Original Article

Hair-to-blood ratio and biological half-life of mercury: experimental study of methylmercury exposure through fish consumption in humans

Kozue Yaginuma-Sakurai^{1,2}, Katsuyuki Murata³, Miyuki Iwai-Shimada^{1,2}, Kunihiro Nakai¹,
Naoyuki Kurokawa¹, Nozomi Tatsuta¹ and Hiroshi Satoh¹

¹Environmental Health Sciences, Tohoku University Graduate School of Medicine, 2-1 Seiryō-machi,
Aoba-ku, Sendai 980-8575, Japan

²Research Fellow of the Japan Society for the Promotion of Science, 8 Ichiban-cho, Chiyoda-ku,
Tokyo 102-8472, Japan

³Department of Environmental Health Sciences, Akita University Graduate School of Medicine, 1-1-1 Hondo,
Akita 010-8543, Japan

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ABSTRACT — The hair-to-blood ratio and biological half-life of methylmercury in a one-compartment model seem to differ between past and recent studies. To reevaluate them, 27 healthy volunteers were exposed to methylmercury at the provisional tolerable weekly intake (3.4 µg/kg body weight/week) for adults through fish consumption for 14 weeks, followed by a 15-week washout period after the cessation of exposure. Blood was collected every 1 or 2 weeks, and hair was cut every 4 weeks. Total mercury (T-Hg) concentrations were analyzed in blood and hair. The T-Hg levels of blood and hair changed with time ($p < 0.001$). The mean concentrations increased from 6.7 ng/g at week 0 to 26.9 ng/g at week 14 in blood, and from 2.3 to 8.8 µg/g in hair. The mean hair-to-blood ratio after the adjustment for the time lag from blood to hair was 344 ± 54 (S.D.) for the entire period. The half-lives of T-Hg were calculated from raw data to be 94 ± 23 days for blood and 102 ± 31 days for hair, but the half-lives recalculated after subtracting the background levels from the raw data were 57 ± 18 and 64 ± 22 days, respectively. In conclusion, the hair-to-blood ratio of methylmercury, based on past studies, appears to be underestimated in light of recent studies. The crude half-life may be preferred rather than the recalculated one because of the practicability and uncertainties of the background level, though the latter half-life may approximate the conventional one.

Key words: Methylmercury, Biological half-life, Blood-to-hair concentration ratio, Kinetics, Experimental study

INTRODUCTION

Methylmercury (MeHg) is well known to be a widespread environmental neurotoxicant to which we are exposed mainly via fish consumption (National Research Council, 2000; WHO, 1990). As the developmental effects of prenatal exposure are of great concern (Grandjean *et al.*, 1997, 2004; Strain *et al.*, 2008; Suzuki *et al.*, 2010), recommendations for the amount of MeHg intake were proposed by the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA, 2003) and the

Japan Food Safety Commission (2005) as the provisional tolerable weekly intake (PTWI) of 1.6 µg of mercury/kg body weight (BW)/week and tolerable weekly intake (TWI) of 2.0 µg of mercury/kg BW/week for pregnant women, respectively. In the calculation of PTWI/TWI, however, various assumptions (*e.g.*, the hair-to-blood ratio of 250 and elimination constant of 0.014) and different uncertainty factors for interpersonal pharmacokinetic variability have been utilized, but the former values seem to differ between past and recent studies. For endorsing the validity of such assumptions, it is crucial to reevaluate the kinetics of MeHg transfer in human blood and hair.

Correspondence: Hiroshi Satoh (E-mail: h.satoh@ehs.med.tohoku.ac.jp)

Some experimental studies of the MeHg kinetics in humans were conducted several decades ago (Aberg *et al.*, 1969; Birke *et al.*, 1972; Hislop *et al.*, 1983; Kershaw *et al.*, 1980; Miettinen *et al.*, 1971; Sherlock *et al.*, 1984; Smith *et al.*, 1994). The exposure settings of those studies may differ from our current state in general, *i.e.* long-term exposure at low levels. In addition, the hair-to-blood mercury concentration ratio shows wide variation in not only animal experiments (Mottet *et al.*, 1987) but also epidemiological studies (Berghlund *et al.*, 2005; Budtz-Jørgensen *et al.*, 2004). However, no one has revised the above conventional assumptions. To scrutinize the hair-to-blood ratio and biological half-life of mercury, we conducted an experimental study on low-level exposure to MeHg in humans.

