

Final Report for:

**Speciation of Mercury in Environmental Samples by Chromatography
Coupled with Mass Spectrometry**

Project Officer: Randy England

Submitted by: Brian T. Buckley PhD

Abstract:

The research described in this report was performed to develop methods for mercury speciation in environmental samples. Mercury speciation will be defined for this work as the differentiation and quantification of the relevant chemical species of mercury. Specifically methods were developed to quantify the dication, mono-methyl and mono-ethyl forms. Although the method seemed to be applicable to both the dimethyl form and the reduced inorganic form (Hg^0), they were not thought to be major contaminants in the ecosystems studied so further refinement of this capability was not pursued. The method was applied to water samples from various lakes in the state, soils, sediments and even fish tissue. Finally the method was used in conjunction with studies of the methylation process in anaerobic sediment microcosms where it was discovered that two different organisms could act symbiotically to methylate the dication form of mercury via an interspecies electron transfer mechanism.

Summary of Results:

The speciation technique was used to measure lake water concentration to roughly 20 ppt and soil concentration in the 1 ppb range. The fish tissue concentrations were measured by difference and it is expected that the technique can be used for the selective measurement of organo mercury species and applied to a variety of biological as well as vegetation matrices once the proper modifications are made to the extraction conditions. The use of mercury stable isotopes both as internal standards and as isotope tracers in a microcosm was seen as a significant enhancement to the method and it is expected that these methods will see continued and broadening application in the future. It was also discovered the supercritical fluid extraction technique was capable of methylating the dication form under some conditions. Although this was seen as a non-desirable effect of the extraction method, its utility as a potential cleanup of contaminated soils and sediments may still be explored.

A. Objectives:

The primary objectives accomplished by this research were:

- 1) To quantify Hg species to ng/ml level or lower.
- 2) To develop a specie intact extraction method for all of the species found in solution.
- 3) To use stable isotopes for internal standards in the extraction step to account for recoveries > 100%
- 4) To use stable isotopes and mercury speciation method to measure forward and reverse rates of the anaerobic mercury methylation process in soils and sediments
- 5) To demonstrate the mercury extraction and speciation method for lake water samples, soils, sediments and tissue samples.

B. Background:

The speciation of mercury has become increasingly more important as medical data has indicated toxicological differences between the organo-mercury and metal cation forms. In addition the relative amounts of the different species are not easily correlated to any particular event or source. In fact studies have shown that there is biologically mediated interconversion of the mercury and the methyl-mercury species in some matrices.^{1,2} Because mercury exists in the environment as both an inorganic and an organic contaminant, the speciation task becomes more difficult. Both species cannot be separated with either a strictly organic or inorganic chromatographic technique so a mixture of techniques is necessary for acquiring useful speciation information.

The most recent methods for the speciation of metals, including arsenic, tin, and mercury, are usually carried out with a chromatographic system (typically CE, IC or HPLC) coupled to an element specific detection system (typically ICP-AES or ICPMS).³⁻⁷ Mass spectrometry has proven to be improved by the use of an direct injection nebulizer, which also decreases the washout time of the mercury (a major problem in chromatographic studies).⁸ A review of this information has been critical in attempting to optimize the methods that were developed.

The speciation of mercury is also difficult because the ambient concentrations require preconcentration of some type before the species can be measured. Previous work has been accomplished with instrumental techniques such as gas chromatography to quantify the organic species or cold vapor atomic absorption to quantify the inorganic species with a separate extraction protocol. The single step extraction of the two mercury species without interconversion was actually more difficult than quantifying each of the individual species.

The extraction of metal species with supercritical fluids has not received much attention because of the limited solubility of metal cations in the more commonly used supercritical fluids (such as carbon dioxide). It has been shown through that organo-metallic, metal cations have been extracted from samples such as fish and soil by the addition of a complexing agent to the supercritical fluid.^{10,11} Ultimately it may be possible to separate the organic mercury species because of their differing solubilities.

The speciation of mercury was carried out in two separate steps. The first was the separation and quantification of both species using an Ion chromatography - Inductively Coupled Plasma Mass Spectrometric technique (IC-ICPMS). The specie intact extract was performed using both liquid solid extractions with a mixed mobile phase and supercritical fluid extraction (SFE). To obtain the necessary sensitivity for the environmental samples required the use of ultra-clean techniques (some of which were possible some of which were not) and preconcentration or mercury complexation methods. The recoveries from water samples were adjusted using stable isotope methods and measurement of both species at ambient concentrations was carried out.

C. Experimental:

I. Separation of mercury species

These are the recommended operational parameters for the system described, the optimization of these parameters will be discussed in the following stepwise procedure. The optimum operational parameters differ slightly from column to column because it was found that the columns used in the separation are not reproducibly manufactured.

Ion Chromatography System-Dionex DX-300 system

Replicate injections of the dication, monomethyl and monoethyl mercury under the optimized conditions can be seen in figure 1a. The monoethyl mercury was added to the separation analytes because it was believed that the monoethyl form could be used as an internal standard in many of the experiments.

Flow rate: 1 ml/min. (the recommended flow rate for the columns used)

Injection loop: 300 microliters, Lower detection limits can be achieved with a 1.3 ml injection loop but this causes greater tailing of the mercury dication peak. chromatograms with the two different sized injection peaks are shown in figure 1b. The larger injection loop can be used when complete separation is not required.

Separation Column: Omnipac PCX500 guard column (the Omnipac PCX500 “separation” column is too big and would result in very long elution times for the mercury species) Eluent: 70 % methanol and 1 N HCl or HNO₃. The acid concentration and methanol fraction can vary considerably from column to column because these guard columns are not tested for reproducibility like the analytical columns. Methanol fractions as low as 30% and acid concentrations as high as 1.2 M have also been used.

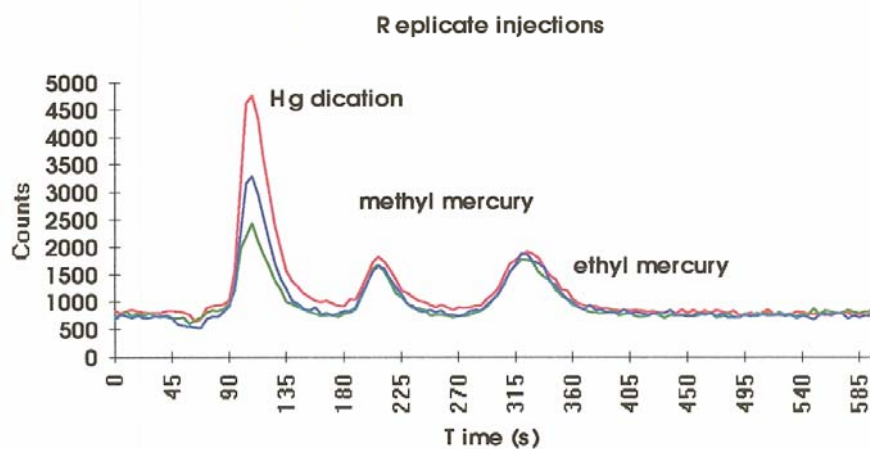


Fig. 1a.

