3) | 0.24 | 0.17 |
| | docosahexaenoic (C22:6$n$-3) | 0.27[c] | 0.21 |
| fetal ($n$ = 63) | linoleic (C18:2$n$-6) | −0.06 | −0.04 |
| | linolenic (C18:3$n$-3) | 0.06 | 0.10 |
| | dihomo-γ-linolenic (C20:3$n$-6) | −0.08 | −0.08 |
| | arachidonic (C20:4$n$-6) | −0.23 | −0.20 |
| | eicosapentaenoic (C20:5$n$-3) | 0.36[b] | 0.32[c] |
| | docosahexaenoic (C22:6$n$-3) | 0.33[b] | 0.35[b] |

[a] The associations between maternal and fetal fatty acids were studied by correlation analysis. [b] $p < 0.01$. [c] $p < 0.05$.

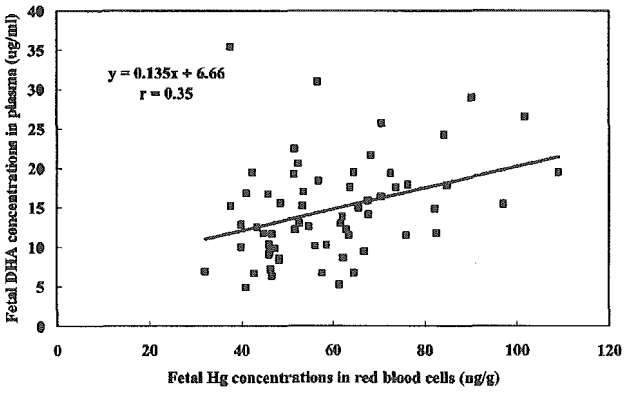


**FIGURE 3. Correlations between RBC-Hg concentrations and plasma DHA concentrations in 63 fetuses.**

zero exposure. However, the exposure is through fish, which is an important source of protein especially for Japanese and other Asian people. Fish also contain $n$-3 PUFA and other nutrients (*31*). Therefore, this study was designed mainly to determine the relationship between MeHg exposure and $n$-3 PUFA concentrations in fetus to consider the risks and benefits of maternal fish consumption during the gestation period.

The RBC-Hg level in umbilical cord blood was about 1.6 times higher than those in the mothers, and there was a significant correlation between them. This suggests that MeHg actively transfers to the fetus across the placenta via a neutral amino acid carrier, as demonstrated by previous studies (*9*, *10*). This higher Hg accumulation in the fetuses than in mothers is widely acknowledged from human and animal studies (*1–3*, *8–10*). However, the individual fetal/maternal RBC-Hg ratio varied from 1.08 to 2.19, indicating the individual differences in MeHg concentrations between maternal and fetal circulations at late gestation. This will be partly explained by the individual differences in MeHg transfer from mother to fetus through the placenta. The maternal MeHg level tends to be influenced by the latest meal. On the other hand, blood/organ ratios of MeHg concentration will be settled at parturition in fetal circulation. The results suggest that not the maternal side biomarker but the fetal side biomarker is much more advantageous to evaluate the subtle effects of MeHg exposure on the fetus during gestation.

DHA and AA are abundant in the brain (*22–25*, *28*), and the DGLA concentration is higher than those of LN, EPA, and LnN (*22*). During rapid brain growth, large amounts of DHA and AA from maternal circulation must reach the fetus to meet its needs for development (*23*, *25*, *33*). The rapid quantitative accretion of both DHA and AA during the third trimester of pregnancy was noticed in human brain (*25*, *28*, *33*). Breast milk also contains these fatty acids (*22*, *33*). The result of the high fetal/maternal ratio of DHA, AA, and DGLA (Table 2) also may indicate that the fatty acids which are important for the brain and its growth were selectively transferred from maternal circulation to fetal circulation, as was demonstrated in the previous study by Sakamoto and Kubota (*34*).

There were significant correlations in the EPA ($r$ = 0.39, $p < 0.01$) and DHA ($r$ = 0.37, $p < 0.01$) concentrations between maternal and fetal plasma (Table 2), indicating that EPA and DHA in fetal circulation which originated from fish consumption reflected the existence of these fatty acids in maternal circulation. Maternal RBC-Hg concentrations had significant correlation coefficients with both fetal plasma ($r$ = 0.36, $p < 0.01$) and DHA ($r$ = 0.33, $p < 0.01$) levels; further, fetal RBC-Hg concentrations had significant correlation coefficients with both fetal plasma EPA ($r$ = 0.32, $p < 0.05$) and DHA ($r$ = 0.35, $p < 0.01$) levels, indicating that both the MeHg and these $n$-3 PUFAs existing in fetal circulation showed a positive correlation (Table 3 and Figure 3). This is, to our knowledge, the first report to indicate significant correlation coefficients between the MeHg level and these fatty acids originating from fish consumption. These two results indicate that both MeHg and DHA, which act contrary to the normal growth and function of the developing brain, were taken into maternal circulation through maternal fish consumption and transfer to fetal circulation, 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 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 to confer the benefits. The different outcomes of the two main cohort studies in the Faroe Islands (*11*) and Seychelles Islands (*13*) regarding the effect of fetal MeHg exposure on children's development may be partly explained by the difference in the amount of DHA. However, the average MeHg exposure level was slightly higher in the Seychelles Islands than in the Faroe Islands. The Seychelles study (*13*) concluded there was no adverse effect from MeHg exposure through fish consumption, whereas the Faroe Islands study (*11*) demonstrated a negative developmental effect due to MeHg exposure. In any event, DHA concentrations in the fetal biomarker should be measured as a confounding factor to examine the subtle effects of MeHg exposure from fish consumption.

