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Inter-laboratory validation of EPA method 3200 for mercury speciation analysis using prepared soil reference materials

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Determinations of the concentration of individual mercury species from environmental samples have increased significantly over the past decade. The techniques used for the determination of mercury species in soils or sediments generally involve a series of analytical steps (extraction, separation, detection) that may all be prone to systematic errors. An inter-laboratory validation study of the EPA draft method 3200 was conducted under the auspices of the United States Environmental Protection Agency on two specifically prepared soil matrices. The study was performed successfully by a limited number of participating laboratories. Evaluation of the data demonstrates that the method is more highly efficient for extracting the highly toxic methylmercury than inorganic mercury. The proposed method does not induce transformation of methylmercury to inorganic mercury. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: mercury speciation; mercury species; speciation; validation; inter-laboratory; methylmercury

INTRODUCTION

The interest in determining the concentration of an individual chemical species, as opposed to determining the total elemental concentration, has increased significantly in recent years. This is true especially where these species are known to be very toxic to humans and biota.[1] The toxicity, bioavailability and environmental mobility of mercury in soil, sediments and water are very dependent on its chemical species. Methylmercury in many matrices can be an order of magnitude more mobile (bioavailable) than the corresponding inorganic mercury species and, thus, more toxic and more readily bio-accumulated.[2] The toxic impact of methylmercury on man was observed for the first time in Minamata, Japan in 1955, when the ingestion of fish contaminated with methylmercury resulted in hundreds of poisonings and a thousand fatalities.[3,4] During the 1970s, the ingestion of wheat flour produced from seeds treated with organic mercury also led to large-scale poisoning and many deaths in Iraq.[4] Therefore, it is essential to be able to determine the exact concentration of inorganic mercury and methylmercury from environmental, biological and food samples.

It is observed that most mercury speciation methods available in the literature are either based on a chromatographic separation technique, or they obtain the amount of inorganic mercury from the difference of total mercury and methylmercury. Similarly, the amount of methylmercury can be obtained from the difference between total mercury and inorganic mercury. These are often analytically unreliable methods of speciation. Also, there is a chance of obtaining biased results, positively or negatively, from this kind of analysis. Such results do not provide any information about the source of methylmercury or inorganic mercury, that is, whether a reported amount of methylmercury or inorganic mercury is actually present in the analyzed sample, or merely a result

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of species transformation or interconversion. Although there are a few published papers available in the literature based on simultaneous extraction and separation for mercury speciation analysis, it is a growing concern to develop new speciation methods that are capable of maximizing extraction efficiency for various mercury species with minimum species transformations.

In order to resolve the aforementioned problems in mercury speciation analysis, a new extraction procedure using acidic ethanol solution has been developed to extract alkyl mercury and soluble inorganic mercury from soil and sediment matrices.[5] The method was then submitted to the United States Environmental Protection Agency (US EPA) for acceptance as an official method for mercury speciation from soils and sediments (EPA draft method 3200).[6] As the developing laboratory of the draft method 3200, the extraction efficiency of the draft method was compared with some of the published literature on methylmercury and mercury speciation methods. A description of each method studied, along with the final results, is reported elsewhere.[7] Heretofore, the technique for determining the speciation of mercury in soil and sediment samples was a succession of analytical steps: extraction, separation and detection. The results obtained have been 'operationally defined' using a given procedure. Results are useful only if they correspond to well-defined and accepted procedures. In other words, the only means of achieving sound interpretation of, and a basis for, decisions is when results are comparable using the same method in a similar homogeneous matrix. The prerequisites for comparability are agreement of the procedure to be used, its testing and validation, and its possible implementation as a standard procedure. Therefore, an inter-laboratory validation study for evaluating the performance of the EPA draft method 3200 has been organized by the US EPA and Science Applications International Corporation (SAIC). It was recognized that, in order to arrive at sound conclusions on the analytical performance of EPA draft method 3200, there was a strong need to use a similar matrix in the study. Therefore, two different types of reference soils were prepared and distributed for analysis among the participating laboratories.