Effect of Injection volume on analyte elution and retention times

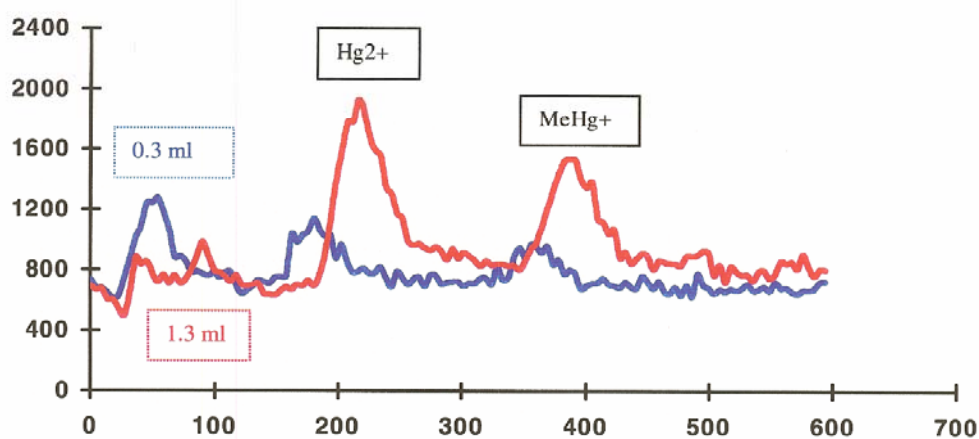


Fig.1b. Number of counts or signal intensity vs. time in seconds. Effect of injection loop volume on the separation of Hg^{2+} and CH_3Hg^{+} . The first peak is caused by the sample plug

Elution times:

The dependence of the species elution time on chromatographic conditions is listed in table 1. The use of this chromatographic system to remove organic impurities and the organic solvent from the sample material should allow for different solution matrices and possibly different extraction methods without major changes to the ICP/MS detection system.

Table 1 Chromatographic conditions

Conditions	Hg ²⁺ retention time (s)	HgCH ₃ ⁺ retention time (s)	Hg(CH ₃) ₂ retention time (s)	HgCH ₂ CH ₃ ⁺ retention time (s)
0-1 M HCl/HNO ₃ 10 min. gradient and 30 % Methanol	410	800	1350	2300 ¹
1M HCl/HNO ₃ and 30 % Methanol	100	695	1320	1900 ¹
1 M HCl and 50 % Methanol	100	350		780
1 M HCl/HNO ₃ and 50 % Methanol	100	350		760
1.3 M HCl and 50 % Methanol	100,210 ²	360		790
1 M HCl and 60 % Methanol	100,210 ²	320		580
1 M HCl/HNO ₃ and 70 % Methanol	110	185		290

1. Approximate numbers, peak appeared after ion monitoring ended

2. Results for two acquisitions, equilibrium with mobile phase may not have been obtained for second run

Dimethyl mercury:

Originally it was proposed that the mercury dication, methyl-mercury cation, phenyl-mercury cation, and dimethyl mercury could all be significant environmental contaminants. In order to test the chromatographic separation the mercury dication, methyl-mercury cation, ethyl-mercury cation, and dimethyl mercury have been studied, as these species would be the most difficult to separate (owing to there similar polarities). The dimethyl mercury proved to be unstable in even

slightly acidic water solutions. This is evident in fig 2, where dimethyl mercury (in methanol) is analyzed. The carrier solvent was a mixture of dilute acid and methanol (see table 1, in this report, for conditions). Methyl-mercury cation elutes under these conditions at approximately 800 s, and it can be inferred from this plot that during the analysis the dimethyl mercury converts into the monomethyl form. Consequently dimethyl mercury is probably not an important component in the water analysis because of the acidic nature of the lake water.

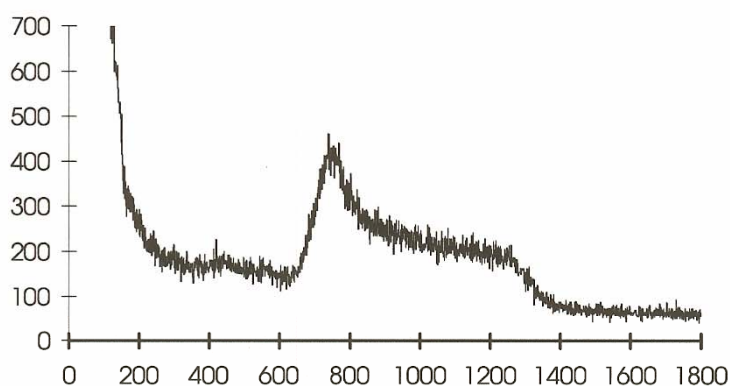


Fig 2. Chromatogram of dimethyl mercury

ICPMS Detection System-VG Plasmaquad

Delivered Power: 1600 W

Spray chamber temperature: -8 C or colder if possible The cooling removes organic vapor from the spray chamber which can be carried to the plasma and reduce its stability. Unfortunately, the addition of large amounts of organic solvent to the eluent causes a cooling of the ICP plasma, and resulted in a reduction in all around sensitivity. This fact is highlighted by the data contained in table 2, where the mercury signal dependence on methanol concentration is shown.

Table 2 Effect of Methanol on Mercury Signal

Methanol (v/v%)	Nebulizer Flow (L/min.)	Reflected Power (W)	Signal for 10 ppb Hg ²⁺ (raw counts/200 msec)
0 %	0.83	0	70,000
15 %	0.67	1-2	2,000
30 %	0.62	2-3	4,000
50 %	0.62	2-4	8,500
70 %	0.62	7-8	1,500

Data Collection: single ion monitoring of the 202 isotope, integration time is 4.5 seconds.
 For isotope ratio measurements the 200, 201, and 202 isotopes are all monitored using
 Fisons TR Vision software, the longest integration time allowed with the software is 0.5s.