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# Methylmercury Exposure in the Tohoku Pregnant Women

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**Abstract:** Methylmercury (MeHg) is a potent neurotoxicant to the developing fetal brain. Since the major source of MeHg is through fish intake, MeHg exposure is a great concern in the fish-eating population. However, few recent data on exposure of the pregnant women are available in Japan. The present study undertook a study of MeHg exposure in the pregnant women in the Tohoku district in Japan to investigate the present status of MeHg exposure and to identify predictors of elevated exposure. Pregnant women were recruited through a large obstetrician clinic at each four cities. Hair sample was analyzed for total mercury. A questionnaire including fish-consumption frequency and hair cosmetic treatment was also administered. The results showed that 2-3 % of the pregnant women had levels in a range of possible concern for adverse developmental effects (> 8 ppm). We decided to initiate a cohort study to examine the effects of prenatal exposure to MeHg on the neurobehavioral development in the children.

**Key words:** hair mercury, methylmercury, pregnant women

## INTRODUCTION

Methylmercury (MeHg) is well known neurotoxicant. One of the main target organs is the central nervous system for adults and fetus. The developing brain is much more vulnerable and sensitive to the effects of MeHg. Actually, it was shown that prenatal MeHg exposure causes the delay of development of cognitive functions in Faroe Islands (GRANDJEAN, 1997). Therefore, the Japanese Ministry of Health, Labour and Welfare recommends pregnant women and women who would be pregnant refrain from consuming several kinds of fish species (MHLV, 2003). However, little is known about the actual exposure level of pregnant women at present in Japan. The present study was aimed at evaluating the exposure levels to MeHg by analysis of hair collected from pregnant women living in Tohoku, Japan, where the main mercury source is expected to be the consumption of fish and the related products.

## METHODS

From June 2002 to August 2002, healthy pregnant women were recruited with their

informed consent at obstetrical wards of hospitals at four cities in Tohoku, Japan. All cities face the Pacific Ocean, and two (city A and city D) of four cities have a large fishery port. The major products at these fishery ports include tuna, swordfish, and bonito. Hair samples and questionnaire data were collected from approximately 100 pregnant women at each city. The study protocol was approved by the Medical Ethics Committee of the Tohoku University Graduate School of Medicine.

Hair samples were cut next to the scalp, in the nape area, with stainless steel scissors. Several types of questionnaire were administered after the delivery. To assess the fish-intake status of the pregnant women, a food-intake frequent questionnaire (FFQ) regarding the consumption of fish and the related products, and another questionnaire regarding hair treatments including bleaching, permanent wave and coloring were administrated. Total mercury analysis was carried out by cold vapor atomic absorption spectrometry. Briefly, without washing the hair samples, each sample was acid digested with $HNO_3$, $HClO_4$ and $H_2SO_4$ at 200 °C for 30 minutes. The resultant ionic mercury was then reduced to mercury vapor by tin chloride to a flameless atomic absorption monitor (HG-201, Sanso Co., Ltd., Tokyo). Analytical accuracy was ensured by analyzing the Human Hair Reference Material NIES CRM No. 13 from the National Institute of Environmental Studies (Lot #650, Tsukuba). In fish-eating populations, total mercury in hair consists mostly of MeHg. Indeed, a few samples were analyzed to know the exact MeHg concentration by the method of AKAGI AND NISHIMURA (1991). MeHg in hair first extracted with hydrochloric acid and then with benzene. The organic layer was subjected to electron-capture detection gas chromatography (ECD-GC) at the National Institute for Minamata Diseases. The concentration of MeHg was confirmed to be more than 95 % of the total mercury content.

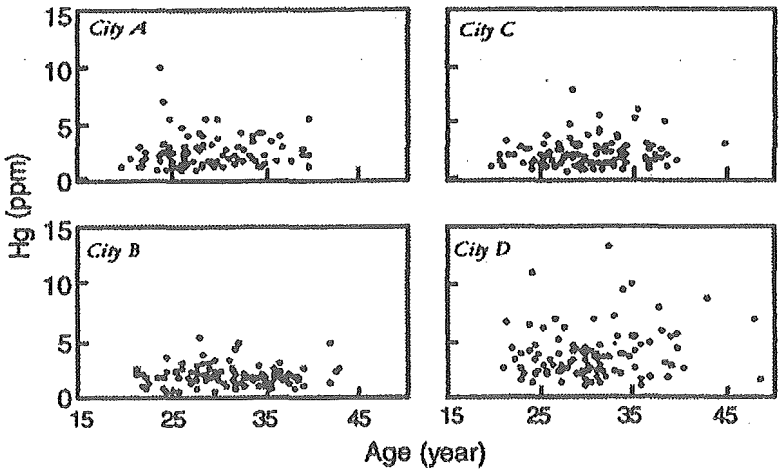


**Figure 1.** Distribution of hair total mercury content of pregnant women in the four cities in Tohoku, Japan. N=94-100.

## RESULTS AND DISCUSSION

Total mercury levels in hair samples obtained from pregnant women at four cities are shown in Figure 1. Hair mercury content was 2.33±1.48 ppm (mean±SD, N=94) for city A, 2.30±1.27 (N=100) for city B, 1.99±0.92 (N=99) for city C, and 3.79±2.25 (N=100) ppm for city D. Our results indicated that mercury levels in hair samples differed among the four cities surveyed, and mean mercury levels was highest in city D. These regional differences appeared to reflect differences in the amount of fish and shellfish consumption.

The U.S. Environmental Protection Agency proposed a safe level (reference dose, RfD) for MeHg intake at 0.1 μg/kg (body weight)/day for pregnant women (US EPA, 1997, 2001). This dose seemed to correspond to the hair mercury concentration of approximately 1 ppm. In the present study, we found that 92 % of pregnant women in Tohoku had levels that exceeded this concentration. Based on the findings of the cohort studies at Faroe, the lower confidence bounds of benchmark doses (BMDLs) for maternal hair mercury concentration were shown to be 6.1 ppm for the attention measure and 9.1 ppm for the language test (BUDTZ-JORGENSEN, 2000). These suggest that some neurobehavioral adverse effects were found in children when their maternal hair mercury concentrations were higher than 8-10 ppm. Our results also showed that the percentage of pregnant women who exceeded these levels (> 8 ppm) was 2-3 %; this number was increased to be 6 % in city D. Additional study is strongly required to determine the effects of these lower levels of MeHg exposure in pregnant women in Japan. We, therefore, recently initiated a birth cohort study, the Tohoku Study of Infant Development, in Japan (NAKAI, 2004).