EXPERIMENTAL

Preparation of the reference soil

Environmental Resource Associates® (ERA) (Arvada, CO, USA) prepared one set of soil samples (labeled as lot no. 0313-01-01-3: mixed mercury) by spiking an equal mixture of HgO and CH_3HgCl in 100% processed topsoil. The soil sample was shipped to Duquesne University (DU) on 30 March 2001 for evaluation and concentration verification. In this identical soil matrix, total mercury content was determined using a direct mercury analyzer-80 (DMA-80) and the concentration was 100 μg kg^{-1}, which is very small and difficult to detect by most available instruments after extraction. Therefore, it was decided not to use this sample for the inter-laboratory validation study, but to prepare instead a new set of samples with higher concentrations.

ERA then prepared a new set of sample (lot no. 0501-01-09) by spiking higher amounts of the different mercury species as described in the previous paragraph. This was again shipped to DU for evaluation on 10 May 2001. While the analysis found that this sample indeed contained higher concentrations of the different mercury species, the measured value was 6.00 mg kg^{-1} (3.00 mg kg^{-1} as inorganic mercury and 3.00 mg kg^{-1} as methylmercury), which is 1000 times smaller than their purported values. ERA at that point rechecked the sample and revised their certificate on 23 May 2001. It was decided to use this sample for the inter-laboratory validation study. ERA was also asked to prepare another set of soil sample by adding 20–25% silica (as matrix) and higher mercury species concentrations. ERA then prepared two sets of soil samples and labeled them material 1 (100% processed topsoil) and material 2 (75% processed topsoil and 25% Ottawa sand), lot no. 0611-01-02. These soil samples were shipped to DU for evaluation on 11 July 2001 and analyzed. These soil samples (material 1 and material 2) were then distributed to three participating laboratories (including DU) on 9 August 2001 for inter-laboratory validation of the EPA draft method 3200. ERA also shipped the same two materials [but from a different lot (lot no. 0416-03-01)] to three other participating laboratories on 16 April 2003 for validation study (Table 1).

Participating laboratories

The following laboratories participated in the inter-laboratory validation studies: the Center for Microwave and Analytical Chemistry at Duquesne University (DU); PDC Laboratories, Inc. (PDC); APPL Inc. (APPL); Severn Trent Laboratories Inc. (STL); Brooks Rand LLC (BRLLC); and Environmental and Occupational Health Sciences Institute at Rutgers University (EOHSI/RU) (see the Acknowledgments).

RESULTS AND DISCUSSION

Stability test for prepared reference soil materials

The shelf-life and the species composition in the specifically prepared soil samples were evaluated at DU periodically using speciated isotope dilution mass spectrometry (SIDMS) protocol along with extraction by the EPA draft method 3200. The SIDMS analysis procedure is described in detail elsewhere.[7–13] The amount of inorganic mercury and methylmercury determined in material 1 was 2.68 ± 0.34 and 2.20 ± 0.29 μg g^{-1}, respectively, on 28 October 2002 and 2.85 ± 0.47 and 2.25 ± 0.10 μg g^{-1}, respectively, on 3 November 2003. The amount of inorganic mercury found to be converted to methylmercury in material 1 during extraction or analysis at the mentioned dates was 0 ± 3 and 3 ± 1%, respectively. The amount of methylmercury converted to inorganic mercury in material 1 during extraction or analysis for the same

Table 1. Summary of reference materials used in the validation study

Material	Sample name	Lot no.	Date shipped	Mercury species	Reported concentration
Test material (Later material 1)	Mixed mercury	0313-01-01.3	30 March 2001	HgO	49.8 μg kg^{-1}
				CH_3HgCl	50.0 μg kg^{-1}
	Mixed mercury	0501-01-09	10 May 2001	HgO	3000 mg kg^{-1}
				CH_3HgCl	3000 mg kg^{-1}
	Mixed mercury	0501-01-09	10 May 2001	HgO	3000 μg kg^{-1}[a]
				CH_3HgCl	3000 μg kg^{-1}[a]
	Mixed mercury	0611-01-02	11 July 2001	HgO	3000 μg kg^{-1}
				CH_3HgCl	3000 μg kg^{-1}
	Mixed mercury	0611-01-02	11 July 2001	HgO	4500 μg kg^{-1}
				CH_3HgCl	4500 μg kg^{-1}
Material 1	Mixed mercury	0416-03-01	16 April 2003	HgO	3000 μg kg^{-1}
				CH_3HgCl	3000 μg kg^{-1}
Material 2	Mixed mercury	0416-03-01	16 April 2003	HgO	4500 μg kg^{-1}
				CH_3HgCl	4500 μg kg^{-1}