Procedure:

Step 1: Mercury is readily adsorbed on the glassware and tubing of the ICPMS sample introduction components. In order to clean out the system and prevent excessive peak tailing the sample introduction system is "cleaned" by running a 0.05% solution of iodine monochloride in an acetic acid solution through the nebulizer. The mercury, which is already on the glassware, is replaced, probably by the iodine, and partially blocked from absorbing during the chromatographic run. The ICl is pumped through the ICPMS for approximately 5-10 minute. This cleaning should be repeated every hour or if the background goes above 100 cps. This cleaning procedure is not quite as important if higher levels of methanol (60-70%) are used in the eluent because of the high methanol vapor pressure. This cleaning does cause the signal levels to decrease slightly, probably by lowering the amount of mercury vapor.

step 2: The ICPMS parameters must be optimized for the eluent matrix. The system is optimized with a solution of 1 ppb mercury made with the same solution matrix as the chromatographic eluent. The coolant gas flow is typically 16l/min and the auxiliary flow is set to minimize reflected power. Greater methanol concentrations result in lower nebulizer flow rates, typically 0.75 ml/min for a 60 % methanol solution and about 0.1 ml/min, higher at 30 % methanol. The plasma position and ion optic settings also must be re-optimized, though typically the extraction and collection voltages are the only ion optic parameters that need to be optimized carefully.

step 3: The output of the IC is removed from the UV detector input and attached to the nebulizer tubing with a piece of Tygon tubing. The IC should be operated for 10-20 minutes to allow the column to stabilize.

step 4: Follow standard procedures for injection and analysis using the Dionex Ion Chromatograph. The optimization of the system has been discussed previously. There are a few special problems encountered when the IC instrument is attached to the ICPMS.

- a. The solution being analyzed should have a lower acid concentration and methanol fraction than your eluent. This leads to concentration of your analyte on the front of the column during the injection and consequently better peak shape. This is especially important with the larger injection loops.

- b. The initial plug of solvent may cause the impedance matching to change, make sure the reflected power is back to normal (by varying auxiliary gas flow) before the dication peak elutes.

step 5: The column should be stored with the eluent diluted ten fold with deionized water because of the high acid concentrations used. The system should be flushed with the diluted eluent for 5-10 minutes after the final sample is analyzed.

Two basic methods were being developed in this project, the extraction of mercury species from soil and sediment samples and the speciation of mercury. The speciation method has already been described the results are summarized in table 3.

Table 3: Detection Limits

Sample Period	Hg ²⁺ Signal and Noise	Hg ²⁺ Detection Limit	CH ₃ Hg ⁺ Signal	CH ₃ Hg ⁺ Detection Limit
1 s	S=305 N=6.1	60 ppt	S=105	170 ppt
3 s	S=875 (1 pt) N=8.2 S=5100 (7 pts)	28 ppt 13 ppt	S=300	82 ppt 42 ppt
9 s	S=2550 N=40	48 ppt	S=845	140 ppt
4.5 s & 1.3ml ¹	S=1230 N= 50 S=5530 (5 pts)	12 ppt 6.1 ppt	S=830 S=3928	18 ppt 8.5 ppt

1. 100 ppt solution/column 2

II. Speciated mercury extractions:

The specie intact extraction was expected to be accomplished using a supercritical fluid extraction protocol. After extensive trials with dynamic extraction protocols, the system was modified so that static extractions and a complexing agent can be added to the supercritical fluid. Both modifications were necessary for the extraction of the mercury dication. The principal problem with this method was of clogging of the instrument's restrictors with high concentrations of organic material from the sample matrix. The mercury dication was expected to be extracted with either a methanol/water cosolvent or with a complexing agent added to the cosolvent.

The extraction of mercury dication from a soil matrix using an organic chelate to increase its solubility in the supercritical fluid was carried out. The results are shown in figure 3a (masses 198-202). As is evident in this figure the mercury dication is relatively insoluble until the addition of the chelate. The mercury extracted from the soil is

approximately 1-2% of the total present in the sample (NIST Montana soil, 32 ppm Hg, total extracted Hg is 3.8 ug). It is also evident that chelation of other ions also occurs, in particular zinc seems to bond well. The major concern with this chelation extraction is that soil samples generally contain large amounts of other ions, which will chelate preferentially compared to the mercury. In particular, in the study of mercury interconversion between the dication and methylmercury, CuSO_4 is added to the soil to stop the methylation reaction. Fortunately it appears that the copper (354 ug total weight in sample, mass 63 and 65 in figure 3b) did not chelate as readily as mercury (approximately 90 counts/ug for Hg and 1 count/ug for Cu in this analysis).

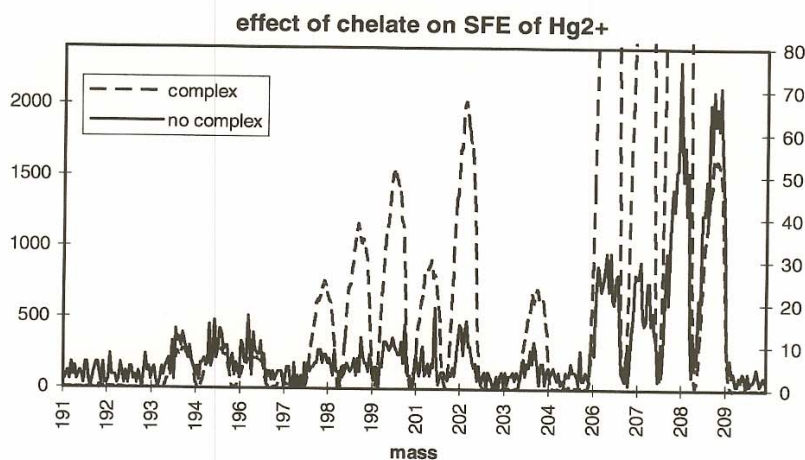
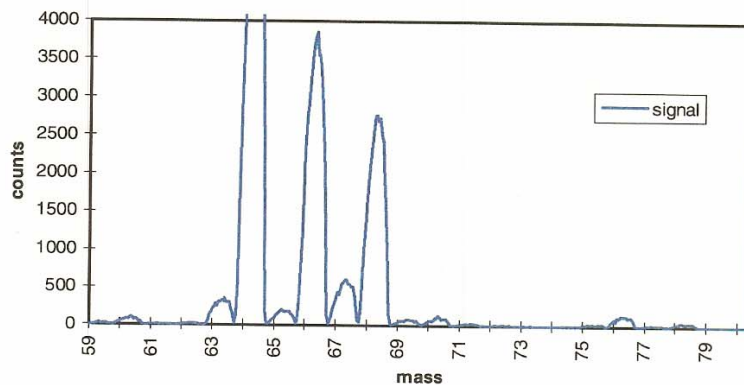


Fig. 3a

Fig. 3b



The quantitative extraction of mercury and methyl mercury from the soil samples without interconversion had proven to be very difficult. Quantitative extractions were performed, without regard to interconversion, to confirm extraction recoveries using the specie intact extraction techniques. For the study of the mercury species in soil two methods were used. Method one involved the quantitative (or reproducible) extraction of the soil using a concentrated acid. In the second method, the soil was doped with the isotopically labeled mercury dication and the methylmercury. Isotope ratio measurements of the extracted mercury species were carried out, and the deviation from the natural ratio was used to calculate the original concentrations of the two species. The second method was a backup to compensate for recoveries of less than 100%. The number of samples that can be analyzed with the second method would be limited by the amount of isotopically labeled material available. Because the sediment interconversion study (discussed in a subsequent section) had a sample taken at the zero time point these soils will be analyzed with this second method as a matter of course.

