Versatile Combustion-Amalgamation Technique for the Photometric Determination of Mercury in Fish and Environmental Samples

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1.0 Overview

Total mercury in a variety of substances is determined rapidly and precisely by direct sample combustion, collection of released mercury by amalgamation, and photometric measurement of mercury volatilized from the heated amalgam. Up to 0.2 g fish tissue is heated in a stream of O_2 (1.2 L/min) for 3.5 min in one tube of a two-tube induction furnace. The released mercury vapor and combustion products are carried by the stream of O_2 through a series of traps (6% NaOH scrubber, water condenser, and $Mg(ClO_4)_2$ drying tube) and the mercury is collected in a 10 mm diameter column of 24-gauge gold wire (8 g) cut into 3 mm lengths. The resulting amalgam is heated in the second tube of the induction furnace and the volatilized mercury is measured with a mercury vapor meter equipped with a recorder integrator. Total analysis time is approximately 8 min./sample. The detection limit is less than 0.002 µg and the system is easily converted for use with other biological materials, water, and sediments.

Concern over mercury contamination in the environment has resulted in a rapid proliferation of methods in which the principle of "flameless atomic absorption" is used for the determination of mercury (1-4). Common to all of the flameless methods is the production of an elemental mercury vapor which can be measured photometrically. Methods generally differ only in the means by which the mercury is released from the sample and in the steps taken to remove interferences. Mercury is commonly released by acid digestion, followed by reduction and aeration (5, 6) or amalgamation and heating (7, 8); direct combustion (9, 10); or various combinations of these and other less common techniques (11-15). Our experience has shown that present flameless methods tend to be deficient in one or more of the following areas: sensitivity, accuracy and precision, effort or time required for analysis, ease of adaptability to various sample matrices, freedom from error due to sample or reagent contamination, and safety of operation.

This paper describes a method in routine use at the Great Lakes Fishery Laboratory for the determination of total mercury in fish and other environmental samples. The method combines several proven techniques into a unit that is simple in design and operation and adequately meets the criteria defined above. Mercury is volatilized from the sample in a stream of oxygen by means of combustion in a high frequency induction furnace. The mercury vapor is carried along with combustion products and other volatilized materials from the sample by a gas stream through a series of traps (sodium hydroxide, water condenser, and magnesium perchlorate) to reduce interferences. Final separation of mercury from possible interferences is accomplished by amalgamation on gold. The amalgam is then heated in the induction furnace and the released mercury is measured in a mercury vapor meter. Total analysis time is about 8 min./sample and a single analyst can make up to 40 determinations in eight hours.

2.0 Method

2.1 Apparatus and Reagents

2.1.1 High frequency induction furnace.--Laboratory Equipment Corp. Model 523000 (Leco) two-tube induction furnace equipped with Type "L" conversion for combustion sulfur analyses of hydrocarbons and Type "C" conversion for gasometric or gravimetric carbon analyses of iron and steel (Type "C" combustion tube removed). See Fig. 1.

2.1.2 *Induction furnace accessories*.--Leco No. 84 variable transformer for control of power input to induction furnace. Ceramic crucibles (Leco No. 528035) and Vycor insert crucibles (Leco No. 550183) with silicon carbide crucible covers (Leco No. 763212).

2.1.3 *Spectrophotometer*.--Laboratory Data Control Model 1235 mercury monitor, with Beckman Model 1005, 10 mv recorder equipped with Disc integrator.

2.1.4 Sodium *hydroxide gas wash* trap.--125 mL gas washing bottle (Corning No. 31770) containing ca 35 mL 6 % (w/v) NaOH in distilled water. Renew daily. Solution should be prepared before use (1 L) and aerated to remove traces of mercury found in reagent NaOH.

2.1.5 *Water condenser*.--28 X 200 mm od separable vacuum trap (Corning No. 7729) with tube portion immersed in ice bath. Empty as needed to prevent bubbling.

2.1.6 *Drying tube*.--18 X 150 mm od drying tube filled with anhydrous magnesium perchlorate ($Mg(CIO_4)_2$. Replace daily.

2.1.7 Amalgamator.--Quartz tube (Fig. 2) containing 8 g 24-gauge gold wire cut into short lengths (ca 3 mm) supported by a very coarse glass disk over a layer of silicon carbide chips (used to preheat gas stream).

