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Development of Human Hair Reference Material Supporting the Biomonitoring of Methylmercury

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A certified reference material, NIMD-01, was developed for the analysis of mercury speciation in human hair. We collected the hair of Vietnamese males from a barbershop in Hanoi in 2016 and prepared 1200 bottles containing 3 g of sieved and blended hair powder. The certified value was given on a dry-mass basis, with the moisture content obtained by drying at 85°C for 4 h. Certified values with the expanded uncertainties (coverage factor, $k = 2$) were as follows: methylmercury, 0.634 ± 0.071 mg kg^{-1} as mercury; total mercury, 0.794 ± 0.050 mg kg^{-1}; copper, 12.8 ± 1.4 mg kg^{-1}; zinc, 234 ± 29 mg kg^{-1}; selenium, 1.52 ± 0.29 mg kg^{-1}. An indicative arsenic concentration of 0.17 ± 0.03 mg kg^{-1} was measured. Extended uncertainties were estimated by sample homogeneity, long- and short-term stabilities, and a characterization from measurements made by collaborating laboratories.

Keywords Human biomonitoring, reference material, methylmercury, mercury speciation

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Introduction

Mercury (Hg) is classified by the World Health Organization as a chemical of major public health concern[1]. Once Hg enters the environment, it cycles between the air, land, and water. Some inorganic Hg is converted into more toxic organic Hg (methylmercury [MeHg]), which can undergo magnification in the food web and adversely affect the highest consumer, humans. The Minamata Convention on Mercury, entered into force on 16 August 2017, is a multilateral agreement that obligates parties to manage and control Hg so as to reduce human and environmental exposure. The convention includes several articles on the monitoring of health risks-related exposure and effectiveness evaluation.

Human biomonitoring provides evidence of actual exposure to environmental chemicals[2], and contributes directly to the assessment of an individual's exposure by measuring biomarkers that may be the exposure substances themselves or their breakdown products. These biological markers are useful in assessing human exposure, and this type of biomonitoring also contributes to public health by evaluating the effects of exposure.

MeHg exposures can be estimated by measuring Hg levels in body fluids and tissues such as blood, hair, umbilical cord, and nails.[3] Hair is the preferred biomonitring matrix for MeHg because its testing is non-invasive and hair retains Hg well: MeHg levels remain constant in hair samples over many years under dry and dark conditions at room temperature.[4] MeHg has high affinity for sulfur-containing anions, particularly the thiol groups of the amino acid cysteine.[5] The MeHg-L-cysteine conjugate, which is structurally similar to the amino acid L-methionine, is transported freely throughout the body and sequestered by hair matrix cell during its formation.[6] Once incorporated into hair, MeHg does not return to the blood and its levels therefore correlate directly with blood MeHg levels.[7] MeHg is the main chemical form of Hg in hair (80% or more) in general populations,[8] and the analysis of total mercury (THg) in hair, which is less time-consuming and inexpensive than analysis of MeHg, is thus generally accepted as a substitute for MeHg measurements. Although hair is a suitable medium for monitoring human intake levels, since it provides a simple, integrative, and non-invasive sample, the THg content in hair may not reflect actual exposure in special populations, such as artisanal gold mining communities, which are also exposed to external Hg vapor and inorganic Hg; speciation analysis is therefore required in these cohorts.

Certified reference materials (CRMs) for assessing Hg are required, as an increasing number of surveys are conducted to evaluate the effectiveness of the Minamata Convention.[9,10] Although CRMs of human hair are currently available from the International Atomic Energy Agency (IAEA) and National Institute for Environmental Studies (NIES),[11] their homogeneity is not sufficient when measuring a small sample. However, the required weight/volume of a sample has decreased with improvements to instrument sensitivity, thereby reducing the donor burden in human biomonitoring.

The current paper fully describes the preparation and certification of a new human hair CRM for Hg speciation. Considering the demands of human biomonitoring of hair for other elements, we extended the certification to cover several elements with toxicological and nutritional significance. Special attention is paid to minimizing contamination from the grinding vessel so that the prepared material is representative of human hair.