[a] Made-to value was revised by ERA on 23 May 2001. Material 1, 100% processed topsoil, and material 2, a mixture of processed topsoil plus Ottawa sand in a ratio of 75 : 25, respectively.

period was 0 ± 9 and $0 \pm 5\%$, respectively. The analysis shows that the concentrations of both methylmercury and inorganic mercury in material 1 were stable and the amounts of their interconversion were statistically indistinguishable over time. In the case of material 2, only the SIDMS data for 2002 October 28 are available; this material was unavailable for testing on 3 November 2003. The amount of inorganic mercury and methylmercury determined in material 2 by the SIDMS method was 3.55 ± 0.61 and 2.79 ± 0.29 μg g^{-1}, respectively; the amount of inorganic mercury converted to methylmercury was $2 \pm 2\%$; and that for methylmercury to inorganic mercury was $6 \pm 5\%$. The concentration of methylmercury in materials 1 and in 2 determined by SIDMS (spiking occurring before extraction) was less than its reported value, probably due to the incomplete extraction or lack of proper equilibration between sample and spike species.

Statistical evaluations of the data obtained from participating laboratories

After obtaining the final data from the six participating laboratories, several observations were made. First, laboratories 1, 4, 5 and 6 reported three separate mercury concentration measurements in materials 1 and 2 for each of the following four categories: extractable inorganic mercury; extractable organic mercury; semi-mobile mercury; and non-mobile mercury. From these measurements, the total extractable mercury and total mercury were calculated in straightforward fashion, and considered as the additional two categories. Laboratory 2 reported four separate mercury concentrations for materials 1 and 2 in only one category (total extractable mercury), but no measurement for other categories. The remaining laboratory, laboratory 3, reported their data in percentage recovery (not in concentration) for both materials across all six categories. Laboratory 3 did not report individual mercury concentration measurements nor how many times each measurement was taken.

Table 2 summarizes the percentage recovery results of various mercury fractions in materials 1 and 2 calculated by the authors based on the reported mean concentration measurements. Margins of error given for each percentage recovery were calculated using a standard 95% confidence limit (CL) for an unknown mean. Without knowledge of individual measurements, no margins of error could be given for the percentage recoveries reported by laboratory 3. Note in Table 2 the additional percentage recovery results for laboratory 4. Laboratory 4 measured mercury concentrations with and without separation (all other laboratories measured with separation only). The first set of percentage recoveries (82.8 ± 4.8 for total extractable mercury, 22.8 ± 4.8 for semi-mobile mercury, and 0.5 for non-mobile mercury in material 1; 71.9 ± 6.9 for total extractable mercury; 18.1 ± 6.4 for semi-mobile mercury, and 0.3 for non-mobile mercury in material 2) for laboratory 4 represents measurements taken without separation. The second set of percentage recoveries (7.6 ± 6.2 for inorganic extractable mercury, 63.3 ± 14.3 for organic extractable mercury, 35.4 ± 7.6 for total extractable mercury, 22.8 ± 4.8 for semi-mobile mercury, and 0.5 for non-mobile mercury in material 1; 14.4 ± 11.6 for inorganic extractable mercury, 59.3 ± 6.4 for organic extractable mercury, 36.9 ± 4.5 for total extractable mercury, 18.1 ± 6.4 for semi-mobile mercury, and 0.3 for non-mobile mercury in material 2) was based on the mercury measurements taken with separation.

