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# Significance of fingernail and toenail mercury concentrations as biomarkers for prenatal methylmercury exposure in relation to segmental hair mercury concentrations

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ABSTRACT

*Objective:* To investigate the appropriateness of mercury (Hg) concentrations in fingernails and toenails at parturition for detecting prenatal exposure to methylmercury (MeHg).
*Methods:* Total Hg concentrations were measured in 54 paired samples of fingernails, toenails, maternal blood, and maternal hair (1 cm incremental segments from the scalp toward the tip) collected at 4th weeks of (early) pregnancy, and the same specimens and cord blood collected at parturition.
*Results:* Strong correlations were observed between Hg concentrations in fingernails and toenails at early pregnancy ($r=0.923$, $p<0.01$) and at parturition ($r=0.895$, $p<0.01$). At early pregnancy, Hg concentrations in fingernails and toenails showed the strongest correlations with those in hair 3–4 cm from the scalp ($r=0.818$ and $r=0.747$, $p<0.01$, respectively) among the 1 cm incremental hair segments. Mercury concentrations in fingernails and toenails at parturition represented strong correlations with those in cord blood ($r=0.803$, $p<0.01$ for fingernails and $r=0.792$, $p<0.01$ for toenails, respectively). At parturition, Hg concentrations in fingernails had the highest correlation with those in hair 0–1 cm from the scalp ($r=0.918$, $p<0.01$), and Hg concentrations in toenails showed the highest correlation with those in hair at 2–3 cm from the scalp ($r=0.872$, $p<0.01$). In addition, Hg concentrations in both finger and toe nails at parturition had equally high ($p<0.01$) correlation coefficients with hair segments at 0-1, 1-2, and 2-3 cm from the scalp.
*Conclusions:* Mercury in fingernails and toenails at early pregnancy reflected the maternal Hg body burden level approximately 5 months retroactively. At parturition, Hg levels in fingernails and toenails also showed strong correlations with those in cord blood. In addition, Hg levels in fingernails and toenails at parturition reflected more recent MeHg exposure, compared with those at early pregnancy. These results suggest that fingernails and toenails at parturition are useful biomarkers for prenatal MeHg exposure for mothers and fetuses, especially during the third-trimester of gestation.


## 1. Introduction

Methylmercury (MeHg) is a widespread environmental neurotoxin. The organ targeted by MeHg exposure during gestation is the fetal brain, especially the developing brain during the third trimester (Rice and Barone, 2000). For this reason, biomarkers reflecting the MeHg exposure level in the fetus during the third trimester are very important for predicting the effects of MeHg on child development. In the Faroe Islands study, cord blood mercury (Hg) concentration was the preferred biomarker for MeHg exposure, although maternal hair Hg was also analyzed (Grandjean et al., 1997; Murata et al., 2004). In the Seychelles study, maternal hair Hg concentration was used as the only biomarker for fetal exposure (Davidson et al., 1998). Each biomarker has its

*Abbreviations:* ; Hg, mercury; MeHg, methylmercury; CV, coefficient of variation; CVAAS, cold vapor atomic absorption spectrophotometry; ICP-MS, inductively coupled plasma mass spectrometry

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advantages and disadvantages. Cord blood circulates in the fetal body and can directly reflect the MeHg concentrations in the fetal organs, including the fetal brain at birth (Cernichiari et al., 1995a; NRC, 2000). Although hair Hg analysis is associated with a number of variables such as the hair's growth rate, density, color, waving, external contamination, and permanent treatment (WHO, 1990), segmental analysis of maternal hair is able to provide time-course information, because the average hair growth rate is commonly assumed to be about 1 cm per month (Boischio et al., 2000; Cernichiari et al., 1995a). However, because the hair follicle grows out of the skin surface after about 3 weeks, Hg concentrations in the first hair segment proximal to the scalp (0–1 cm from the scalp) will only reflect the MeHg exposure from 3 weeks before (Cernichiari et al., 1995a; Phelps et al., 1980; Yaginuma–Sakurai et al., 2012). Therefore, maternal hair Hg concentrations at parturition may not reflect the prenatal MeHg exposure during the last 3 weeks of gestation.

A number of studies have employed Hg concentrations in toenails and/or fingernails as biomarkers for MeHg exposure (Alfthan, 1997; Hinners et al., 2012; Morton et al., 2004; Ohno et al., 2006; Rees et al., 2007; Suzuki et al., 1989; Yoshizawa et al., 2002). In most of these studies, toenails rather than fingernails were preferred, because toenails are often less contaminated than fingernails, especially among dental personnel and gold miners who handle Hg amalgam (Morton et al., 2004). Nevertheless, the time-lag for nail growth from the nail matrix to the nail edge was not considered for analyzes in the above-mentioned studies. Furthermore, the appropriateness of using fingernails and toenails as biomarkers for maternal and fetal MeHg exposure at parturition has not yet been validated.