MATERIALS AND METHODS

Subjects

Twenty-seven healthy volunteers (14 men and 13 women) were recruited among students and graduates of universities in Sendai, Japan. The main exclusion criteria of this study were obvious diseases such as heart, neurological and metabolic disorders and, for women, confirmed or suspected pregnancy, intended pregnancy and nursing. All subjects were provided with a complete study protocol, and gave written informed consent for participation.

Study protocol

The study protocol was approved by the Ethics Committee of the Tohoku University Graduate School of Medicine. The detailed method and design were described previously (Yaginuma-Sakurai *et al.*, 2010). Although a control group unexposed to MeHg was set up in that study, only the subjects of the experimental group were analyzed in the present study. The subjects of the experimental group were exposed to MeHg at the PTWI for Japanese adults (3.4 µg/kg BW/week) from the consumption of bigeye tuna and swordfish, containing approximately 1.0 µg/g MeHg, during the initial 14-week period (the exposure period), and after that, observation continued for another 15 weeks (the washout period). The amount of fish consumption was calculated on the basis of each subject's BW (*e.g.*, a subject whose BW was 60 kg consumed 204 g/week of bigeye tuna or swordfish). A mean of 210 (range, 131–310) g of fish was given to each subject once a week. No restriction was placed on the cooking method because frying, boiling or steaming of fish does not lead to any significant loss of MeHg (Sherlock *et al.*, 1984). Consumption of fish containing high levels of MeHg was restricted during the whole period of 29 weeks.

Blood was collected every week during the exposure period and every 2 weeks during the washout period. After the blood was collected and the hematocrit measured, the blood was centrifuged for 5 min at 12,000 rpm. Until mercury analysis, separated red blood cells (RBCs) and plasma were stored at -80°C. Hair growing newly was cut close to the scalp in the same 1 cm × 1 cm square of the occipital area every 4 weeks, and the median length in the hair rearranged from the shortest to the longest one was measured as the growth rate. The hair samples were kept in a desiccator until mercury analysis. Since artificial hair-waving and hair-straightening may affect the total mercury (T-Hg) level in hair (Dakeishi *et al.*, 2005; Ohba *et al.*, 2008), they were banned during the whole period of this study.

Measurements

Analysis of the T-Hg content of RBCs, plasma and hair was carried out by cold vapor atomic absorption spectrometry according to the method of Akagi and Nishimura (1991). The samples were acid digested with HNO₃, HClO₄, and H₂SO₄ at 200°C for 30 min. The resultant ionic mercury was then reduced to mercury vapor by adding SnCl₂ to a flameless atomic absorption monitor (HG-201 Sanso Co., Ltd., Tokyo, Japan). The T-Hg concentration in whole blood (*W*, ng/g) was calculated from the formula: $W = R \times H/100 + P \times (1 - H/100)$, where *H* is the hematocrit and *R* and *P* are the concentrations in RBCs (ng/g) and plasma (ng/g), respectively. T-Hg analyses of RBCs and plasma were conducted by IDEA Consultants, Inc. (Tokyo, Japan). The analysis of blood samples was conducted after the samples were arranged at random to avoid systematic bias.

Accuracy of determination was confirmed by using certified reference materials. Human blood material (Seronom trace elements whole blood level-2 201605 and serum level-1 201405; SERO AS, Billingstad, Norway) was used for T-Hg in RBCs and plasma, and human hair material (NIES CRM No.13; National Institute for Environmental Studies; Ibaraki, Japan) was used for T-Hg in hair. The determined value (mean ± S.D.), for T-Hg in Seronom 201605 was 7.86 ± 0.49 ng/ml (*n* = 28; certified value 7.4 ~ 10.0 ng/ml), that for T-Hg in Seronom 201405 was 1.01 ± 0.11 ng/ml (*n* = 24; 0.9 ~ 1.3 ng/ml), and the T-Hg level in NIES CRM No. 13 was 4.46 ± 0.14 µg/g (*n* = 27; 4.42 ± 0.20 µg/g).