Experimental

Sample collection and cleaning

Scalp hair that had not been permed or dyed was collected from Vietnamese males in a barbershop in Hanoi between June and September in 2016. The total quantity of hair (10 kg) was washed well by hand five times in a 0.3% neutral detergent (Contaminon N, Wako Pure Chemical Industries, Osaka, Japan). The detergent was removed using tap water and then rinsed out with distilled water. The clean hair was dried in an oven at 60°C overnight.

Preparation of hair powder

The most crucial aspects of the preparation of a large amount of homogeneous hair powder are the pulverizing efficiency and uncertain contamination from the grinding apparatuses. Hair has a fibrous structure and is easily entangled, and thus difficult to pulverize by a cutting mill.[12,13] We therefore tested the following grinding apparatuses, depicted in Fig. 1, in terms of pulverizing efficiency and contamination:

(1) Rolling ball mill. A 50-g sample of hair was ground by a roll rolling ball mill (7 L, 95% alumina; Kankyo Tech Co. Ltd., Fukuoka, Japan) at room temperature for 1 h.
(2) Cutting mill. A 5-g sample of hair was pulverized at 20000 rpm for 2 min by a rotor mill (P-14, Fritsch Idar-Oberstein, Germany) with dry ice.
(3) Roll crusher and pin mill. The hair sample was incrementally pulverized with a roll crusher (RP-300, Seishin Co. Ltd., Fukuoka, Japan) and a pin mill (Pin Mill-4, Seishin Co. Ltd.) in pre-grinding with liquid nitrogen. The hair sample was pressed between the two rotating rolls of the roll crusher with dry ice. The pre-ground hair powder was then pulverized by a series of hardened embedded steel pins on the two rotating disks of the pin mill.
(4) Air jet mill. The hair sample, pre-ground by the roll crusher and pin mill, was incrementally pulverized with a cryogenic air jet mill with a classifying rotor (Turbo Mill, Toho Reinetsu Co. Ltd., Aichi, Japan) and liquid nitrogen. Each hair sample was pressurized by particle-to-particle collision in the air stream.

Large-scale preparation

We considered the combined action of the roll crusher, pin mill, and air jet mill to be technically superior for the material preparation. We pulverized 7.8 kg of hair using the roll crusher and pin mill for pre-grinding. After 6.0 kg of hair powder was sieved through a 500-μm mesh screen, it was then pulverized using the air jet mill. The obtained 3.7 kg of hair powder with a particle size of less than 74 μm (as classified by the rotor) was then placed in a stainless-steel barrel in one lot and blended for 24 h using a rocking mixer (TMHS-100S, Seiwa Giken Co. Ltd., Osaka, Japan). Three-gram aliquots of the homogenized hair powder were poured into 1200 precleaned borosilicate bottles and sterilized by γ-irradiation. The bottles were stored at –20°C in the dark.

Moisture content

A 150-mg aliquot of hair powder was placed in a capped weighing bottle and dried for 4 h at 85°C in an electric drying oven. The bottle was then transferred into a silica-gel desiccator, cooled for 30 min, and the percentage of the weight lost was calculated as the moisture volume.