The supercritical extraction of the mercury species was carried out with sand doped with a single species (either methyl mercury or the mercury dication). With these experiments the relative solubility of each species was determined. The results of these experiments are shown below in table 4.

Table 4 Supercritical Extraction of Mercury Species

Species	conditions	amount extracted	percent extracted
Hg ²⁺	100% CO ₂	6.5 ng	6.5 %
Hg ²⁺	95% CO ₂ /5% methanol	5.2 ng	5.6 %
Hg ²⁺	75% CO ₂ /25% methanol	5.5 ng	6.3 %
Hg(CH ₃) ⁺	100% CO ₂	36 ng	36 %
Hg(CH ₃) ⁺	95% CO ₂ /5% methanol	33 ng	51 %
Hg(CH ₃) ⁺	75% CO ₂ /25% methanol	24 ng	77 %
blank	not applicable	4.7 ng *	not applicable

All extractions were run for 15 minutes at 75 °C and 250 atm

The samples contained 100 ng of a single species and each was first extracted with CO₂, then 5% methanol, then 25% methanol

* the amount which would correspond to the observed signal

This initial data indicates a great difference in solubility between the organo-mercury forms and the mercury dication with an apparent increase in solubility for the methyl mercury as the methanol concentration is increased.

III Analysis of Lake Water Samples:

Measurement of trace amounts of mercury in water was adapted to be used to measure mercury in lake water. The necessary first step for this part of the project, though, was sample preconcentration. Because the total mercury concentration is typically on the order of a single ppt (and the methyl mercury concentration is possibly two orders of magnitude lower) a minimum of a hundred fold preconcentration of the mercury species was necessary to estimate the relative amount of the two species.

Sample Collection: The sample bottles (1L) were prepared using a method presented by Nicholas Bloom in the journal Environmental Lab (March/April 1995, p. 20). The sample bottles are washed out with DI water, soaked in 4 N hydrochloric acid (trace metal grade, Fisher) for two days, and then stored in 0.5 N HCl until lake water

collection. After the samples are brought back to the lab they are acidified with 10 ml of concentrated hydrochloric acid (0.8-1% v/v). This allowed the samples to be stored for up to 6 months. Three sample preconcentration procedures that were considered: solid phase extraction, organic extraction, and cold room evaporation. The Harrisville lake samples were allowed to evaporate in a cold room along side a standard bottle of acidified DI water and 10 ppt of methylmercury and the mercury dication. The main concern with this method was the possible evaporation of the mercury species. Solid phase extraction of the mercury species with a c18 solid phase had proven unsuccessful without complexation, and an ion exchange solid phase extraction filter was also attempted. The organic extraction of the mercury species was not attempted because of the difficulty involved with achieving a 100-fold concentration.

Water Samples Collected to date (2 liters each)

Harrisville	11/6/95	Morksville	11/28/95
Wanque	11/28/95	Assunpink	11/28/95
Mountain Lake	11/30/95	Merrill Creek	11/30/95

Water Analysis

Goal: The analysis of lakewater samples is challenging because of the low concentration of mercury and methyl mercury in the system. It is estimated that quantity of methylmercury in the lakewater is approximately 0.1 ppt. Though detection limits on the order of a single ppt have been achieved with the IC/ICPMS system, quantitation of the mercury species should only be attempted at concentration of at least an order of magnitude greater than the detection limit. Therefore the methyl mercury in the sample needed to be concentrated by a factor of 100 in order to measure the methylmercury in the water samples. The limits of the IC/ICPMS system have been discussed in previous reports, therefore it was decided that in order to analyze the water samples a preconcentration step is necessary before the actual analysis. Several methods, including distillation and liquid extraction, have been proposed in other publications for the preconcentration of mercury and methylmercury in water. These methods suffer from the possibility of contamination and low concentration factors. Solid phase extraction is a possible

route for the mercury extraction, unfortunately mercury in water is usually bonded strongly to chlorine ions, and ion exchange resins rarely work. Concentration of methylmercury on sulfhydryl groups bonded to a glass fiber support has been used in the past to achieve large concentration factors and low detection limits for methylmercury. This extraction works so well because of the strong bond between mercury and these sulfur compounds. In this work a soluble sulfur containing organic complex of the mercury species was formed and the ions are extracted out of the water solution by trapping the complex on a C18 column. The mercury species were complexed with the diethyldithiocarbamic ion, a sulfur containing compound. This organic molecule is readily extracted with the C18 organic resin. The procedure for this work is outlined below.

Experimental: Instrumentation for this section is not complex, the diethyldithiocarbamic acid sodium salt and the ODS C18 SPE resin can both be purchased from Fisher Scientific.

Procedure

step 1: Hydrochloric acid is added to the lakewater after collection in order to stabilize it (final HCl concentration is 1 % vol./vol.) and the lakewater is refrigerated.

step 2: 550 ml of the lakewater is filtered through a 0.2 micron filter.

step 3: An internal standard is added to the lakewater (25 ppt $^{200}\text{Hg}^{2+}$ and 0.5 ppt $^{202}\text{CH}_3\text{Hg}^+$) The internal standard is added in this step in order to avoid loss of the internal standard in the filtration step. If the internal standard is added to the water before the filtration it may end up complexed to a larger molecule which may be lost in filtration. This would make the actual concentration of the free mercury appear too high. If the mercury was added before the filtration step, and allowed to equilibrate with the water we could generate a number that would be indicative of the suspended and free mercury concentration in the water.

step 4: 500 ml of the lakewater (50 ml is put aside) is neutralized with sodium hydroxide to a pH of 9. The pH can be buffered if a buffer without mercury contamination can be obtained (many pH buffers have been found to have significant mercury concentrations, possibly part of the fungicide). Before actual lakewater analysis a blank run should be made to determine the level of contamination in the procedure.

step 5: diethyldithiocarbamic acid sodium salt is added to the neutralized solution (0.16 g, 0.001 M final concentration), and stirred for an hour. A solution of diethyldithiocarbamic acid (DEDTC) can be made for convenience but this may increase the possibility of contamination because the DEDTC is most stable in a basic buffered solution. If ion content is too high in the water, recoveries may go down and greater amounts of DEDTC may be needed.

step 6: The resulting solution is filter through 0.3 g of a c18 resin, which bonds well with the DEDTC/hg complex. In addition other organic complexes should be bound as well. Overloading of the column may occur and greater amounts of resin should be used if the mercury yield decreases dramatically.