Statistical analysis

The time-dependent changes of T-Hg concentrations of whole blood and hair and the hair-to-blood T-Hg concentration ratio were tested by two-way analysis of vari-

Table 1. Characteristics of the study subjects (14 men and 13 women) at week 0

Characteristics	Mean $\pm$ S.D.	Range
Age (year)	25.2 $\pm$ 4.1	(20 ~ 35)
Body mass index (kg/m ²)	22.2 $\pm$ 3.2	(17.6 ~ 30.5)
Hematocrit (%)	43.2 $\pm$ 3.9	(36 ~ 50)
Total mercury in red blood cells (ng/g)	13.5 $\pm$ 6.4	(7.4 ~ 39.7)
Total mercury in plasma (ng/g)	1.4 $\pm$ 0.5	(0.5 ~ 2.8)
Total mercury in whole blood (ng/g)	6.7 $\pm$ 3.3	(3.2 ~ 19.8)
Total mercury in hair (μ g/g)	2.3 $\pm$ 1.1	(1.1 ~ 6.5)
Ratio of hair to blood total mercury	351 $\pm$ 54	(263 ~ 478)

ance (ANOVA) to control for interpersonal variations and by a trend test using the cumulative χ^2 method because they were regarded as an approximately normal distribution (Hosmer-Lemeshow test, $p > 0.1$). The difference between two slopes of the increasing changes in whole blood and hair was tested by ANOVA (Snedecor and Cochran, 1967). All statistical analyses, with two-sided p values, were performed using the Statistical Package for the Biosciences (Murata and Yano, 2002) and the statistical significance was set at $p < 0.05$.

RESULTS

The characteristics of the 27 subjects at week 0 are summarized in Table 1. The participants had neither weight loss nor subjective symptoms during the whole period of 29 weeks, and the laboratory data for blood pressure, liver function, total cholesterol, triglycerides, and hemoglobin were within the normal ranges (Yaginuma-Sakurai *et al.*, 2010). Although the interpersonal variation in hematocrit was significantly large (ANOVA, $p < 0.001$), no significant changes in the hematocrit were seen during the entire period of 29 weeks ($p > 0.2$). The mean T-Hg level in whole blood was significantly higher for men (8.0 ± 4.0 ng/g) than for women (5.3 ± 1.5 ng/g) (Student t test, $p < 0.05$); but, there was no significant sex difference either in the hair-to-blood ratio ($p > 0.2$) or biological half-lives in blood ($p > 0.8$) and hair ($p > 0.6$). Hence, the statistical analyses were conducted without separating the subjects by sex. Most of the subjects (81.6%) ate the bigeye tuna/swordfish twice or three times a week, by dividing it into two or three portions. Similarly, 14.2% of them ate it in one helping, and the remaining did so in four or five. They also consumed other fish containing low levels of MeHg a mean of 1.0 (range, 0 ~ 5) times a week during the exposure period, and a mean of 1.3 (range, 0 ~ 6) times a week during the

washout period.

Temporal patterns of T-Hg levels in whole blood and hair of the 27 subjects are presented in Fig. 1. The blood T-Hg level changed significantly with time ($p < 0.001$). The trend test showed that the increasing trend in the period from weeks 0 to 14 and the decreasing trend in the period from weeks 14 to 29 were statistically significant ($F = 262$, $p < 0.001$ and $F = 108$, $p < 0.001$, respectively). The T-Hg level at week 0 was 6.7 ± 3.3 ng/g. It reached the maximum (26.9 ± 6.8 ng/g) at week 14 (*i.e.*, the last week of the exposure period), and decreased to 11.9 ± 4.2 ng/g at week 29. Similarly, the hair T-Hg level changed significantly with time ($p < 0.001$), increasing from 2.3 ± 1.1 μ g/g (week 0) to 8.8 ± 2.0 μ g/g (week 17) ($F = 233$, $p < 0.001$ by the trend test), and then decreasing to 4.9 ± 1.6 μ g/g (week 29) ($F = 40.8$, $p < 0.001$). The slopes of increasing trends in the 27 subjects were 1.39 ng/g/week in whole blood and 0.498 μ g/g/week in hair, and the two slopes differed significantly ($F = 71.7$, $p < 0.001$).