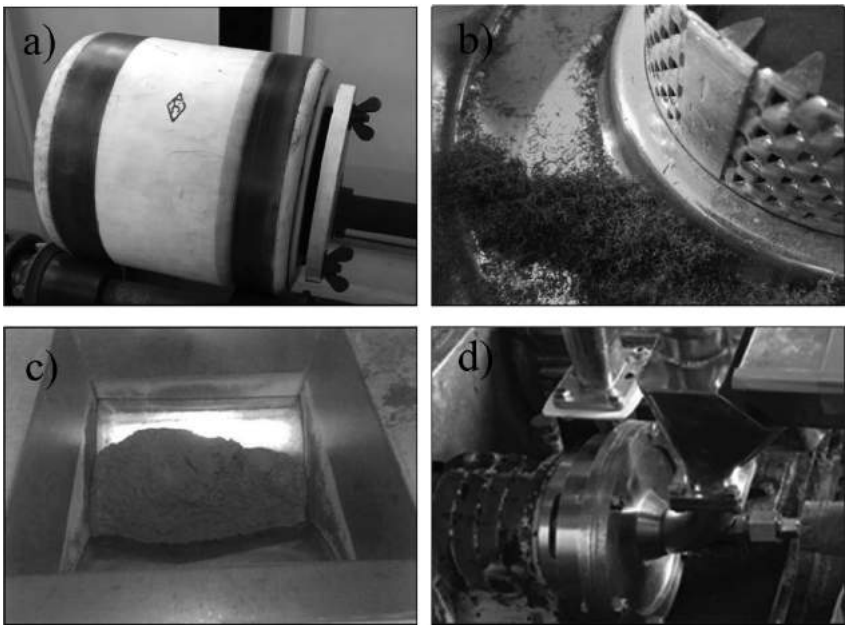

Fig. 1 Grinding apparatuses assessed in this study. a) Rolling ball mill; b) cutting mill; c) roll crusher and pin mill; d) air jet mill.

Minimum sample test

From the lot of 1200 bottles, 10 bottles were used at regular intervals for the minimum sample test. Sixty aliquots (two aliquots for each weight category from a bottle) weighting approximately 10, 20, and 50 mg were analyzed for THg content by cold-vapor atomic absorption spectrophotometry (CV-AAS) after acid digestion with H_2SO_4, HNO_3, and $HClO_4$ at 230°C using an open pressurized system.[14] Twenty 150-mg aliquots from each bottle were analyzed for moisture content.

Homogeneity test

A homogeneity test was carried out for the candidate CRM (NIMD-01) following the ISO Guide 35.[15] Twelve of the 1200 bottles were selected at regular intervals for the minimum sample test. Twenty-four aliquots (two aliquots from each bottle) weighing approximately 20 mg were analyzed for MeHg by gas chromatography with an electron capture detector (GC-ECD).[14] The THg concentration was determined by CV-AAS (Hg-201, Sanso Seisakusho Co. Ltd., Tokyo, Japan).[14] The concentrations of Cu, Zn, Se, and As were determined by inductively coupled plasma quadrupole mass spectrometry (ICP-QMS, 7500cx, Agilent Technologies, Santa Clara, CA, USA) after microwave digestion with HNO_3 at 190°C. To reduce the variability of the measurement, three replicate analyses were performed for each sample.

The standard uncertainty from homogeneity, U_{hom}, was calculated as the root of the variance of the between-bottle homogeneity. The within-bottle mean square (MS_{within}) of the mean square (MS_{among}) and repeatability standard deviation (S_r) were statistically estimated in an analysis of the variance. The data were input into Eqs. (1) – (3) to obtain the between-bottle standard uncertainty (S_{bb}) and the between-bottle variance incorporating the effect of analytical variation (U_{bb}):

$$S_{bb} = ((MS_{among} - MS_{within})/n)^{0.5}, \quad (1)$$

$$U_{bb} \leq (S_{bb}^2 + (S_r^2/n))^{0.5}, \quad (2)$$

$$U_{bb} = (MS_{within}/n)^{0.5} \times (2/\nu_{MSwithin})^{0.25}, \quad (3)$$

where n is the number of aliquots ($n = 2$) and $\nu_{MSwithin}$ is the degree of freedom of MS_{within}.

U_{hom} was considered to be S_{bb} if S_{bb}^2 was positive, while U_{hom} was considered to be the larger of the right sides of Eqs. (2) and (3) if S_{bb}^2 was negative.

Stability assessment

The stability of the material hair was assessed by measuring the concentrations of MeHg, THg, Cu, Zn, Se, and As. Long-term stabilities were evaluated to determine suitable temperature for the storage of this material as a CRM. Five bottles were maintained at each temperature of –20, 5, and 35°C for 12 months and analyzed at regular intervals (0, 3, 6, 9, and 12 months). Short-term stabilities were evaluated for transporting at normal temperature. Five bottles were maintained at each temperature of –20, 5, 20, 35, and 60°C for 4 weeks and analyzed at weekly intervals (0, 1, 2, 3, and 4 weeks). To reduce the variability of measurement, three replicates were analyzed for each sample. Sample digestion and measurements were carried out in a manner similar to that used in the homogeneity test.

