Total Mercury in the Hair of Children by Combustion Atomic Absorption Spectrometry (Comb-AAS)

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Abstract

A simple and rapid procedure for measuring total mercury in human hair was evaluated and compared with a conventional technique. An Advanced Mercury Analyzer (AMA-254) based on sample catalytic combustion, preconcentration by gold amalgamation, thermal desorption, and atomic absorption spectrometry (AAS) (Comb-AAS) was assessed for the direct determination of milligram quantities of human hair. Precision (% relative standard deviation) was < 7% and accuracy was determined by using two human hair reference materials (i.e., NIES No. 13 and IAEA-086) that were within the certified range. In comparison to conventional graphite-furnace atomic absorption spectrophotometry (GF-AAS), we found that our method obtained statistically equivalent results. Because total analysis time per sample was less than 10 min, the Comb-AAS method was in fact much faster than the GF-AAS method. In addition, Comb-AAS does not generate waste products and could be mainly useful for the analysis of a large amount of samples. Then, the authors suggest that this quick method could be useful for measuring mercury in human hair. Therefore, the mercury content in hair for a non-exposed group of children (n = 40) living in Spain was evaluated. The mean and median hair mercury levels for the subjects under study were found to be lower than the value of 1 µg/g, corresponding to the reference dose of 0.1 µg of methylmercury per kilogram body weight set by the U.S. Environmental Protection Agency.

Introduction

Over the past century, an increasing awareness throughout the world of the environmental exposure and human health impacts of toxic metals, such as mercury (Hg), has occurred. Among all of the mercury species, methylmercury (MeHg) is the most toxic form that is produced in the environment by biotic and abiotic methylation of inorganic form. MeHg is a neurotoxicant that can produce adverse effects on human neurological and cardiovascular function when individuals are exposed to it at high-levels (acute exposure) or at lower doses over long periods of time (chronic exposure), which can also cause measurable effects (1). Because of mercury's bioaccumulation and biomagnification in the aquatic food chain, fish and shellfish contain the highest Hg concentrations (2,3), and fish consumption is the main route of human chronic MeHg exposure (4–7).

In order to estimate the individual exposure to mercury, several studies have been carried out analyzing different human body fluids such as blood and urine or tissues such as nail or hair (8–12).

Human hair is a widely accepted biomarker for MeHg exposure (1). Whereas results from the other body specimens can only be used as a measure of recent exposure, hair, in contrast, can provide a historical exposure record. Mercury levels in hair have been found to be a suitable indicator of dietary, environmental, and occupational human exposure (13–15). Furthermore, hair mercury levels are at least 200–250 times higher than the corresponding concentrations in the blood. In addition, hair collection is non-invasive, and samples are easily collected and stored. Consequently, hair has been used in a variety of epidemiological and toxicological studies in several populations (6,16–18).

Once collected, hair is routinely washed and rinsed with acetone to remove external contamination before digestion in order to measure the Hg by several methods. It is generally considered that the oxidative conversion of all forms of mercury, in an ionic mercury (Hg^{2+}) sample is necessary prior to a reduction to elemental mercury (Hg^0) and its subsequent measurement by various detection techniques (19).

To accomplish that, digestion of hair matrix is frequently performed by using strong acids (HCl, H_2SO_4, HNO_3), strong bases (KOH, NaOH), oxidants (H_2O_2, $KMnO_4$, $K_2S_2O_8$), microwave (20), and elevated temperatures are used (21–24).

The most frequently instrumental techniques employed to measure mercury concentrations in hair include cold-vapor atomic absorption spectroscopy (25), cold-vapor atomic fluorescence spectroscopy (26), inductively coupled plasma atomic emission spectrometry (27), and inductively coupled plasma

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mass spectrometry (24). Moreover, mercury content in hair has also been measured by neutron activation analysis (28) and X-ray fluorescence (29).

In general, all the methods for the determination of mercury in human hair are, to some extent, tedious and time-consuming. As an alternative approach, total Hg can be measured directly in many solid and liquid matrices by means of automated commercial instruments, such as the Advanced Mercury Analyzer (AMA-254), the Trace Mercury Analyzer, or the Direct Mercury Analyzer. These systems integrate combustion of the sample and preconcentration of mercury by amalgamation with gold trap followed by atomic absorption spectroscopy (Comb-AAS).

