Journal of Exposure Analysis and Environmental Epidemiology **(1999) 9, 393–401**

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Analysis of mercury in hair of EPA Region V population

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A scoping study, the National Human Exposure Assessment Survey (NHEXAS) was conducted in EPA Region V from July 1995 to May 1997. This probability-based population study provided an opportunity to examine the mercury levels in 182 participants who provided hair samples. A sensitive analytical procedure based on atomic fluorescence spectrometry was developed and evaluated for the analysis of Hg in approximately 5 mg of human hair. The correlation coefficient (r), the precision, and bias were 0.9983, $\leq$1.6%, and $\leq$8%, respectively, for standard curves in the hair matrix. The method detection limit (MDL), recovery of Hg in a certified sample (NIES-13), precision (% RSD) for duplicate extract analysis, and precision for duplicate sample analysis averaged 12 ppb (range 4 to 22 ppb), 100±3% (N=27), 4.6±2.8 (N=18), and 12.5±7.4 (N=17), respectively, over the 7 to 8 months of sample analysis. The low MDL yielded 95% of the samples with measurable values, permitting the entire distribution of Hg levels to be characterized. Comparison of annualized Hg distribution in hair with and without background correction revealed a negligible bias on the distribution (1.47% at the 90th percentile). Also, a comparison of the unweighted and nonannualized weighted Hg levels throughout the percentile distribution indicated a small deviation in the upper tail (95th percentile) and is attributable to the small sample size (N=182). The mean, median, and maximum of the annualized Hg levels in hair were 287, 204, and 3505 ppb, respectively. The 75th percentiles were 335 and 368 ppb for the weighted annualized and unweighted distributions, respectively. The percent of individuals in three age categories (0–24, 25–49, and 50 years and older) who exceeded the 75th percentile showed a linear increase with age. Males (N=81) had 10% and 20% lower mean levels than females (N=101) for unweighted and annualized weighted Hg data, respectively. The application of this methodology for characterizing hair Hg levels in fish-eating populations is discussed.

Keywords: *analysis, annualized distribution, hair, mercury, method, statistics.*

Introduction

The hazards of environmental mercury contamination were first recognized in the 1950s and 1960s when a massive outbreak of methylmercury poisoning occurred in Minimata, Japan, following the consumption of fish heavily contaminated with mercury (Harada, 1995). Subsequent studies have shown that most forms of mercury entering the aquatic environment can be transformed by the action of microorganisms to methylmercury, which accumulates in fish (Callahan et al., 1979; ATSDR, 1995; Mason et al., 1995). Although human exposure to mercury can result from a number of sources and through various pathways, the general population is predominantly exposed to mercury in the diet, with methylmercury residues in fish being the dominant dietary source of exposure (Stopford et al., 1978; Simpson et al., 1974). The general population is also exposed to mercury vapors released from dental fillings (Engle et al., 1992).

Several reliable and accurate methods have been developed to measure mercury levels in the body. Measurements of total mercury and methylmercury levels in blood and hair are both used to characterize exposures from fish (Haxton et al., 1979; Sherlock et al., 1982; Airey, 1983; Suzuki, 1988; Oskarsson et al., 1990; ATSDR, 1995). Under steady dietary conditions, blood and hair may be used to predict possible health effects of methylmercury exposure since the concentrations are directly proportional to the levels of methylmercury that occur in the brain (Phelps et al., 1980; Cernichiari et al., 1995a). Studies wih methylmercury labeled with radioactive ^{203}Hg have shown that methylmercury is nearly all absorbed from the diet, is distributed throughout the body within a few hours, and attains peak blood levels within 4–14 hours. After a rapid clearance phase of about 20–30 hours, blood levels decline exponentially with a half-life of about 50 days (Miettinen et

1. Abbreviations: NHEXAS, National Human Exposure Assessment Survey; MDL, method detection limit; ppb, parts per billion; ATSDR, Agency for Toxic Substances and Disease Registry; PSU, probability sampling unit; DI, deionized water; HEPA, high-efficiency particle air filter; ACS, American Chemical Society; CVAFS, cold vapor atomic fluorescence spectrometry; NIST, National Insititute of Standards and Technology; DOLT-2, dolphin liver tissue; ppt, parts per trillion; pg/ml, picograms per milliliter; QC, quality control; NIES-13, National Institute of Environmental Studies, Japan.

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al., 1971). By allowing for completion of the initial clearance phase, changes in blood-mercury levels have been shown to be directly proportional to the levels of methylmercury ingested, with >95% of the mercury found in blood in the form of methylmercury (Sherlock et al., 1984).