The mean and median concentration ratios of hair-to-blood T-Hg, calculated using hair and blood samples collected at the same time (*i.e.*, unadjusted ratios), are shown in Fig. 1 and Table 2. The ratios changed significantly with time ($F = 180$, $p < 0.001$). The mean ratio ($\pm$ S.D.) for the entire period was 345 ± 102 (median 356), and the 5th and 95th percentiles were 176 and 492, respectively. Next, since the time lag (*i.e.*, the time to transfer mercury) from blood to hair on the scalp was estimated to be 3 weeks from the rising slope of hair T-Hg (Yaginuma-Sakurai *et al.*, 2010), the adjusted hair-to-blood ratios were calculated by means of interpolation. The mean value for the entire period was 344 ± 54 (median 343), and the 5th and 95th percentiles were 260 and 447, respectively. Although the adjusted ratio changed significantly with time ($F = 19.4$, $p < 0.001$), the S.D. of the adjusted ratios for the entire period became smaller than that of the

Fig. 1. Temporal changes of total mercury (T-Hg) concentrations in whole blood (●) and hair (■) and of hair-to-blood T-Hg concentration ratios in 27 subjects (mean and SD). The symbols of △ and ▲ represent the time lag-adjusted and unadjusted hair-to-blood ratios, respectively.

Table 2. Unadjusted and adjusted ratios of the hair-to-blood total mercury concentrations in 27 subjects

Week	Unadjusted ratios		Adjusted ratios*	
	Median	(5 and 95 percentiles)	Median	(5 and 95 percentiles)
0	354	(267, 475)	354	(267, 475)
5	194	(139, 249)	315	(244, 413)
9	241	(144, 375)	303	(225, 443)
13	279	(212, 396)	321	(264, 451)
17	399	(316, 512)	353	(294, 474)
21	433	(344, 571)	370	(300, 489)
25	421	(335, 551)	352	(287, 472)
29	411	(300, 569)		

* In these ratios, total mercury concentrations in hair were adjusted for the time lag from blood to hair on the scalp (3 weeks) by using interpolation.

Table 3. Biological half-lives of mercury in whole blood and hair calculated from three types of data*

Biological half-lives	Whole blood ⁺		Hair ⁺	
	Mean $\pm$ S.D.	Range	Mean $\pm$ S.D.	Range
Crude half-life				
Background value $\times 0$	94 $\pm$ 23	(58 ~ 155)	102 $\pm$ 31	(60 ~ 192)
Recalculated half-life after subtracting the below background values from the raw values				
Background value $\times 0.5$	76 $\pm$ 18	(42 ~ 122)	83 $\pm$ 24	(46 ~ 138)
Background value $\times 1$	57 $\pm$ 18	(22 ~ 112)	64 $\pm$ 22	(14 ~ 126)

* Mean half-lives in whole blood and hair changed significantly according to the background values (*i.e.*, $\times 0$, $\times 0.5$, and $\times 1$) ($F = 120$ and $F = 79.2$, respectively, $p < 0.001$ by two-way ANOVA).

⁺ There were significant differences in half-lives between whole blood and hair ($t = 3.15$ for $\times 0$, $t = 3.74$ for $\times 0.5$, and $t = 4.09$ for $\times 1$, $p < 0.01$ by paired-sample t test).

unadjusted ones.

The elimination of T-Hg can be expressed as a single exponential function, and the concentration (C) in an organ at any time (t) is as follows (Task Group on Metal Accumulation, 1973; WHO, 1976): $C = C_0 \times e^{-bt}$, where C_0 and b are the initial concentration in the organ and the elimination constant, respectively. The relation between the elimination constant and the biological half-life (T) is: $T = \ln 2/b$, where $\ln 2$ (natural logarithm of 2) is 0.693. Table 3 presents the mean half-lives calculated from three background levels (*i.e.*, $\times 0$, $\times 0.5$, and $\times 1$) to examine the impact of a certain background level. The mean half-lives calculated from raw data (*i.e.*, not using background levels) of the 27 subjects were 94 ± 23 days in whole blood and 102 ± 31 days in hair. The crude half-life in whole blood was significantly shorter than that in hair (paired-sample t test, $p < 0.01$). On the other hand, the half-lives, recalculated after subtracting the background level (*i.e.*, the T-Hg concentration at week 0) from the actual T-Hg concentrations in the decreasing phase of this experiment, were 57 ± 18 days in whole blood and 64 ± 22 days in hair, and gradually became shorter according to subtraction of the background level (Table 3).