2.1.8 Flow meters.--Two gas controller-flow meters with 0-5 L/min. capacity (Matheson Model 62OBBU).

2.1.9 Heating tape.--2.5 X 610 cm "Briskeat" heating tape (96 watts) connected to STACO, Inc., Type 500B (120 v) variable transformer.

2.1.10 Mercury standard solution.--(1) Stock solution.--1000 µg/mL. Dissolve 0.1358 g $HgCI_2$ in 100.0 mL water or use commercially available standard solution. *(2) Working solution*.--As required to give desired µg in 0.05-0.2 mL water for particular range of sensitivity.

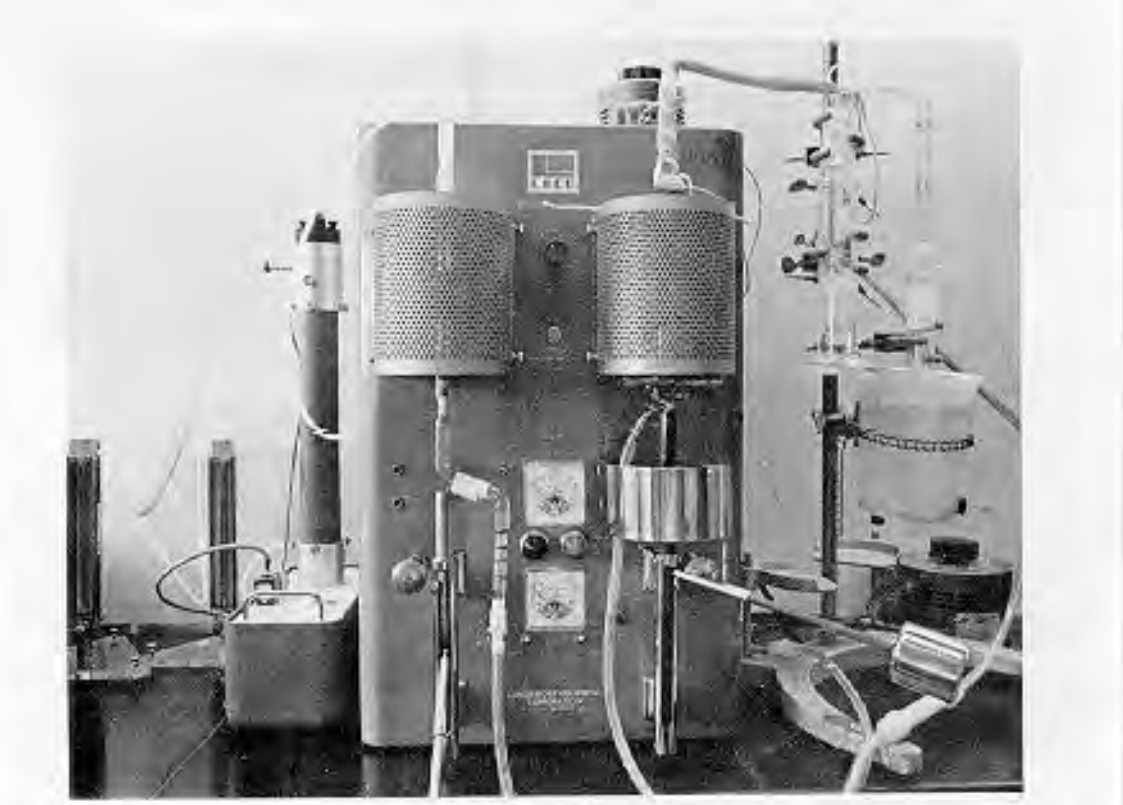

FIG. 1—Induction furnace.

