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THE MEASUREMENT OF TOTAL AND ORGANIC MERCURY IN MARINE SEDIMENTS, ORGANISMS, AND WATERS

Robert P. Eganhouse, Jr.

SOUTHERN CALIFORNIA COASTAL WATER RESEARCH PROJECT
1500 East Imperial Highway, El Segundo, California 90245

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ABSTRACT

The Coastal Water Research Project has conducted studies to determine the best methods for measuring total and organic mercury in a variety of marine samples. Acid digestion of organic material was used in the preparation of total mercury samples, and liquid/liquid extraction was used to isolate organomercurials. All measurements were made by cold-vapor atomic absorption. The analytical techniques provide an absolute detection limit of approximately 3 ng with a coefficient of variation of ±5.8 percent. These methods appear useful for the routine measurement of mercury in marine samples.

INTRODUCTION

Efforts to measure mercury in natural systems intensified in the 1960's with the recognition that environmental mercury pollution could pose a serious health hazard (Kurland et al. 1960). Since then, analytical capabilities have been developed to the state that high sensitivity and reasonable accuracy and precision are attainable with relatively simple and inexpensive equipment (Hatch and Ott 1968; Hawley and Ingle 1975).

In most cases, published data on mercury pollution have been presented as "total" mercury, which means the sum of organic, inorganic, and elemental forms. (The term "organic mercury" encompasses a group of compounds in which mercury is chemically bonded to organic, principally alkyl, substituents. Examples are methyl mercuric chloride (MeHgCl), dimethyl mercury, and phenyl mercuric acetate.) However, the distinction between these forms is important from both analytical and toxicological standpoints (Sumino 1968).

Because of the differences in the chemical properties of inorganic and organic forms of mercury, and the variation of matrices in natural samples, the analytical procedures for organic forms are quite different from those for inorganic forms. As a result of its greater chronic toxicity, high affinity for tissues, and tendency to concentrate at higher trophic levels, organic mercury is considered more harmful than inorganic or elemental mercury. Consequently, a full understanding of mercury pollution depends

upon measurement of not only the total amount of mercury in environmental samples but also the organic fraction (Rivers et al. 1972; Andren and Harriss 1973; Jernelöv et al. 1975).

This paper deals with the methodology developed at the Coastal Water Research Project for the measurement of total and organic mercury in sediments, animal tissues, and water samples. Sampling procedures and precautions are reviewed and analytical procedures are presented in detail. The advantages, difficulties, and limitations of the methods are discussed in a technique development section. Appendices contain information on chemicals, computations, and water analyses.

SAMPLING

Sediments

Because of the volatility of organic forms of mercury, sediment samples must be handled with extreme care (Bisogni, Olson, and McCarty, personal communications*). Grab samples should be split into two subsamples, one for total mercury analysis and one for organic mercury analysis. The samples should be kept cold or frozen in tightly sealed conventional polyethylene, polystyrene, or glass containers. When brought to the laboratory, the samples should either be analyzed immediately or stored frozen until analysis is possible.

Past studies have shown that repeated freezing and thawing may result in the loss of up to 96 percent of the organic mercury in a sediment sample. Published results (Andren and Harriss 1973; Olson and Cooper 1974) indicate that organic mercury often constitutes less than 0.1 percent of all mercury found in marine sediments; thus, the loss from thawing and refreezing may not always be a serious problem in the analysis for total mercury. However, in one sample collected off the Palos Verdes Peninsula, we found 0.8 mg/dry kg organic mercury--59 percent of the total mercury in the sample. In samples such as this, the loss of mercury can be significant. Consequently, chemical processing of the sediment sample should be initiated when it is fresh or frozen.

In studies of sediments collected around the Whites Point municipal wastewater outfall off Palos Verdes during March 1975, gravity cores yielded consistently lower values for both total and organic mercury than grab samples taken at the same stations. On the average, values for total mercury in the upper 4 to 5 cm of the cores were only about 36 percent of those in the same layers of the grab samples. We suspect that the use of this type of coring device results in the loss of the thin surface layer of sediment where most of the mercury is located.

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*J.J. Bisogni, Cornell University, Ithaca, New York. B.H. Olson, University of California, Irvine. T. McCarty, Stanford University, Palo Alto, California.

Biological Tissues

Each specimen must be thoroughly washed with fresh seawater and placed alone in a tightly sealed polyethylene bag. It is important to avoid contamination with sediments. The animals should be kept cold until they can be brought to the laboratory where they are to be dissected. After dissection, the tissues can be frozen and stored in polyethylene containers until analysis is possible. Because mercury binds strongly to animal tissue, samples may be thawed without incurring losses.

LABORATORY PROCEDURES

Total Mercury in Sediments

Approximately 0.3 to 0.6 g of fresh or frozen sediments are weighed into a 250-ml flat-bottomed boiling flask, and 20 ml of freshly prepared "aqua regia" are added to the sediments. The flask is attached to a high-efficiency reflux condenser positioned above a hotplate. The mixture is then refluxed at 200°C (Setting 5 on the Corning PC-100 hotplate) for a period of 2 to 3 hours and cooled to room temperature. Next, the condensers, which have small watch glasses atop them to prevent outside contamination from reaching the flask contents, are rinsed with several milliliters of distilled water (the quantity is not critical).

