Biomonitoring of Mercury Exposure with Single Human Hair Strand

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Hair samples continue to be used extensively for biomonitoring of mercury (Hg) exposure. Routine methods require a bundle of 100–150 hair strands and involve chemical digestion. Recently, Hg analyzers that combine combustion, gold amalgamation, and atomic absorption spectrometry (C-GA-AAS) became commercially available. This method was shown to provide quick and sensitive measurements in solid samples such as hair. The objective of this study was to validate C-GA-AAS for measuring total Hg in single hair strands as an alternative method for Hg biomonitoring. Hair samples from 12 women with a wide range of Hg exposure were obtained from two projects conducted in Brazil and Canada. A 1:1 relationship was observed between C-GA-AAS and the established cold vapor atomic absorption spectrometry (CV-AAS) for analysis of 1-cm segments from a bundle of hair. For individual hair variability, the average relative standard deviation (RSD) of Hg between hair strands was $6.5 \pm 2.8\%$, thus justifying the use of single hair strand for biomonitoring. With a limit of quantification of 0.10 ng of total Hg, a single hair strand can be used to assess monthly exposure. This technique will facilitate routine biomonitoring and thus help prevent Hg poisoning among the public.

Introduction

Neurotoxic effects of Hg, particularly methylmercury (MeHg) on humans and wildlife, have been well documented (*1*). Neurodevelopment of children is considered the most sensitive toxic endpoint and in utero exposure the most sensitive period of exposure (*2*). Analysis of the most recent 1999–2001 National Health and Nutrition Examination Survey (NHANES) data showed that at least 300 000 newborns each year in the United States have blood Hg concentrations higher than those considered to be without increased risk of adverse neurodevelopmental effects associated with MeHg exposure (*3*). On the basis of results of several large epidemiological studies (*4*, *5*), the Joint FAO/WHO Expert Committee on Food Additives (JECFA) recently reduced the

provisional tolerable weekly intake (PTWI) from 3.3 to 1.6 μg MeHg per kg body weight per week (*2*). This intake guideline may pose a restriction on fish consumption among fish eating populations (*6*). Therefore, it is important to have reliable data for exposure assessment as conflicting classification of exposure may lead to poor development of dose–response relationships (*7*) and compromise public health decisions and recommendations (*8*).

Blood and hair are the most commonly used media for biomonitoring of MeHg exposure and body burden. In adults, the ratio of hair to blood has been modeled with hair containing approximately 250 times more Hg (*9*). However, the ratios obtained within and between populations are variable as the time period of exposure represented by blood and hair are often different (*10*). This is particularly relevant in regions with clear variation in seasonal exposure as in the Brazilian Amazon (*11*). Hair analysis is the preferred biomarker of exposure because, in addition to correlating with levels in the brain (*12*), it presents the following advantages. At a growth rate of approximately 1 cm a month (*13*), segmental analysis can retrace weeks and months of exposure depending on the length of the hair of the individual (*11*). Hair samples are easily obtained making it a practical biomonitoring tool for large populations (*10*) and it is less invasive compared to blood collection. Fetal exposure during gestation can be assessed with monitoring of maternal hair samples. Therefore, hair has been extensively used as a biomarker of exposure in large epidemiological studies (*4*, *5*, *10*).

The most common methods to measure Hg include CV-AAS, cold vapor atomic fluorescence spectrometry (CV-AFS), and inductively coupled plasma mass spectrometry (ICP-MS) (*14*). These methods usually measure Hg in solution samples only. Therefore, chemical digestion using a combination of strong acids (HCl, H_2SO_4, HNO_3), strong bases (NaOH), and oxidants (H_2O_2, $KMnO_4$, $K_2S_2O_8$) coupled with microwave and elevated temperatures are used to extract the Hg from solid samples such as hair (*14*). Detection limits range from about 0.04 mg/kg up to 0.4 mg/kg in hair (*15*, *16*). Therefore, a minimum amount of hair of at least 5–10 mg (or about 100–150 hair strands depending on the hair thickness) is usually required for biomonitoring purposes. The major disadvantages of this method include intrusiveness to participants because of the large requirement of strands and lengthy extraction procedures which reduce throughput.