step 7: The C18 resin is allowed to dry, and then extracted with 3 ml of methanol. Two ml of 1 N HCl are added to the extractant. The acid portion is necessary in order to achieve good peak shape for the methylmercury during IC analysis. If the acid is not added clogging of the precolumn filter may also occur (due to breakdown of the DEDTC in the eluent stream).

step 8: (total mercury determination 1) The 50 ml of solution with the internal standard which was not preconcentrated was directly analyzed for total mercury content with the ICPMS instrument. The instrument was placed in peak jumping mode, and three isotopes, 200, 201, and 202 were analyzed. The signal acquired during this analysis could come from either mercury in the sample solution or mercury in the sample introduction system, so a background spectrum should be acquired first. This analysis is an initial check in case the mercury content is fairly high.

step 9: (total mercury determination 2) One ml of the 5 ml concentrated solution is removed and diluted 10 fold This solution is analyzed in the same manner as step 8. If the concentration of hg in the water is found to be much less in this solution then in the previous solution it can be assumed that the instrument background was the cause of the high reading in the previous sample. In addition because we now have two unknowns and two spectra it should be possible to determine the effect caused by the instrument. Again it is very important to run a preceding blank in order to test whether contamination is a problem.

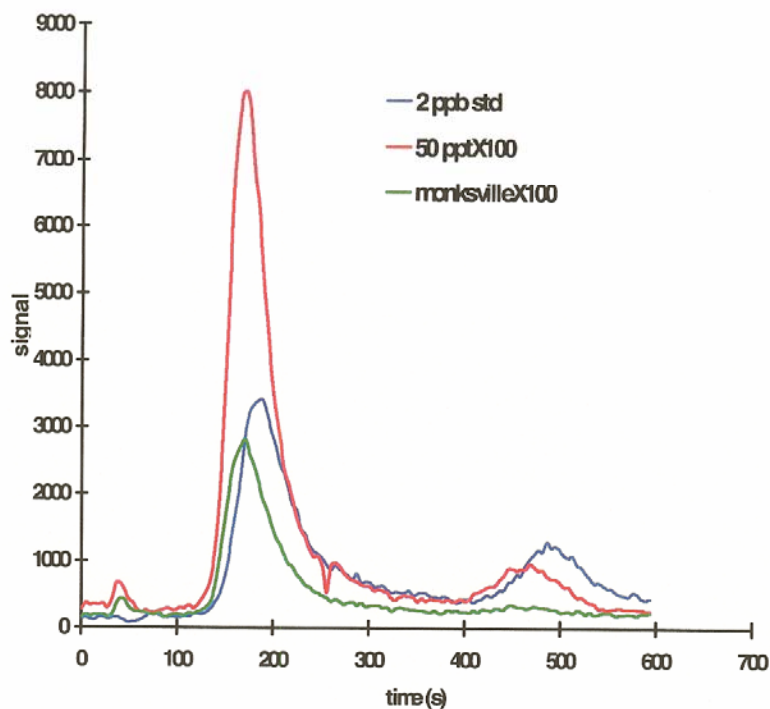
step 10: Speciation of the four ml of remaining concentrated water is then carried out (2 runs) using the procedure discussed in the section entitled procedure for hg speciation. The three

isotopes mentioned above, should all be measured. The dication and methylmercury concentrations can be calculated from the internal standard concentrations. Special conditions, which could be required, are as follows. If the methylmercury concentration is still very low in the solution a 1.3 ml injection loop can be used. This can lead to instability in the plasma as the high concentration of methanol goes through and the initial eluent flow could be diverted away from the plasma.

Results

Several standard solutions have been analyzed with this method. The extraction results for a 50 ppt standard of Hg^{2+} and CH_3Hg^+ in deionized water, a Monksville lake sample and a 2 ppb standard (not extracted) are shown in figure 4. From the spectra in figure 4 we can see that the dication is extracted by a factor of roughly 90 % from the standard while the methylmercury was extracted to a lesser extent (25-30%). There is no evidence of methylmercury in the Monksville samples. Harrisville and Assunpink samples have also been analyzed for total mercury and mercury dication concentration. No significant concentration of methylmercury has been measured in these samples either. The yield for the dication in the extraction step has dropped from 100 % in the standard samples to 30-40% in the lakewater samples. Greater DEDTC and C18 amounts were used to compensate for the interferences in the water samples. Greater methanol volumes have been used to increase the extraction of the methylmercury from the C18 column. The major problem with the analysis was the differentiation between the analyte signal and mercury contamination. Extraneous mercury can be added, inadvertently, during the sample workup, storage, and during the analysis (instrumental background). At present blank signal levels (Fisher optima grade water) have ranged from 10-50 ppt. These contamination levels are too high for the water samples that were analyzed.

Fig. 4



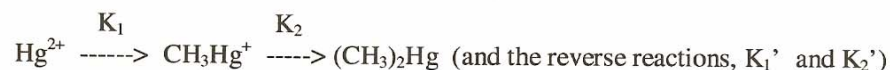
IV. Isotopic analysis and kinetics measurements of sediment interconversion

Mercury sediment interconversion studies: The mercury interconversion studies were carried out in the laboratory of Dr. Bartha. Dr. Bartha had successfully converted the mercury 202 oxide into the methyl mercury. The first study of soil collected was carried out on sediments collected from Harrisville lake. In this study the sediment was prepared in an aerobic chamber with each species of mercury labeled with a different stable isotope. The dication was labeled with ^{200}Hg and the MeHg was labeled with ^{202}Hg . With these labels and the speciation technique, the reversible interconversion (carried out in the anaerobic sediment columns) of the mercury could be observed as it changed from the Hg^{2+} to MeHg^+ and back again.

The microbes in the samples were killed by the addition of CuSO_4 , and the samples were frozen until they were extracted and analyzed. Because the samples were spiked with the stable isotopes, a reproducible extraction was not required. Extraction efficiencies on the order of 30%

for the methyl mercury, and roughly 1 % for the mercury dication were achieved with SFE extractions. Because the mercury dication is doped in at a much higher concentration, these extraction efficiencies were adequate although less than optimal.

Kinetics study goals: The mercury reaction being studied is shown below:



The goal of this part of the project was to measure the relative reaction rates shown above. Because of the ability of the ICPMS system to measure multiple masses precisely, isotopic dilution was deemed a good method for following the reactions.