A teflon-coated stirring bar and 145 ml of distilled water are added to the flask, which is then attached to the purge system (Figure 1). After 5.0 ml of the stannous chloride solution have been injected into the flask, the magnetic stirrer is turned on for 60 seconds, and the N_2 flow is started. Elemental mercury is carried by the nitrogen through the sample cell, and the absorbance at the 254-nm wavelength is displayed on a strip chart recorder in the form of a peak. The amount of mercury in the sample is determined by comparing the integrated peak area to a standard curve (see Appendix A). In our work, peak area has been found to be a more precise measure of the mercury present than peak height. For each day's run, a standard curve is constructed from data obtained for five processed standard samples ranging from 25 to 500 mg of mercury as mercuric chloride ($HgCl_2$). A blank is also run. The standards are prepared daily using a concentrated stock solution (at about 100 ppm) by serial dilution. One ml of concentrated HNO_3 is added for every 100 ml of standard solution to minimize loss of mercury by absorption or vaporization. The 100-ppm stock solution can be used effectively for 1 week. Moisture content is determined by weighing approximately 3 to 5 g of wet sediment in an aluminum pan, drying the sediment at 70 to 80°C for 48 hours, and reweighing.

Total Mercury in Tissues

Up to 10 g of tissue are homogenized as a 50/50 mixture with distilled water in a homogenizer. Using a medicine dropper or spatula, 0.2 to 1.0 g of homogenate is transferred to a 250-ml

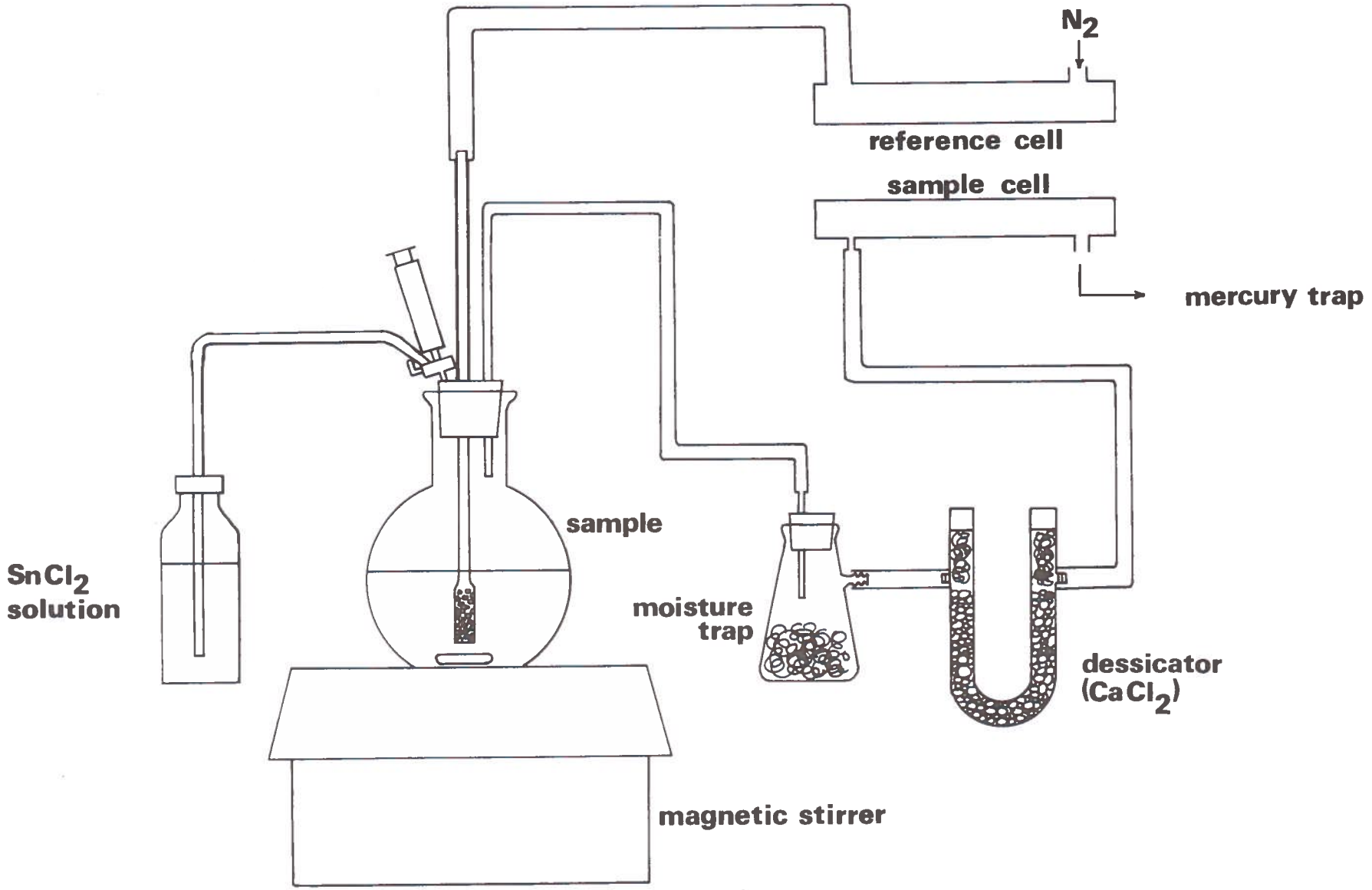

Figure 1. Cold-vapor atomic absorption (CVAA) spectrophotometer with purge system for mercury analysis.

flat-bottomed boiling flask. Twenty ml of a 3:1 H_2SO_4/HNO_3 solution are added to the flasks, which are then attached to the hotplate condenser apparatus.

The samples are digested at 200°C for 15 to 20 minutes and cooled to room temperature. A pale yellow to colorless solution should result. The flasks are then detached from the rinsed condensers and are reheated (in a fume hood) at 200°C until all NO_x gases have been released. This usually takes from 15 to 30 minutes. The samples are then cooled to room temperature and analyzed in the manner described above.

(Analysis of total mercury in water is described in Appendix B.)