Collaboration measurements

After the homogeneity and short-term stability of the material were confirmed at the National Institute for Minamata Disease, collaborative analysis for the certification of MeHg and the other elements was undertaken with 21 laboratories using a protocol and reporting format that met the criteria of ISO Guide 35.[15] Two bottles were sent to each laboratory with a document describing how the material had been prepared along with instructions for its handling.

Participating laboratories were asked to provide individual analytical results of two replicates for each bottle by using their routinely used methods over 2 days. Further, their validation results were provided from analyzing a CRM (IAEA 086 or NIES CRM No. 13), a blank test with seven replicates,

Table 1 Evaluation of contamination by the grinding apparatus (mg kg^{-1})

Grinding apparatus		Cr	Fe	Ni	Al	Si
Rolling ball mill	Before	0.2	10	3.6	26	380
	After	3.0	270	2.7	26000	3300
Cutting mill	Before	0.2	14	0.3	23	340
	After	81.0	130	11.0	21	890
Roll crusher	Before	0.3	22	0.5	—	—
	After	0.88	68	1.0	—	—
Air jet mill	After	1.9	32	1.5	—	—

a recovery test (spiking a blank sample with standard reagent) with seven replicates, and the moisture content after drying at 85°C for 4 h.

Uncertainty

The uncertainty associated with the certified value of the candidate CRM (NIMD-01) can be expressed as

$$U_{CRM} = (U_{hom}^2 + U_{sts}^2 + U_{lts}^2 + U_{char}^2)^{0.5}, \quad (4)$$

where U_{hom} is the uncertainty component from batch homogeneity, U_{sts} is the short-term stability uncertainty, U_{lts} is the long-term stability uncertainty, and U_{char} is the uncertainty from characterization.

Results and Discussion

Pulverizing efficiency

Table 1 summarizes the results of contamination assessments for the various grinding apparatuses. Grinding with the rolling ball mill was fast and simple, but was not considered further because the hair powder contained alumina debris and the acid solution had a milky, cloudy appearance after digestion. The digested solution was highly contaminated by Al and Si. The cutting mill was also eliminated from consideration because of serious contamination by Cr, Fe and Ni from the stainless-steel blade. Furthermore, a low yield of powdered hair was obtained because of tangling.

The combined use of the roll crusher and pin mill resulted in negligible contamination (Table 1). However, this combination produced only 2.0 kg of hair powder with a particle size of less than 74 µm from 7.8 kg of hair. To increase the yield, the fraction with a particle of size less than 500 µm fraction (6 kg), sieved through a mesh screen, was then pulverized with an air jet mill. No further contamination from the grinding vessel was observed for the obtained 3.7 kg of hair powder with a particle size of less than 74 µm (as classified by the rotor). The pulverizing efficiency of the three mills was a satisfactory 47% (Fig. 2).

Homogeneity

The between-bottle variations in the samples containing form 10 to 50 mg had a relative standard deviation of lower than 1%. Although no heterogeneity was detected in THg analysis of 10-mg samples, we recommend a minimum sample weight of 20 mg, while considering the effects of any analytical variation and the equipment used (Table 2). To assure the homogeneity of the NIMD-01 CRM, all subsequent tests were conducted using a minimum of 20 mg. Table 3 summarizes the results of the homogeneity tests. The uncertainty component from the batch homogeneity was less than 1.6%.

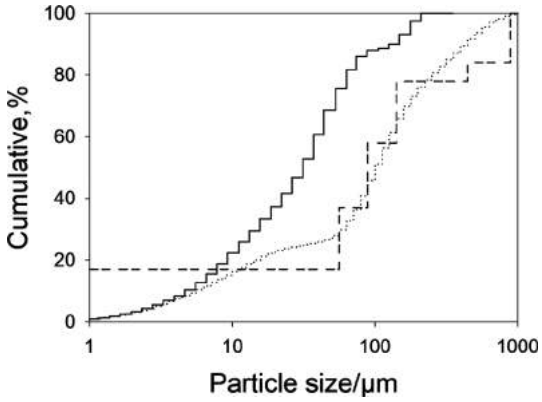

Fig. 2 Cumulative particle size. Broken, dotted, and solid lines indicate the rolling ball mill, roll crusher and pin mill, and combination of roll crusher and pin mill and air jet mill, respectively.