In the case of hair, the concentrations are about 250 times greater than the blood-mercury concentrations at the moment the hair is formed. Once formed, the hair strand grows at a rate of approximately 1 cm/month, providing a record of previous mercury exposures that remains unchanged for periods up to at least 11 years (Suzuki, 1991). Approximately 80% of the mercury present in the strand is methylmercury (Phelps et al., 1980; Cernichiari et al., 1995b). Although both hair and blood samples can be used to document methylmercury exposure, hair-strand analysis is preferred because it provides a simple, noninvasive sampling procedure that allows monitoring of methylmercury intake. Since total hair mercury and methylmercury levels are linearly related, total mercury determination can also be used to characterize methylmercury exposure from fish.

The National Human Exposure Assessment Survey (NHEXAS), a scoping study, was conducted in EPA Region V from July 1995 to May 1997 (Pellizzari et al., 1995). This probability-based population study provided an opportunity to examine the mercury levels in participants who provided hair samples. This paper describes a sensitive analytical procedure that was developed and evaluated for the analysis of total Hg in a few strands of hair (5 mg). The method was applied to the analysis of hair samples collected in the NHEXAS study, and the distribution of mercury levels in the general population are presented here.

Methods

Study Design

The target population selected for study was the EPA Region V Great Lakes area, which includes Minnesota, Wisconsin, Illinois, Indiana, Ohio, and Michigan. This region has a relatively large population with a proportion of races and ethnic groups, socioeconomic distribution, and other demographic characteristics similar to the national profile (Pellizzari et al., 1995).

The survey design was previously described in (Pellizzari et al., 1995). A stratified, four-stage probability-based design was implemented. The first-stage sample involved 32 primary sampling units (PSUs) (usually counties) selected with probabilities proportional to 1990 census counts of occupied housing units; these PSUs were grouped into four analysis strata (i.e., temporal categories indicating when data collection was scheduled). Three or four sample areas (as defined by census blocks) were then selected within each sample county as second-stage units. For third-stage units a random sample of 24 housing units (the third-stage units) was identified in each segment. Nine participants within each PSU were then selected at the fourth stage. One member was sampled from a household for collection of biological samples such as hair. Approximately equal person-level probabilities of selection were achieved in this design even though there were unequal numbers of persons in the sample households.

Hair Collection

An alcohol wipe (Kendall Webcol Alcohol Prep No. 6818) was used to clean all cutting surfaces of a stainless-steel scissors and a plastic comb. Powder-free gloves were worn while taking the hair sample. A bundle of hair approximately the size of a pencil eraser (0.75–1 cm in diameter) in the occipital region of the head was isolated. The bundle was cut as closely as possible to the scalp and placed in a plastic Ziploc® bag. If the hair was more than 5 cm in length, a plastic paper clip kept the bundle intact before cutting and during storage. If shorter than 5 cm, the hair was cut directly into the plastic bag. The protocol targeted the collection of 25–50 mg of hair represented in 3-cm lengths for multiple analysis. The hair samples were shipped to the laboratory and stored at 4°C until ready for analysis.

Analytical Laboratory Procedures

Preparation of Labware New labware was soaked for a minimum of 48 h (two 8-h days at 65–75°C and overnight at room temperature) in a 4 N HCl acid bath, rinsed five times with deionized (DI) water, and dried under high efficiency particle air (HEPA) filtered air. An additional soak in 1% HCl for a minimum of 16 h was required for sample storage vials. Recycled labware was soaked in a solution of low-metal, phosphate-free Alconox Detergent 8 and rinsed with DI water and then acid soaked as new labware. Plastic pipet tips were rinsed with DI water, dried under HEPA filtered air, and stored in sealed plastic bags in a Class 100 laboratory.

Plastic autosampler cups were soaked in a 50% nitric acid bath for a minimum of 16 h, drained, rinsed five times with DI water, dried under HEPA filtered air, and stored in double-sealed plastic bags. Autosampler cups were reused after cleaning. After use, cups were rinsed with DI water and soaked in water or water containing low-metal, phosphate-free detergent, rinsed with DI water, and soaked in the 50% nitric acid bath as described above.

Teflon capliners were cleaned as described for labware. Caps without liners for scintillation vials were rinsed with DI water three times, soaked in DI water for about 1 h, rinsed again with DI water three times, and dried under HEPA filtered air. Clean caps were stored in double-sealed

plastic bags. Teflon-faced silicone septa (22-mm diameter) were rinsed and soaked in DI water as described for caps.