Organic Mercury in Sediments

The following analysis is essentially a modification of the techniques described by Westöö (1966) and Rivers et al. (1972). To a 250-ml centrifuge bottle with a teflon-lined cap, 10 g NaCl, 70 ml of distilled water, and 15 ml of concentrated HCl are added. The mixture is shaken to dissolve the salt, and 5 to 10 g of fresh or frozen sediment is added. This may be conveniently done by collecting the samples in 3-dram containers, which are weighed, emptied of frozen sediment, and reweighed. After 65 ml of benzene has been placed in the bottle, the mixture is shaken on a wrist-action shaker for at least 10 minutes. The contents are then centrifuged at 1,200 rpm for 20 minutes or until the benzene layer is clear. A pipette is used to transfer 50 ml of the benzene extract to a 60-ml separatory funnel. Seven ml of a cysteine solution (1.0 g cysteine HCl, 0.744 g Na acetate, 12.0 g Na_2SO_4, and 100 ml water) is added to the funnel, and the mixture is shaken for 3 minutes. Once the phases have separated (centrifugalization may be necessary), the cysteine extract is drained into a test tube. The extracts may be stored for at least 10 days in sealed test tubes or centrifuge tubes.

Two ml of the cysteine extract solution are transferred by pipette to a 250-ml flat-bottomed boiling flask. After 20 ml of aqua regia have been added to the flask, the contents are digested (as previously described) for 1 hour at a setting of 3.5 (135°C). The flasks are cooled, condensers rinsed, and samples analyzed in the same manner described for total mercury. The standards are made up with distilled water using a stock solution at 100-ppm MeHgCl. The dilute standards must be prepared daily, but the stock solution is usable for 3 to 4 days.

Organic Mercury in Tissues

Up to 10 g of tissue are homogenized as a 50/50 mixture with distilled water using a homogenizer. Five to 10 g of the homogenate are immediately weighed into a 250-ml centrifuge bottle and extracted, digested, and analyzed in the manner described in the section on organic mercury in sediments.

GENERAL PROCEDURES

All glassware is kilned at 1,000°C for 6 hours following thorough rinsing with 6N HNO_3, tap water, and distilled water. Reagent-grade chemicals are used without further purification (see Appendix C). Usually, reagent blanks yield peaks of about 1.0 to 1.5 ng of mercury.

In our laboratory, absorbance readings are obtained with a Laboratory Data Control Model 1235 mercury monitor equipped with dual 30- by 0.75-cm PVC cells and a Hewlett Packard Model 7123A recorder. Five standards, one blank, and up to 18 samples can be analyzed for both total and organic mercury in one day.

TECHNIQUE DEVELOPMENT STUDIES

The following sections review the series of experiments that led to the development of the techniques described above.

Total Mercury in Sediments

Range of Linearity. Aqueous standards of $HgCl_2$ were prepared and analyzed to determine the range of linearity for the Laboratory Data Control mercury monitor. Figure 2 shows that over the range of 0 to 1,000 ng, the instrument provides linear response with acceptable precision: Thus, analyses may be performed on sediments containing up to 5.0 µg/dry g of mercury when a 0.2 g (dry) sample is used.

Digestion Studies. Experiments were conducted to determine the optimum digestion time and reflux rate using aqua regia. Aqua regia is a commonly used digestion reagent for sediments (Dow Chemical Company 1970; Iskandar et al. 1972; Jacobs and Keeney 1974). In the study, sediment samples were digested at 135° and 200°C for periods of up to 4.0 hours. Figure 3 illustrates the data from this experiment. After 3 hours at 135°C and 2 hours at 200°C, the recovery seemed to stabilize. Based on this work, all subsequent sediment digestions were performed at a setting of 5.0 (200°C) for a period of at least 2 hours to ensure maximum recovery of mercury.

Adsorption Studies. Applequest et al. (1972) have suggested that mercury may readsorb to residual undigested siliceous material during the reduction of Hg^{++} to Hg^{0} with stannous chloride. This could result in depressed results. To avoid the possibility of this problem, some researchers have filtered out the silicate residue (Häkanson and Uhrberg 1973). However, because the additional handling of the digests during filtration could introduce contamination or result in loss of mercury due to adsorption, the filtration step is undesirable (Thompson and McComas 1973). To discover if readsorption occurs, we conducted two experiments.