Table 3 displays percentage recoveries of total mercury for the five reporting laboratories (laboratory 2 did not report the results of non-extractable mercury in materials 1 and 2 that would permit calculation of percentage recovery of total

Table 2. Final results from different participating laboratories for the validation of EPA draft method 3200: percentage recovery with 95% confidence level

Laboratory	Material	Percentage recovery for extractable			Percentage recovery for semi-mobile mercury	Percentage recovery for non-mobile mercury
		Inorganic mercury	Organic mercury	Total mercury		
1	Material 1	28.8 ± 5.4	102.7 ± 14.5	65.8 ± 8.5	34.2 ± 11.2	1.0 ± 0.3
	Material 2	43.9 ± 5.9	85.4 ± 6.3	64.7 ± 4.3	33.7 ± 2.6	0.5 ± 0.04
2	Material 1	NR	NR	52.7 ± 16.3	NR	NR
	Material 2	NR	NR	34.8 ± 8.9	NR	NR
3	Material 1	30.0	73.4	51.7	45.7	2.6
	Material 2	29.8	56.8	43.3	51.9	4.9
4	Material 1	NR	NR	82.8 ± 4.8	22.8 ± 4.8	0.5
	Material 2	NR	NR	71.9 ± 6.9	18.1 ± 6.4	0.3
	Material 1	7.6 ± 6.2	63.3 ± 14.3	35.4 ± 7.6	22.8 ± 4.8	0.5
	Material 2	14.4 ± 11.6	59.3 ± 6.4	36.9 ± 4.5	18.1 ± 6.4	0.3
5	Material 1	4.0 ± 6.8	54.6 ± 1.6	29.3 ± 3.5	40.8 ± 4.3	0.6 ± 0.1
	Material 2	9.4 ± 7.0	47.5 ± 4.2	28.4 ± 4.1	30.8 ± 4.3	0.6 ± 0.1
6	Material 1	17.7 ± 9.0	94.7 ± 10.0	56.2 ± 6.7	33.0 ± 9.4	0.3 ± 0.2
	Material 2	12.9 ± 9.2	76.1 ± 6.9	44.5 ± 5.7	20.0 ± 4.2	0.2

NR, not reported.

Table 3. Total percentage recovery from inter-laboratory validation study

Laboratory	Lot no.	Material	Reported value ($\mu g\ g^{-1}$)	Total percentage recovery[a]
1	0611-01-02	Material 1	6.0	101.0 ± 14.1
		Material 2	9.0	98.9 ± 5.0
3	0611-01-02	Material 1	6.0	100
		Material 2	9.0	100.1
4	0416-03-01	Material 1	6.0	58.7 ± 8.9 (106.1 ± 6.8)
		Material 2	9.0	55.3 ± 7.8 (90.3 ± 9.4)
5	0416-03-01	Material 1	6.0	70.7 ± 5.5
		Material 2	9.0	59.9 ± 5.9
6	0416-03-01	Material 1	6.0	89.4 ± 11.6
		Material 2	9.0	64.7 ± 7.1

[a] Values in parentheses represent the total percentage recovery considering the total extractable results obtained from direct analysis. Uncertainties are expressed with 95% confidence level, $n = 3$.

mercury). Laboratories 1 and 3 obtained 100% total mercury for both materials after mass balance. Laboratory 6 obtained 100% recovery for material 1 and 65% recovery for material 2. Laboratory 5 did not obtain 100% recovery and lost 29 and 40% mercury for materials 1 and 2, respectively. For laboratory 4, if the reported value for the total extractable mercury is considered, then they obtained 100% recovery from both the studied materials. However, if the speciation value is used, then laboratory 4 obtained approximately 60% recovery for both materials and lost almost 40%. The reason behind these losses is probably the poor recovery of mercury during the sulfydrylated cotton fiber (SCF)-aided solid-phase separation steps.