Reagents Solutions were prepared with ACS reagent grade or 'trace metal' grade chemicals, and stored at 4°C. Deionized water was generated from a Hydro Pico System yielding 18 megohm water quality.

A 10% (v/v) HCl solution was prepared by adding 100 ml of concentrated HCl to 200 ml of DI water in a 1-l volumetric flask, mixing well, and bring it to volume. The solution was transferred and stored in a glass bottle with a Teflon-lined cap. A 2% (w/v) Sn(II) chloride solution in 10% HCl was made by weighing 20 g of $SnCl_2 \cdot 2H_2O$ into a tared glass beaker, adding 60–80 ml of 100 ml concentrated HCl to dissolve the $SnCl_2$, transferring the solution to a 1-l volumetric flask, and then adding the remaining HCl to the volumetric. The beaker was rinsed several times with DI water, the rinses were added to the volumetric, and the volumetric brought to volume with DI water. The $SnCl_2$ solution was stored in a glass bottle with a Teflon-lined cap.

To prepare a 12% (w/v) hydroxyl amine HCl solution, 12 g of $NH_2OH \cdot HCl$ was weighed into a 100-ml volumetric flask, and DI water was added to fill the flask partially. The solids were dissolved by swirling the solution, and then the flask was brought to volume. The hydroxyl amine solution was placed in a glass bottle with a Teflon-lined cap.

The bromide/bromate solution was prepared by weighing separate aliquots of KBr (5.95 g) and $KBrO_3$ (1.39 g) into small glass beakers. The beakers were covered with watch glasses and placed in a muffle oven at 250°C for approximately 8 h. After cooling in a desiccator, the solids were combined into a single 500-ml volumetric flask. The beakers were rinsed with DI water and rinses transferred to the volumetric flask, which then was brought to volume. The bromide/bromate solution was transferred to a glass bottle with a Teflon-lined cap.

The extracting solution consisted of 30% sulfuric in nitric acid. This solution was made by slowly adding 60 ml sulfuric acid to 140 ml nitric acid with the nitric acid cooled in an ice bath in a Class 100 exhaust hood. Since this mixture generates considerable heat and caustic fumes, caution was required.

Sample Preparation A 3-cm segment of hair nearest the scalp end was analyzed, representing approximately 3 months of integrated exposure to Hg. For lengths greater than 3 cm, the segment nearest the scalp was determined under a dissecting microscope and cut. For lengths less than 3 cm the entire length was analyzed. The segment was transferred to a clean glass vial for storage at −20°C. The vial was sealed with an airtight, Teflon-faced silicone septum screw cap. At the time of analysis, a 5-mg hair sample was transferred to a labeled, preweighed polyvial with a plastic cap, and 0.5 ml of acetone was added to dissolve any Hg present on the surface of the hair strands. After standing for 5 min, the acetone was decanted from the vial, and the hair was dried for approximately 3 h under HEPA filtered air. The vial was reweighed to determine the precise amount of hair sample. The hair was transferred by tapping into a 20-ml glass scintillation vial fitted with an airtight plastic screw cap lined with a Teflon-faced silicone septum. The polyvial was rinsed with 1 ml of reagent water to transfer any remaining hair segments to the scintillation vial.

One milliliter of 30% sulfuric in nitric acid was added to the hair sample. The vial was sealed with an airtight cap and placed in an oven at 90°C for 6–8 h to digest the hair. After cooling, 2 ml of DI water was added to the vial and mixed. Two milliliters of this solution was transferred to a 25-ml glass volumetric flask, and 1 ml each of concentrated HCl and bromide/bromate solution was added to the flask, capped, and allowed to sit overnight in a Class 100 hood at room temperature to convert all ionic forms of Hg to Hg(II). Hydroxyl amine hydrochloride solution (90–150 μl) was then added to oxidize the excess bromide/bromate and decolorize the sample. The solution was then brought to volume with DI water. The sample was analyzed using a cold vapor atomic fluorescence spectrometer (CVAFS) on the same day that the hydroxyl amine hydrochloride solution was added. The remaining 2 ml of the original solution was transferred to a clean, 15-ml low-density polyethylene bottle, sealed with Teflon tape and stored at 4°C.

Reagent blanks consisted of acid extraction solvent carried through the sample preparation procedure along with each batch of samples analyzed. Analysis of blanks provided information on contamination that resulted from reagents used in the sample extraction and preparation steps.

Method controls consisted of acid extraction solvent spiked with a certified Hg reference solution (National Institute of Standards and Technology, NIST, traceable) at levels in the calibration range and treated the same as reagent blanks. Analysis of these provided information on recoveries of the target analyte from sample extraction and preparation steps.