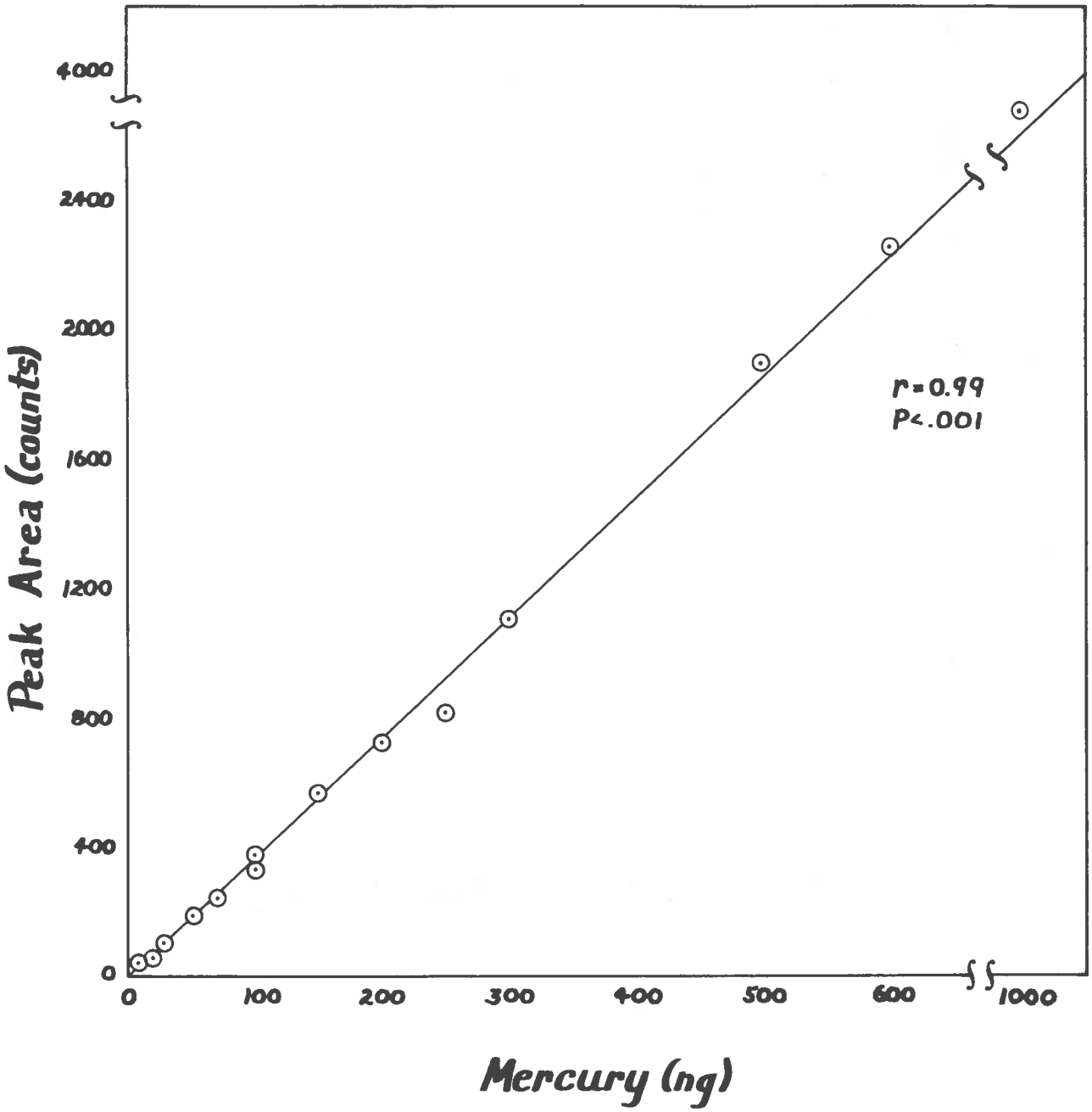

Figure 2. Peak area versus nanograms of mercury for aqueous solutions of mercuric chloride.

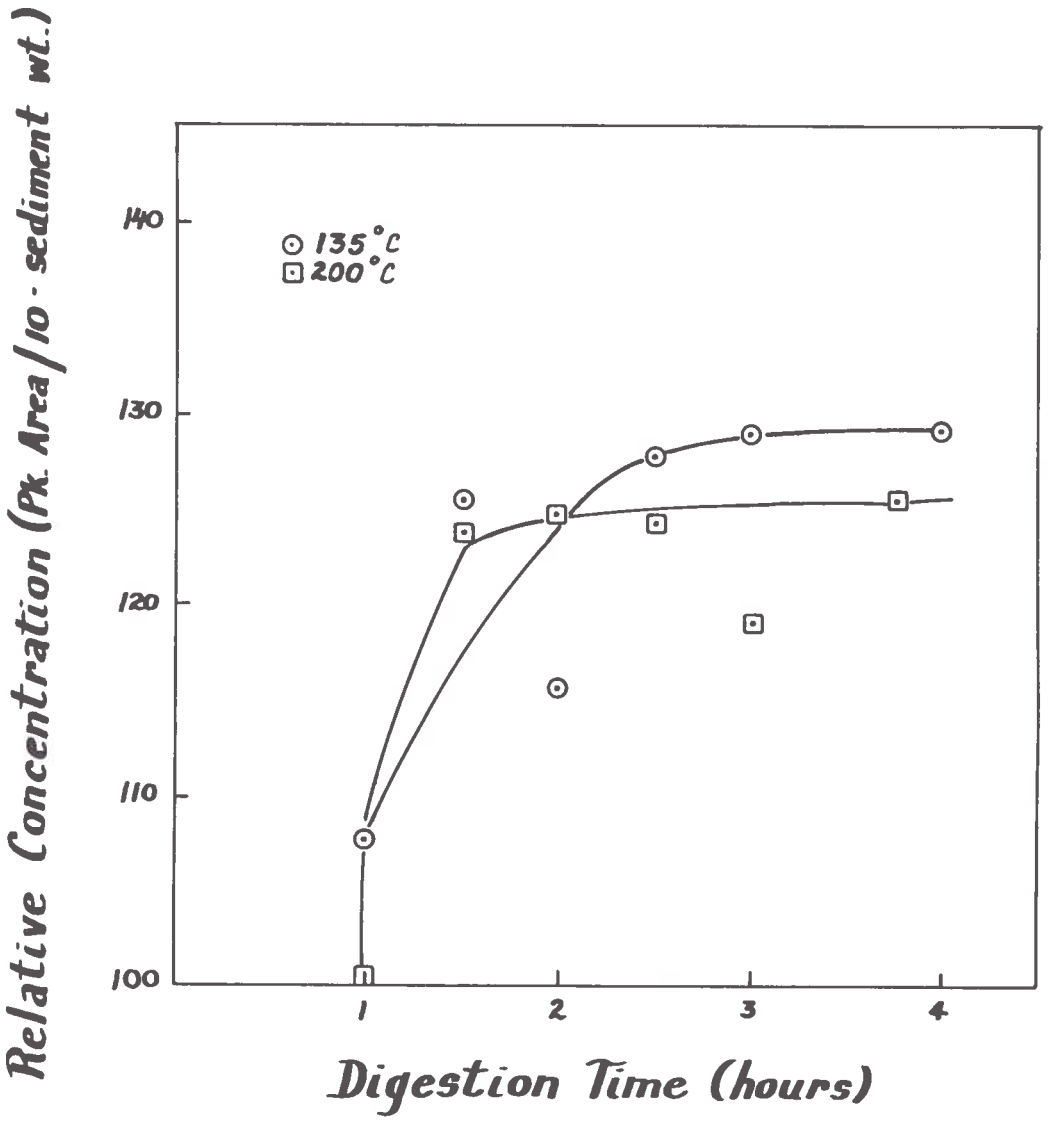

Figure 3. Relative mercury concentration as a function of digestion time at 135°C and 200°C.