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# Effects of Methylmercury Exposure on Human Reproduction

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**Abstract:** We examined relation between environmental methylmercury exposure via fish consumption and human fecundity. Time to pregnancy (TTP), a measure of fecundity, was investigated by self-administrated questionnaire from parturient women at two hospitals in Sendai, Japan. Total mercury concentration in their hair was determined. So far the result suggests that the subject with longer TTP had higher hair mercury level.

**Key words:** Mercury, Time to pregnancy, Fecundity

## INTRODUCTION

Fish has been major protein source of Japanese from ancient time and its healthiness has recently been highlighted in many countries. It is important, however, to evaluate the health risk posed by consuming fish because it is well known that fish can be a major source of hazardous chemicals as well. Methylmercury is one such chemical and concern has been posed that its excess intake via fish consumption in pregnant woman can result in developmental deficit in the child. Although reproductive effects of methylmercury were indicated in the previous studies, they have been scarcely investigated in human population to date. This paper examines possible relationship between environmental methylmercury exposure via fish consumption and human fecundity, a biological ability to reproduce, in Japanese maternal cohorts.

## BACKGROUND OF THE PRESENT STUDY (ARAKAWA ET AL., 2003)

We examined time to pregnancy (TTP), time taken by a couple to get pregnant after cessation of contraception, as a measure to assess human fecundity for use in environmental reproductive health survey. A delayed TTP can be considered to reflect an injury in reproductive processes (BAIRD ET AL., 1986).

Since TTP has never been measured in Japan, we carried out a preliminary study aimed at establishing a Japanese version of the TTP questionnaire and determining if TTP could be reliably measured in Japan. We prepared a self-administered questionnaire that consisted of questions on TTP, sexual life and dietary habits and it was tested on 92 pregnant women, gestational age 2-3 mo., in Tokyo.

The result indicated that TTP could be reliably measured in Japanese based on the findings that 1. high response rate, and 2. the distribution of the TTP reported by our subjects was similar to that reported in previous studies in the USA and European countries and 3. the shape of the distribution seemed biologically plausible. Moreover, we found a statistical association between delayed TTP and frequent fish eating habit in that study, as well as that between TTP and smoking. This result suggested the adverse effect of chemicals on fecundity, and we intended to extend the TTP study to larger population with the study method involving not only questionnaire on fish-eating frequency but also hair mercury measurement.

## MATERIALS AND METHODS

We employed self-administered questionnaire that had been developed in our preliminary study. The subjects, who had a baby born in two different hospitals in Sendai, Japan, were asked to fill the questionnaire on 3 days postpartum at the hospitals during the period of January 2002 to March 2004. Hair samples were obtained from the subjects on 2 days postpartum. We used total hair mercury level as an indicator of methylmercury intake (SUZUKI ET AL., 1993). Total mercury concentration in the hair samples was determined by cold vapor atomic absorption spectrometry (AKAGI AND NISHIMURA, 1991). Certified reference material from NIES was concurrently analyzed for quality assurance of hair mercury analysis.

The subjects were classified into two groups according to TTP: Group 1 (TTP ≤ 6 months) and Group 2 (TTP > 6 months). Biological attributes and life style parameters (dietary habits, smoking, drinking, intake of caffeine etc) of both female subject and her partner were compared between the two groups by chi-square test and U-test. Binominal logistic regression analysis was also employed.

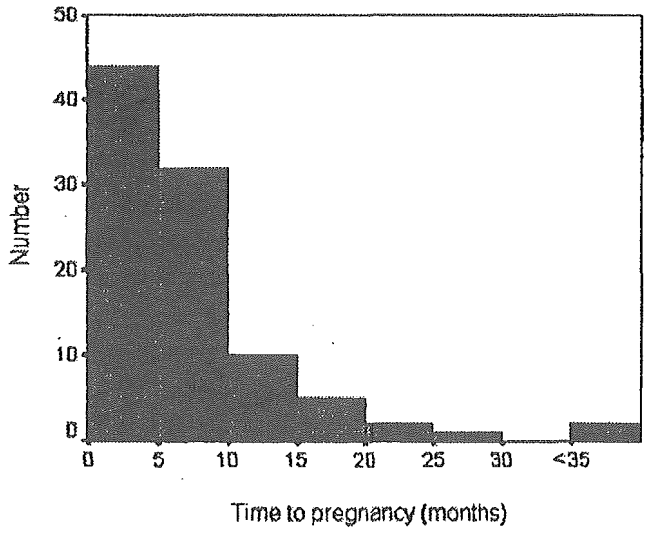


**Figure 1.** Histogram of time to pregnancy.

## RESULTS AND DISCUSSION

Of the 154 women approached, 106 (68.8 %) could report TTP. We excluded subjects who had infertility treatment from data analysis (10 women) because their TTP may not present intact fecundity.

Figure 1 shows the TTP distribution of the present subjects, which was similar to those reported so far (e. g., CURTIS ET AL., 1999).

Of all of the variables tested for the association with TTP, frequency of fish consumption by female subject was the only variable with significance: there were statistically significantly more frequent fish eaters in Group 2 than in Group 1 ($p=0.02$, chi-square test).

So far we have obtained hair mercury concentration for only 48 subjects and we carried out a tentative analysis on this limited data set. As shown in Figure 2, hair mercury level in Group 2, 2.9 mg/kg, was statistically higher than that of Group 1, 2.2 mg/kg ($p<0.05$, U-test). Although the data analysis so far suggests that fish consumption is associated with human fecundity, as has been reflected in the association between high mercury exposure level and delayed TTP, it does not necessarily mean that mercury is the causative agent to impair human fecundity. It is well recognized that fish consumption is associated with elevated intake of a variety of chemicals such as dioxins and PCBs as well as mercury. We have to specify which agent is involved in the impaired fecundity.