The main advantage is that no sample preparation is needed, making it much more rapid than conventional methods. In addition, the technique eliminates reagent waste because the sample is analyzed without liquid reagents and reduces the potential for contamination. Despite those advantages, these analyzers have not extensively been used for the determination of Hg, except in a variety of matrices such as hair (30), fish tissue (31), bullfrogs (32), waterfowl (33), or even matrices associated with a coal-fired power plant, including coal, fly ash, bottom ash, and flue gas desulfurization material (34).

In this article, results evaluating the applicability of the Comb-AAS technique for human hair samples (35), focusing on the performance of the method in terms of precision, accuracy, and detection limits have been evaluated. Thus, the objectives were to determine the feasibility of using small amounts of hair (5–20 mg) to characterize the T-Hg concentration and to evaluate the overall performance of a combustion analyzer for measuring mercury in human hair. Moreover, mercury levels in hair from a non-exposed group of children were also assessed. In this study, we report a survey of the hair mercury levels in a Spanish subpopulation to estimate the current mercury exposure levels in Spain during early infancy.

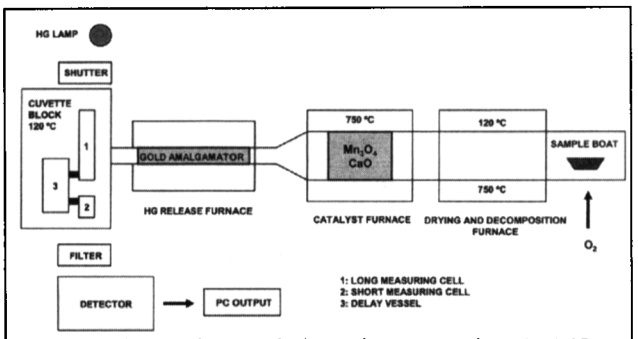

Figure 1. Schematic diagram of Advanced Mercury Analyzer AMA-254.

Experimental

Instrumentation

A schematic diagram of the AMA-254 from Leco Corp. (Altec, Praha, Czech Republic) instrument based on Comb-AAS is shown in Figure 1. Following the automated introduction of the sample into a quartz combustion tube by means of a nickel boat, the instrument self-seals, and oxygen begins flowing over the sample at a rate of approximately 200 mL/min. Then the sample is dried at 120°C and thermally and chemically decomposed at 750°C. Gaseous combustion products are carried in an oxygen stream through the catalytic converter where a Mn_3O_4/CaO-based catalyst (kept at 750°C) allows complete oxidation and halogens, nitrogen, and sulfur oxides are trapped. Then the different chemical species of Hg are converted to elemental Hg vapor. Elemental mercury (Hg^0) and the remaining decomposition products are inserted into a tube containing gold-coated sand (gold-based amalgamator) that selectively traps mercury. While other products are flushed out of the system, Hg^0 is selectively trapped and forms an amalgam with gold. During a pre-specified time interval (120–150 s), the system is flushed with oxygen to remove any remaining gases or decomposition products and the amalgamator is rapidly heated (700°C), releasing mercury vapor. Later in the cycle, the flow of oxygen quantitatively carries the mercury, which is transported to a cuvette heated at 120°C to prevent condensation and to minimize carry-over effects prior to analysis by an AAS. A low-pressure mercury vapor lamp is used as the light source at the working wavelength of 253.65 nm. The detector is connected to a computer for data acquisition and analysis. Usual operating conditions are drying time, 30 s; decomposition time, 150 s; and waiting time, 40 s. The total analysis time is about 5 min.