Table 1 lists data from the first experiment. Sediments that had been kilned at 1,000°C (to remove mercury and most of the organic matter) were spiked with $HgCl_2$, digested, and compared to processed standards. Any decrease in the amount of elemental mercury for the spiked sediments would have indicated readsorption; however, no decrease was detectable.

To ensure that this result was not due to the use of kilned sediments, unkilned sediments were also analyzed. Two sets of four replicates of the same sediment were digested in aqua regia as usual. After the digests had cooled, one set of four replicates was filtered and the filtrates were analyzed. Comparison of the filtered and unfiltered digests (Table 2) revealed no significant differences, substantiating the belief that readsorption does not occur during the reduction step. All subsequent analyses were performed without a filtration step.

Sediment Charge Study. An experiment was performed to determine if recovery of mercury from sediments depends upon sediment charge. Sediment samples ranging in size from 0.1 to 0.5 g were digested and analyzed for total mercury. The data in Table 3 demonstrate the fact that, at higher sediment charges, the measured concentration of mercury decreases. It is not clear if this decrease is due to ineffective digestion, loss of mercury, or experimental variation. However, because the recovery appeared to be highest and remain constant at low sediment charges (i.e., from 0.1 to 0.2 g), all subsequent analyses were made with samples in this range.

Storage of Digests. For routine processing of large numbers of samples, it is advantageous to be able to store digested sediments. In this way, large blocks of time can be devoted to digestion, with analyses being performed at a later date. To determine the feasibility of digest storage, sediment samples were digested along with standards and compared at various storage intervals. Digested samples stored without processed standards were run in the same manner. All digests were stored in the 250-ml digestion flasks and plugged with ground-glass stoppers.

The results, listed in Table 4, show that the loss of mercury that occurs during storage periods of up to at least 4 days is compensated for when digested standards are stored with the samples. Thus, if digested sediments are to be stored, standards should be digested on the same day and stored along with them.

Recovery Study. Percent recoveries can be calculated by spiking a sediment sample with a known amount of $HgCl_2$ and comparing standards, blanks, and spiked samples. The results of one such study are given in Table 5. While the recovery for the sample spiked with 26.5 mg mercury is low, the results probably reflect a lack of precision at this concentration. The other recovery values appear to be acceptable relative to published results for sediments.

Table 1. Comparison of spiked, kilned sediments and digested and undigested standards.

Sample Description	Peak Area (Counts)	Average Count*
Undigested standard samples containing 100 ng Hg as $HgCl_2$.	330 358 358 368	353.5 ± 16.0
Digested standard samples containing 100 ng Hg as $HgCl_2$, corrected for blank.	360 320 344 334	339.5 ± 16.5
Kilned sediments spiked with 100 ng Hg as $HgCl_2$, corrected for blank.**	362 373 357 359	362.8 ± 7.0

*Precision measure is the 95 percent confidence interval.
**0.1 g of sediment was used for each sample.

Table 2. Comparison of filtered and unfiltered sediment digests.

Sample Description	Peak Area (Counts*)	Average Count**
Unfiltered digests of sediments.	111.0 118.0 110.0 113.0	113 ± 3.5
Filtered digests of sediments.	107.0 100.0 112.0	106.3 ± 6.8

*All values corrected for reagent and/or filter blanks.
**Precision measure is the 95 percent confidence interval.

Table 3. Dependence of yield for total mercury upon sediment charge.

Sediment Weight (g)	Total Mercury (µg/dry g)*
0.099	0.548
0.110	0.594
0.246	0.546
0.402	0.473
0.522	0.466

*The coefficient of variation for the analysis of total mercury in 0.1 g of these sediments was ±5.8 percent.

Table 4. Comparison of digested sediment samples stored with standards and those stored without standards.*

	With Standards	Without Standards
Day Zero		
Total mercury (µg/dry g)	0.551 0.577 0.516 0.632	0.506 0.552 0.601 0.674 0.522 0.596
Average	0.569 ± 0.048	0.577 ± 0.049
Day 1		
Total mercury (µg/dry g)	0.570 0.604 0.548 0.520	0.535 0.498 0.544 0.556 0.521 0.503
Average	0.560 ± 0.035	0.527 ± 0.018
Percent change**	-1.6	-8.7
Day 4		
Total mercury (µg/dry g)	0.590 0.539 0.668 0.541	0.482 0.575 0.476 0.433 0.524 0.484
Average	0.584 ± 0.059	0.497 ± 0.039
Percent change**	+2.6	-13.9

*Precision measure is the 95 percent confidence interval.
**Values based on percent change from Time Zero.

Table 5. Recovery of mercury from sediments spiked with mercuric chloride.*

Mercury Added (ng as $HgCl_2$)	Mercury Recovered (ng)	Percent Recovery
26.5	20.4	77.2
53.0	49.9	94.1
106.1	109.6	103.3

*Literature values for percent recovered are 92.0 (Jacobs and Keeney 1974), 95.0 (Riley and Aston 1972), and 96.0 (Potter et al. 1975).

Comparative Sediment Studies. To determine whether or not this technique could reproduce results obtained earlier (Johnson 1974), sediments collected by a Shipek grab sampler at stations around the Whites Point outfall (Figure 4) in 1973 were reanalyzed for total mercury. The sediments had been stored frozen for over a year. The results of the two analyses are listed in Table 6: In general, the values agree quite well. Earlier and later data for the same stations have been included in the table for comparison.

Detection Limits, Precision, and Accuracy. The absolute limit of detection for this analysis is approximately 3 ng mercury. This is equivalent to a concentration of 0.015 µg/dry g for a sample of 0.2 g dry sediment. Precision was determined by analyzing 10 replicate subsamples from a sediment sample taken on the Palos Verdes shelf. A value of 0.573 ± 0.033 µg/dry g was obtained (the precision is calculated as the 95 percent confidence interval).