Analytical Method Performance

The extraction method was validated prior to any sample analysis. Six matrix standards (9.9, 19.5, 28.9, 47.1, 64.4, and 72.7 pg/ml Hg) were prepared by spiking 5-mg aliquots of composite hair sample with mercury reference standards, with triplicate preparations of both the lowest and highest concentrations and a single preparation of concentrations at the middle levels. Three unspiked hair samples were also prepared. These samples were carried through the extraction procedure and analyzed along with eight aqueous Hg calibration standards (5, 10, 20, 30, 50, 70, 80, and 95 pg/ml

Hg) and a calibration blank. Linearity, accuracy, and recovery were assessed from the analytical results. A Canadian-certified reference material (dogfish liver tissue, DOLT-2) was also processed and analyzed to demonstrate method performance. A standard reference hair material (NIES-13) certified for Hg levels became available for use as a performance evaluation sample during hair-sample analysis.

The CVAFS was calibrated with aqueous solvent standards, and the matrix standards were analyzed as samples. Two quality control (QC) check samples (a calibration blank and a calibration standard at 50 pg/ml Hg) were analyzed immediately after the calibration, after every 10 samples, and at the end of the analysis.

The fluorescence intensity was measured for each calibration standard, and a least-square linear regression calibration curve was constructed as follows:

$$y = a + bx$$

where, y=net fluorescence intensity; a=y intercept; b=slope; x=analyte concentration (pg/ml).

Method detection limit (MDL) was defined as three times the standard deviation of reagent blanks:

$$\mathrm{MDL} = 3 \times \mathrm{SD_{bl}}$$

where MDL=the method detection limit in pg/ml or ppt; $\mathrm{SD_{bl}}$=standard deviation of reagent blanks in pg/ml or ppt.

Instrumentation and Calibration

Instrumentation A PS Analytical Merlin Plus atomic fluorescence spectrometer was used that consisted of a random access autosampler (PSA 20.100), automated continuous vapor generator (PSA 10.003) with a gas–liquid separator for Hg and a Perma Pure dryer tube, a Merlin Hg-specific fluorescence detector (PSA 10.023), and a dedicated personal computer.

The formation of Hg (0) vapor was carried out by the addition of a reducing agent—tin(II) chloride ($SnCl_2$)—to an acidified solution containing the Hg(II). The formed Hg(0) was passed directly to a Hg-specific fluorescence detector in a stream of argon gas. The free Hg atoms were excited by an intense excitation source (Hg lamp), and the resultant fluorescence was measured using a 254-nm interference filter and a photomultiplier tube detection system.

Two instrument performance checks were conducted prior to sample analyses. A lamp check was done before starting the liquid or argon flow. A direct reading of the signal intensity from the lamp was measured, and a value between 750 and 1300 was considered acceptable. The system sensitivity was also checked using a 100-ppt Hg standard under typical running conditions. The sensitivity was displayed in a QC chart with a signal of 145 counts±3 SD considered acceptable for sample analysis to proceed (Figure 1).

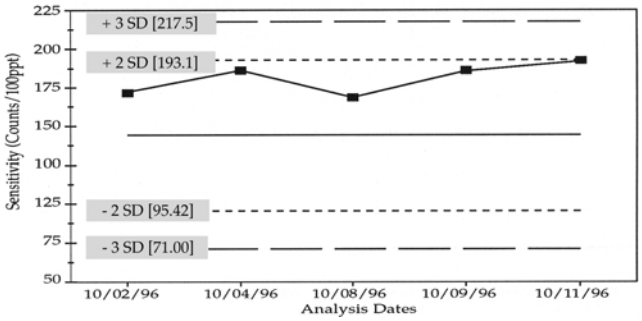

Figure 1. Instrument performance control chart for Hg.

Calibration Instrument performance, primarily sensitivity, was assessed prior to the analysis of any samples as described above. Once the acceptance criteria were met, an initial calibration was performed. At the beginning of each day of analysis, a new calibration curve was constructed. Calibration blank and calibration standards were analyzed for Hg starting from the lowest concentration to the highest.

The fluorescence intensity was measured for each calibration standard, and a least-square linear regression calibration curve was constructed. The linearity of the calibration curve was verified to be greater than 0.99 for acceptability. The concentration of the analyte in the calibration standards was calculated using the measured signal and the calibration curve was ±20% of the nominal concentration for the lowest calibration standard and ±10% for all other calibration standards.