Recently, commercial instruments capable of measuring total Hg directly in solid and liquid matrixes have become available. The principle of the methodology combines combustion, Hg collection with gold amalgamation, and detection with atomic absorption spectrometry (C-GA-AAS). Direct introduction techniques eliminate sample pretreatment, produce very little waste, and lower potential contamination. As a result, the throughput of samples is increased. This method has been used to determine total Hg in a variety of fish and food reference materials (*17*), in fish tissues (*18*), and in waterfowl (*19*). The only analysis of hair using C-GA-AAS was reported by (*20*) where total Hg was determined in hair powdered reference material, in composite human hair and horse fur.

Single hair strand Hg analysis presents an advantage over the traditional bundle analysis because the collection is less invasive to participants (especially for children) and the subsequent segmentation is more efficient. C-GA-AAS holds potential to measure Hg in single hair strand with detection suited to assess natural levels, but validation of the meth-

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odology is required. Thus, the primary objective of this study was to validate the use of C-GA-AAS for the direct determination of total Hg in single human hair strands by comparing results of split samples with an established CV-AAS method (*16*). The secondary objective was to investigate whether a single hair strand is a valid representation of the body burden of an individual by determining the variation of Hg between single hair strands within individuals.

Materials and Methods

A commercial Hg analyzer that integrates combustion, Hg collection by gold amalgamation, and detection by atomic absorption spectrometry (C-GA-AAS) technology was used for this study (MA-2000, Nippon Instruments Corporation, Osaka, Japan). Milligrams of hair samples were placed in ceramic boats between two layers of additives containing a mixture of sodium carbonate and calcium hydroxide (1:1). The additives absorb byproducts such as sulfates and halogens that may be produced during combustion and may reduce signal noise. The ceramic boats are directly introduced to a high-temperature combustion tube (850 °C) where the material is thermally decomposed and then converted to elemental Hg. The free Hg vapor is collected by a gold-coated diatomite particle support and then heated to release atomic Hg. The released total Hg is detected using atomic absorption at a wavelength of 253.7 nm in the absorption cell of the detector. The entire cycle is completed within 7 min.

This technique was criticized for the potential interference due to memory effects which occurs when Hg vapor remains in the combustion tube, amalgamator, or absorbance cell and then is released in subsequent analysis resulting in a positive bias (i.e., when a high Hg sample precedes a low Hg sample) (*21*). In this study, blank readings were typically less than 0.1 ng, even following samples containing a relatively high amount of Hg (i.e., over 20 ng). The instrument can be calibrated over two ranges (0−20 and 20−1000 ng of Hg). Calibration curves were established using external liquid standards which were prepared daily. For the low range, the standards included 0, 0.25, 0.5, 0.75, 1.0, 2.5, 5, and 10 ng. For the high range, it included 0, 25, 50, 100, and 150 ng of Hg. The R^2 was consistently at or above 0.999.

Method detection limit (MDL) was defined as 3 times the standard deviation of the blanks divided by the slope: $MDL = (3 \times SD_{bl})/m$. Method quantification limit (MQL) was defined as 10 times the standard deviation of the blank divided by the slope: $MQL = (10 \times SD_{bl})/m$. The standard deviations of the blank, the MDL, and the MQL are expressed as nanograms (ng) of Hg. The limit of quantification for hair was determined to be 0.10 ng of total Hg.

All analytical procedures were conducted at the Center for Indigenous Peoples' Nutrition and Environment, Macdonald Campus, McGill University, Ste-Anne-de-Bellevue, Québec, Canada.

Hair Samples

The objective was to obtain hair samples with a wide range of Hg concentrations. Hair samples collected from 12 individuals from two previous studies (*22*, *23*) were used.

Hair from eight women residing in the village of Brasilia Legal, Brazil with fish as a dietary mainstay was obtained. The hair was collected in the context of a larger interdisciplinary project (*22*). For confidentiality, the women were assigned a number from 1 to 8. A second set of hair samples with lower Hg concentrations (below 0.4 mg/kg) was obtained from women residing in the Bay of Fundy, New Brunswick, Canada as part of another large interdisciplinary project (*23*). Hair from these women were labeled from A to D. In both studies, a bundle of hair (approximately 100−150 strands) was isolated and cut from the occipital region, placed in a polyethylene bag, and fastened with two staples near the scalp end. The hair bundle was cut into 1-cm segments, weighing a minimum of 10 mg, digested with a base solution, and total Hg measured with CV-AAS using the Farant et al. (1981) (*16*) method at the Laboratory Services of the Research and Environmental Health Division, Health Canada, Ottawa, Ontario, Canada. The Hg concentrations of the 12 women, as determined by CV-AAS using the Farant et al. methodology, ranged from less than 0.4 mg/kg to 27.2 mg/kg.