Total number of subjects of this study cohort is 298 and we will analyze total data set with logistic regression analysis including hair mercury level as independent variable.

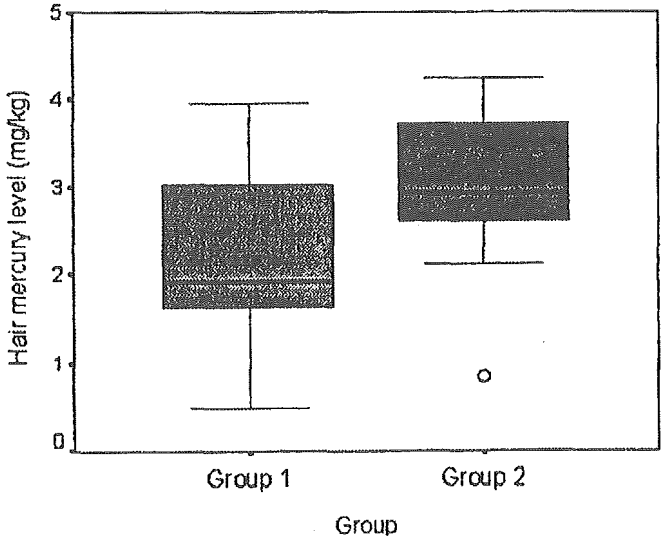


**Figure 2.** TTP of Group and hair mercury level.

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特　集

# 水銀と健康問題
## ―過去と現在―

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### 摘　要

水銀は常温・常圧で液体である唯一の金属元素で，金属そのものまた各種の無機・有機水銀化合物として利用されてきた。毒性はそれぞれ異なるが，中でもメチル水銀はとりわけ強い中枢神経毒性を有し，水俣やイラク等世界各地で多数の中毒患者を発生させた。ことに妊娠中に母親がメチル水銀に曝露されると，母に影響の無い濃度であっても出生後の児の発育・発達に影響が及ぶこと知られている。

水銀はごく低濃度であるが自然界に存在し微生物の作用でメチル水銀が生成され，さらに食物連鎖により人が食べる大型の肉食魚や歯鯨等海棲哺乳類に蓄積する。したがって，魚介類等を多食する人集団で胎児期メチル水銀曝露の生後の影響が懸念されていくつかの研究が行われている。しかし，影響の有無について異なった結果が得られている。

若い人々が魚を食べなくなったとは言われつつも，やはり日本人は魚を食べている集団であることは間違いない。その意味で，日本でもメチル水銀の曝露量とその影響の調査が必要である。本特集では，現在問題視されている魚介類摂取によるメチル水銀の胎児期曝露とその生後の影響の可能性について理解いただくため，水銀問題を主に健康の観点から取り上げた。


**キーワード**：水銀，メチル水銀，生物濃縮，胎児期曝露，発達への影響，健康影響

## 過去―水銀とその化合物とそれらの健康影響

### 1．水銀とその化合物

水銀（Mercury，元素記号 Hg，原子番号 80，原子量 200.61）は常温・常圧で液体である唯一の金属元素で，亜鉛やカドミウムと同じく12族に属する。沸点は 356.7 ℃，融点は－38.88 ℃ であり，常温でも蒸発し水銀蒸気（$Hg^0$）となる。水銀は他の金属と容易にアマルガムを形成することから各種金属の精錬に利用され，銀とのアマルガムは歯科治療にも用いられてきた。また，体温計・気圧計・血圧計などの計測機器や照明器具や乾電池などの電気製品に使われてきた。現在我が国では利用されていないが，電気分解による苛性ソーダの生産にも触媒として使われることもある。各種の水銀化合物は農薬や防カビ剤等様々な薬品として利用されてきた[1]。

水銀化合物の化学形態としては，無機水銀化合物と水銀原子が有機分子中の炭素と結合している有機水銀化合物の２種類がある。さらに，イオン価や有機分子部分の違いや，単体の水銀（金属水銀とその蒸気）を加えて，表－１のように分類される[2~4]。しかし，１価の無機水銀化合物は，容易に単体の水銀と２価の無機水銀になると考えられており，短鎖アルキル水銀以外の有機水銀化合物も生体内や環境中で容易に分解し，代謝や動態は無機水銀と同様だと考えられている[4]。

### 2．水銀の健康影響

中毒学の立場から考えると，金属水銀の蒸気，2価の無機水銀，および短鎖アルキル水銀の毒性が人などの高等生物にとって重要な問題と考えられる。この３種の水銀（化合物）は，それぞれ独特の毒性を示す[4]。

本特集でも，金採掘・精錬に伴う曝露について述べているが金属水銀の場合，問題となるのは発生する蒸気の吸入である。高濃度の蒸気の吸収では，曝

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露後数時間で化学的肺臓炎と呼ばれる肺間質の炎症をおこし，呼吸困難におちいる。その後腎不全（後述）もおこすことがある[5]。それより低い濃度のくり返し曝露では，著明な振戦（身体の一部に不随意的に出現する律動的振動，典型的水銀中毒では手指の振戦が見られる）を伴う神経・心理学的症状を示す。特に短気になりすぐに怒る様子は，“mad as a hatter”と言う英語の表現があるくらい有名で，「不思議の国のアリス」にも mad hatter として登場する[6]。それは，フェルトの帽子を製造する時に水銀化合物に浸漬した材料を成形して加熱するのであるが，その時水銀蒸気が発生するからである。さらに低濃度の曝露では，上記のような典型的な症状は出現しないが，機器を使って検出できるような細かい振戦や尿細管上皮のごく軽度の傷害を示すような低分子蛋白尿が出現するとの報告がある[3,7]。

無機水銀化合物（塩）の場合は，その溶液を誤ってあるいは自殺を企図して飲んだ場合に中毒が起きる。濃度が高いと口腔内や食道等が腐食され，ショック状態になることもある。消化管での吸収率は高くはないが，充分量が吸収されれば腎不全が起きる[2]。この腎不全は尿細管の壊死によるもので回復することもある。前述の高濃度の水銀蒸気曝露後におきる腎不全も，体内で蒸気（$Hg^0$）からイオン化した水銀（$Hg^{++}$）よっておきると考えられている[4]。