The performance of the initial calibration was verified prior to any sample analysis by comparing the results of the previous QC check with the current QC check. A difference of 10% or less between the two values was deemed acceptable. Daily performance test results for sensitivity as peak height/pg/ml were plotted against time (in days).

For sample analysis, the CVAFS was calibrated with aqueous solvent standards and the matrix standards (0, 10, 30, 50, and 80 pg/ml Hg) were analyzed as samples. Matrix standards were used to calculate the matrix correction factor. Two check standards, a calibration blank, and a calibration standard of 50 pg/ml Hg, were analyzed immediately after the calibration.

Analysis of Sample Extracts

Prior to sample analysis, a set of calibration standards were analyzed and the calibration curve constructed. The

Table 1. Comparison of demographics among overall Region V sample, and subset of participants providing hair.

Demographics	Census percent	Overall sample		Sample providing hair	
		Sample size (total=326)	Percent (S.E.)[a]	Sample size (total=182)[a]	Percent (S.E.)
Gender					
Male	49	131	46.54(4.21)	81	49.95(4.85)
Female	51	195	53.46(4.21)	101	50.05(4.85)
Minority					
Yes	14.0	53	16.46(4.70)	15	9.58(3.34)
No	86.0	272	83.38(4.72)	167	90.42(3.34)
Missing[b]	–	1	0.16(0.17)	0	0
Age (yr)					
0–14	18.0	55	21.04(3.03)	29	22.19(4.21)
15–21	9.0	13	7.42(2.14)	7	6.75(2.66)
>21	72.0	258	71.54(3.71)	146	71.06(4.86)
Income ($)					
<20K	31	47	18.28(2.77)	25	15.95(3.59)
20–49.9K	44	130	40.03(3.58)	74	38.51(3.71)
50–99.9K	20	59	15.71(2.16)	34	17.69(3.38)
>100 or more	4	7	1.19(0.48)	3	1.52(1.11)
Missing	1	83	24.79(4.78)	46	26.33(5.40)

[a]SE=standard error.
[b]Questionnaire data missing, or not provided.

calibration standard at 50 pg/ml Hg also subsequently served as the QC check standard.

Sample preparation and analysis were carried out in batches consisting of 25–30 samples, 4–5 matrix standards (0, 10, 30, 50, and 80 pg/ml Hg), triplicate reagent blanks, triplicate performance evaluation (PE) samples, and one method control. The order of analysis was as follows: calibration blank, QC check standard, reagent blank, method control, PE sample (National Institute of Environmental Studies, Japan, NIES-13), matrix standards, and samples. A subset of samples (about 12%) was prepared and analyzed in duplicate. A subset of sample extracts also was analyzed in duplicate. These replicate subsets provided information about the precision of the method and the instrumental measurement step.

The QC check standard was analyzed after every 10th sample and at the end of the analysis set. All QC check standard results had to meet an acceptance criteria of ±10% of the nominal or the initial concentration to be valid. The reagent blank results were used to calculate the MDL for each batch of samples analyzed. A PE sample (reference hair sample certified at 4.42±0.20 μg/g for Hg, according to NIES-13), was prepared in triplicate with each batch of samples and analyzed. The method control and the PE sample were used to monitor the recovery of Hg, and biases

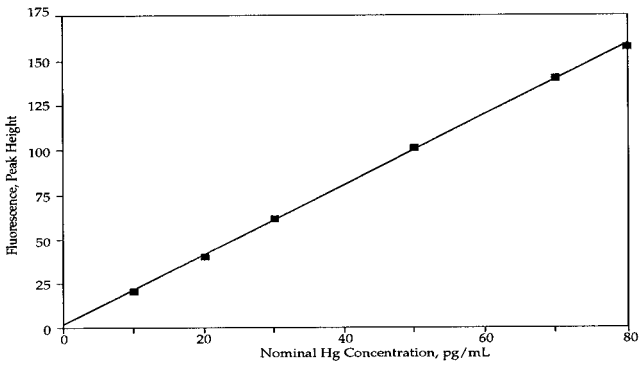

Figure 2. Method performance evaluation curve for Hg in solvent. Linear regression: $y = 1.9659x + 1.3872$. Correlation coefficient (r): 0.9997.