To determine the accuracy of the analytical procedure, National Bureau of Standards (NBS) orchard leaves (No. 1571) were digested and analyzed. We obtained a concentration of 0.172 ± 0.009 µg/dry g total mercury (the NBS value was 0.155 ± 0.015).

Total Mercury in Tissues

Recovery Study. The recovery of both $HgCl_2$ and MeHgCl from Dover sole flesh was studied. Homogenized fish samples were spiked with approximately 25, 50, and 100 ng of $HgCl_2$ and MeHgCl. Blanks, standards, and spiked tissues were analyzed. The results, listed in Table 7, show that recoveries were in the range of values obtained by other researchers.

Detection Limit, Precision, and Accuracy. If 0.5 g of tissue is used, down to 6 ng/wet g of mercury can be detected. The precision and accuracy of the technique were determined by analyzing six samples of NBS tuna homogenate (No. 1578). The results obtained were 0.99 ± 0.04 µg/g. Table 8 lists values obtained for this material by other researchers.

Organic Mercury in Sediments

Extraction and Digestion. At first, we attempted to extract aqueous standards of MeHgCl using the procedure of Rivers et al. (1972). Our experiments showed that the procedure was reproducible up to the digestion step; however, the cysteine compound could not be digested. The digestion mixture of HCl and $KMnO_4$ consistently turned brown, giving off a vapor that smelled like chlorine (presumably as a result of an oxidation/reduction reaction). When HNO_3 was substituted for HCl, the Mn^{+7} was no longer reduced; however, no digestion of the cysteine was effected. Consequently, we attempted a 1-hour aqua regia digestion at 135°C. This system seemed to yield excellent standard curves ($r \geq 0.999$, $p < 0.001$) without loss of mercury.

Table 6. Comparison of data on total mercury in Palos Verdes shelf sediments.*

Station**	1972 Gravity Cores	1973 Shipek Grabs		1975 Van Veen Grabs
		Original Values	Reanalysis Results	
1B	0.64	0.64	0.71	-
1C	2.08	1.87	1.85	-
1D	0.22	0.21	0.26	-
3B	-	-	0.54	-
3C	3.42	2.70	2.83	-
3D	0.71	0.63	0.50	-
5B	3.24	3.12	4.05	-
5C	-	-	3.80	-
5D	0.95	0.74	0.35	-
6B	2.92	3.03	2.30	-
6D	0.35	0.99	1.17	-
9B	4.78	4.67	3.61	3.16
9C	2.54	2.77	2.90	2.84
9D	0.88	1.76	0.87	0.55
10B	0.71	0.43	0.54	1.22
10C	0.84	2.08	1.92	1.17
10D	0.96	0.91	0.98	-

*1972 samples collected 22 Jun; 1973 samples collected 5 Sep (reanalysis performed 18 Feb 75); 1975 samples collected 19 Mar.
**For station locations, see Figure 4.

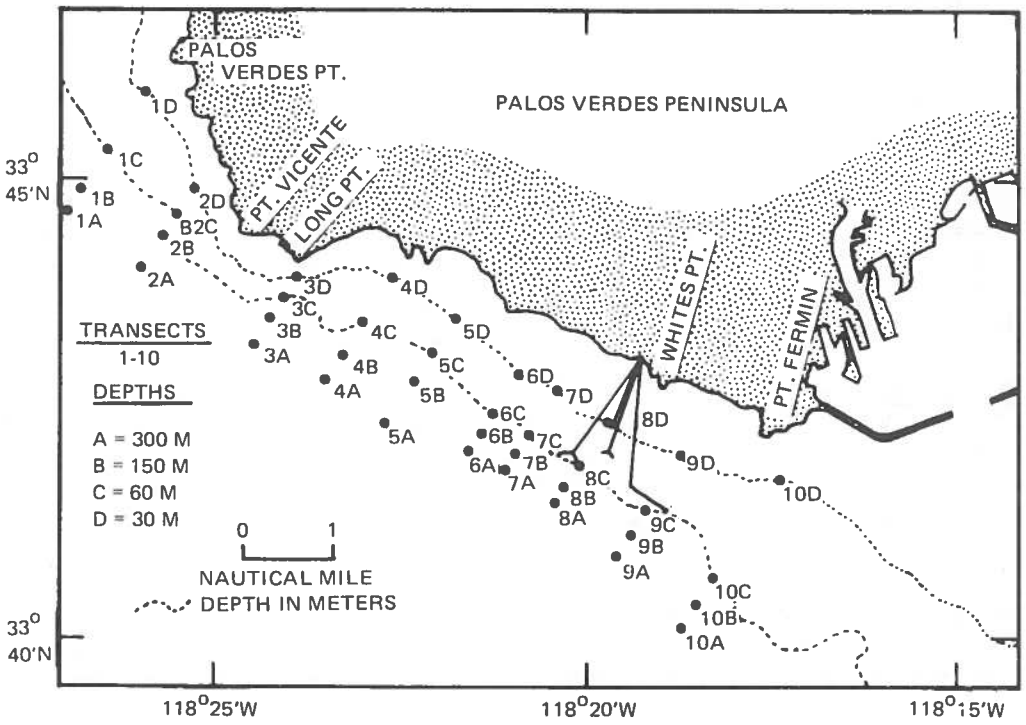

Figure 4. Sediment sampling stations, Palos Verdes shelf.