Kinetic study extraction method: The kinetics of the interconversion of mercury and methyl mercury was studied. A sediment sample taken from Harrisville lake was doped with 1 ppm $^{200}\text{Hg}^{2+}$ and 0.1 ppm $^{202}\text{CH}_3\text{Hg}^+$. The biologically dependent interconversion was allowed to occur for several weeks, with samples collected each day. The interconversion was terminated in each of the individual samples by the addition of CuSO_4 . A blank (no isotopically labeled mercury added) was first analyzed to determine the concentration of the mercury in the original soil sample, this concentration can also be verified by measuring the 201 isotope abundance in the spiked soils. The isotope abundances at S0, which was the sample taken right after the isotope spikes were added, were analyzed in order to determine the validity of the method (the isotope ratio measurement method is described below). The result for the measurement of the samples is shown in figure 5a. It can be seen that the lower response of the speciation method for the methyl mercury and the lower extraction yield of the organo mercury result in the peak tailing of the dication interfering with the methyl mercury measurement. The mercury tail is subtracted from the methyl mercury signal using points along an exponential fit of the dication tail. To summarize the findings: the dication does not appear to be created from the labeled methyl mercury in the soil, it is obvious the mercury dication is readily converted to the methyl mercury. The amount converted should be measurable with the current method precision.

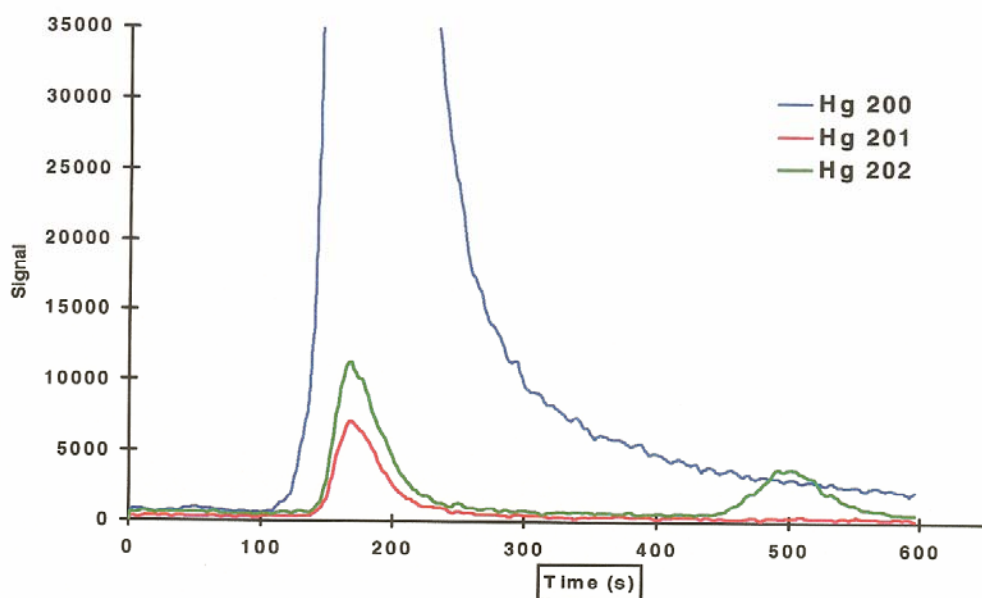


Fig. 5a The acid extraction and resulting speciation of S0, a spiked Harrisville sediment (1 ppm $^{200}\text{Hg}^{2+}$ and 0.1 ppm $^{202}\text{CH}_3\text{Hg}^+$).

There are two possible reaction outcomes that may occur, consequently, our analysis method will depend on the relative reaction rates. In the simplest scenario $K_1 \gg K_2$ or $K_2 \ll K_2'$ and the dimethyl mercury concentration is insignificant. Because this is a biologically mediated methylation the relative reaction rates are difficult to predict, and the isotope ratio of each methyl species must be measured. If there are significant amounts of dimethyl mercury in the sample then it must be determined. It is known, however, that dimethyl mercury is unstable at low pH values, and the molecule is rapidly converted back to the monomethyl form under these conditions. Therefore in lake systems with acidic pH values the dimethyl form can be ignored.

To determine whether there was a significant concentration of dimethyl mercury in the sample two samples of S14 (spiked soil, reacted 14 days) were extracted, one with 4N HCl and one with diethyldithiocarbamic acid. The extraction mechanisms are very different, with the HCl likely to break the dimethyl mercury down into the monomethyl form. Therefore if the methyl mercury isotope ratios are the same the concentration of the dimethyl is small enough to ignore. The monomethyl mercury isotope ratio (202/200) calculated for the HCl extraction of S14 is 2.0 ± 0.2 , and for the DEDTC extraction it is 1.6 ± 0.1 . The dimethyl mercury would be expected

to have a higher 202/200 ratio than the monomethyl form. Therefore, the higher ratio found in the HCl extraction may be caused by the breakdown and subsequent extraction of the dimethyl form as the monomethyl form. Other factors may also influence the isotope ratio: the acid extraction may be more prone to breaking humic acid/mercury bonds (only important if the isotope ratio for the “free methyl mercury” is different than the “bound” methylmercury), or the conversion of the dication (mass 200) to the methylmercury may be an ongoing process even when the sample is frozen (the DEDTC extraction took place after the acid extraction by several days). This second possibility was demonstrated by extracting the S14 sample simultaneously with both methods. The data from this experiment is presented in figure 5b.

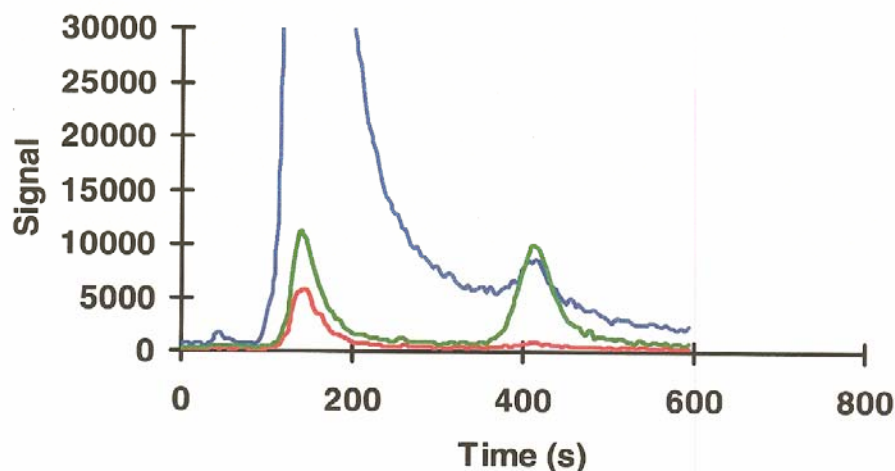


Fig 5b The same sediment sample 14 days later. The formation of methylmercury is evident.

Kinetics study-isotope ratio measurement: The isotope ratios of the mercury and methyl mercury species were measured during the chromatographic separation. This measurement was accomplished on the Fisons system by using the TR vision software package, which is offered with this system. During speciation three mercury isotopes are monitored (masses 202, 201, and 200). The 200 and 202 isotopes were used to follow the reaction while the 201 isotope was used to correct for natural mercury impurities in the sample solution.