Table 2 Minimum requirement for measuring the total mercury

Sample amount/mg	Mean/ mg kg^{-1}	MS_{among}/ mg^2 kg^{-2}	MS_{within}/ mg^2 kg^{-2}	S_r, %	S_{bb}, %
10	0.709	0.00042	0.00033	2.6	0.9
20	0.724	0.00010	0.00004	0.8	0.8
50	0.698	0.00015	0.00004	0.9	1.0

MS_{among}, among-bottle mean square; MS_{within}, within-bottle mean square; S_r%, relative repeatability standard deviation in analysis of the variance; S_{bb}%, relative between-bottle standard uncertainty.

Table 3 Homogeneity of 20-mg samples

Element	Concentration/ mg kg^{-1}	MS_{among}/ mg^2 kg^{-2}	MS_{within}/ mg^2 kg^{-2}	S_r, %	S_{bb}, %	U_{bb}, %
MeHg as Hg	0.665	0.00002	0.00002	0.7	0.2	—
THg	0.722	0.00008	0.00008	1.2	0.1	—
Cu	12.1	0.113	0.090	2.5	0.9	—
Zn	233	26.6	28.5	2.3	—	1.6
Se	1.42	0.00043	0.00087	2.1	—	1.0
As	0.149	0.00002	0.00001	2.1	1.1	—

MS_{among}, among-bottle mean square; MS_{within}, within-bottle mean square; S_r%, relative repeatability standard deviation in analysis of the variance; S_{bb}%, relative between-bottle standard uncertainty; U_{bb}%, relative between-bottle variance incorporating the effect of analytical variation.

Since the analytical sensitivity has improved, the required weight/volume of a sample has decreased, reducing the donor burden. Human biomonitoring studies of MeHg exposure have used 3.0 – 3.5 mg of hair for thermal decomposition-atomic absorption spectrophotometry (TD-AAS),[9] 5 – 10 mg of digested hair for CV-AAS,[3] and 10 – 50 mg for ICP-QMS.[16] For Hg speciation analysis, 10 – 25 mg of hair have been used in the liquid-liquid extraction of GC-ECD[4] and TD-AAS,[17] and a 100-mg sample was used in high-performance liquid chromatography.[18] Homogeneity is crucial in a CRM; the minimum sample is small enough (NIES No. 13: 120 mg; IAEA-086: 50 mg) so that NIMD-01 was adequately homogeneous for analytical standards.

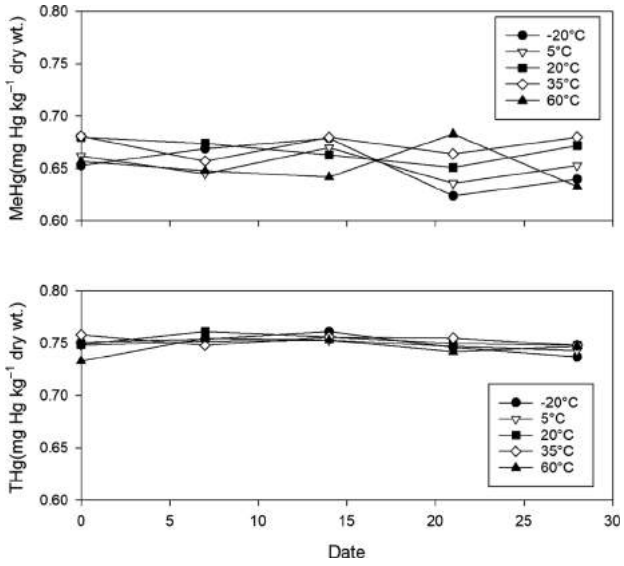

Fig. 3 Short-term stability assessment of methylmercury (MeHg) and total mercury (THg).