### 3．水俣病とイラクのメチル水銀中毒禍

有機水銀化合物の中でもメチル水銀はとりわけ強い中枢神経毒性を有し，我が国の水俣やイラク等世界各地で多数の中毒患者を発生させたことで知られている。我が国の公害のひとつで多数の犠牲者を出した水俣病は，これまで発生した水銀の健康影響の中でもっとも悲惨な事件のひとつである。原因となったメチル水銀化合物は，触媒として水銀を使用していたアセトアルデヒド製造過程で生じた副生成物が工場排水と混じって流出した。それが水俣湾を汚染し，生態系の食物連鎖を通じて魚介類の体内で生物濃縮・蓄積され，その魚を食する事によって住民が曝露されたのであった[8]。

イラクでは水俣の場合と異なり，カビの発生を防ぐためにメチル水銀で処理された種播き用小麦を食べてしまったことによって，メチル水銀中毒が発生した。患者数は水俣病の犠牲者を上回る六千名以上と言われている[9]。

メチル水銀は消化管から高率に吸収され，血液脳関門を通過して中枢神経系に侵入する。曝露が継続し中枢神経系組織内の濃度がある程度まで上昇すると神経細胞の脱落（「死」）をおこし，そのために種々の神経症状が発生すると考えられている（このような濃度を「臨界濃度」と言う）。成人における典型的なメチル水銀中毒は，知覚障害，運動失調，求心性視野狭窄を３主徴とする Hunter-Russell 症候群と称される[10]。水俣病では，これらの主要症状の他にも様々な神経症状が認められ，曝露量の違いによる症状の差異もある。例えば，中毒の初期や軽度の例では，四肢末端・舌や口唇周囲の錯感覚をともなう知覚障害が認められた[11]。

妊娠中に母親がメチル水銀に曝露されると，出生後の児の発育・発達に影響が及ぶことが水俣病で明らかになった[12]。メチル水銀は血液脳関門ばかりでなく，さらに胎盤関門も通過して発育・発達の途中の胎児の中枢神経系に作用するが，その影響は成人のそれと比べ遙かに重篤になる。母体には明らかな症状を呈するほどに到らない曝露量で，児には重篤な症状を引き起こした胎児性水俣病が発生した。その理由はいくつか考えられるが，胎児期の曝露は神経細胞の遊走（細胞が組織の中で移動することを言うが，ここでは発達中の胎児脳で神経細胞が表面に向かって移動し層状構造を構築することを意味している）に影響し，中枢神経系組織の構築そのものに影響を与えることも一つであろう[13]。胎児性水俣病

**表－1　水銀とその化合物**

| | |
|---|---|
| 金属水銀（metalic mercury）<br>（単体の水銀，水銀蒸気）<br>（elemental mercury, mercury vapor） | $Hg^0$ |
| 無機水銀化合物<br>（inorganic mercury compounds） | 1価 mercurous mercury; $Hg^+$<br>2価 mercuric mercury ; $Hg^{++}$ |
| 有機水銀化合物<br>（organic mercury compounds） | アルキル水銀 alkyl mercury<br>フェニル水銀 phenyl mercury<br>他 |

の臨床像は成人の場合とは異なり，中枢神経系全般が障害されたことを示す非特異的な脳性麻痺症状（失調性運動障害や様々な精神障害や小脳症状も含む）で，出生後の発育・発達に伴い明らかとなる[12]。イラクにおいても胎児期曝露の患者がおり，この時の調査結果がその後のリスク評価に用いられている（本特集にて後述）。

## 現在－水銀への曝露

### 4．一般集団の水銀への曝露

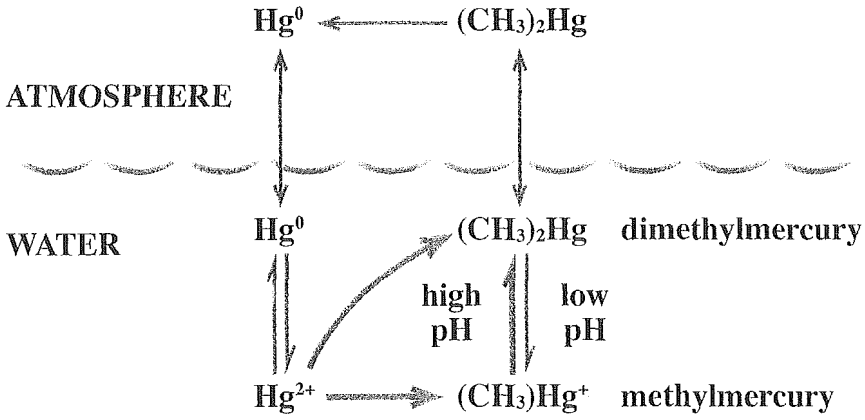
水俣病のような公害やイラクのメチル水銀中毒禍は，現在では過去の話となってしまったが，世界の各地では水銀に曝露されている人々が皆無になったわけではない。それは，水銀がごく低濃度ながら岩石圏ばかりでなく大気圏や水圏等自然界に存在し，主に微生物の作用でメチル水銀が生成するからである（図－1）。生成されたメチル水銀は，さらに水中の生物圏で食物連鎖と生物濃縮によって，人が食べる大型の肉食魚や歯鯨等海棲哺乳類に蓄積する。したがって，これらの魚介類等を多食する人はメチル水銀曝露量も大きいと考えられている。なお，化石燃料の燃焼も，大気圏への水銀の負荷を高めていると考えられており，その影響を考慮する必要もあろう。

さて，前述のイラクのメチル水銀禍の調査結果によるリスク評価で，WHO[14]は「妊娠中の母親の毛髪水銀値が10-20 ppmで胎児に及ぶ影響のリスクが5％である」とした。しかし，イラクの事例では発症レベル付近の毛髪水銀濃度をもつ母親とその児のペアー数が少なかったので，閾値や最大無作用量の推定を行う上で充分な精度を得られていないと考えられている。その一方で，様々な魚多食集団でメチル水銀曝露量の調査の結果，10-20 ppmをこえる人々の存在が示された。そこで，実際に胎児期曝露の影響が見られるか，調査が行われるようになった（本特集にて後述）。