Another observation made upon data examination is that the detection technique varied among the six laboratories (Table 4). Two, laboratories 1 and 6, used liquid chromatography coupled with inductively coupled plasma mass spectrometry (LC-ICP-MS), while four others, laboratories 2, 3, 4 and 5, used cold vapor atomic absorption spectrometry (CV-AAS). Close inspection of the data summarized in Tables 2 and 3 reveal higher percentage recoveries on average for laboratories using LC-ICP-MS technology. In the following subsections, data is analyzed in the categories of

Table 4. List of separation and detection techniques used by different participating laboratories

Laboratory	Separation technique	Detection technique
1	HPLC	ICP-MS
2	SPE-SCF	CV-AAS
3	SPE-SCF	CV-AAS
4	SPE-SCF	CV-AAS
5	SPE-SCF	CV-AAS
6	HPLC	ICP-MS

HPLC, high-performance liquid chromatography; SPE-SCF, solid-phase extraction with sulfydrylated cotton fiber; ICP-MS, inductively coupled plasma mass spectrometry; and CV-AAS, cold vapor atomic absorption spectrophotometry.

extractable organic mercury, extractable inorganic mercury, total extractable mercury and total mercury, to determine whether a significant difference exists between measurements from the two detection techniques. Direct analysis of semi-mobile and non-mobile mercury measurements are of lesser interest, due to their low solubility and toxicity. These categories were not considered explicitly in the data analyses. Other investigations of interest, including an examination of differences between labs sharing the same detection technique, are also included in the following subsections.

Extractable inorganic mercury

The measurements of extractable inorganic mercury were separated into two groups: those obtained from LC-ICP-MS and those obtained from CV-AAS. Six LC-ICP-MS measurements (three from laboratory 1 and three from laboratory 6) and six CV-AAS measurements (three from laboratory 4 and three from laboratory 5) for materials 1 and 2 were analyzed with two-sample *t*-tests. The comparison of material 1 concentrations yielded a *p*-value less than 0.0004. This indicates that the average LC-ICP-MS measurement is significantly higher than the CV-AAS material 1 measurement. Applying the same test to the material 2 data gave a *p*-value of 0.0331. This was further evidence (although not as strong as with material 1) suggesting a difference between detection techniques. Noticing that laboratory 6 consistently reported lower concentration measurements than laboratory 1, while laboratory 5 consistently reported lower concentration measurements than laboratory 4, a two-sample *t*-test comparing the lower LC-ICP-MS measurements (laboratory 6) with the higher CV-AAS measurements (laboratory 4) was conducted. The LC-ICP-MS technique was for material 1 again found to yield a significantly higher measurement (p-value $= 0.0102$) than CV-AAS. For material 2, however, the LC-ICP-MS technique did not yield significantly greater measurements than CV-AAS (p-value $= 0.6619$). Table 3 provides support for these results: the difference in percentage recovery between laboratories 6 and 4 is much greater for material 1 than for material 2.

It is worthwhile recognizing the measurement variation between laboratories using the same detection technique. Measurements from laboratories 1 and 6, although based on the same detection technique, suggest that the average laboratory 1 measurement was significantly higher than that of laboratory 6 (p-value $= 0.0085$ for material 1, 0.0003 for material 2). The analogous comparison between laboratories 4 and 5 was not significant (p-value $= 0.0850$ for material 1, 0.0990 for material 2). These results are a reflection of Table 2, where the percentage recovery differences between laboratories 1 and 6 are larger than those between laboratories 4 and 5. One explanation for any between-laboratory, within-detection technique variation could be the difference in the laboratory chemist's familiarity with EPA draft method 3200. Similar analyses for data from categories that follow show that results from laboratory 1 were not always significantly greater than those from laboratory 6, while results from laboratory 4 were sometimes significantly greater than those from laboratory 5. These analyses were omitted and any significant difference between laboratories 1 and 6, and between laboratories 4 and 5, attributed to the variability of laboratory chemists' experience with method 3200. The overall recovery data for extractable inorganic mercury from participating labs averaged $17.6 \pm 3.8\%$ for material 1 and $22.1 \pm 4.4\%$ for material 2.