The instrument must first be optimized for isotope ratio measurements. This optimization was similar to the procedure used for other isotope ratio methods when the signal is not a chromatographic transient. Before optimizing the data collection parameters gas flows, the

ion optics settings should be optimized in order to get the best signal precision (low noise/high signal) for a constant mercury signal. In addition, there are four data collection parameters which need to be optimized for good isotope ratio precision, including: number of points at each mass, how far apart these points are on the mass axis (measured in DAC steps), dwell time at each mass, and settle time between isotope changes. It has been found that the signal *rsd* depends strongly only on dwell time. The optimal parameters used in these studies: number of points at each mass equals three, number of DAC steps equals five, and the settle time was set at a minimum value of 1 ms. The dwell time of 0.5 s is used because it is the longest dwell time allowed by the software. Longer dwell times could result in better *rsd* values, but could also skew the ratio results because of the transient nature of the signal. Therefore it was decided to average the data points after the analysis is finished in order to increase signal precision.

In addition to isotope ratio precision, the accuracy of the method must also be verified. Corrections must be made if the instrument response has any mass bias or detector (dead time correction) bias. The detector bias is corrected first by measuring two natural mercury samples. Since the natural isotope ratios are very well known a mercury dication solution is made with two concentrations 5 ppb and 50 ppb. The speciation method is carried out with the system optimized to measure the three isotopes discussed in the preceding section. The 202/200 and 202/201 isotope ratios are calculated for the two chromatograms. If the ratios for the two samples are the same the dead time need not be corrected (do not worry that they are not equal to the natural isotope ratio, this will be corrected later). If the lower concentration solution has greater isotope ratios (outside the standard deviation of the measurement) the dead time should be set higher. The deadtime should be changed incrementally until the two isotope ratios match. There is nothing that can be done to the instrument to counteract the mass bias, which causes the measured ratio to differ from the natural isotope ratio. Therefore, a mass bias correction factor is determined from the natural isotope ratio measurement and multiplied by all subsequent isotope ratio measurements to obtain the "real" isotope ratio. A new correction factor should be determined each day before running the real samples.

Alkylation of Mercury

The alkylation of inorganic mercury was studied using isotopically enriched mercury and methyl mercury species. The reaction kinetics of the methylation was determined by measuring isotope ratios of methyl and inorganic mercury as they elute during speciation. In the initial experiments the instrumental parameters were optimized to achieve the most precise measurements. When the settings of the quadrupole mass analyzer were optimized it was found that by far the most

important criteria for optimizing the measurement precision was optimizing the mercury signal level (signal noise appeared to be relatively constant). Shown in table 5 is the dependence of signal and signal rsd on mercury concentration.

Table 5.

concentration	signal (peak height of Hg200 isotope)	Hg200:Hg202 ratio (Actual=0.778)	standard deviation (5 pts across the peak)
20 ppb mercury dication	2000 counts	0.747	0.04
200 ppb mercury dication	32,000 counts	0.771	0.007

The preceding data were collected without optimizing the chromatography, roughly 10-20 times greater signals can be acquired if the volume injected and the integration time are increased. Typically the mercury concentration in the test soil is 1-5 ppm, with the mercury diluted 20 fold during the extraction into the liquid phase (assuming 1 g soil extracted). Consequently, 1 % rsd or better were achieved for the isotope ratio precision. Method development demonstrated that background subtraction and detector mass calibration, must be carried out using the isotopically enhanced solutions.

Kinetics study-isotope ratio calculations: The isotope ratios are calculated in a spreadsheet by transporting the TR vision files in CSV format. As indicated above, three points are collected every 0.5 second. This leads to a separation in collection time between the 200 and 202 isotopes of only 0.33 s. The peaks are approximately 2 minutes wide, and the data treatment requires a minimum of fifteen points for each peak. Therefore ten consecutive points are added to achieve a total integration time of 5 second. The reason longer integration times are not used in the original data collection (besides the inadequacies of the software) is due to the transient nature of the signal. If each isotope measurement is taken too far apart temporally the isotope ratio will be skewed. By averaging the data post data collection the different isotopes are temporally separated at most by only 0.33 seconds.

Two background subtractions must be carried out in order to determine the real isotope ratio. The constant instrument background can be taken from the constant background measured before the mercury species elutes, this background isotope ratios should not be expected to correspond to natural abundances. The tailing of the dication peak may cause the background to

drift before the methylmercury elutes. Therefore, an exponential fit of the data should be done and the exponential data points subtracted from the methylmercury peak. The second background subtraction involves the 201 isotope, and is used to determine how much natural mercury is in the sample. The natural abundances of the 202 and 200 isotopes of mercury can be calculated from the mercury 201 abundance. This subtraction is typically very small, and can be used to estimate the natural mercury content in the sediment.

After background subtraction the isotope ratio is calculate for all of the points in the chromatogram. Six points are taken from the peak of each of the two species and an average and rsd values are calculated. Three samples are run for each sample, and the rsd value calculated is the overall speciation precision. Three extractions are carried out for each sample and the three different isotope ratios are averaged and the precision calculated again (this is a check because of the incomplete extraction of the heterogeneous sample).

V. Measurement of mercury in fish tissue:

The extraction of mercury from fish was accomplished using supercritical fluid extraction of the tissue. The tissue was initially mixed with sand, in a 50:50 weight mixture, to create a large surface extraction area. The mixture was then placed in the extraction cells and extracted with the methanol cosolvent for different extraction periods. The previous selectivity experiments suggested that the only mercury species that would be extracted was the organic form. The fish was analyzed after the extraction to determine the residual mercury concentration in the tissue and consequently the recovery. The trapped extract was also analyzed to determine the trapping efficiency. The results are reported in table 5.

Table 5. Extraction and trapping efficiency of mercury from fish tissue.

Extraction Time (min)	1 g Mako Digest	Extracted Mercury	Trapped Mercury
0	850 ng	NA	NA
15	650 ng	200 ng	9 ng
60	480 ng	370 ng	52 ng
15-mixed	515 ng	335 ng	45 ng
60-mixed	350 ng	500 ng	29 ng

Although the SFE technique appears to be an efficient means for extracting the mercury from the fish tissue and the selectivity for the organic form has already been demonstrated. The problem remains that the mercury is not trapped efficiently and therefore the utility of the method

can only be demonstrated with a difference measurement. It may find an application in studies of the methylation process due to the selectivity of the method but it is doubtful that the method will see general application.