ところで，なぜ母親の毛髪中水銀値が指標になっているのか？その理由は次のように考えられる。まず，メチル水銀はケラチンの豊富な毛髪中に取り込まれやすく，いったん毛髪に取り込まれるとその後の移動はないと考えられるからである。そうすると，毛髪の生え際に近いところは現在の曝露を示すことになるが，それだけでなく生え際から先端になるに従ってより過去の曝露を示すことにもなる。その結果，毛髪をセグメント状に切ってそれぞれの部分の水銀濃度を測定すると，曝露の歴史がわかることになる。もし，ひと月に1 cm毛髪が伸びるとすると，20 cmもあれば1年以上過去の曝露からほぼ現在の曝露までが推定できることになり，妊娠期間を充分にカバー可能である。したがって出産後に採取しても胎児期の曝露が推定出来るので，毛髪水銀濃度はこのような調査には適している。

実際には，血液中水銀濃度と毛髪中水銀濃度の比の変動，髪伸長速度の個人差や民族差，パーマネントをはじめとする毛髪の美容上の処理で水銀濃度が低下してしまう可能性など，様々な変動要因がある。そこで，妊娠中の母体血や出産時の臍胎血の方がより正確な曝露の指標になるとの議論もあるが，母体血や臍胎血の採取は困難である場合が多い。

人々が曝露されるのはメチル水銀だけではない[15]。歯科用アマルガムは，我が国ではあまり使わ




図－1　環境中での水銀の動態

れてはいないようではあるが，しかし，アメリカ合衆国や北欧ではまだ齲歯治療で利用されている。その歯科用アマルガムの表面から水銀蒸気が発生することは容易に想像されるが，チューインガムを噛んだり歯ブラシで磨くと発生量が著増することが知られるようになった[16]。

我が国ではあまり話題にならなかったが，数年前アメリカ合衆国ではワクチン用注射液のチメロサールが取り除かれることになった。チメロサールは，水銀を含む薬品で主に消毒・防腐を目的にほとんどのワクチン用注射液に含有されていた。チメロサールが分解することによって分子構造の一部であったエチル水銀が生ずる[15]。このエチル水銀は，メチル水銀の毒性と類似している考えられ，さらに自閉症と関連づける見方もあり，そのためにアメリカ合衆国ではチメロサールの使用を取り止めたのである。

## 5．胎児期メチル水銀曝露の生後の影響に関するコホート研究

胎児性水俣病やイラクの胎児期曝露の研究結果を受けて，1980年代から，魚介類を多食する人口集団で，胎児期水銀曝露の影響についていくつかの疫学的研究がなされてきた。その代表例が，フェロー諸島前向き研究（Faroe Islands Prospective Study: FIPS）とセイシェル小児発達研究（Seychelles Child Development Study: SCDS）の二つである（図－2）。この二つの研究については，本特集の別稿で詳しく紹介しているが，対象集団の規模，曝露量，および児の神経行動学的検査を主として観察していると言う点で比較的類似していると考えられるにも関わらず，メチル水銀の影響の有無となると異なった結果が得られている。この理由としては異なる曝露源，対象集団の民族的背景の違い，言語の違い，曝露濃度の微細な違い等が考えられているが，最終的な解決を得ていない[17,18]。

この外にも，カナダ，ニュージーランド，マデイラ諸島，アマゾン川流域での調査等いくつかの調査があるが，前向きコホートであると言う意味では，現在のところ上記の二つの研究の意義が大きいと考えられる。

## 6．おわりに：魚食の制限を巡る混乱

我が国の厚生労働省は2003年6月に歯鯨類を含むいくつかの魚種の妊婦の摂食制限の注意喚起行った。そのために摂食制限魚種のひとつの「キンメダイ」が売れなくなって漁師が困ったとの報道もあった。妊婦等に対する摂食制限は我が国の政府だけでなく，アメリカ合衆国，英国，ニュージーランド等各国の政府も出している（表－2）。

制限魚種が異なるのは各国の事情もあるであろうが，根拠とするメチル水銀の耐用摂取量もそれぞれである。アメリカ合衆国では政府機関によって異なっていたりする。国際機関のJoint FAO/WHO Expert Committee on Food Additives(JEFCA)も，2003年6月に新たな耐用週間摂取量を提案した[19]。このような混乱は，魚を食している人々やあるいは水産業に従事している者にとっては，好ましいものではない。しかし，これまでの研究結果が一致して


図－2　フェロー諸島とセイシェル共和国

表－2　各国での食事指導の比較

| | 米国 | 英国 | カナダ | オーストラリア／ニュージーランド | ノルウェー |
|---|---|---|---|---|---|
| 機　関 | FDA | Food Standard Agency | Health Canada | ANZFA (Australia NewZealand Food Standards) | SNT（食品衛生監視局） |
| 実施日 | 2001年1月 | 2002年5月<br>2003年2月 | 2002年5月 | 2001年1月 | 2003年5月 |
| 魚　種 | サメ，メカジキ，サワラ，アマダイ（日本のアマダイとは異なる魚種） | サメ，メカジキ，マカジキマグロの缶詰，マグロステーキ | メカジキ，サメ，マグロ | サメ，エイ，カジキ，バラマンディ，ギンサワラ，オレンジラフィー，リング，ミナミマグロ，地熱水域で捕獲される魚 | 鯨，川カマス，パーチ（25 cm 以上），マス及びイワナ（1 kg 以上），サメ，カジキ，エイ，マグロ |
| 対象者 | 妊婦や妊娠を考えている女性<br>また，授乳中の母親や乳幼児も同様 | 妊婦，妊娠を考えている女性，乳児，16才以下の子供 | すべての人<br>更に，幼児，妊娠可能年齢の女性 | 妊婦，妊娠を考えている女性 | 妊婦，授乳中の母親 |
| 指導内容 | 上記の魚の摂取を避けると共に，その他の魚種は週に12オンス（340 g）とすべき | 上記の者はサメ，メカジキ，マカジキの摂取を避けるべき | 上記の魚の摂取は週に1食とすべき，また，幼児，妊娠可能年齢の女性は月に1食とすべき | 週に4食以下とすべき（1食約150 g） | 妊婦，授乳中の母親は鯨を食べるべきではない。<br>また，妊婦は鯨以外の上記の魚についても食べるべきではない |