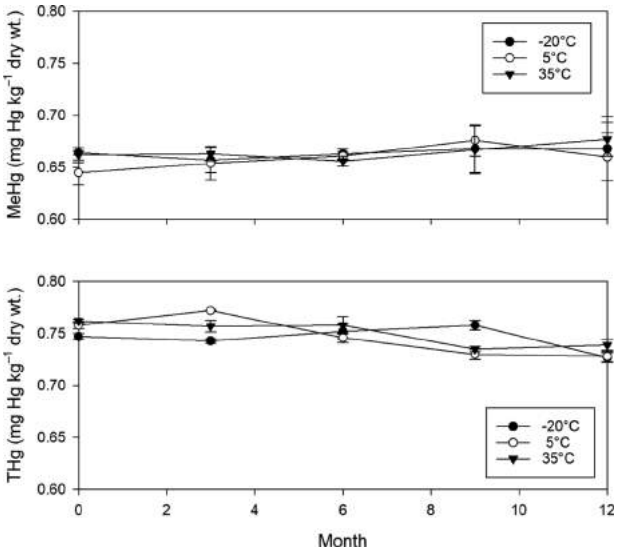

Fig. 4 Long-term stability assessment of methylmercury (MeHg) and total mercury (THg).

Stability

The stability of the hair powder was assessed by measuring the concentrations of MeHg and other elements. Figures 3 and 4 show the short- and long-term stability of MeHg and THg, respectively. No systematic trends were detected; Table 4 lists the data uncertainty associated with the slope, calculated by regression, and the short-term and long-term uncertainty contributions.

We concluded that the candidate CRM, NIMD-01, is stable. The CRM can be transported at room temperature (<60°C) for up to 4 weeks. The expected shelf life is 12 months. The material should be kept in a tightly closed original bottle and stored in a clean location away from light and high temperatures, preferably below 35°C and frozen or refrigerated. We plan to monitor the material at regular intervals to check the stability of MeHg and other elements.

Table 4 Budgets of the combined relative uncertainties (%)

Element	U_{hom}	U_{sts}	U_{lts}	U_{char}	Combined relative, U_{CRM}	Laboratories (n)
MeHg as Hg	0.2	4.4	1.8	3.0	5.6	8
THg	0.1	1.5	2.1	1.7	3.1	11
Cu	0.8	2.3	5.0	0.9	5.6	10
Zn	1.6	2.1	5.5	1.5	6.2	7
Se	1.0	5.1	7.8	2.6	9.7	6
As	1.0	4.1	7.8	1.9	9.0	8

U_{hom}, the uncertainty component from batch homogeneity; U_{sts}, the short-term stability uncertainty; U_{lts}, the long-term stability uncertainty; U_{char}, uncertainty from characterization; U_{CRM}, uncertainty associated with the certified value of the candidate certified reference material.

Certification

The participants in our inter-laboratory collaboration provided datasets of the measured values and validation information for the hair CRMs, blanks and recovery test. The validation data were evaluated concerning the accuracy of the available CRMs' measured values, the variation of values form blanks, the mean recovery, and the variation of recovery. The Smirnov–Grubbs test (significance level 0.05) was used to detect any outliers, which were rejected. Table 5 lists the analytical methods used.

The results for MeHg were provided by 10 laboratories, and eight laboratories reported satisfactory validation, and eight laboratories had no outlier value. Eight of the 10 laboratories supplied MeHg analytical data derived using GC-ECD preceded by HCl leaching[14] or NaOH digestion[19] (Table 5). The mean value ± 2SD of the mean quantity (0.562 ± 0.019 mg kg^{-1} dry weight as Hg obtained by aqueous phase ethylation–GC–atomic fluorescence spectrophotometry [AFS] preceded by HNO_3 leaching and by CH_2Cl_2 extraction)[20] agreed well with the GC-ECD values. The value of 0.593 ± 0.018 mg kg^{-1} dry weight as Hg obtained by chemiluminescence with high-performance liquid chromatography coupled with HCl leaching and toluene extraction[21] also agreed well with the other values. The certified value for MeHg was determined to be 0.634 ± 0.07 mg kg^{-1} dry weight as Hg. No other organomercury species was detected by any of the laboratories. We thus consider MeHg to be the only organomercury species present in the candidate CRM.