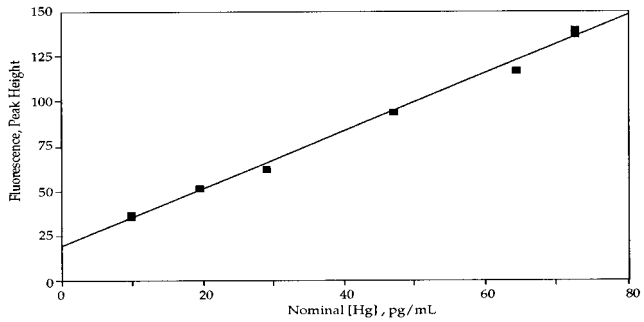

Figure 3. Method performance evaluation curve for Hg in human hair matrix. Linear regression: $y = 1.5951x + 19.433$. Correlation coefficient (r): 0.9983.

Table 2. Method detection limit and method quantification limit for Hg in human hair.

Analysis time (week no.)	*N*	MDL (ppb)	MQL (ppb)
1	3	12	39
2	3	11	36
3	3	11	38
4	3	22	72
4	3	15	49
13	3	9	31
14	3	4	12
22	3	11	38
34	3	15	50
	Average	12	41
	Standard Deviation	5	15

greater than ±10% of the certified value required a reanalysis of the extract. Matrix standards were used to calculate the matrix correction factor.

Population Weighting Adjustments

Sampling weights applicable to the population during the data-collection period were developed using weighting class nonresponse adjustments. These weighting procedures are described in (Clayton et al., 1998). Weights applied to the Hg concentration data allowed inferences to be made to the population during the period of data collection. Population estimates of means, medians, and other percentiles were generated using weighted data analysis techniques. Confidence intervals for such population parameters were generated using SUDAAN software, to properly account for the sampling design (SUDAAN, 1989). Sampling weights applicable to the population both during the data collection period and on annualized basis were estimated. The initial weights for the sample and subsamples were adjusted for nonresponse using weighting-class adjustment procedures; longitudinal models for propensity to respond; and exponential, generalized ranking models (Folsom, 1991; Kalton and Maligalig, 1991). Seasons were used to form weighting classes, forcing each to be equally represented, and these weights were used to produce annualized estimates.

Table 3. Percent recovery for Hg in quality control samples.

	N	Ave+SD	Median	Min	Max
Method control[a]	27	103±6	103	91	113
DOLT-2 certified standard[b]	12	105±4	104	101	110
NIES-13 certified standard[c]	21	100±3	100	97	106

[a]Certified standard (50 ppm) analyzed in nine batches over an 8-month period.
[b]Certified dogfish liver standard (1.99 ppm) analyzed in four batches over an 8-month period.
[c]Certified human hair standard (4.41 ppm) analyzed in five batches over a 7-month period.

Table 4. RSDs for duplicate extract and sample analysis.

	N	Mean±SD	Min	Max
Duplicate extract analysis	18	4.6±2.8	0.6	10
Duplicate sample analysis	17	12.5±7.4	0	14.8

Results and discussion

Demographics of Study Population

A comparison of demographics among the Census, the overall study participants providing questionnaire information for EPA Region V, and the participants providing hair samples for mercury analysis is given in Table 1. The demographic (gender, age, etc.) distributions for participants providing hair samples were in good agreement with the census and the overall study population. Some divergence between the groups was observed for minorities. The minority category includes white Hispanic, African-American, and miscellaneous 'other.' The percentages for all three minority groups were underrepresented relative to the nonminority group (white non-Hispanic). Because the number of individuals in each minority group is small, one or two nonrespondents substantially changes the percent representation, and inferences cannot be made accurately on these data. In general, the demographics indicate that participants providing hair samples were representative of the general population of EPA Region V.

Analytical Performance

Method Performance Method performance was evaluated for total Hg measurement in human hair. Plots of response

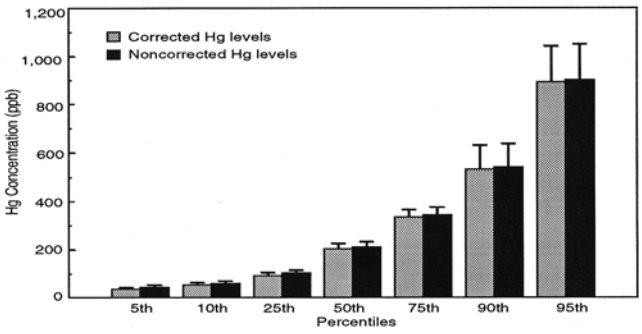

Figure 4. Comparison of method background corrected and non-corrected distributions for Hg in hair (annualized weighted data).

Table 5. Nonannualized and annualized weighted distribution of Hg levels (ppb) in hair for Region V population.