Extractable organic mercury

The measurements of extractable organic mercury were separated into two groups according to detection technique (in an analogous fashion to the previous extractable inorganic mercury analysis). The test for a difference in mean material 1 concentrations was significant (p-value < 0.0001), as was the test for material 2 (p-value < 0.0001). These results are consistent with those from the inorganic analysis: LC-ICP-MS technique yields a higher average concentration measurement than CV-AAS technique. When comparing laboratories 6 and 4 (lowest average from LC-ICP-MS with highest average from CV-AAS), the analysis of measurements from both materials revealed significant results (p-values of 0.0012 and 0.0007 for materials 1 and 2, respectively). Extractable organic mercury data completely supported the superiority of LC-ICP-MS over CV-AAS. The overall recovery data for extractable organic mercury from participating laboratories averaged $77.7 \pm 5.7\%$ for material 1 and $65.0 \pm 3.3\%$ for material 2.

Total extractable mercury

Recall that laboratory 2 reported four total extractable mercury measurements for materials 1 and 2. These measurements were grouped with those from the two CV-AAS laboratories (laboratories 4 and 5), and the resulting sample of 10 measurements compared with the six measurements from LC-ICP-MS labs (laboratories 1 and 6). When examining material 1, the total extractable mercury measurements based on LC-ICP-MS showed a significantly higher average than measurements based on CV-AAS (p-value $= 0.0003$). This result was also obtained when comparing material 2 measurements (p-value $= 0.0021$). The overall recovery data for total extractable mercury from participating laboratories averaged $56.4 \pm 2.5\%$ for material 1 and $47.9 \pm 2.1\%$ for material 2.

Total mercury

Total mercury measurements (summarized in terms of percentage recovery in Table 4) were grouped according to detection-technique and analyzed for significant differences between group means. Since the laboratory 2 did not report total mercury, the sample size for both detection-technique groups was again six. A test of material 1 measurements revealed a significantly higher average measurement from LC-ICP-MS laboratories as compared with CV-AAS laboratories (p-value $= 0.0006$). The average LC-ICP-MS laboratory measurement for material 2 was also

Table 5. Mean and median recoveries for different mercury fractions across laboratories for each reference material

Mercury fraction	Minimum value (%)	Maximum value (%)	Mean (%)	Median (%)	95% CL[a]
EIM1	4.0	30.0	17.6	17.7	3.8
EIM2	9.4	43.9	22.1	14.4	4.4
EOM1	54.6	102.7	77.7	73.4	5.7
EOM2	47.5	85.4	65.0	59.3	3.0
ETM1	29.3	82.8	56.4	54.4	2.5
ETM2	28.4	71.9	47.9	43.9	2.1
TM1	70.7	106.1	93.4	100.0	5.0
TM2	59.9	100.1	82.8	90.3	3.5

[a] These uncertainty values (95% confidence level) are calculated for the pooled standard deviation for each species fraction reported by all participating laboratories. **EIM1**, extractable inorganic mercury in material 1; **EIM2**, extractable inorganic mercury in material 2; **EOM1**, extractable organic mercury in material 1; **EOM2**, extractable organic mercury in material 2; **ETM1**, extractable total mercury in material 1; **ETM2**, extractable total mercury in material 2; **TM1**, total mercury in material 1; **TM2**, total mercury in material 2

found to be greater than that for CV-AAS with a moderately significant *p*-value of 0.0480. The overall recovery data for total mercury from participating laboratories averaged $93.4 \pm 5.0\%$ for material 1 and $82.8 \pm 3.5\%$ for material 2.

The analysis demonstrates that data for the various mercury fractions reported by different laboratories are widely spread around their arithmetic mean and are not distributed normally. Also, the number of participating laboratories is very limited (only six). In this type of asymmetric data set, the presence of any extreme value (larger or smaller) will unduly influence the arithmetic mean. Therefore, it is more appropriate to use the median instead of the mean to find the most probable value and its confidence limits. Comparison of the calculated arithmetic mean and median, along with the 95% CL, is displayed in Table 5. It was found that, in most cases, the mean and median were statistically indistinguishable at their 95% CL.