The results for THg were provided by 19 laboratories, and data from 12 laboratories exhibited satisfactory validation (Table 5). Twelve of the 19 laboratories reported no outliers. Eleven laboratories provided analytical THg values by TD-AAS. The mean value ± 2SD was 0.764 ± 0.023 mg kg^{-1} dry weight. CV-AAS data were provided by four laboratories as 0.752 ± 0.022 mg kg^{-1} dry weight. Data derived from cold-vapor atomic fluorescence spectrophotometry (CV-AFS, N = 1, 0.776 ± 0.084 mg kg^{-1} dry weight) was in good agreement with data derived from TD-AAS or CV-AAS. The analytical values obtained by ICP-QMS (N = 3) were 0.879 ± 0.111 mg kg^{-1} dry weight, slightly higher concentrations than those determined by other analytical techniques. These laboratory values were considered to be outliers and were excluded from the certification.

Extensive analysis was carried out for Se and heavy metals, such as Cu, Zn, and As, because the levels of these elements in human hair may have nutritional and toxicological implications. Table 5 lists the analytical methods used in the determination by 12 collaborating laboratories. Of the 12 laboratories, only three laboratories provided satisfactory validation for As.

Table 5 Analytical methods used in the analyzing methylmercury (MeHg, mg kg^{-1} dry weight as mercury), total mercury (THg), and other elements (mg kg^{-1} dry weight) in NIMD-01 human hair by collaborating laboratories

Element	Instrumental analysis	Sample preparation	Number of laboratories delivering data	Number of laboratories satisfactory results	Concentration of satisfactory results
MeHg	GC-ECD	HCl leaching; NaOH digestion/toluene extraction	8	6	0.653 ± 0.048
	GC-AFS	HNO_3 leaching/ethylation	1	1	0.562
	HPLC-CL	HCl leaching/toluene extraction/EDTA extraction	1	1	0.593
THg	TD-AAS	N/A	11	8	0.807 ± 0.023
	CV-AAS	H_2SO_4, HNO_3 $HClO_4$; H_2SO_4, HNO_3, $HClO_4$, HCl/hot plate	3	2	0.761 ± 0.023
	CV-AFS	H_2SO_4, HNO_3, $HClO_4$/hot plate	1	1	0.776
	ICP-QMS	HNO_3; HNO_3, HF; HNO_3, HCl/hot plate; microwave	3	0	—
Cu	ICP-QMS	HNO_3; HNO_3, HF; HNO_3, H_2O_2; HNO_3, HF, $HClO_4$, HCl/hot plate; microwave	12	10	12.8 ± 0.1
Zn	ICP-QMS	HNO_3; HNO_3, HF; HNO_3, H_2O_2; HNO_3, HF, $HClO_4$, HCl/hot plate; microwave	12	7	234 ± 3
Se	ICP-QMS	HNO_3; HNO_3, HF; HNO_3, H_2O_2; HNO_3, HF, $HClO_4$, HCl/hot plate; microwave	12	6	1.52 ± 0.04
As	ICP-QMS	HNO_3; HNO_3, HF; HNO_3, H_2O_2; HNO_3, HF, $HClO_4$, HCl/hot plate; microwave	12	3	0.166 ± 0.003

GC-ECD, gas chromatography with an electron capture detector; GC-AFS, gas chromatography with atomic fluorescence spectrophotometry; HPLC-CL, chemiluminescence with high-performance liquid chromatography; TD-AAS, thermal decomposition-atomic absorption spectrophotometry; CV-AAS, cold-vapor atomic absorption spectrophotometry; CV-AFS, cold-vapor atomic fluorescence spectrophotometry; ICP-QMS, inductively coupled plasma quadrupole mass spectrometry.

The uncertainty from characterization (U_{char}) is essentially impossible to calculate from three datasets, therefore the concentration of As is indicative value but not certified value. The U_{char} of As was calculated from the values reported by eight laboratories that passed the Smirnov–Grubbs test (Table 4).