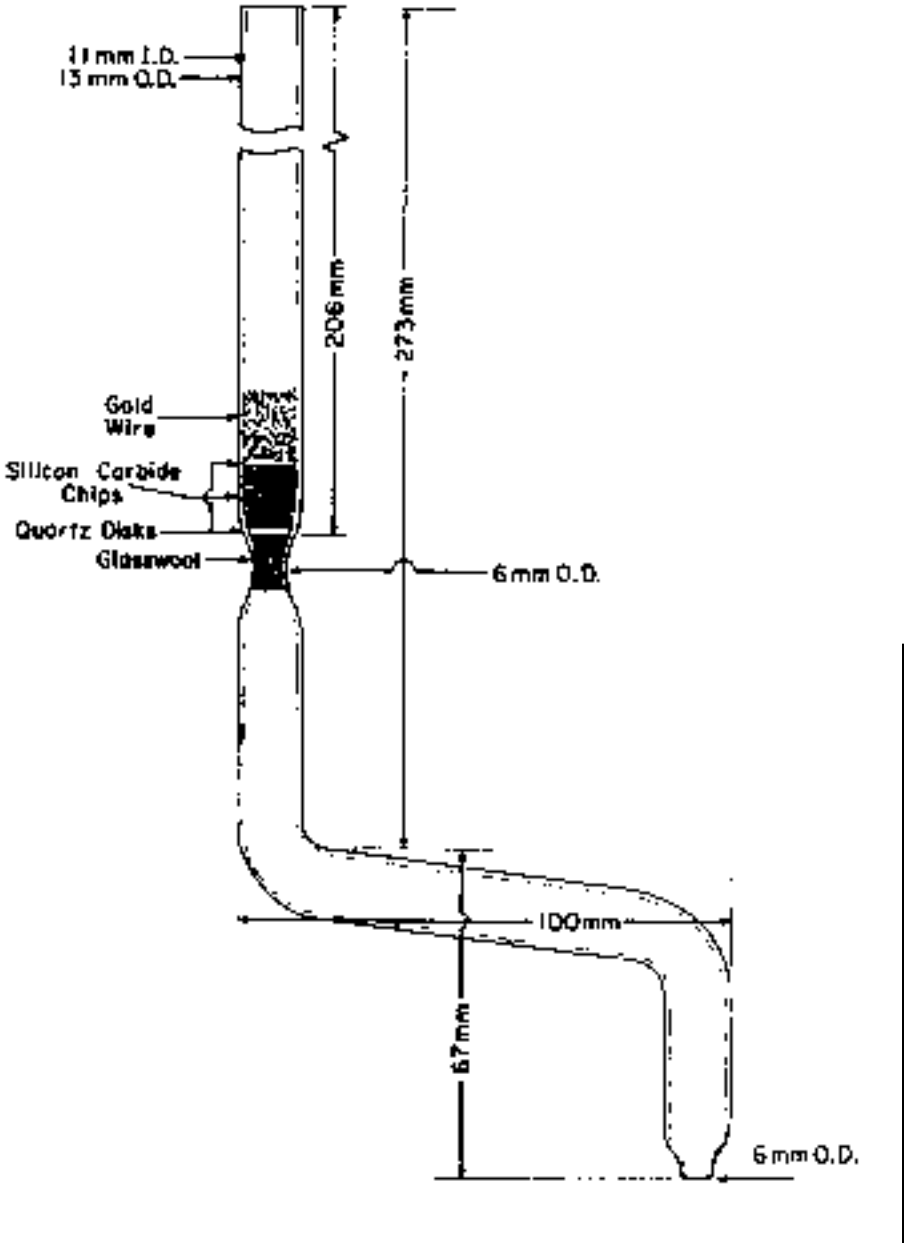

Fig. 2—Detailed drawing of gold amalgamator.

3.0 Assembly of Apparatus

Connect a regulated cylinder of compressed oxygen, using flexible tubing so that the gas stream passes through controller-flow meter, the reference cell of the mercury vapor meter, and the gas inlet post of the tray assembly on the Type "L" arm of the induction furnace (Fig. 3). Connect the Type "L" tube, with ignitor installed, to the NaOH gas wash bottle with Teflon or glass tubing. Wrap exposed portion of combustion tube and connecting tube to gas wash with heat tape controlled by a 110v variable transformer. Maintain internal temperature of connecting tube in excess of 100°C to prevent condensation in line leading to the NaOH gas wash. Connect NaOH gas wash in series to the water condenser, drying tube, and bottom of the amalgamator, using Tygon tubing.

Remove lower guard, tray assembly, and combustion tube from Type "C" arm (carbon analysis) of induction furnace. Install amalgamator in modified arm by attaching it to center post of raising-and-locking assembly for that arm. Curved metal spatula ("Scoopula") bent at 90° and inserted into center post serves as adequate bed for attachment of amalgamator. Adjust height of amalgamator on center post so that entire column of gold and silicon carbide is completely within induction coil when locked in raised position. Connect top of amalgamator to absorption cell of mercury analyzer with flexible tubing and run exhaust tube from outlet of absorption cell to fume hood; install second flow meter in this exhaust tube to detect possible leaks in system.