In this study, we conducted simultaneous analyzes of fingernails, toenails, maternal blood, umbilical cord blood, and maternal hair segments to investigate how Hg concentrations in nails are related to the other biomarkers, especially at parturition. We measured total Hg concentrations as a surrogate for MeHg concentrations in the samples. The overall hypothesis is that Hg concentrations in both toenails and fingernails at parturition strongly reflect the fetal exposure to MeHg during the third trimester.

## 2. Materials and methods

### 2.1. Sample collection

Fifty-four healthy Japanese pregnant women without any occupational exposure to Hg compounds and planning to undergo delivery at Fukuda Hospital, Kumamoto, Japan from 2006 to 2007, provided informed consent at their first pregnancy check-up to participate in the study. Their ages ranged from 21 to 41 years (mean: $29.6 \pm 4.4$ years). None of the women had undergone permanent hair treatments for at least 6 months prior to participation, and they were asked not to do the treatments during gestation. Fasting venous blood samples were collected from the participants at the 4th week of (early) pregnancy in the morning and 1 day after parturition. Umbilical cord venous blood was collected at parturition. Blood samples were obtained by venipuncture and collected into heparin-Na-containing vacutainer tubes. All samples were stored at $-80$ °C until analysis. Approximately 50 full-length strands of maternal hair were collected at early pregnancy and parturition by cutting the strands close to the scalp in the occipital area. Each maternal hair strand was cut into 1 cm incremental segments from the scalp end toward the tip. Approximately 1 mm clippings of the free edges of fingernails and toenails were also collected at early pregnancy and at parturition. Nail polish found on one sample at early pregnancy was removed using acetone. Hair and nail samples were stored at room temperature until analysis. The study protocol was reviewed and approved by the Ethics Committee of the National Institute for Minamata Disease, Minamata, Japan.

### 2.2. Hg analytical methods

The total Hg concentrations in approximately 0.5 ml of blood, 5–10 mg of hair, and 10–13 mg of nails were determined by cold vapor atomic absorption spectrophotometry (CVAAS) according to a previously described method (Akagi et al., 2000) using a mercury analyzer Model Hg-201 (Sanso Seisakusho Co. Ltd., Tokyo, Japan). The method involved sample digestion with $HNO_3$, $HClO_4$, and $H_2SO_4$ followed by reduction to $Hg^0$ by $SnCl_2$. The method detection limit was 0.01 ng/g. The accuracy of Hg analysis in blood samples was ensured using a reference blood material (Level 2, MR9067; Nycomed Co., Oslo, Norway). The mean value ($n=5$) of the determined Hg was 7.5 μg/L, which was within the recommended range of 6.8–8.5 μg/L as measured by inductively coupled plasma mass spectrometry (ICP-MS). Reference human hair (NIES CRM No. 13 Human Hairs; National Institute for Environmental Studies, Environmental Agency of Japan) was also measured as quality control. The mean value ($n=5$) of the determined total Hg was 4.32 ng/mg, which was within the certified range of 4.22–4.62 ng/mg. A coefficient of variation (CV) of 0.8% was obtained from 5-time repeated measurements of a standard solution (50 ng Hg/ml) analysis. Hg levels were expressed as concentrations (ng/g) in blood, hair, and nail samples.

### 2.3. Statistical analysis

Mercury concentrations for all biomarkers were expressed as geometric means and 25th–57th percentiles because the values were log-normally distributed. For the statistical analysis, logarithmically-transformed Hg concentrations were used. Differences in Hg concentrations were evaluated by a paired *t*-test. The strength of the relationships among the Hg concentrations in blood, hair segments, and nail samples was analyzed by the Pearson product-moment correlation coefficient. Values of $p \leq 0.05$ were considered as statistically significant.

## 3. Results

### 3.1. Hg in nails and other biomarkers at early pregnancy and parturition

The geometric means and 25th–75th percentiles of the Hg concentrations (ng/g) in maternal blood, cord blood, fingernails, toenails, and 1 cm hair segments from the scalp to the tip at early pregnancy and at parturition are summarized in Table 1. Among the maternal specimens, the highest Hg concentration was observed in hair, followed by fingernails, toenails, and blood at both sampling periods. Mercury ratios of 3 specimens (hair 1–3 cm from the scalp, fingernails, and toenails) to maternal blood (mean and range in parentheses) were 307 (118–606), 130 (63.1–241), and 111 (61.6–198) at early pregnancy, and 348 (148–506), 134 (47.8–231), and 114 (35.0–224) at parturition, respectively. At parturition, Hg level in cord blood was significantly ($p<0.01$) higher than that in maternal blood, with an average ratio of 1.84. Mercury concentrations in maternal blood, fingernails, and toenails at parturition were significantly lower than those at early pregnancy ($p<0.05$ for fingernails, and $p<0.01$ for maternal blood and toenails). Fig. 1 depicted the correlations of Hg concentrations between fingernails and toenails at early pregnancy (A) and at parturition (B). Mercury concentrations in fingernails