いないことによって，様々な関係者から充分に支持される耐用摂取量を提案することは極めて困難であると言えよう。

若い人々が魚を食べなくなったとは言われつつも，やはり日本人は魚を食べている集団であることは間違いない。その意味で，日本でもメチル水銀の曝露量とその影響の調査が必要である。食品中にはメチル水銀だけでなくPCB類や農薬類等ほかの環境汚染物質も存在するので，それらの影響も考慮する必要もある[18]。さらにそれら汚染物質の濃度は低く，様々な環境要因からの交絡作用を受けやすい。例えば魚の不飽和脂肪酸の一部は胎児期の脳の発達に必須であると考えられている。実際に母親の毛髪水銀値が高い児の方が良い成績を示す検査があったことを示す報告もある（本特集で後述）。したがって調査は魚の栄養学的な価値も含んだ我が国の食文化や栄養摂取パターンに配慮した独自のものでもあるべきである。そう言う意味では，文化や人々の背景の異なる外国での調査結果をそのまま日本に適応するのは難しいと考えられる。また単に環境汚染物質等の負の影響を見ると言う調査でなく，広い意味での環境と発達の調査そのものであるべきだとも考えられる。

本特集では，水銀問題を主に健康の観点から取り上げたが，すべての面に触れられたわけではない。現在各国で問題視されている魚介類摂取によるメチル水銀の胎児期曝露とその生後の影響の可能性の問題について，多少でも御理解いただければ幸いである。

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# Environmental exposure to mercury and its consequences on human health: the history and the present situation

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## Abstract

Mercury is only a metal that is a liquid in room temperature and its compounds have been extensively used. Toxic effects are various depending on the chemical forms and methylmercury is most toxic to the central nervous system of humans. The fetal toxicity by maternal exposure during gestation was shown in disasters of Minamata Disease and methylmercury poisoning in Iraq. Since then it is considered that fetuses are more susceptible than adults.

Mercury exists in the natural environment at low concentrations and is partly methylated by microorganisms. Generated methylmercury is accumulated in predatory fish and sea mammals at the concentration that is of concern for the people who eat large amount. Developmental effects after fetal exposure are especially concerned.

Although fish consumption among young Japanese has been decreased, Japanese still eat relatively large amount of fish and sea food. Therefore, it is needed to investigate exposure level and possible effects of methylmercury exposure among Japanese population. In this special series of reports, current issues on health effects of exposure to mercury and its compounds are described for better understanding of the problems.

**Key Words:** Mercury, Methylmercury, Biological Concentration, Fetal Exposure, Developmental Effects, Health Effects

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特　集

# メチル水銀と健康問題 ～未来～

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## 摘　要

メチル水銀に関する健康問題は，日本の水俣病やイラクのメチル水銀禍に端を発し，その後世界各地で展開されたコホート研究に繋がり，メチル水銀のリスク評価からリスク管理に移す過程に至っている。リスク管理への移行に際しては，基礎的研究の不十分さを楯にして，科学的データの下に政策的配慮を加味して不確実係数が定まり，幾つかの基準値が米国ないし世界機関から出されている。しかしながら，メチル水銀の健康影響に関する研究結果が世界中の有識者の間で議論される中で，各国固有の食文化についてどれほどの配慮がなされたのか疑問に思われる。本稿は，メチル水銀の基準摂取量（*RfD*）および暫定的耐容週間摂取量（PTWI）の作成過程に垣間見られる問題点を吟味し，今後のわが国のリスク管理の方向性を提言する。


**キーワード：**メチル水銀，基準摂取量（*RfD*），暫定的耐容週間摂取量（PTWI）

## 1. はじめに

米国環境保護庁（EPA）は，メチル水銀に感受性の高い特定集団（特に，妊娠中に曝露を受けた胎児）の健康を脅かす有害影響を防止する目的で，メチル水銀の基準摂取量（毎日摂取しても人体に影響を及ぼさないとされる量 reference dose, *RfD*）を 0.1μg/kg 体重/日と 1995 年に定めた[1]。EPA はこの *RfD* の改訂作業を，米国科学アカデミー（NAS）諮問委員会の勧告に従い，フェロー諸島前向き研究（Faroe Islands Prospective Study）[3]の成績に基づいて行った[4]。しかし，フェロー諸島前向き研究の 7 歳児データから算出されたメチル水銀の臨界濃度（有害影響が現れ始める濃度）はイラクのデータから算出された数値とほぼ一致していたことから，改訂後の *RfD* は 1995 年に算出した数値と同じである。

一方，オーストラリア・ニュージーランド合同食品基準協議会は 2001 年 1 月に「魚は妊娠や授乳に有用な栄養素の良好な供給源であるが，水銀なども含まれているので，科学的根拠は今後の課題であるものの魚摂取を週 600 g 未満にすることが望ましい」と報じた。同様の勧告はカナダ食品検査局（2002 年 5 月），英国食品基準局（2002 年 5 月），米国食品医薬品局（2003 年 12 月）でも行われ，わが国の厚生労働省もこれらの動向に追随する形でキンメダイなど数種の魚と幾つかの歯鯨類の摂食制限を妊婦に呼び掛けた（2003 年 6 月）[5]。このような時流のなか，2003 年 6 月に開催された第 61 回 FAO/WHO 合同食品添加物専門家委員会（JECFA）は，暫定的耐容週間摂取量（provisional tolerable weekly intake, PTWI）として 1972 年に定めた 3.3μg/kg 体重/週を，1.6μg/kg 体重/週に変更した[6]。この JECFA の会議では，EPA が臨界濃度として採用した頭髪（以後，毛髪と表す）水銀濃度 11μg/g でなく，セイシェル小児発達研究（Seychelles Child Development Study）[7]とフェロー諸島前向き研究[3]から算出された値を採用し，しかも EPA と異なる不確実係数（uncertainty factor, UF）を用いた。