Table 7. Recovery of methyl mercuric chloride and mercuric chloride as total mercury from homogenized fish tissue.*

Sample	Mercury Added (ng)	Mercury Recovered** (ng)	Percent Recovery**
MeHgCl spike	110.7	117.8	106.4
	55.4	57.4	103.6
	27.7	29.4	106.1
$HgCl_2$ spike	91.5	87.0	95.1
	45.7	43.6	95.4
	22.9	24.3	106.1

*Rivers et al. (1972) reported 101.4 percent recovery of mercury from samples spiked with MeHgCl. Literature values for recovery from samples spiked with $HgCl_2$ are 83.2 percent (Bache et al. 1971), 89.0 percent (Pappas and Rosenberg 1966), 105.0 percent (McKone et al. 1971), and 102.5 percent (Uthe et al. 1970).
**Average of duplicate determinations.

Table 8. Total mercury in NBS tuna homogenate (No. 1578).*

Value (µg/g)	Source
0.99 ± 0.04	Coastal Water Project
0.99 ± 0.04	J.H. Martin and G.A. Knauer
0.67 ± 0.009	R. Carpenter
0.94 ± 0.04	R. Kishore and V.P. Guinn
0.92 ± 0.06	H.L. Windom
0.95 ± 0.05	NBS uncertified value

*All values other than that for the Coastal Water Project are from "Baseline studies of pollutants in the marine environment," background papers for a workshop sponsored by the National Science Foundation's Office for the IDOE, Brookhaven National Laboratory, 24-26 May 1972.

Recovery Study. Sediments and NBS orchard leaves were spiked at three levels with MeHgCl. These samples were analyzed and compared to processed standards and blanks to determine percent recoveries. The results are given in Table 9. The recoveries are in line with values reported by other researchers for marine sediments and microbial biomass samples.

Additivity Experiment. Bisogni and Lawrence (1974) point out the problems of inorganic mercury carryover in the benzene extraction. In fact, the purpose of adding NaCl in the first extraction is to minimize such carryover by aiding the formation of complexes such as $NaHgCl_3$

Table 9. Recovery of organic mercury from marine sediments and NBS orchard leaves spiked with methyl mercuric chloride.*

Sample	Mercury Added (ng as MeHgCl)	Mercury Recovered (ng as MeHgCl)	Percent Recovery**
Marine sediments	127.0	112.1	88.2
	63.0	62.5	99.2
	25.3	24.6	97.2
NBS orchard leaves	107.1	98.7	92.1
	53.6	47.6	88.8
	21.4	19.9	93.0

*Literature values for recovery from marine sediments are 92.9 percent (Olson and Cooper 1974), 80.0 percent (Andren and Harriss 1973), and 93.3 (Bisogni and Lawrence 1974). We know of no other values for recovery of organic mercury from NBS orchard leaves.
**Average for duplicate determinations.

Table 10. Determination of inorganic, organic, and total mercury in aqueous standard solutions of methyl mercuric chloride and mercuric chloride.

Mercury Added (ng)		Peak Area* (counts)				
As $HgCl_2$	As MeHgCl	Inorganic Mercury	Organic Mercury	Sum, Inorganic and Organic	Total Mercury	Percent Inorganic and Organic
1,015	996	4,600.5	3,849.3	8,449.8	9,017	95.7
508	498	2,340.5	1,769.9	4,110.4	4,217	97.5
254	249	1,112.5	700.7	1,813.2	1,953	92.8

*Values have been corrected for blanks.

or Na_2HgCl_4. In our experiment, standard solutions at three concentrations were analyzed for inorganic mercury, organic mercury, and total mercury. Inorganic mercury was determined by direct reduction of the standard using $SnCl_2$ and analysis by CVAA. The results are summarized in Table 10. The agreement between the values for total mercury and the sum of organic and inorganic mercury was gratifying. However, as Bisogni and Lawrence (1974) emphasize, the inorganic mercury carryover depends upon the nature of the sample and the concentrations of inorganic and organic mercury in the sample.

In studies of sediments collected in March 1975 on the Palos Verdes shelf, we found the least organic mercury (from nil to less than 1 percent of the total mercury) in regions near the

outfalls where organic carbon and total mercury levels were high. In contrast, the highest percentages of organic mercury were found in regions of moderate organic carbon and total mercury content. This evidence suggests that little, if any, inorganic mercury is carried over in the extraction of organic mercury.

Sample Handling Studies. Because of the volatility of organomercurials, especially MeHgCl and Me_2Hg, we experimented to determine if organic mercury could be lost during the freezing and thawing of sediments. Measurements of total mercury were also made to determine if the decrease in total mercury was attributable to the volatilization of organomercurials. These data indicate that the amount of organic mercury lost does account for the decrease in total mercury.

Sediments known to contain organic mercury were analyzed, both wet and after air-drying in a desiccator, for total mercury. The values were then compared with data on the organic mercury content. Table 11 shows that the decrease in total mercury upon air-drying is almost exactly equal to the amount of organic mercury present. The implication is that organic mercury can be easily lost, and that careful handling of sediments is necessary if such losses are to be avoided.

Storage Study. The storage of cysteine extracts in capped test tubes was studied over a 2-week period. A series of five standards and one blank were analyzed at suitable intervals. Linear regression analysis of the data revealed that the linearity of the standard curve did not decrease, although slope and intercept values changed, presumably due to variation of instrument response. Table 12 lists these data, along with correlation coefficients and other pertinent statistics.

Detection Limits, Precision, and Analysis. Using the absolute detection limit of 3 ng, this technique is capable of measuring organic mercury in sediments at the level of 2.7 ng/g for a 5-g sample. Precision was not determined.

We were not able to obtain standard samples of marine sediments for accuracy determination.

Recovery of Organic Mercury in Tissues

Recovery of MeHgCl in homogenized fish tissue was determined as described in the previous section. The results are presented in Table 13. The recoveries are excellent and compare well with the published results of others.

ACKNOWLEDGMENTS

I am indepted to Joseph Johnson, whose efforts laid the groundwork for much of the methodology discussed here. Special thanks are due to Dr. David Young and Theadore Heesen for their helpful comments and criticisms.