The first experiment performed with C-GA-AAS included analysis of total Hg of 36 1-cm hair segments from eight individuals. Each segment weighed approximately 2 mg, comprising a bundle of 20−30 hair strands. Pearson correlation was performed comparing total Hg measured with C-GA-AAS and the traditional CV-AAS.

The second experiment included analysis of five single hair strands from eight individuals to determine strand-to-strand variation. Five single hair strands from each of the eight women ($n = 40$) were selected and individually cut to obtain a standard length of 12 cm away from the root. This would represent an annual exposure assuming hair growth of 1 cm per month. Individual hair strands were chosen on the basis of visual criteria: the longest and thickest strands were selected. Each 12-cm hair strand was independently weighed with a 0.01 mg precision balance (Sartorius, Germany) and total Hg was measured by C-GA-AAS. Relative standard deviation (RSD) was computed to determine strand-to-strand variation for Hg content within each individual.

The last experiment was conducted to measure Hg in 1-cm segments within one hair strand. One 12-cm hair strand from five women was cut into 12 1-cm segments ($n = 60$ segments) and total Hg was determined by C-GA-AAS in each 1-cm segment independently. Because of the lack of sensitivity of the balance, the 1-cm segments were not independently weighed. Rather, the weight of the 12-cm segment was divided by 12 with assumption of homogeneity of weight within a single hair strand. Pearson correlation was performed between total Hg of 1-cm single hair strand determined with C-GA-AAS and 1-cm hair bundle determined with CV-AAS.

Quality assurance and quality control measures were taken to ensure data quality. Blank (empty ceramic boat) was always below detection limit. Check standards and certified reference material (CRM) for Hg in hair (IAEA (International Atomic Energy Agency) - 086, Vienna, Austria) were run every 10 samples. Recovery of the check standard and the CRM were consistently above 95% and 90%, respectively.

All data were imported and analyzed with Microsoft Office 10 Excel 2000. All *p* values below the 0.05 alpha were considered significant.

Results

Table 1 presents the average and standard deviation of Hg measurements of 1-cm segments from a bundle of hair from the same individuals using both methods. A 1:1 relationship was observed. The correlation coefficient was 0.99 and the slope was 1.02 for concentrations ranging from 3.3 to 22.4 mg/kg. Weaker but significant correlations were noted within the higher (THg > 10 mg/kg, $r = 0.87$, slope = 1.02) and the lower (THg < 10 mg/kg, $r = 0.7$, slope = 1.00) range. Hair segments with concentrations below the CV-AAS detection limit of 0.4 mg/kg showed wide variations with a minimum concentration of 0.10 mg/kg..

The weight of individual 12-cm hair strands ranged from 0.25 to 0.98 mg with an average of 0.62 mg. Figure 1 shows the individual Hg results for each 12-cm hair strand from eight women ($n = 40$ hair strands). The RSD between hair strands within an individual ranged from 2.2 to 11.0% with an average of 6.5%.

TABLE 1. Average and Standard Deviation of Total Hg Measurements of 1-cm Sequential Segments from a Bundle of Hair of the Same Individuals Using Both Methods

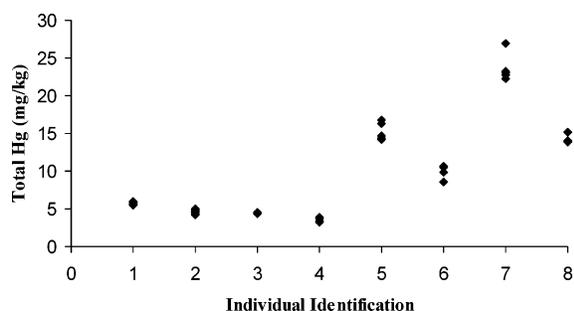| individual ID	sequential segments (*n*)	THg[a] (mg/kg) CV-AAS average ± SD	THg[a] (mg/kg) C-GA-AAS average ± SD
A	4	<0.4 ± N/A	0.36 ± 0.04
B	4	<0.4 ± N/A	0.11 ± 0.01
C	4	<0.4 ± N/A	0.28 ± 0.03
D	4	<0.4 ± N/A	0.34 ± 0.06
1	5	3.9 ± 0.4	4.18 ± 0.36
2	5	4.4 ± 0.6	4.16 ± 0.83
3	5	16.1 ± 1.9	16.61 ± 1.82
4	5	19.7 ± 2.3	20.06 ± 1.86

[a] THg = total mercury.