Before using system, ensure that all connecting lines and glassware are free of mercury contamination or residues of acid. Flush sample combustion tube and connecting line to NaOH gas wash bottle with 6% (w/v) NaOH daily. This precaution is required to prevent accumulation of acids in line which may trap mercury and reduce recoveries.

4.0 Determination

Establish 1.2 L/min flow of oxygen in system, using flow meter on gas inlet line. Check for gas leaks by comparing readings with exhaust flow meter. Weigh 0.05-0.1 g sample into silica crucible and place silicon carbide cover over crucible. Place covered crucible on ceramic pedestal of Type "L" arm of induction furnace. Adjust setting on variable transformer that controls power to induction furnace to read 80. Lift raising mechanism on Type "L" arm, locking sample into position in induction coil, and heat for 3.5 min. Second arm of furnace (amalgamator) must be in lowered position with gold out of induction coil during this period. After combustion of the sample, lower crucible from coil and place crucible and cover in safe place to cool.

Re-establish gas flow in system by locking raising mechanism of Type "L" arm into raised position without crucible. Adjust variable transformer to setting of 60, raise and lock gold amalgamator into position, and heat until recorder peak returns to baseline (1-2). Lower gold amalgamator and allow to cool (ca 1 min). Count integrator sweeps under peak and compare with standard curve prepared by heating standard solutions, using same operating procedures as above. Because of porosity of silica crucibles, use of Vycor liner is required for standard solutions and other liquid samples. It is recommended that volume of standards used in system not exceed 0.2 mL.

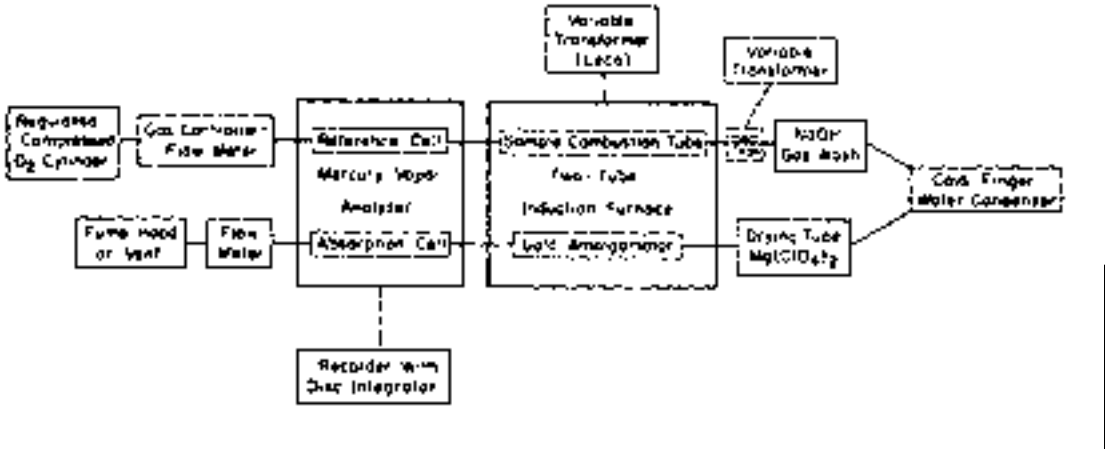

Fig. 3—Combustion amalgamation system for the photometric determination of mercury.

Silicon carbide covers can be used for an indefinite number of samples. For rapid analysis of several samples, two covers are alternated to allow adequate time for cooling between samples. Silica crucibles are also reusable; however, it is recommended they be fired at 800°C for two hours in a muffle furnace between uses, to prevent accumulation of organic residue.

5.0 Results and Discussion

Operating Procedures

The described rate of oxygen flow and settings used on the variable transformer that controls the rate of heating in the induction furnace give a good balance between sensitivity, precision, and time of analvsis for samples of fish containing 0.02-5.0 ppm mercury. Samples in this range are analyzed by attenuating the mercury analyzer and adjusting the sample size. Typical analytical ranges used are 0.002-0.01, 0.01-0.05, and 0.05-0.25 µg. Fish samples larger than 0.2 g can overload the system and greatly reduce accuracy of the results.