Statistic	Level (S.E.)[a]	
	Nonannualized	Annualized
Mean	304(23)	287(24)
5th percentile	46(10)	36(7)
10th percentile	66(8)	54(9)
25th percentile	106(12)	93(12)
50th percentile	214(19)	204(23)
75th percentile	359(26)	335(30)
90th percentile	564(100)	531(100)
95th percentile	980(149)	893(150)
Min	12	14
Max	3505	3505
N=182		

[a]S.E.=standard error.

versus concentration of Hg were constructed for both the aqueous standards (Figure 2) and the hair matrix standards (Figure 3). The correlation coefficient r was 0.9997 and 0.9983 for the aqueous and matrix regressions, respectively; thus, linearity was confirmed for the matrix curve. Precision, which was estimated by calculating the percent relative standard deviation (% RSD) from triplicate standards, was $\leq$1.60%. The bias between actual and found amounts for the matrix spike was $\leq$8%.

Mercury spikes in the hair matrix were recovered at 69.9% to 81.8% with an average recovery of 75.7% for concentrations ranging from 9.9 to 72.7 pg/ml of Hg. Two method controls were prepared in duplicate by spiking reagent blanks with Hg reference standards at 9.9 and 72.7 pg/ml of Hg. The Hg spikes in method controls were recovered at 75.3% to 84.4% with an average of 80.3%. Triplicate analysis of Canadian-certified reference material (DOLT-2) by this method resulted in 1.95±0.03 μg/g of Hg, which is 98.2% of the certified value (1.99±0.10 μg/g).

Based on these results, acceptable method performance was demonstrated. Analysis of samples was then conducted using the described method.

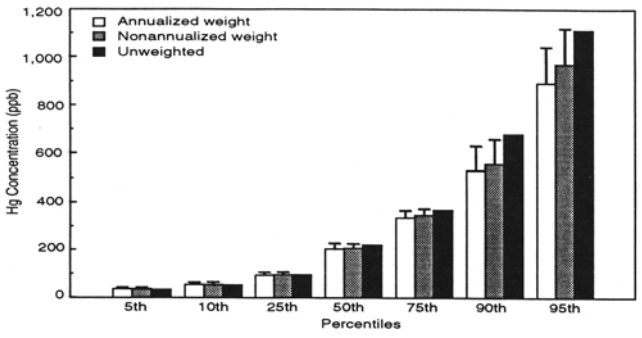

Figure 5. Comparison of weighted (annualized and nonannualized) and unweighted distributions for Hg in hair.

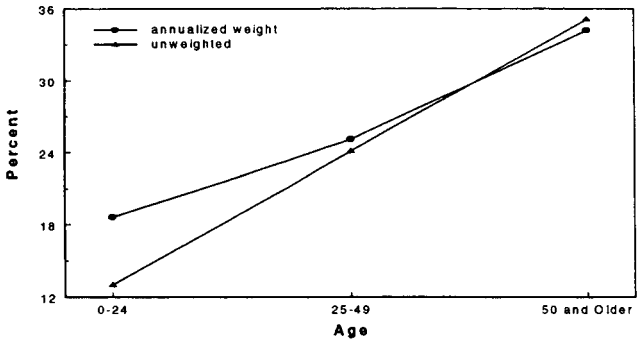

Figure 6. Comparison of the percent of persons in each age group exceeding Hg hair level 75th percentile.

Quality Control Results The analysis of hair for Hg occurred over a 9-month period, and the performance of the method was comparatively assessed within and between each analysis batch. Table 2 lists the method detection limit and method quantification limit achieved during the course of the study. The MDL range was 4 to 22 ppb with an average of 12 ppb. This low MDL resulted in only nine samples out of 182 with nondetectable levels of Hg. Thus, the use of as little as 5 mg hair sample was sufficient to measure Hg.

The percent recovery for Hg in QC samples was determined in each analysis batch in triplicate. The results for the QC samples from the nine batches are given in Table 3. The average percent recovery of Hg for the method control and DOLT-2 and NIES-13 certified standards were 103%, 105% and 100%, respectively. All of the results were within the ±10% acceptability limits, indicating a low measurement bias.

The precision of analysis was assessed from duplicate extracts (i.e., a re-analysis of the same extract at a later time) and from duplicate hair samples (i.e., a second aliquot of hair processed through the entire procedure) analyses. These results are given in Table 4. The mean precision for duplicate extract and sample analyses was 4.6% and 12.5%, respectively. These results were within the acceptable limits of ±10% and ±15% for duplicate extract and sample analysis, respectively.