FIGURE 1. Total Hg (mg/kg) in five independent 12-cm hair strands for eight women showing strand-to-strand variation (*n* = 40 hair strands).
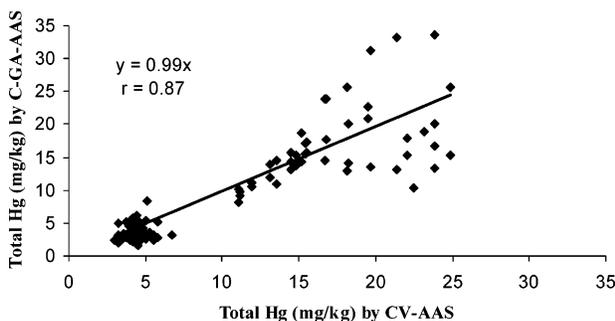

FIGURE 2. Correlation of 1-cm from a single hair strand measured by C-GA-AAS with the matched 1-cm hair bundle measured by CV-AAS (*n* = 60 segments).

One-centimeter segmental analysis with single hair strand measured by C-GA-AAS also correlated (coefficient of 0.87 with a slope of 0.99) with 1-cm hair bundle measured by CV-AAS (Figure 2).

Discussion

High-temperature combustion coupled with gold amalgamation and AAS for the direct determination of Hg in single human hair strands has been validated. Good agreement was demonstrated with certified reference material, and statistically equivalent results were found between C-GA-AAS and conventional CV-AAS.

Analysis of 1-cm segment bundle with C-GA-AAS required a lesser number of hair strands as compared to CV-AAS (*16*) and CV-AFS (*15*) while offering improved detection limit. The MQL of the Farant et al. (*16*) method for total Hg is 0.4 mg/kg using 10 mg of hair, while that of Pellizzari et al. (*15*) is 0.04 mg/kg using 5 mg of hair. C-GA-AAS offers improved MQL over both chemical digestion methods with a MQL 40 times less than CV-AAS and half that of CV-AFS.
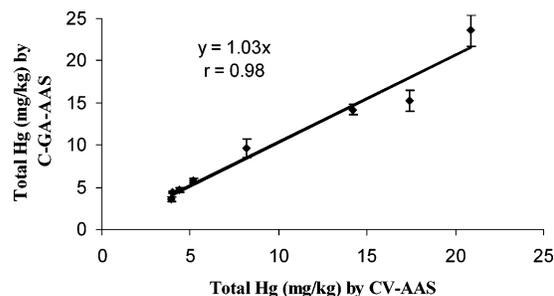

FIGURE 3. Mean ± standard deviation of five 12-cm strands measured by C-GA-AAS plotted against the mean of the matched 12 1-cm segments measured by CV-AAS (*n* = 8 individuals).

The most conservative guideline for total Hg in hair was derived by the United States Environmental Protection Agency (USEPA) and set at 1 mg/kg (*24*). Thus, the 0.4 mg/kg detection limit of the Farant et al. method, while acceptable for exposure assessment purposes, has limited statistical use when data are labeled below detection limit. Indeed, data below detection limit are either assigned zero, half the value of the detection limit, or the value of the detection limit. Table 1 shows that values below the limit of detection varied by at least a factor of 4 which is not consistent with any of the proposed methods for data below detection limit.

Other direct solid introduction techniques include X-ray fluorescence (XRF) (*25*) and proton-induced X-ray emission photometry (PIXE) (*26*). XRF, a nondestructive method capable of spatial resolution of 2 mm on single hair strands, was useful to assess the Hg profile of a woman exposed to dimethylmercury following a laboratory spill (*27*) and of women exposed to MeHg from frequent fish consumption during pregnancy (*12*). PIXE, capable of spatial resolution of micrometers, was used to determine depth and longitudinal profiles of several elements in single hair strands (*28*). Although these two methods offer improved spatial resolution compared to C-GA-AAS, the instrumentation requirement for PIXE is expensive and the operation is complicated, while the Hg detection limits of 3−5 mg/kg for XRF (*12, 25*) are not sensitive for detecting natural levels in hair.