Table I. CRM Hair Hg Determinations by Combustion-AAS

	IAEA-086			NIES-CRM 13			
Amount (mg)	*n*	Mean*	RSD (%)	Amount (mg)	*n*	Mean*	RSD (%)
Intra-assay precision							
5	10	0.631 ± 0.095	15.7	5	10	4.640 ± 0.510	11.0
7.5	10	0.589 ± 0.062	10.6	7.5	10	4.290 ± 0.380	8.9
10	10	0.563 ± 0.045	8.3	10	10	4.260 ± 0.180	4.2
15	10	0.555 ± 0.039	7.2	15	10	4.312 ± 0.168	3.9
20	10	0.562 ± 0.036	6.3	20	10	4.390 ± 0.173	3.9
Interassay precision†							
1st day	5	0.563	8.0		5	4.260	4.9
2nd day	5	0.552	7.6		5	4.320	5.1
3rd day	5	0.579	7.8		5	4.200	4.3
Mean		0.565				4.260	
SD		0.044				0.203	
RSD(%)		7.8				4.8	
Certified value‡	0.573 ± 0.039					4.420 ± 0.200	

* Mean value ± SD in micrograms per gram, based on dry weight.
† The hair sample weight used every day was 10 mg.
‡ Mean value ± 95% confidence interval (CI) in micrograms per gram, based on dry weight.

Analysis of T-Hg were also detected by a Perkin Elmer atomic absorption spectrophotometer 1100 equipped with a HGA-300 heated graphite furnace system (GF-AAS) (Perkin Elmer, Norwalk, CT).

Sample analysis and quality assurance

Hair samples were precisely weighed (5 to 20 mg) in a nickel boat by means of an analytical balance (model GA110, Ohaus, Union, NJ) with 0.1 mg readability. Then, the boat containing the sample was then placed into the instrument which is automatically introduced into the instrument's quartz combustion tube. The stability of calibration of the AMA-254 analyzer is very high. The AMA was regularly calibrated over two ranges (0.05–40 and 40–500 ng of mercury). This calibration is usually valid for a period of months and then it is necessary to calibrate with a certified reference material (CRM). In our case, the entire analytical procedure was validated by analyzing two human hair CRMs, the NIES CRM No. 13 and the IAEA-086, and applied to human hair samples from urban inhabitants. Both CRMs were obtained from the National Institute of Environmental Studies, Environmental Agency of Japan and the International Atomic Energy Agency (IAEA) of Austria, respectively. Analyses of both CRMs at the beginning and end of each set of samples (typically 10) ensured that the instrument remained calibrated during the course of the study.

Precision for the CRM analyses, as measured by relative standard deviation, was above 7% (Table I). Besides demonstrating accuracy and precision, these results indicate that the AMA-254 is able to provide a stable calibration over an extended period of time. Detection and quantification limits were calculated from blank measurements following the recommendations of the International Union of Pure and Applied Chemistry (IUPAC) (36), being these values 0.027 and 0.090 ng of Hg, respectively. The limit of detection was calculated to be 0.0027 µg/g mercury, based on three times the standard deviation of the blank and a sample hair mass of 10 mg, whereas the limit of quantization is 0.009 µg/g mercury.

A blank (i.e., an empty sample nickel boat) was analyzed periodically to verify that mercury was not being carried over between samples. Blank readings were typically < 0.0003 absorbance units, corresponding to values < 0.009 ng of Hg. About every 100 samples, a new boat was used after heating it twice to remove potential traces of mercury.

Total mercury (T-Hg) analysis carried out by the AMA in human hair was evaluated and compared with a conventional technique such as graphite-furnace atomic absorption spectrophotometry (GF-AAS). Then T-Hg was measured using 25 mg of hair according to the method described by Chen et al. (37) and determined using minor modifications. Quantification of hair samples was carried out by external calibration with $r^2 > 0.99$. The LOD obtained was 0.09 µg/g, and the limit of quantification of the method was estimated at 0.160 µg/g.

All statistical analyses were conducted with SPSS, version 12.01 for Windows (SPSS, Chicago, IL). All means are arithmetic unless otherwise stated. Statistical significance was defined as $p \leq 0.05$. Cases with values above or below 1.5 times the interquartile range were not considered for the statistical analysis, as defined by the Tukey method.

Human samples

Human hair samples were obtained from children living in Menorca island, located in the northwestern Mediterranean Sea where the main income is from tourism and there is relatively low industrial activity. The Menorca group includes children born between January 1997 and March 1998. A total of 482 children (94% of those eligible) were subsequently enrolled, and 470 (97.5%) provided complete outcome data up to the fourth year visit. Among these, 40 (8.5%) were selected randomly to measure mercury in hair. The mothers of selected children agreed to participate, and consent was obtained. This study was approved by the Ethics Committee of the Institut Municipal d'Investigació Mèdica (IMIM, Spain).