For analysis of samples outside the range of 0.02-5.0 ppm a change of operating procedure is required. Sensitivity can be increased by reducing the rate of oxygen flow or reduced by increasing the rate. This technique, within limits, effectively increases or decreases the concentration arid the retention time of the mercury vapor in the absorption cell of the mercury analyzer, resulting in the altered sensitivity. Installation of a stream splitter in the gas line between the sample combustion tube and sodium hydroxide gas wash also effectively reduces sensitivity. Precision may be reduced and time required for analysis increased, however, at altered flow rates.

The heating rate and temperature of the gold amalgamator can be varied by adjusting the variable transformer attached to the induction furnace; this results in a large alteration of observed peak height on the recorder for a given amount of mercury atid oxygen flow rate. The integrator counts vary only slightly, however, for settings on the transformer between the values of 50 and 100%. It is this characteristic which makes it mandatory to use area measurement (integrator counts) in place of peak height to ensure reproducible results. The heating rate and temperature obtained in the induction field at a given power setting are functions of geometry, mass, density, and purity of

the amalgamator. After repeated use, the heating characteristics of the amalgamator slowly change, as evidenced by increased peak width and lowered height. This change is probably due to a gradual accumulation of oxides and organics on the amalgamator (8, 13). Periodic removal of the amalgamator (semi-monthly) and firing of the gold in a muffle furnace at 800°C for two hours generally restores the amalgamator to its original characteristics. The use of an integrator compensates for the changing characteristics of the amalgamator while it is in use.

6.0 Precision and Accuracy

The average recoveries of mercury ($HgCl_2$) from fortified samples of fish muscle, lake sediment, aquatic vegetation, and bituminous coal were 94-105% for fish muscle and 94-112% for all materials tested (Table 1). The average recovery for all materials and levels tested was 102.7% (coefficient of variation, 5.5%). Our inability to obtain truly homogenous sediments of constant mercury background undoubtedly contributed to the wider variation in recoveries from sediment. Limited testing of fish samples fortified with methyl mercuric chloride gave recoveries within the range obtained with mercuric chloride. The accuracy of the method was determined on samples of fish flesh and sediments analyzed concurrently by several laboratories (Table 2). Values obtained by the described method (Method CA) agree well with average values obtained by other laboratories using acid-digestion, flameless atomic absorption, and neutron activation techniques.

To determine the reproducibility of the method over a period of time, we collected, composited, and homogenized large amounts of fish muscle containing different levels of mercury contamination to form a source of uniform reference material for analysis. Samples of this material were analyzed daily along with unknown samples. Table 3 presents a statistical treatment of data accumulated from these repeated analyses. The values given should include nearly every source of routine error that can be expected when this method is used, since they represent over 100 days of testing. Sample replication on any given day generally gave a coefficient of variation less than 10% and routinely 5% or less, as indicated in Table 1. Failure of standard solutions or check samples to give at least ±10% agreement indicates that the analytical system should be dismantled and cleaned before routine analyses are performed.

Table 1. Recoveries of mercury ($HgCl_2$) added to samples of fish muscle, lake sediment, vegetation, and coal

Sample and amount of mercury added, μg	No. of replicates	Av. amount recovered,[a] %	Coeff. of var., %
Fish muscle (coho salmon)			
0.01	6	94.4	5.8
0.03	6	104.9	3.7
0.06	6	98.5	3.1
0.20	6	104.0	4.7
Sediment (Lake Michigan)			
0.03	3	112.2	2.1
0.06	3	96.2	11.5
Vegetation (chara)			
0.03	3	102.2	5.8
0.06	3	106.8	0.6
Coal (bituminous)			
0.03	3	109.8	2.4
0.06	3	99.3	3.1

[a] Values corrected for following background (ppm) in samples: fish muscle, 0.093; sediment, 0.032; vegetation, 0.016; and coal, 0.081.