A prerequisite for use of single hair strand analysis is the determination of the variation in Hg content between hair strands within the same individual. Hair undergoes several stages of growth leading to strands with various thickness and length (*13*). The low RSD for Hg content despite the larger RSD for weight showed that one single hair strand is sufficient to represent the occipital region of the head. Thus, one 12-cm hair strand may be used to assess yearly exposure to Hg, assuming a growth rate of 1 cm per month. In this population, there are wide variations in Hg concentrations between each 1-cm segment within individuals, reflecting seasonal variability in the amount and species of fish intake ( ). Nevertheless, the Hg concentration of a 12-cm hair strand significantly correlated (*r* = 0.98 with a slope of 1.03; Figure 3) with the average of the 12 1-cm segments measured by CV-AAS, thus further supporting that a single hair strand is a good indicator for average annual exposure.

Analysis of 1-cm segments within single hair strand, however, showed a weaker correlation than that obtained for single 12-cm analysis. Because of the limitations of the precision of the balance, the weight of each 1-cm segment was obtained by dividing the 12-cm hair segment by 12. The assumption of homogeneity of weight within a hair strand may not be justified which would explain the observed variation in Hg content. Thus, future studies using C-GA-AAS to determine Hg in 1-cm segments of single hair strand must utilize a balance that provides precision at 0.001 mg level to weigh each segment. The alternative is to use multiple hair strands for this purpose. Table 2 summarizes the number

TABLE 2. Number of Hair Strands Required for Various Hg Concentration in Hair and for Various Exposure Periods

Hg concentration in hair (mg/kg)	minimum weight required (mg)[a]	minimum # strands for monthly exposure (1 cm)[b]	minimum # of strands for seasonal exposure (3 cm)[b]	minimum # of strands for annual exposure (12 cm)[b]
0.1	1	20	7	2
0.2	0.5	10	4	1
0.4	0.25	5	2	1
0.8	0.125	3	1	1
1.6	0.063	2	1	1
3.2	0.031	1	1	1
6.4	0.016	1	1	1

[a] Limit of quantification is 0.1 ng of Hg. [b] Average weight of a 1-cm hair strand is 0.05 mg.

of hair strands required to determine Hg by C-GA-AAS for various exposure periods. Assuming that the average weight of 1-cm segment hair strand is 0.05 mg, one hair strand can be used to determine yearly exposure if the Hg concentration in hair is equal to or above 0.2 mg/kg and monthly exposure if the hair Hg concentration is equal to or above 3 mg/kg. To screen for exposure using the USEPA guideline of 1 mg/kg (24) as cutoff, one hair strand is required for annual exposure estimation and three hair strands are required for monthly exposure estimation.

Large surveys such as the 1999–2001 NHANES have included Hg assessment for 838 children 1–5 years of age and 1726 women 16–49 years of age (10). Exposure to Hg was determined with dietary questionnaires, blood, hair, and urine. Approximately 100 hair strands were collected from the occipital region of the scalp and the first cm closest to the scalp was analyzed for total Hg by CV-AFS as described in ref 15. Use of C-GA-AAS in large studies would reduce the traditional lengthy time analysis since the method calls for direct solid introduction (i.e., no chemical digestion). With the minimal hair strand requirement, participation rate would be enhanced and inclusion of neonates and infants would be favored.

Primary health care providers also require easily accessible and reliable tools for prevention of Hg accumulation and its associated effects particularly for women of childbearing age, pregnant women, and children. Data from six commercial laboratories that process 90% of samples submitted for mineral analysis in the United States yielded inconsistent results (8) with Hg values for a split hair sample of one individual ranging from 0.1 to 2 mg/kg. The variation was attributed to differential laboratory sample preparation and calibration standards (8). The need for quick and accurate results was exemplified in the study by physicians Hightower and Moore (29) where urban residents from the San Francisco Area were shown to accumulate unsafe levels of Hg following consumption of carnivorous fish like swordfish.

As of 2002, 48 of the 50 states in the United States have issued health advisories for sport fish consumers which included 39 chemicals with Hg being the most commonly identified (30). Assessment of the effectiveness of the advisories can be achieved by measuring Hg in hair (31). This strategy was successfully demonstrated on the Faroe Islands where Hg concentrations in hair were determined pre- and postrecommendations (32).