DISCUSSION

This is the most recent report examining the kinetics of mercury on the basis of an experimental study. As stated in our previous paper (Yaginuma-Sakurai *et al.*, 2010), the exposure procedure was successfully achieved and no participants had weight loss or any symptoms such as tremor or finger numbness during the whole period of 29 weeks. As shown in Fig. 1, fish consumption at the PTWI level for 14 weeks elevated the T-Hg concentrations in whole blood and hair, though the exposure duration of MeHg was not long enough to reach a steady state. Under

the hypothetical steady-state condition, the blood mercury level will be estimated to be 18.3 ng/g by substituting the MeHg intake of 3.4 $\mu\text{g/kg BW/week}$ into a one-compartment pharmacokinetic model (National Research Council, 2000), and the mercury level in hair will be 4.6 $\mu\text{g/g}$ by using the hair-to-blood concentration ratio of 250, which was established in a study of various populations exposed to MeHg at fairly constant levels (JECFA, 2003; US EPA, 2001; WHO, 1990). However, the observed values of increments in our study were considerably higher than the above expected ones. This disagreement implies that some constants of the pharmacokinetic model may not be appropriate.

All the median ratios of the time lag-adjusted/unadjusted hair-to-blood T-Hg, except those at the 5th and 9th weeks, exceeded 250 as shown in Table 2, whereas the published ratios lay within the range of 140 ~ 370 (JECFA, 2003). The median ratios in Faroese children were 370 at 7 years of age and 264 at 14 years (Budtz-Jørgensen *et al.*, 2004). Sakamoto *et al.* (2007) reported that the average hair-to-blood ratio in Japanese pregnant women was about 350, probably because of the lower hematocrit during gestation. The median ratio in Swedish men and women was 373 (Berglund *et al.*, 2005). Taken together, these data suggest that the previous ratio of 250, used for calculation of the PTWI/TWI (Japan Food Safety Commission, 2005; JECFA, 2003) and tolerable daily intake (National Research Council, 2000), is too small compared with the recent ones. In the advanced world of science and technology, further discussion is required to decide whether the hair-to-blood ratio of MeHg should be raised to around 350 on the basis of these recent data, not the conventional ones.

As can be seen in Table 2 and Fig. 1, the unadjusted hair-to-blood T-Hg concentration ratio declined briefly during the 14-week period of bigeye tuna/swordfish

intake. A similar finding has been shown by a cross-sectional study reporting that the hair-to-blood ratio seemed to decrease with increasing MeHg in blood due to more frequent fish consumption (Berglund *et al.*, 2005). This could be accounted for by the fact that the rise of T-Hg during the exposure period is faster in whole blood than in hair, as proven both by the present study and by another study using toxicokinetic model simulations (Gosselin *et al.*, 2006). The temporal decline of the ratio adjusted for the time lag of 3 weeks was indeed minimized as compared to the unadjusted ratio. Similarly, a wide range of hair-to-blood ratios can be explained by the time lag; that is, most of the previous studies may not have considered this effect in calculating the hair-to-blood ratio, and the frequency of fish consumption is very changeable seasonally (Murata *et al.*, 2007). As a result, the uncertainty factor relevant to risk management of MeHg should be lessened based on this evidence, although it differs among several organizations for risk management (EFSA, 2004; Japan Food Safety Commission, 2005; JECFA, 2003; Legrand *et al.*, 2010; US EPA, 2001).

The biological half-lives of T-Hg calculated from raw data of this study were approximately 100 days, and the elimination constant would become half of the current one (*i.e.*, $\ln 2/50$). If this crude half-life is considered to be too long, it may have been attributable partly to fish consumption during the washout period after the cessation of exposure. Actually, our restriction of fish consumption was not so strict and some of our subjects ate a small amount of fish containing low levels of MeHg, such as sardines, salmon, and eel, during the washout period, while their docosahexaenoic and eicosapentaenoic acids in plasma gradually, but not significantly, decreased from 141 $\mu\text{g/ml}$ at week 0 to 115 $\mu\text{g/ml}$ at week 29 (Yaginuma-Sakurai *et al.*, 2010). At least, the minimum values for the crude half-lives of the 27 subjects were 58 days for whole blood and 60 days for hair, and therefore more than the 50-day conventional value.