Hair specimen collection

A minimum of 5–10 mg of scalp hair was required for the hair analysis assay. A small lock of hair was cut as close as possible to the occipital region of the scalp and placed in small plastic resealable bags until analysis. Once in the laboratory, hair samples were rinsed well with deionized water, followed by two washes with purity acetone to remove external contamination. The dried hair was cut in small pieces (< 2 mm) and weighed in the nickel boat.

Results and Discussion

Method evaluation, precision, and accuracy

As can be seen in Figure 1, two cells can be used for measuring the absorbance of mercury, that is, a small and a larger cell for higher and lower concentrations, respectively.

This facility allows the AMA-254 to determine the concentration of mercury in two different ranges that have been used in this work. For the lower range (0.05–40 ng), the apparatus was validated using IAEA-086, and for the higher range (40–500 ng), the NIES-CRM 13 was employed.

The capability of the method for quantification of mercury concentration in both CRMs was also tested. Different amounts were employed to prove the feasibility of the use of small amounts of hair in the determination of mercury. Therefore, samples of 5, 7.5, 10, 15, and 20 mg were selected for the measurements. Determinations were carried out on a given day, and the obtained results are summarized in Table II. The results were very satisfactory and showed a good agreement with the certified values. The values were acceptable considering the low amount of sample weight, even in the case of 5.0 mg (RSD > 15%).

The intra-assay precision measured variation of results on a

Table II. Children's Hair Hg by GF-AAS and Comb-AAS

Method	Mean ± SD (µg/g)	Median (Range) (µg/g)
GF-AAS (n = 40)	0.766 ± 0.730	0.476 (0.225–3.826)
Combustion-AAS (n = 40)	0.760 ± 0.722	0.484 (0.178–3.778)

given day, whereas between-run precision measured the overall precision for the analysis, which also reflected day-to-day deviations. Both intra-assay and interassay precision were measured. Determination of intra-assay precision was accomplished by replicate analyses (n = 10) of a given amount of CRM sample (5, 7.5, 10, 15, and 20 mg) on a given day. On the other hand, interassay precision was measured by replicate analyses (n = 5) of the same amount of CRM (10 mg) on three non-consecutive days. Table I shows the intra- and interassay precision results. For CRM IAEA-086, the intra-assay precision the RSDs were < 16% and the overall interassay precision, RSDs < 8%. However, both RSDs values were much lower for NIES-13. This can be explained by the fact that the concentration of T-Hg in IAEA-086 was closer to the method detection limit. On a given day, relative standard deviation for IAEA-086 (5 mg) was approximately 15%, which may be due to weight imprecision considering the low amount of sample weighed.

Comparison data for Comb-AAS and GF-AAS

The same number of hair samples (n = 40) from children living in Menorca were analyzed by the two selected methods, Comb-AAS and GF-AAS. Statistically equivalent results (p > 0.05) and high correlations (r^2 = 0.998) were observed between both methods and the linear regression is shown on Figure 2. Results for hair samples from Spanish children by the two methods were in very good agreement. The mean value of T-Hg concentration (in µg/g, dry weight mass) ± SD was 0.760 ± 0.722 for Comb-AAS and 0.766 ± 0.730 for GF-AAS (Table II). Median values and range for Comb-AAS and GF-AAS were 0.476 (0.225–3.826) µg/g and 0.484 (0.178–3.778) µg/g, respectively

The equation of the regression line between the Comb-AAS method (y) and the GF-AAS method (x) was y (µg/g) = 0.9887x + 0.0028. The slope and the intercept were not significantly different (p > 0.05) from 1.0 and zero, respectively.