Elemental composition of NIMD-01 human hair

Table 6 compares the elemental composition of the newly prepared NIMD-01 human hair and hair from the NIES No. 13 and IAEA 086 CRMs. NIMD-01 contains less THg and MeHg than NIES No. 13, likely because of dietary differences between current-day Vietnamese and Japanese donors 40 years ago. The NIES No. 13 was prepared from Japanese scalp hair collected in 1980; the donors are believed to have been exposed to MeHg in excess of the provisional tolerable weekly intake levels,[22] 1.6 μg Hg kg^{-1} body weight/week, corresponding to a hair Hg level of 2.2 mg kg^{-1}. The Hg levels in the hair of 47% of Japanese donors were shown to exceeded the equivalent level of the provisional tolerable weekly intake for MeHg because of the habitually high consumption of fish by the Japanese population in those days.[23] Although the per capita consumption volume of fishery products in Japan is still high, it is currently 75% of the levels in 1980.[24] A recent study measuring Hg levels in the hair of Vietnamese donors found that 2% of the samples exceeded the equivalent level of the provisional tolerable weekly intake for MeHg.[25] The MeHg levels in the NIMD-01 material are 2.5-times higher than the levels in IAEA 086 samples prepared from Indian scalp hair,[26] again attributable to a difference in the fish consumption between countries.[24]

MeHg typically constitutes at least 80% of THg in hair from fish consumers without external exposure.[8] However, inorganic Hg in hair can increase with the adhesion of Hg vapor through artisanal gold mining activities or the use of Hg-containing skin lightening creams and soaps. Hg salt is a common ingredient in skin-lightening cosmetics in some African and Asian nations including India, but this use has not been reported in Vietnam.[27]

The long-term stability of CRMs is essential. γ-Irradiation is recommended for stabilizing MeHg to eliminate bacteria as a potential source of instability, even if irradiation reduces the MeHg content of the material. A study of the effects of the γ-irradiation of biologicals did not record any significant effects on the MeHg levels.[28] NIMD-01 was subjected to γ-irradiation, but NIES No. 13 was not, so as to avoid MeHg decomposition. The percentage of MeHg in NIMD-01 was slightly lower than in NIES No. 13 (Table 6). Since the percentage of MeHg is approximately 80% or more of THg in hair in the general population,[8] γ-irradiation may therefore be considered to exert no significant effects on the MeHg content in hair. Although the γ-irradiation process partially decomposed MeHg, the remainder was stable and homogeneous in the NIMD-01 material.

Table 6 Comparison of element concentrations between the certified reference materials NIMD-01, and NIES No. 13, and IAEA 086

Element	NIMD-01	NIES No. 13	IAEA 086
MeHg as Hg	0.634 ± 0.071	3.8 ± 0.4	0.258 ± 0.021
THg	0.794 ± 0.050	4.42 ± 0.2	0.573 ± 0.039
Cu	12.8 ± 1.4	15.3 ± 1.3	17.6 ± 0.9
Zn	234 ± 29	172 ± 11	167 ± 7
Se	1.52 ± 0.29	1.79 ± 0.17	1.00 ± 0.20
As	0.17 ± 0.03a	0.10[a]	—
Me%	80	86	45

mg kg^{-1} dry weight.
a. Indicative value.

Conclusions

The demand for CRMs for Hg in human hair is growing. We developed and validated a human hair CRM, NIMD-01, in collaboration with multiple laboratories, as well as a standard operating procedure to prepare the CRM for testing the levels of MeHg and other elements. NIMD-01 has been certified as

being in compliance with ISO Guide 35, and is provided as a gray powder packed in an amber glass bottle. Each bottle contains approximately 3 g of the reference material, and the minimum amount required for analysis is 20 mg, so as to ensure homogeneity. Unlike other hair CRMs, this minimum quantity is small enough to be adequately homogeneous for the current analytical standard techniques. The long-term stability of NIMD-01 was confirmed over a period exceeding 12 months, but it must be stored unopened and under satisfactory temperature and in the dark. We continue to monitor the long-term stability of the CRM, and will update the effective expiry date from future findings. Revisions to the NIMD-01 protocol and stability information will be posted online (http://nimd.env.go.jp/english/crm/index.html).

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