The application of **FLOW INJECTION TECHNOLOGY** to automating cold vapor **MERCURY** analyses

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This document describes the application of flow injection technology to cold vapor mercury analyses. It can be obtained from PerkinElmer by requesting method number ENVA-100. Appendices 1 and 2 show letters from the US EPA indicating acceptability through the Alternate Test Program for both drinking water and wastewater sample analysis.

1.0 Scope and Application

1.1 This method measures total mercury (organic plus inorganic) (CAS Registry No. 7439-97-6) and is applicable to waters regulated under the National Primary Drinking Water Regulation (NPDWR) and National Pollutant Discharge Elimination System (NPDES) monitoring. These waters include drinking waters, effluents, indirect discharges, and effluent samples containing high chlorides.

1.2 This method is suitable for the determination of total mercury content in the concentration range between 0.01 and 20 µg/L Hg, depending on the instrument configuration and sample loop size used. Table I shows the method detection limits achievable for different configurations of the flow injection system. The range may be extended above or below the normal range by increasing or decreasing sample aliquot size taken for digestion, but changes cannot be made without first demonstrating the performance requirements (i.e., accuracy, precision, and MDL) may be achieved. The actual method detection limit and linear dynamic range will be dependent on the sample matrix, type of instrument configuration, and selected operating conditions.

2.0 Summary of Method

2.1 A known portion of a water sample is transferred to a BOD bottle, equivalent ground glass stoppered flask or other suitable closable container. It is digested in diluted potassium permanganate - potassium persulfate solutions and oxidized for 2 hours at 95 ^{o}C. Mercury in the digested water sample is reduced with stannous chloride to elemental mercury and measured by the conventional cold vapor atomic absorption (CVAA) technique.

The CVAA procedure is a physical method based on the absorption of radiation at 253.7 nm by mercury vapor. The analyst may use a Flow Injection Mercury Analysis System (FIAS) in conjunction with an atomic absorption spectrometer or a stand-alone Flow Injection Mercury System (FIMS). Organic mercury compounds are oxidized and the mercury is reduced to the elemental state and aerated from solution in a closed system. The mercury vapor passes through a cell positioned in the light path of an atomic absorption spectrometer. Alternatively, the stand-alone mercury analyzer can be used which contains a light source and detector specific for mercury and does not require the use of a separate atomic absorption spectrometer. Figure 1 shows a general schematic of the technique. Absorbance (peak height) is measured as a function of mercury concentration and recorded in the usual manner.

2.2 The organo-mercury compounds will not respond to the cold vapor atomic absorption technique unless they are oxidized and converted to mercuric ions. Potassium permanganate oxidizes many of these compounds, but recent studies have shown that a number of organo-mercurials, including phenyl mercuric acetate and methyl mercuric chloride, are only partially oxidized by this reagent. Potassium persulfate has been found to give approximately 100% recovery when used as the oxidant with these compounds. Therefore, a persulfate oxidation step following the addition of the permanganate has been included to ensure that organo-mercury compounds, if present will be oxidized to the mercuric ion before measurement.

3.0 Definitions

3.1 Calibration Blank - A volume of reagent water acidified with the same acid matrix as in the calibration standards. The calibration blank is a zero standard and is used to auto-zero the instrument.

3.2 Calibration Standard (CAL) - A solution prepared from the dilution of stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.3 Field Reagent Blank (FRB) - An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to the sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.4 Instrument Performance Check (IPC) Solution - A solution of the method analyte, used to evaluate the performance of the instrument system with respect to a defined set of method criteria.

3.5 Intermediate Stock Standard - A standard diluted from the stock standard which is used to prepare working calibration standards.

3.6 Laboratory Duplicates (LD1 and LD2) - Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicates precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.7 Laboratory Reagent Blank (LRB) - An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, and internal standards that are used with other samples. The LRB is used to determine if the method analyte or other interferences are present in the laboratory environment, reagents, or apparatus.

3.8 Laboratory Fortified Sample Matrix (LFM) - An aliquot of an environmental sample to which a known quantity of the method analyte is added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentration of the analyte in the sample matrix must be determined in a separate aliquot and the measured value in the LFM corrected for background concentration.

3.9 Laboratory Fortified Blank (LFB) - An aliquot of LRB to which a known quantity of the method analyte is added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control and whether the laboratory is capable of making accurate and precise measurements.

3.10 Linear Dynamic Range (LDR) - The concentration range over which the instrument response to an analyte is linear.

3.11 Method Detection Limit (MDL) - The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero.

3.12 Quality Control Sample - A solution of the method analyte of known concentration, which is used to fortify an aliquot of LRB or sample matrix. The quality control sample is obtained from a source external to the laboratory and different from the source of calibration standards. It is used as a quality control check sample to verify laboratory and instrument performance.

3.13 Stock Standard Solution - A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

4.0 Interferences

4.1 Interferences have been reported for waters containing sulfide, chloride, copper, and tellurium. Organic compounds which have broad band UV absorbance (around 253.7 nm) are confirmed interferences. The concentration levels for interferences are difficult to define. This suggests that quality control procedures (Sect. 9) must be strictly followed.

4.1.1 Possible interference from sulfide is eliminated by the addition of potassium permanganate. Concentrations as high as 20 mg/L of sulfide as sodium sulfide do not interfere with the recovery of added inorganic mercury from distilled water.

4.1.2 Copper has also been reported to interfere; however, copper concentrations as high as 50 mg/L had no effect on recovery of mercury from spiked samples.