Risk assessment

Children are more vulnerable than adults to environmental exposures. It is well known that MeHg is neurotoxic, being the developing and infant brain both much more sensitive to MeHg than the mature brain. A monograph from the International Programme on Chemical Safety (IPCS) that focuses on the risks to human health from MeHg (38) describe the fact that its effects on the adult differ both quantitatively and qualitatively from effects seen after prenatal and, possibly, postnatal exposure. Prenatal exposure to MeHg through maternal seafood diet has been associated with an increased risk of neurodevelopmental deficit (39). Recent prospective epidemiologic studies from the Faroe Islands, the Seychelles Islands and New Zealand cohorts assessed the developmental effects of lower MeHg exposure in fish consuming populations resulting from maternal and fetal exposures to MeHg (29,40,41). The U.S. Environmental Protection Agency decided to rely on the Faroe Islands study (42) and a benchmark analysis dose was established and converted into a maternal intake of 1.1 µg/kg body weight (bw) a day. Finally, applying a safety factor of 10, a reference dose (RfD) of 0.1 µg/kg bw was recommended. Then the corresponding Hg level in hair would be 1 µg/g (42). In this regard, risk assessment in the European Union has followed a similar pathway (43), recommending an RfD of 0.1 µg/kg bw a day. Although the RfD applies to all populations, the main groups at risk at these levels are pregnant women and their fetuses and, of course, children. Exposure information for women of childbearing age and young children is important because of fetal sensitivity to adverse effects and continuing neurobehavioral development during childhood.

Analysis performed by Comb-AAS found that T-Hg concentrations in the study participant hair ranged from 0.178 to 3.778 µg/g and the median concentration was 0.484 µg/g. Among the subjects we studied, approximately 23% of the children had hair mercury levels above the 1 µg/g EPA health advisory, and about 55% had levels that were greater than one-half the EPA advisory level (i.e., > 0.5 µg/g).

Fish consumption was assessed using a food frequency questionnaire, including detailed information about consumption of different fish species, reflecting average intake over one

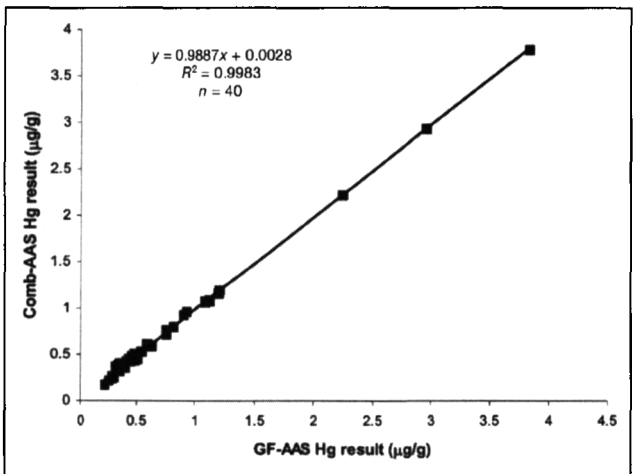

Figure 2. Linear regression for the mercury data (n = 40, in micrograms per gram, dry weight) obtained from Comb-AAS and GF-AAS.

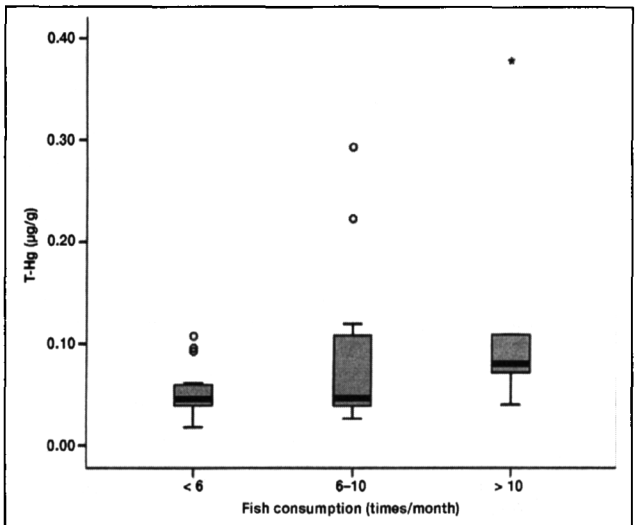

Figure 3. T-Hg in children's hair (micrograms per gram, dry weight) related to frequency (times/month) of fish consumption. Boxes depict 25th, 50th, and 75th percentiles and whiskers minimum and maximum, excluding outliers (O) and extreme values (*).

month. The average total fish consumption, as reported in the food frequency questionnaire, was approximately 7.4 times/month (range 0–16 times/month). Our results could suggest that the concentration of T-Hg in hair slightly increased with the consumption of fish (Figure 3). These results are in agreement with other studies, indicating that there is a significant association between the frequency of fish consumption and the amount of mercury in hair (5,9,16,44–46).