Table 2. Average mercury content (ppm) in fish flesh and sediments as determined by the described method and other methods of analysis

Sample[a]	Method[b] CA	FAAS	NAA
Fish flesh			
1	0.22 (1)	0.17 (12)	---
2	0.83 (1)	0.76 (12)	0.92 (2)
3	2.1 (1)	2.4 (12)	2.5 (2)
Sediment			
4	0.18 (1)	0.17 (9)	---
5	107 (1)	110 (20)	120 (1)
6	45 (1)	45 (20)	44 (1)

[a] Samples 1-4: Mercury in Fish and Sediment Round-Robin-1971, Ontario Water Resources Commission, Division of Laboratories. Samples 5 and 6: Mercury in Sediment Round-Robin-1972, Environmental Protection Agency, Region IV, Surveillance and Analysis Division.
[b] Methods used: CA, combustion-amalgamation; *FAAS*, flameless atomic absorption (variety of digestion mixtures); *NAA*, neutron activation analysis. Numbers in parentheses show the number of laboratories participating.

Table 3. Accumulated data on the precision of replicate analyses of fish flesh during several weeks of testing

Sample	No. of Analyses	No. of Days	Av. Concn, PPM	Std dev.	Range	Coeff. var %
A	258	58	0.0883	0.0123	0.0563 - 0.141	13.9
B	60	16	0.399	0.0485	0.310 - 0.569	12.2
C	65	16	0.774	0.0837	0.596 - 1.09	10.8
D	186	49	2.82	0.214	2.24 - 3.20	7.6

7.0 Sample Adaptation

The data given in this paper were derived primarily from "clean" samples of fish muscle, vegetation, lake sediments, and coal. We found, however, that some samples of individual tissues, as well as of whole fish, caused an acidification in the lines, resulting in low recoveries of mercury. The problem was eliminated by air-drying the samples overnight at room temperature to remove most of the water. We have adopted this practice for all samples of whole fish. Care must be taken, however, to ensure that no mercury contaminated air or dust reaches the samples during this drying period.

Since the heat supplied by the silicon carbide cover used during sample combustion may not be adequate for samples other than those tested (e.g., geological materials or metals), we also tested a quartz-enclosed silicon carbide crucible (Leco No. 550182) in place of the silica crucible. This crucible was satisfactory for samples of low organic content but not for biological materials because combustion was apparently too rapid and caused erratic results. The addition of "activators" of metal (copper and iron) to the sample gave the same results; they, too, were unsatisfactory for biological materials and, in addition, had high and variable background levels of mercury.

Water can be analyzed directly in the system, with a sensitivity as low as about 10 P.B., by using the same analytical procedure used for standard solutions. However, a superior and much more sensitive method is a modification of the procedure described by Kala (13). In this modification, a 100 mL water sample is heated with sulfuric acid, nitric acid, potassium permanganate, and potassium persulfate; decolorized with hydroxylamine hydrochloride; reduced with stannous chloride; and aerated in a gas wash bottle (16). The released mercury vapor is passed through magnesium perchlorate and amalgamated on gold as described earlier in this paper. A sensitivity of less than 0.02 P.B. can be obtained with this technique.

Of the various materials we have tried in the system, only those which are volatile or explosive in nature could not be analyzed. Samples of volatile solvents have been analyzed after evaporation to dryness, but this technique is subject to loss of mercury from the sample. Extraction and concentration of the mercury into an aqueous system which can be analyzed similarly to standard solutions is a useful technique.

8.0 Conclusions

The described method has been found to be a reliable and rapid technique for the precise determination of total mercury in a variety of samples. Materials that do not lend themselves to direct analysis by the method as described can be accommodated by minor changes in equipment or sample preparation. Routine use of the method at the Great Lakes Fishery Laboratory has shown it to be superior to previously tested methods for use with fish tissue, and preliminary work strongly suggests that it is superior for numerous other types of samples as well.

Major advantages of the method over the normal acid-digestion, flameless atomic absorption techniques include: simplicity of operation; speed of complete analysis; high sensitivity, precision, and accuracy; small sample size required; freedom from rigorous and sometimes hazardous acid digestion procedures; freedom from reagent and glassware contamination; and comparatively low cost of equipment.

Disadvantages of the method as described include: somewhat limited usable range of sensitivity (0.02-5.0 ppm); a general inability to analyze highly contaminated samples (>5.0 ppm) without the use of a gas stream splatter or an extremely small sample; necessity for frequent changes in attenuation of mercury vapor meter, unless previous knowledge permits grouping of samples within ranges having less than a five-fold difference, in concentration; and increased emphasis on the need for well homogenized representative samples because of the small sample size used. It is expected that minor changes in the system or substitution of a less sensitive mercury vapor meter would overcome many of these disadvantages when they are restrictive for a particular use.

9.0 Acknowledgments

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