In contrast, the mean half-lives of T-Hg, recalculated after subtracting the background T-Hg concentration, were 57 days for whole blood and 64 days for hair, which may be apparently accordant with past studies. Kershaw *et al.* (1980) and Sherlock *et al.* (1984) reported that the mean half-lives of T-Hg in whole blood of volunteers exposed to high doses of MeHg from fish consumption were 52 ± 4 (S.E.) days (mean dose of 20.0 $\mu\text{g/kg BW/day}$) and 50 ± 1 days (1.6 $\mu\text{g/kg BW/day}$), respectively, whereas Smith and Farris (1996) obtained a half-life of 44 days from other data. However, the formula $C = C_0 \times e^{-bt}$ does not specify that the elimination constant (b) should be calculated after subtracting the background

level from C_0 and C (Al-Shahristani *et al.*, 1976; WHO, 1976); because, the background levels are uncertain in most cases. For instance, the fact that the average blood lead levels of American populations in 1976-1980 were considerably high, *i.e.* 10 ~ 15 $\mu\text{g/dl}$ as the background level (Mahaffey *et al.*, 1982), may be cited. Most human studies using an isotope-labeled metal (Aberg *et al.*, 1969; Miettinen *et al.*, 1971; Smith *et al.*, 1994) ignored the background levels or did not exactly present them. Rather, the above formula initially must have been practical in light of the background uncertainties (Rabinowitz *et al.*, 1976). Thus, the half-life of mercury was suggested to be longer than we expected.

In the present study, the crude/recalculated half-life of T-Hg was significantly longer in hair than in whole blood. In past studies, the half-life in whole blood was around 50 days (Stern, 2005), as mentioned above. The average half-life in hair was calculated to be 72 (range, 35 to 189) days in Iraqi patients with high blood levels of T-Hg (Al-Shahristani and Shihab, 1974), and that in hair was 80 (33 to 120) days in volunteers having high MeHg intakes from fish (Birke *et al.*, 1972). In any case, the half-life of mercury is organ-dependent, similar to other metals such as lead.

Concerning factors influencing the half-life of mercury, we showed that subtraction of the background level modified the half-life drastically (Table 3), though it is debatable whether such subtraction is necessary. Stern (2005) reported that the mean half-lives of four studies increased with increasing MeHg doses, *i.e.* there was a positive correlation between the exposure dose and half-life, but our crude/recalculated half-lives under the exposure dose of 0.486 $\mu\text{g/kg BW/day}$ did not seem to correspond with that result. Apart from the half-life of mercury, O'Flaherty *et al.* (1982) found that the half-life of blood lead was positively correlated both with pre-strike blood lead (initial dose) and the duration of lead exposure in 68 primary smelters. However, they thought that the dependence of half-life on pre-strike blood lead was a reflection of inter-personal differences in lead kinetics, and only the direct dependence of half-life on duration of lead exposure was stressed. Although the compartment model differs somewhat between lead and mercury, the mode of exposure, *e.g.* one-shot, short-term, or long-term exposure, may affect the half-life.

In our study, there may have been some potential limitations. For instance, the hair growth rate might be affected by race, sex, age, and season. In fact, the hair growth rate (about 1.4 cm/month) in Japanese subjects (Yaginuma-Sakurai *et al.*, 2010) differed from those (0.8 ~ 1.04 cm/month) in other populations such as Caucasians and Afri-

cans (Zareba *et al.*, 2008). Also, the sample number in the current study was relatively small. Nonetheless, the mean hair-to-blood ratio of our study within the dynamic range was similar to recent studies (Berglund *et al.*, 2005; Budtz-Jørgensen *et al.*, 2004; Sakamoto *et al.*, 2007), as described above. In addition, as maternal MeHg is selectively transferred to the fetus via the placenta (Sakamoto *et al.*, 2010), such pregnant women were not included among our subjects. The measurement bias would have been minimized because the T-Hg levels were not analyzed in all subjects until the end of the washout period. Thus, it is suggested that our findings were not heavily influenced by sampling, selection or measurement bias.

In conclusion, the hair-to-blood ratio of MeHg based on past studies appears to be underestimated in the light of recent studies. The crude half-life may be preferable to the recalculated one because of the practicability and uncertainties of the background level. If the hair-to-blood ratio and elimination constant are assumed to be 350 and 0.007 ($= \ln 2/100$), respectively, the mean T-Hg level of our subjects would be expected to be 36.6 ng/g in whole blood and 12.8 µg/g in hair, probably after 5-month MeHg intake of 3.4 µg/kg BW/week, which is actually plausible as illustrated in Fig. 1.

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