Conclusions

A simple, reliable method based on Comb-AAS for the routine determination of total mercury in hair samples with suitable levels of accuracy and precision and detection limits was developed. Comparison to a standard method such as GF-AAS found that both methods provide statistically equivalent results. However, the Comb-AAS method is faster and involves less sample manipulation (10 mg versus 25 mg), giving a lower risk of contamination. In addition, this method does not generate waste products, and it should be mainly useful for the analysis of a large number of samples. Therefore, the authors suggest that this quick method could be useful for measuring mercury in human hair.

The mean value of the mercury content in the hair samples analyzed (0.760 µg/g) was below the range (1–2 µg/g) that the WHO considers as normal for populations that do not consume fish with high methylmercury content. Mean and median mercury values for children from Menorca Island are also below the EPA advisory level of 1 µg/g. Finally, it should also be pointed out that the values reported from our investigation were comparable with those cited in the literature for normal subjects.

References

1. NRC (National Research Council), Committee on the Toxicological Effects of Methylmercury 2000. *Toxicological Effects of Methylmercury*. National Academy Press, Washington, D.C., 2000.
2. G. Ysart, P. Miller, M. Croasdale, H. Crews, P. Robb, M. Baxter, C. de L'Arg,y and N. Harrison. 1997 UK Total Diet Study—dietary exposures to aluminium, arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, tin and zinc. *Food Addit. Contam.* **17:** 775–786 (2000).
3. E.H. Larsen, N.L. Andersen, A. Moller, A. Petersen, G.K. Mortensen, and J. Petersen. Monitoring the content and intake of trace elements from food in Denmark. *Food Addit. Contam.* **19:** 33–46 (2002).
4. D. Babi, M. Vasjari, V. Celo, and M. Koroveshi. Some results on Hg content in hair in different populations in Albania. *Sci. Total Environ.* **259:** 55–60 (2000).
5. J.M. Hightower and D. Moore. Mercury levels in high-end consumers of fish. *Environ. Health Perspect.* **111:** 604–608 (2003).
6. A. Yasutake, M. Matsumoto, M. Yamaguchi, and N. Hachiya. Current hair mercury levels in Japanese for estimation of methylmercury exposure. *J. Health Sci.* **50:** 120–125 (2004).
7. J.B. Nielsen, O. Andersen, and P. Grandjean. Evaluation of mercury in hair, blood and muscle as biomarkers for methylmercury exposure in male and female mice. *Arch. Toxicol.* **68:** 317–321 (1994).
8. E. Budtz-Jorgensen, P. Grandjean, P.J. Jorgensen, P. Weihe, and N. Keiding. Association between mercury concentrations in blood and hair in methylmercury-exposed subjects at different ages. *Environ. Res.* **95:** 385–393 (2004).
9. J. Morrissette, L. Takser, G. St-Amour, A. Smargiassi, J. Lafond, and D. Mergler. Temporal variation of blood and hair mercury levels in pregnancy in relation to fish consumption history in a population living along the St. Lawrence River. *Environ. Res.* **95:** 363–374 (2004).
10. W.I. Mortada, M.A. Sobh, M.M. El-Defrawy, and S.E. Farahat. Reference intervals of cadmium, lead, and mercury in blood, urine, hair, and nails among residents in Mansoura city, Nile delta, Egypt. *Environ. Res.* **90:** 104–110 (2002).