本稿は，メチル水銀からヒトの健康を守るために設けられた基準摂取量（EPA の *RfD* および JECFA の PTWI）の算出過程を概説し，今後のメチル水銀規制値を設定する際に考慮すべき事項について検討する。併せて，この過程の中で臨界濃度の推定に用いられる NOAEL（no observed adverse effect

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level，無毒性量）およびbenchmark dose（BMD）の算出法の長所・短所，およびセイシェル小児発達研究とフェロー諸島前向き研究で得られた結果が相違した理由等についても触れる。

## 2．基準摂取量の算出手順

通常の環境基準値あるいは基準摂取量を決める手順は，関連する文献を収集し，曝露集団，曝露指標および影響指標（endpoint）毎に整理することから始まる。次に，影響指標として何を用いるべきか決定するとともに，収集文献の中で最も適切な研究を選択する。この選択された研究データより臨界濃度を推定し，不確実係数UFで除して基準値とする。

EPAはメチル水銀の発がん性について“possible human carcinogen”と分類しているが，小集団での疫学研究のため結果の解釈が困難である[2,8]。入手可能なデータの中でメチル水銀に最も感受性の高い影響指標として，胎児期に曝露を受けた子供の神経発達影響がNAS諮問委員会で是認されている（最近，免疫・心血管系への影響も考慮すべきとされている）[2]。このような理由で，EPAは*RfD*算出に当たってMarshらのイラク研究[9]を採用し，またJECFAはセイシェル小児発達研究とフェロー諸島前向き研究の両者を採用した[6]。同様に，最小リスクレベル（minimal risk level，MRL）を算出した米国有害物質・疾病登録局（ATSDR）はセイシェル小児発達研究を採用した[10]。後者のMRLは，セイシェルの人々が摂取している魚の水銀濃度（0.004～0.75 ppm）が米国人のそれに近いことを意識した結果である。

イラク研究では，妊娠中にメチル水銀で処理された小麦から作ったパンを摂取した女性から生まれた子供の神経発達異常（18ヶ月児の歩行および24ヶ月児の言語の遅れ），脳性麻痺，筋緊張異常，深部腱反射異常等が二人の神経学者によって検査された[1]。また，曝露後の母親の毛髪はX線蛍光分光光度計で分析され，毛髪水銀濃度は1～674$\mu$g/gであった。これらの母子81組のデータを用いてメチル水銀曝露による量－反応関係が検討された。

JECFAで採用されたセイシェル小児発達研究とフェロー諸島前向き研究は，NAS諮問委員会が検討した3つの疫学研究からニュージーランド研究[11]を除いた残りの2つである。これら3研究はいずれも出生時に登録された大規模コホートを対象とし小児神経発達を影響指標としていたが，ニュージーランド研究では対象集団237名の中のひとりが極端に高い毛髪水銀濃度（86$\mu$g/g）を示し，かつそのデータが全体の結果に大きな影響を及ぼしていると判断されたので除外された[6]。

## 3．臨界濃度の推定

非発癌性影響のリスク評価として十分にコントロールされた動物実験から得られるNOAELは，曝露群に統計的あるいは生物学的に有意な毒性影響の増加を生じさせない“最も高い実験的曝露量”と定義されている[2]。しかしながら，このNOAELあるいはLOAEL（lowest observed adverse effect level，最小毒性量）は曝露群と非曝露群との比較（有意差検定）により決定されるため，サンプル数に左右されやすく，近年研究者の間で疑議が唱えられている[12]。

CrumpはNOAELまたはLOAELに絡む問題に対して，有意な量－影響（反応）関係を前提としたBMDという考えを提唱した[13]。すなわち，曝露量に伴って影響指標が有意に増加（あるいは減少）する関係があるとき，非曝露集団の影響指標の95パーセンタイル値（あるいは5パーセンタイル値）を算出し，上限値以上（あるいは下限値以下）を異常値（この限界値をcutoff値）と定義すると，非曝露集団には異常値を示す者が5％いることになる。このとき，非曝露集団の影響指標における異常率$P_0$は5％に設定されたことになる。曝露集団において，当該異常率$P_0$よりさらに$\alpha$％の異常増加（benchmark response，BMR）をもたらす曝露濃度をBMDと定義する（$P_0$およびBMRは研究者により異なるが，通常$P_0 = 0.05$，$BMR = 0.05$が用いられている）[12,14]。これは図1の曝露濃度0の集団（非曝露集団）の正規分布を，有意な量－影響関係を示す関数に沿って右方移動し，正規分布のcutoff値以上の範囲が$P_0$＋BMRとなるときの曝露濃度である。また，BMDの95％信頼下限値をBMDLと呼び，NASの報告によるとNOAELと大体同じ値になると考えられている[2]。しかしながら，対象集団のサンプル数が少ないとBMDLは低くなりやすいし[12]，曝露データに極端に高い値が点在すると量－影響関係がその値に引っ張られて誤った結論を導出しやすくなる[6]。

小児の神経発達影響に及ぼすイラク研究の臨界濃度は，Marsh ら[8]が報告した神経影響を全て考慮した発症率と妊娠期間中の母親の毛髪水銀濃度より，BMD法で推定した[1]。このBMDの算出に当たってWeibullモデルが選択されたのは，このモデルが発育毒性データに最も適しているという最近の研究報告からである[13]。EPAが実際に使用した非連続Weibullモデルは$P(d) = P_0 + (1 - P_0)(1 - \exp[-A_1 \cdot d^{A2}])$であり，dは曝露量，$P_0$はバックグラウンド反応率（＝0.12468），$A_1$は勾配（＝$9.47 \times 10^{-3}$），A2は形状母数（＝1.000）である。ここで算