Hg Levels in Hair

The extent that background correction may affect the distribution of Hg was examined. A plot of annualized

Table 6. Percents exceeding the Hg hair level 75th percentile.

	0–24 years	25–49 years	50 years and older
Annualized weight*	18.6	25.1	34.2
Unweighted**	13.0	24.1	35.1

*p=0.001.
**p=0.01.

Table 7. Comparison of mean Hg level (ppb) between genders.

	Males	Females
Annualized weight	260	315
Nonannualized weight	259	330
Unweighted	309	333

distributional data with and without Hg background correction is depicted in Figure 4. The Hg background resulting from the analytical method used had a minimal impact (e.g., at the 90th percentile, the difference was 1.47%). The potential bias affecting the percentile distribution was negligible. Thus, data analysis was performed with background corrected data.

The annualized and nonannualized weighted distributions of Hg levels in the Region V population are given in Table 5. The mean and median were 287 and 204 ppb, respectively, and the maximum value was 3505 ppb. Although these levels are considerably lower than the weighted mean level reported in U.S. hair samples (2900 ppb) in (Airey (1983)), they are comparable to mean and median levels (460 and 260 ppb, respectively) observed in EPA studies conducted in 1972 in Birmingham, Alabama, and Charlotte, North Carolina (U.S. EPA, 1978). They are also similar to the median levels of methylmercury (360 ppb) observed in a study of U.S. women of child-bearing age who ate fish at least once a month (Smith et al., 1997). In contrast, much higher levels are observed among populations that depend on fish as a principal component of their diet. For example, in a multiyear study in the Seychelles Islands, mean Hg levels among pregnant women were observed in the range of 5900 to 8200 ppb (Cernichiari et al., 1995b).

Weighted (annualized and nonannualized) and unweighted distributions for Hg levels in hair were compared (Figure 5). Throughout the percentile distribution, the deviation between unweighted and weighted data was small, indicating that the survey sampling procedures were effective and that minimal selection biases occurred between the census and the sample. The largest deviation occurred in the upper tail of the distribution and is attributed to the relatively small sample size, which yields larger confidence intervals (i.e., standard errors). The effects of weighting the data are evident in the upper tails of the distribution, where the annualized weight tends to sharpen the distribution for Hg levels in hair.

A comparison of increasing age intervals among the percent of individuals exceeding the Hg hair level 75th percentile is given in Figure 6 with the corresponding percents in Table 6. The 75th percentile was 335 ppb for the annualized distribution and 368 ppb for the unweighted distribution. It is interesting to note the nearly linear increase when moving from one age interval to the next. This linearity is not just an effect of income level, as no linear trend was found between income level and the number of individuals exceeding the 75th percentile. Further study is required to verify and account for this trend.

Previous studies have noted a decreased organic mercury concentration in the hair of individuals who use hair treatments containing thioglycolic acid, such as cold-waving solutions, hair relaxers, and some hair dyes; however, washing or bleaching hair has not been shown to alter Hg levels (Giovanoli-Jzkubczak et al., 1974; Yamamoto and Suzuki, 1978; Suzuki, 1988). A reduction of over 60% can occur for organic mercury with cold-wave treatment, while inorganic Hg levels are not altered (Yamamoto and Suzuki, 1978). This reduced Hg level typically is manifested in women's hair, since women are the primary users of these products. A comparison between the mean male and female Hg levels in hair in NHEXAS is presented in Table 7. Data from this study do not suggest an extensive use of these hair treatments among women in the population studied. In fact, males had mean levels about 20% lower than females for the weighted data and about 10% lower using the unweighted data.

In summary, a sensitive analytical procedure was developed and validated that permits characterization of Hg exposure using human hair. In a small general population study in EPA Region V the Hg levels in hair were low relative to levels observed in populations whose diet is principally fish.

The information captured in the NHEXAS permits further exploratory data analysis. For example, baseline questionnaire, food diary, and follow-up questionnaire data are available (Pellizzari et al., 1995) for examining potential associations between fish consumption and Hg levels in hair.

Acknowledgments

We thank Messrs. K. Thomas, L. Cauble, L. Klemm, M. Hermann, and J. Roberds for their expert collection of biological specimens from participants. We are also grateful to Dr. R. Whitmore, Mr. C.A. Clayton, and Ms. R. Perritt for conducting the statistical weighting of data. We are indebted to the many participants who provided samples of hair. This research was supported by the U.S. Food and Drug Administration and the National Oceanic and Atmospheric Administration (NOAA) under contract NO1-ES-55386 and EPA cooperative agreement CR 821902-01-0.

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