11. A.A. Adimado and D.A. Baah. Mercury in human blood, urine, hair, nail, and fish from the Ankobra and Tano river basins in Southwestern Ghana. *Bull. Environ. Contam. Toxicol.* **68:** 339–346 (2002).
12. K.R. Mahaffey, R.P. Clickner, and C.C. Bodurow. Blood organic mercury and dietary mercury intake: National Health and Nutrition Examination Survey, 1999 and 2000. *Environ. Health Perspect.* **112:** 562–570 (2004).
13. C.D. Carrington and M.P. Bolger. An exposure assessment for methylmercury from seafood for consumers in the United States. *Risk Anal.* **22:** 689–699 (2002).
14. S.A. Katz and R.B. Katz. Use of hair analysis for evaluating mercury intoxication of the human body—a review. *J. Appl. Toxicol.* **12:** 79–84 (1992).
15. A.C. Barbosa, A.A. Boischio, G.A. East, I. Ferrari, A. Goncalves, P.R.M. Silva, and T.M.E. Dacruz. Mercury contamination in the Brazilian Amazon—environmental and occupational aspects. *Water Air Soil Pollut.* **80:** 109–121 (1995).
16. M.A. McDowell, C.F. Dillon, J. Osterloh, P.M. Bolger, E. Pellizzari, R. Fernando, R.M. de Oca, S.E. Schober, T. Sinks, R.L. Jones, and K.R. Mahaffey. Hair mercury levels in US children and women of childbearing age: reference range data from NHANES 1999–2000. *Environ. Health Perspect.* **112:** 1165–1171 (2004).
17. J.G. Dorea, A.C. Barbosa, I. Ferrari, and J.R. De Souza. Mercury in hair and in fish consumed by Riparian women of the Rio Negro, Amazon, Brazil. *Int. J. Environ. Health Res.* **13:** 239–248 (2003).
18. G. Muckle, P. Ayotte, E. Dewailly, S.W. Jacobson, and J.L. Jacobson. Prenatal exposure of the Northern Quebec iuit infants to environmental contaminants. *Environ. Health Perspect.* **109:** 1291–1299 (2001).
19. U.S. Gill, H.M. Schwartz, and L. Bigras. Results of multiyear international interlaboratory comparison program for mercury in human hair. *Arch. Environ. Contam. Toxicol.* **43:** 466–472 (2002).
20. R. Knight, S.J. Haswell, S.W. Lindow, and J. Batty. Determination of mercury in hair by coupled CVAA-ICP-MS. *J. Anal. At. Spectrom.* **14:** 127–129 (1999).
21. A. Campe, N. Velghe, and A. Claeys. Determination of inorganic, phenyl and alkyl mercury in hair. *Atom. Spectros.* **3:** 122–125 (1982).
22. J.P. Farant, D. Brissette, L. Moncion, L. Bigras, and A. Chartrand. Improved cold-vapor atomic absorption technique for the microdetermination of total and inorganic mercury in biological samples. *J. Anal. Toxicol.* **5:** 47–51 (1981).
23. T. Giovanoli-Jakubczak, M. Greenwood, J.C. Smith, and T.W. Clarkson. Determination of total and inorganic mercury in hair by flameless atomic-absorption, and of methylmercury by gas-chromatography. *Clin. Chem.* **20:** 222–229 (1974).
24. M. Harada, S. Nakachi, T. Cheu, H. Hamada, Y. Ono, T. Tsuda, K. Yanagida, T. Kizaki, and H. Ohno. Monitoring of mercury pollution in Tanzania: relation between head hair mercury and health. *Sci. Total Environ.* **227:** 249–256 (1999).
25. C.G. Bruhn, A.A. Rodriguez, C. Barrios, V.H. Jaramillo, J. Becerra, U. Gonzalez, N.T. Gras, O. Reyes, and S. Salud. Determination