C-GA-AAS presents clear advantages for hair Hg analysis as significantly less number of hair strands and no chemical pretreatment are required while maintaining an excellent detection limit. With an autosampler, it takes 7 min per analysis, and the associated cost per sample is less than $5 USD. This method thus provides the high throughput required for large health surveillance purpose.

Literature Cited

(1) United Nations Environment Programme (UNEP). *Global Mercury Assessment*; Geneva, Switzerland, 2002.

(2) Joint FAO/WHO Expert Committee on Food Additives (JECFA). *Summary and Conclusions of the Sixty-First meeting on food additives, Rome*, June 10–19, 2003; http://www.who.int/pcs/jecfa/ jecfa.htm (accessed Dec 10, 2004).

(3) Mahaffey, K. R.; Clickner, R. P.; Bodurow, C. C. Blood organic mercury and dietary mercury intake: National Health and Nutrition Examination Survey, 1999 and 2000. *Environ. Health Perspect.* **2004**, *112* (5), 562–570.

(4) Cox, C.; Breazna, A.; Davidson, P. W.; Myers, G. J.; Clarkson, T. W. Prenatal and postnatal methylmercury exposure and neurodevelopmental outcomes. *JAMA, J. Am. Med. Assoc.* **1999**, *282*, 1333–1334.

(5) Grandjean, P.; Budtz-Jorgensen, E.; White, R. F.; Jorgensen, P. J.; Weihe, P.; Debes, F.; Keiding, N. Methylmercury exposure biomarkers as indicators of neurotoxicity in children aged 7 years. *Am. J. Epidemiol.* **1999**, *149*, 301–305.

(6) Chan, H. M.; Receveur, O. Mercury in the traditional diet of indigenous people in Canada. *Environ. Pollut.* **2000**, *110*, 1–2.

(7) Grandjean, P.; Budtz-Jorgensen, E.; Keiding, N.; Weihe, P. Underestimation of risk due to exposure misclassification. *Int. J. Occup. Med. Environ. Health* **2004**, *17* (1), 131–6.

(8) Seidel, S.; Kreutzer, R.; Smith, D.; McNeel, S.; Gilliss, D. Assessment of commercial laboratories performing hair mineral analysis. *JAMA, J. Am. Med. Assoc.* **2001**, *285* (1), 67–72.

(9) World Health Organization. *Environmental Health Criteria 101*: *Methylmercury*; Geneva International Programme on Chemical Safety; Geneva, Switzerland, 1990; http://www.inchem.org/ documents/ehc/ehc/ehc101.htm (accessed Dec 10, 2004).

(10) McDowell, M. A.; Dillon, C. F.; Osterloh, J.; Bolger, P. M.; Pellizzari, E.; Fernando, R.; Montes de Oca, R.; Schober, S. E.; Sinks, T.; Jones, R. L.; Mahaffey, K. R. Hair mercury levels in U.S. children and women of childbearing age: reference range data from NHANES 1999-2000. *Environ. Health Perspect.* **2004**, *112*, 1165–1171.

(11) Dolbec, J.; Mergler, D.; Larribe, F.; Roulet, M.; Lebel, J.; Lucotte, M. Sequential analysis of hair mercury levels in relation to fish diet of an Amazonian population, Brazil. *Sci. Tot. Environ.* **2001**, *271*, 87–97.

(12) Cernichiari, E.; Brewer, R.; Myers, G. J.; Marsh, D. O.; Lapham, L. W.; Cox, C.; Shamlaye, C. F.; Berlin, M.; Davidson, P. W.; Clarkson, T. W. Monitoring methylmercury during pregnancy: maternal hair predicts fetal brain exposure. *Neurotoxicology* **1995**, *16*, 705–710.

(13) Robbins C. R. *Chemical and physical behaviour of human hair*, 4th ed.; Springer-Verlag: New York, 2002; pp 3 and 8–9.

(14) Gill, U. S.; Schwartz, H. M.; Bigras, L. Results of multiyear international interlaboratory comparison program for mercury in human hair. *Arch. Environ. Contam. Toxicol.* **2002**, *43*, 466–472.

(15) Pellizzari, E. D.; Fernando, R.; Cramer, G. M.; Meaburn, G. M.; Bangerter, K. Analysis of mercury in hair of region V population. *J. Exp. Anal. Environ. Epidemiol.* **1999**, *9*, 393–401.