D. Accomplishments:

Presentations:

Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, March 21, 1997: M. Heintz and B. Buckley, "Speciation of Mercury in Lakewater with an IC/ICPMS"

Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, March 7, 1996: M. Heintz and B. Buckley, "Supercritical Fluid Extraction of Inorganic and Organomercury Compounds"

Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, March 8, 1996: M. Heintz and B. Buckley, "Extraction and Speciation of Mercury Pollutants in and Around Freshwater Lakes"

Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, March 9, 1995: B. Buckley, W. Johnson, W. Fang and M. Heintz, "Speciation of Environmental Mercury and Chromium Samples Using IC"

Federation of Analytical Chemistry and Spectroscopy Societies, October 13, 1998: B. Buckley, W. Johnson, K. Cashman, K. Reuhl and M. Dey, "Can We Think Smarter Using Stable Isotope Labeling for Metal Uptake Studies in Brain Tissue?"

Federation of Analytical Chemistry and Spectroscopy Societies, October 4, 1996: M. Heintz, W. Johnson, W. Fang and B. Buckley, "Isotope Ratio Measurements of Labeled Mercury Species in Soil Using an ICPMS System"

Reports:

Four quarterly reports, in addition to this report, were submitted to the NJDEP, throughout the course of this study. This final report is seen as a summary of the data found in the quarterly reports with additional data included.

Papers:

B.T. Buckley, M.J. Heintz, W. Fang & Willie Johnson, "Determination of Isotope Ratios for Individual Mercury Species" ICP Information Newsletter, In Press

B.T. Buckley, M.J. Heintz, W. Fang & Willie Johnson "Mercury Speciation with an IC/ICPMS System: A Marriage of Convenience" ICP Information Newsletter, In Press

K.R Pak and R. Bartha, "Mercury Methylation by Interspecies Hydrogen and Acetate Transfer between Sulfidogens and Methanogens

Manuscript in Progress see appendix 2

Subsequent Studies:

Development of the current microwave extraction protocols:

The separation and quantification of mercury species from a solution was already seen as a significant methods development accomplishment because previously no such method existed. The inability to extract each the monomethyl and dication mercury from soil and sediment matrices in a single step without the risk of species interconversion was still a significant limitation. The initial work performed in this study enables the development of a successful specie intact extraction method where both of the mercury species could be preserved and extracted quantitatively in a single step. As predicted earlier, the use of a microwave extraction system, when coupled to with the mixed solvent system used in the later stages of this work, was successful. Recoveries of greater than 97% for each species was accomplished with the microwave extraction method once microwave power and time parameters had been optimized. This later project was also funded by the NJDEP and would not have been possible without this initial study. A copy of a draft document describing the experimental, results, discussion and conclusions is included in appendix 2. These speciation methods and the stable isotope labeling method (listed below) were both key components in four additional Federal grant applications including a current superfund project. All of these have received excellent review and will continue to be the focus of different research projects in the future.

Labeled experiments in tissue:

Stable isotope labels were used to measure the forward and backward progression of the mercury interconversion studies in the anaerobic sediments. Subsequent to these studies they have been used to trace the differential uptake of the two mercury species in animals. Specifically, low doses of both the dication and the monomethyl were injected into the stomach of a rat to determine where each species would migrate to and in what ratio. The dication was introduced into the animal as ^{200}Hg and the methyl mercury as ^{202}Hg . The experiments demonstrated that these stable isotopes could be used as markers for the differential uptake of two chemical species within a biological system. The results from one of the brain samples is shown in figure 6.

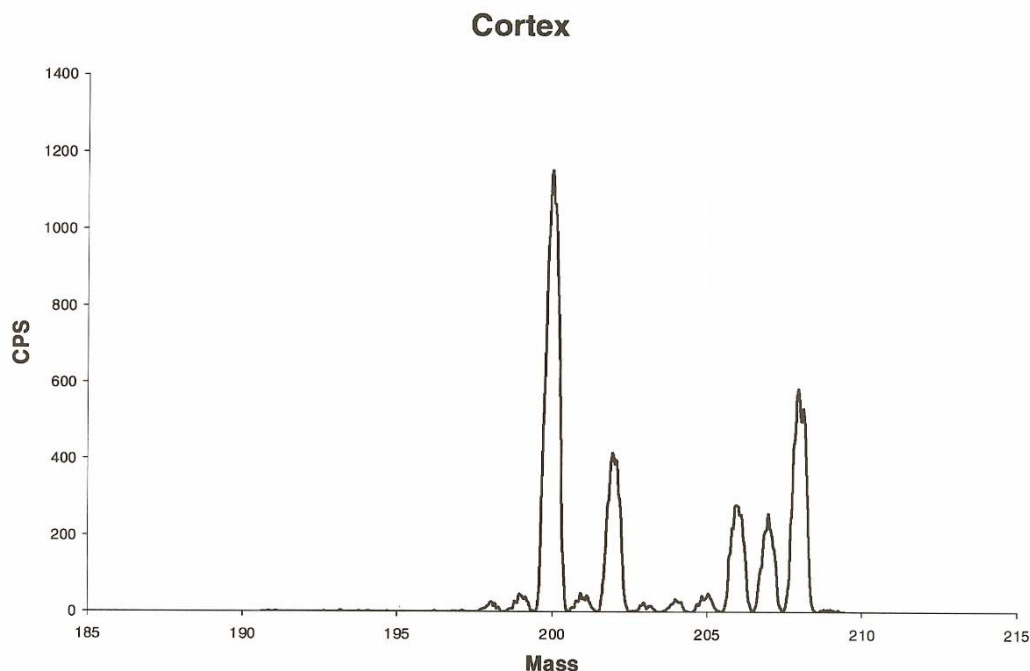


Fig 6. Mass spectrum of the digested cortex of a rat showing the uptake of the two different species of mercury represented as the 200 and 202 peaks.

The cortex was one region of the brain where there was more of the inorganic form present than the organic form. This goes against most conventional wisdom that suggests that almost all of the brain stores the methyl form preferentially. Also present in the plot are the various isotopes of lead, that were present in the brain tissue.

I believe that all of the methods developed, as part of this work will continue to see significant application in the future as they already have. The techniques have been cited in at least five additional grant applications and all of the reviews on the technique were favorable. This technique may also be used in an inter-laboratory study to produce a speciated standard reference material for mercury, something that is desperately needed for future work in the field.

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Appendix 1

Definitions:

Speciation:	The differentiation and quantification of the chemical species of mercury
Hg ⁰ :	Reduced form of mercury
Hg ²⁺ :	Mercury dication
CH ₃ Hg ⁺ :	Monomethyl mercury
(CH ₃) ₂ Hg:	Dimethyl mercury
SFE:	Supercritical Fluid Extraction
ICPMS:	Inductively Coupled Plasma Mass Spectrometer
ICPAES:	Inductively Coupled Plasma Atomic Emission Spectrometry
IC:	Ion Chromatography
GC:	Gas Chromatography
CE:	Capillary Electrophoresis
HPLC:	High Performance Liquid Chromatography

Appendix 2

Manuscripts in progress