Table 11. Comparison of total mercury in wet and air-dried sediments and organic mercury in wet sediments.

Sample (Stations on Figure 4)	Total Mercury (µg/dry g)			Organic Mercury	
	Wet	Air-Dried	Δ*	µg/wet g	Ratio to Δ value**
9A	1.360	0.606	0.754	0.805	1.07
9D	0.553	0.327	0.226	0.197	0.87
10B	1.216	0.876	0.440	0.394	0.90

*Difference between wet and air-dried values.
**Ratio of µg/g organic mercury to Δ value (Column 4).

Table 12. Statistics for stored cysteine extracts of aqueous methyl mercuric chloride standards.

Days Stored	Slope	Intercept	r	t	p
1	4.96	2.2	0.998	49.2	<0.001
5	4.82	3.8	>0.999	310.2	<0.001
7	4.87	-8.4	>0.999	87.0	<0.001
9	4.58	-10.8	>0.999	72.3	<0.001
14	4.52	-12.1	>0.999	81.7	<0.001

Table 13. Recovery of organic mercury from homogenized fish tissue spiked with methyl mercuric chloride.*

Mercury Added (ng)	Mercury Recovered (ng)	Percent Recovery
107.1	110.0	102.7
53.6	55.7	103.9
21.4	20.2	94.2

*Literature values are 79.4 percent (Kamps and McMahon 1972), 95.4 percent (Rivers et al. 1972; average value), and 90.0 percent (Westöö 1966).

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Appendix A

COMPUTATIONS

Step 1, STANDARD CURVE

Sample	Peak Area Minus Blank	Mercury Added (ng)
Standard 1	1,612 counts	270
Standard 2	625 counts	108
Standard 3	301 counts	54
Standard 4	144 counts	27

m = 5.653

b = 5.25

r = 0.9995

t = 91.2

df = 7

Therefore

$$y = 5.65x + 5.2,$$

where y = peak area and x = ng mercury.

Step 2, SAMPLE DATA

Sample	Sample Weight (g)	Peak Area Minus Blank
1	0.1831	344 counts
2	0.1187	593 counts
3	0.1222	815 counts

Step 3, COMPUTATION

Total Mercury

A. Mercury Content (ng)

Sample 1: $x_1 = (y_1 - b)/m$

$= (344 - 5.2)/5.65$

$= 60$ ng

Sample 2: x_2 = 104 ng

Sample 3: x_3 = 143.3 ng

B. Concentration (µg/g)

x_1/sample weight$_1$= 60 ng/0.1831 g = 0.327 µg/g

x_2/sample weight$_2$= 104 ng/0.1187 g = 0.876 µg/g

x_3/sample weight$_3$= 143.3 ng/0.1222 g = 1.173 µg/g

Organic Mercury

A. Mercury Content in nanograms (same as above)

B. Conversion Factor

(x_1) · ((65/50) x (7/2)) = 273 ng organic mercury (x_1')

x_2 · 4.55 = 473 ng (x_2')

x_3 · 4.55 = 652 ng (x_3')

Note: ((65/50) x (7/2)) is the concentration factor due to the analysis procedure.

C. Concentration (µg/g)

x_1'/sample weight$_1$= 1.49 µg/g

x_2'/sample weight$_2$= 3.98 µg/g

x_3'/sample weight$_3$= 5.34 µg/g

Appendix B

CHEMICALS

The following reagents are used in the analyses for total and organic mercury.

Aqua regia. Concentrated HCl is carefully added to concentrated HNO_3 in a 3:1 proportion. The container should not be stoppered as Cl_2 and NO_x forms evolve.

Stannous chloride solution. 50 g $SnCl_2$, 90 ml concentrated HCl, and 360 ml water.

Cysteine solution. 1.0 g L-cysteine hydrochloride, 0.744 g sodium acetate, 12.0 g sodium sulfate, and 100 ml water.

Table B-1 lists the chemicals used and their sources.

Table B-1. Chemical used for total and organic mercury analyses.

Chemical	Source
HCl	Mallinckrodt 2612
HNO_3	Mallinckrodt 2704
H_2SO_4	Mallinckrodt 2876
Benzene	Mallinckrodt 1043
NaCl	Mallinckrodt 7581
L-cysteine hydrochloride	MCB 5188
NaO_2CCH_3	Mallinckrodt 7364
Na_2SO_4	Mallinckrodt 8024
$SnCl_2$	Baker 3980

Appendix C

MEASUREMENT OF TOTAL MERCURY IN WASTEWATERS, STORM RUNOFF AND SEAWATER

Joseph N. Johnson

SAMPLING

Water samples are taken in 2-gallon polyethylene bottles, which have been rinsed with dilute HCl or HNO_3 and distilled water. The containers should also be rinsed several times with the water to be sampled prior to taking the final sample. The water must be kept cool until filtration can be accomplished.

ANALYSIS

The sample is filtered as soon as possible through 0.45-μ Millipore filters. After this, the filter pads are placed into 250-ml flat-bottomed boiling flasks along with 20 ml of fresh aqua regia and refluxed for 2 hours using the hotplate/condenser apparatus described in the "Laboratory Procedures" section of this memorandum. After the flasks have cooled to room temperature, the condensers are rinsed, and 100 ml of distilled water are added to each flask. From this point, the analysis is identical to that described in the "Technique" section.

The filtrate (150-ml aliquots) is placed in a 250-ml flask treated with 2.0 ml of a 6 percent aqueous $KMnO_4$ solution and 2.0 ml of an 80 percent (in water) H_2SO_4 solution. This mixture is refluxed for 2 hours using the hotplate/condenser apparatus. After the flasks have cooled, 1.0 ml of a 10 percent hydroxylamine hydrochloride solution is added to reduce excess permanganate. If the purple color does not disappear, more $NH_2OH \cdot HCl$ is added. The FAA analysis proceeds as previously described.