CONCLUSIONS

From the statistical analyses it was observed that (1) measurements from laboratories using the LC-ICP-MS technique in most cases had a statistically significant higher mean mercury measurement as compared with laboratories using CV-AAS technology (for all categories); (2) the measurements from laboratory 1 were not always responsible for this significant difference. Comparing the 'worst' performing LC-ICP-MS laboratory with the 'best' performing CV-AAS laboratory yielded a significantly higher LC-ICP-MS mean in one or both of the materials for each category; never was the CV-AAS mean significantly higher than that from LC-ICP-MS; (3) the detection technique (LC-ICP-MS) seems to be the only reasonable explanation for the significant results in (1) and (2); and (4) mean mercury measurements between laboratories sharing a common detection technique were sometimes significantly different, and sometimes not, with no pattern to support the hypothesis of material (1 or 2) or category (extractable inorganic, etc.) causality. Any such significant differences were attributed to variation in laboratory chemists and equipment.

The EPA Method 3200 (for mercury speciation) has been validated in a limited validation study using six laboratories. It performed successfully and was applied with relative success by all the participating laboratories. Most of the laboratories do not routinely perform speciated measurements; this was reflected in the data. However, the laboratories without experience in this type of analysis and speciation protocol were able to perform method 3200 adequately to obtain meaningful data. These data are limited to speciation of mercury in two types of reference soil materials; these samples demonstrate that the method is practical and provides a meaningful speciation protocol for the various solubility and toxic forms of mercury. Evaluation of the reported data reveals that the EPA draft method 3200 is efficient for extracting highly toxic methylmercury species (a targeted environmentally significant species of concern) from soils. However, its extraction efficiency for inorganic mercury is not as high as it is for the methylmercury. It was found from a previous study that EPA draft method 3200 had the highest extraction efficiency for both inorganic and organometallic fractions in comparison with other methods that do not induce transformations of the mercury species.[7] The design of the method, including mass balance options, permits the inorganic mercury recovery in multiple ways, for example in later stages as non-extractable mercury, which is mainly the less mobile and less toxic inorganic mercury species.

In the summary compilations (such as in Table 5), the calculated mean and median were statistically indistinguishable at their 95% CL for most of the mercury fractions. In some cases, these two set of values did not overlap, which reflects two main factors: (1) detection method-related bimodal distributions; and (2) the small number of participating laboratories. The average precision and accuracy would probably increase with a commensurate decrease in uncertainty, if the number of participating laboratories was greater. Some of the laboratories obtained lower recoveries of inorganic and organic mercury, which might be due to the loss of these two species during SCF-SPE separation, or may be an indication of some other strategic analytical practices. Laboratories 1 and 6 achieved consistently better recoveries, which reflected their more efficient detection systems. In these two laboratories the use of chromatographic techniques coupled with inductively coupled plasma mass spectrometry for speciated detection appeared to be more efficient. In this case, the extract was directly analyzed by chromatographic and instrumental systems without the use of SCF-SPE separation. Therefore, there was little chance of losing mercury species and less chance of contamination. When laboratory 4 analyzed their

extractable mercury fraction directly with cold vapor atomic absorption spectrometry (CV-AAS), they achieved more than double the recovery obtained from the SCF-SPE separation. Here the detection system was also CV-AAS. Several of the laboratories used CV-AAS for detection followed by SCF-SPE separation.

The SCF-SPE is a method of choice when the concentration of any one of the species differs from the other species by several orders of magnitude, or if a laboratory does not have a chromatographic analysis system to use for speciation. In the latter situation, the mercury species should be separated based on the SCF-SPE technique. The advantage of this technique over the chromatographic technique is that it not only separates, but also pre-concentrates the species. The recovery results obtained from different laboratories based on the SCF-SPE speciation technique demonstrate greater losses and differences among one another due to the lack of experience in employing this technique. It is a recommendation of the EPA draft method 3200 that SCF-SPE cartridges should be prepared *in-situ* by the user, if not available commercially. The surface area of the cartridges made in different laboratories, or in the same laboratory by different analysts, can vary. Consequently, the efficiency of speciation would be different and loss of species due to their retention in the cartridges would also vary from laboratory to laboratory. Therefore, if possible, the SCF-SPE cartridges should be acquired commercially to increase the uniformity of the technique.

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