(16) Farant, J. P.; Brissette, D.; Moncion, L.; Bigras, L.; Chartrand, A. Improved cold-vapor atomic absorption technique for the microdetermination of total and inorganic mercury in biological samples. *J. Anal. Toxicol.* **1981**, *5*, 47–51.

(17) Salvato, N.; Pirola, C. Analysis of mercury traces by means of solid sample atomic absorption spectrometry. *Microchim. Acta* **1996**, *123*, 63–71.

(18) Cizdziel, J. V.; Hinners, T. A.; Heithmar, E. M. Determination of total mercury in fish tissues using combustion atomic absorption spectrometry with gold amalgamation. *Water, Air, Soil Pollut.* **2002**, *135*, 355–370.

(19) Gerstenberger, S. L. Mercury concentrations in migratory waterfowl harvested from Southern Nevada Wildlife Management Areas, USA. *Environ. Toxicol.* **2004**, *19*, 35–44.

(20) Cizdziel, J. V.; Gerstenberger, S. Determination of total mercury in human hair and animal fur by combustion atomic absorption spectrometry. *Talanta* **2004**, *64*, 918−921.

(21) United States Environmental Protection Agency (USEPA). *Method 7473: Mercury in solids and solutions by thermal decomposition, amalgamation, and atomic absorption spectrophotometry*; 1998. http://www.epa.gov (accessed Sept 19, 2004).

(22) Passos, C. J.; Mergler, D.; Gaspar, E.; Morais, S.; Lucotte, M.; Larribe, F.; Davidson, R.; de Grosbois, S. Eating tropical fruit reduces mercury exposure from fish consumption in the Brazilian Amazon. *Environ. Res.* **2003**, *93*, 123−130.

(23) Legrand, M.; Ritchie, C.; Arp, P.; Chan, H. M. Mercury exposure in two coastal communities of the Bay of Fundy, Canada. *Environ. Res.* **2005**, *98*, 14−21.

(24) United States National Research Council (USNRC). *Toxicological effects of methylmercury*; National Academy Press: Washington, DC, 2000; pp 117 and 134.

(25) Toribara, T. Y.; Jackson, D. A.; French, W. R.; Thompson, A. C.; Jaklevic, J. M. Nondestructive X-ray fluorescence spectrometry for determination of trace elements along a single strand of hair. *Anal. Chem.* **1982**, *54*, 1844−1849.

(26) Valkovic, V.; Miljanic, D.; Wheeler, R. M.; Liebert, R. B.; Zabel, T.; Phillips, G. C. Variation in trace metal concentrations along single hairs as measured by proton-induced X-ray emission photometry. *Nature* **1973**, *111*, 251−252.

(27) Nierenberg, D. W.; Nordgren, R. E.; Chang, M. B.; Siegler, R. W.; Blayney, M. B.; Hochberg, F.; Toribara, T. Y.; Cernichiari, E.; Clarkson, T. Delayed cerebellar disease and death after accidental exposure to dimethylmercury. *N. Engl. J. Med.* **1998**, *338*, 1672−1676.

(28) Bos, A. J. J.; van der Stap, C. C. A..H.; Valkovic, V.; Vis, R. D.; Verheul, H. Incorporation routes of elements into human hair; implications for hair analysis used for monitoring. *Sci. Total Environ.* **1985**, *42*, 157−169.

(29) Hightower, J.; Moore, D. Mercury levels in high-end consumers of fish. *Environ. Health Perspect.* **2003**, *111* (4), 604−608.

(30) Anderson, H. A.; Hanrahan, L. P.; Smith, A.; Draheim, L.; Kanarek, M.; Olsen, J. The role of sport-fish consumption advisories in mercury risk communication: a 1998-1999 12-state survey of women age 18-45. *Environ. Res.* **2004**, *95*, 219−226.

(31) Knobeloch, L.; Anderson, H. A.; Imm, P.; Peters, D.; Smith, A. Fish consumption, advisory awareness, and hair mercury levels among women of childbearing age. *Environ. Res.* **2005**, *97*, 219−226.

(32) Weihe, P.; Grandjean, P.; Jorgensen, P. J. Application of hair-mercury analysis to determine impact of a seafood advisory. *Environ. Res.* **2005**, *97*, 200−207.