of total mercury in scalp hair of humans by gold amalgamation cold vapor atomic-absorption spectrometry. *J. Anal. At. Spectrom.* **9:** 535–541 (1994).
26. S. Díez and J.M. Bayona. Determination of methylmercury in human hair by ethylation followed by headspace solid-phase microextraction-gas chromatography-cold-vapour atomic fluorescence spectrometry. *J. Chromatogr. A* **963:** 345–351 (2002).
27. N. Miekeley, M. Carneiro, and C.L.P. da Silveira. How reliable are human hair reference intervals for trace elements? *Sci. Total Environ.* **218:** 9–17 (1998).
28. S.B. Sarmani and I. Alakili. Application of neutron activation analysis for mercury species determination in scalp hair samples from Malaysia, Libya and Jordan. *J. Radioanal. Nucl. Chem.* **262:** 43–48 (2004).
29. E. Cernichiari, T.Y. Toribara, L. Liang, D.O. Marsh, M.W. Berlin, G.J. Myers, C. Cox, C.F. Shamlaye, O. Choisy, P. Davidson, and T.W. Clarkson. The biological monitoring of mercury in the Seychelles study. *Neurotoxicology* **16:** 613–627 (1995).
30. J.V. Cizdziel and S. Gerstenberger. Determination of total mercury in human hair and animal fur by combustion atomic absorption spectrometry. *Talanta* **64:** 918–921 (2004).
31. J.V. Cizdziel, T.A. Hinners, J.E. Pollard, E.M. Heithmar, and C.L. Cross. Mercury concentrations in fish from Lake Mead, USA, related to fish size, condition, trophic level, location, and consumption risk. *Arch. Environ. Contam. Toxicol.* **43:** 309–317 (2002).
32. S. Gerstenberger and R. Pearson. Mercury concentrations in bullfrogs (*Rana catesbeiana*) collected from a southern Nevada, USA, wetland. *Bull. Environ. Contam. Toxicol.* **69:** 210–218 (2002).
33. S.L. Gerstenberger. Mercury concentrations in migratory waterfowl harvested from Southern Nevada Wildlife Management areas, USA. *Environ. Toxicol.* **19:** 35–44 (2004).
34. H.M. Boylan, R.D. Cain, and H.M. Kingston. A new method to assess mercury emissions: A study of three coal-fired electric-generating power station configurations. *J. Air Waste Manag. Assoc.* **53:** 1318–1325 (2003).
35. V. Synek, P. Subrt, and J. Marecek. Uncertainties of mercury determinations in biological materials using an atomic absorption spectrometer - AMA 254. *Accred. Qual. Assur.* **5:** 58–66 (2000).
36. L.A. Currie. International recommendations offered on analytical detection and quantification concepts and nomenclature. *Anal. Chim. Acta* **391:** 103–103 (1999).
37. W.Y. Chen, Y.C. Wang, and M.S. Kuo. Determination of total mercury and methylmercury in human hair by graphite-furnace atomic absorption spectrophotometry using 2,3-dimercaptopropane-1-sulfonate as a complexing agent. *Anal. Sci.* **18:** 255–260 (2002).
38. IPCS, 1990. International Programme on Chemical Safety. Methylmercury. Environmental Health Criteria 101. World Health Organization, Geneva, Switzerland. http://www.inchem.org/documents/ehc/ehc/ehc101.htm, August 7, 2006.
39. U. Steuerwald, P. Weihe, P.J. Jorgensen, K. Bjerve, J. Brock, B. Heinzow, E. Budtz-Jorgensen, and P. Grandjean. Maternal seafood diet, methylmercury exposure, and neonatal neurologic function. *J. Pediatr.* **136:** 599–605 (2000).
40. P. Grandjean, P. Weihe, R.F. White, F. Debes, S. Araki, K. Yokoyama, K. Murata, N. Sorensen, R. Dahl, and P.J. Jorgensen. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicol. Teratol.* **19:** 417–428 (1997).
41. K.S. Crump, T. Kjellstrom, A.M. Shipp, A. Silvers, and A. Stewart. Influence of prenatal mercury exposure upon scholastic and psychological test performance: benchmark analysis of a New Zealand cohort. *Risk Anal.* **18:** 701–713 (1998).
42. U.S. EPA, 1997. Mercury Study Report to Congress. Office of Air Quality Planning and Standards and Office of Research and Development, Washington, D.C. EPA 452/R-97-0003, December 1997.
43. European Commission, 2002. Health effects and risk assessment. In Working Group on Mercury, Ambient Air Pollution by Mercury (Hg), Position Paper on Mercury. Air Quality, Daughter Directives, European Commission (Chapter 6), www.europa.eu.int/comm/environment/air/background.htm#mercury.
44. D. Airey. Total mercury concentrations in human hair from 13 countries in relation to fish consumption and location. *Sci. Total Environ.* **31:** 157–180 (1983).
45. C. Johnsson, G. Sallsten, A. Schutz, A. Sjors, and L. Barregard. Hair mercury levels versus freshwater fish consumption in household members of Swedish angling societies. *Environ. Res.* **96:** 257–263 (2004).
46. T. Agusa, T. Kunito, H. Iwata, I. Monirith, T.S. Tana, A. Subramanian, and S. Tanabe. Mercury contamination in human hair and fish from Cambodia: levels, specific accumulation and risk assessment. *Environ. Pollut.* **134:** 79–86 (2005).

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