4.1.3 Industrial effluents high in chlorides require additional permanganate. During the oxidation step, chlorides are converted to free chlorine, which will absorb radiation at the 253.7 mm mercury wavelength. Care should be taken to assure that free chlorine is absent before the mercury is reduced and swept into the cell (Sect. 11.1.5). This may be accomplished by using an excess of hydroxylamine hydrochloride reagent (as much as 25 mL).

4.2 Low level mercury sample preparation, digestion, and analysis may be subject to environmental contamination if performed in areas with high ambient backgrounds where mercury was previously employed as an analytical reagent in analyses such as total Kjeldahl nitrogen (TKN) or chemical oxygen demand (COD).

5.0 Safety

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be minimized by good laboratory practices. Normal accepted laboratory safety practices should be followed during reagent preparation and instrument operation. Always wear safety glasses or full-face shield for eye protection when working with these reagents. Each laboratory is responsible for maintaining a current safety plan, a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method.

5.2 A reference file of the material safety data sheets, for reagent handling instructions, must be available to all personnel involved in the chemical analysis.

5.3 Mercury compounds are highly toxic if swallowed, inhaled, or absorbed through the skin. Analyses should be conducted using a laboratory exhaust hood. The analyst should use chemical resistant gloves when handling concentrated mercury standards.

5.4 The acidification of samples containing reactive materials may result in the release of toxic gases, such as cyanides or sulfides. Acidification of samples should be done in a fume hood.

5.5 All personnel handling environmental samples known to contain or to have been in contact with human waste should be immunized against known disease causative agents.

6.0 Equipment and Supplies

6.1 Atomic absorption spectrometer: Any atomic absorption spectrometer which has an open sample compartment in which to mount the quartz cell. Instrument settings recommended by the manufacturer should be used. A background correction system is desirable but not necessary.

6.1.1 Radiation source for the determination of mercury, such as a hollow cathode lamp or electrodeless discharge lamp.

6.1.2 Flow injection system (assembled as shown in *Figure 2* for a two-pump system or *Figure 3* for a single-pump system, see also Section 16, references 2-4) consisting of:

6.1.2.1 Autosampler with random access capabilities.

6.1.2.2 Two independently controlled peristaltic pumps: Pump 1 controls the delivery of sample. Pump 2 controls the delivery of the reductant and acid carrier stream. Alternatively, a one-pump system can be used.

6.1.2.3 Switching valve: Multiport valve used for injecting discrete sample volumes, determined by sample loop size, into a hydrochloric acid carrier stream. The carrier stream transports the sample volume to the mixing manifold. The 500 µL sample loop is recommended for the FIAS.

6.1.2.4 Mixing manifold where the sample, reductant, and argon are merged and mixed thoroughly. (*Figure 4*)

6.1.2.5 Gas/liquid separator.

6.1.2.6 Flowmeter: Capable of measuring flows of 0-250 mL/min.

6.1.2.7 Absorption cell: Absorption cell consisting of a quartz cell 14 cm in length, i.d. of 7 mm, with quartz end windows. The cell is positioned in the path of the light beam.

6.1.2.8 Heating mantle: The quartz cell is placed in the heating mantle and the temperature along the length of the cell is maintained at about 10 ^{o}C above ambient. This will prevent condensation within the quartz cell.

6.1.3 Recording device: Any recorder which is compatible with the spectrometer is suitable. Any atomic absorption spectrometer which can print spectral peaks is suitable.

6.2 A stand-alone mercury analysis system such as the FIMS™ (Assembled as shown in *Figure 2* for a two-pump system or *Figure 3* for a single-pump system, see also Section 16, references 5-7) is a suitable substitute for the FIAS/AA combination and consists of:

6.2.1 A flow injection system as described in section 6.1.2. The sample loop for the FIMS can be varied to adjust the linear range desired, as long as adequate detection limits are maintained. Table II shows the trade-off in detection limits vs. linear range for FIMS. The absorption cell consists of a quartz cell 25 cm in length and 4 mm i.d. The cell snaps into position in the path of the light beam.

6.2.2 Radiation source consisting of a low-pressure mercury lamp.

6.2.3 Computer and software for controlling the analysis.

6.2.4 Recording device: Any printer compatible with the FIMS computer and able to print graphics is suitable.

6.3 All Apparatus required for sample digestion described in procedures, including a covered water bath capable of maintaining a temperature of 95 ^{o}C.

6.4 Analytical balance, with capability to measure 0.1 mg, for use in weighing reagents and preparing standards.

6.5 Labware -All reusable labware should be sufficiently clean for the task objectives. Particular attention should be given to all ground glass surfaces during cleaning. Routinely all items should be soaked in 30% HNO_3 and rinsed three times in reagent water. When a previous sample cannot be rinsed from a digestion container, use a detergent solution to clean the container prior to acid cleaning.

6.5.1 Glassware -Volumetric flasks and graduated cylinders.

6.5.2 BOD bottles (or other equivalent suitable closable containers) equipped with stoppers are used for sample digestion. The stoppers should not be sealed during digestion because dangerous pressure buildup and explosion may result.

6.5.3 Assorted calibrated pipettes.

6.6 The sample and reagent flows, through the Flow Injection system, are shown in Figures 2 and 3 and are described here in detail for a two-pump system (Figure 2):

6.6.1 The first channel of Pump 2 transports the 3% HCl acid carrier stream through the Flow Injection valve to the mixing manifold.

6.6.2 The Flow Injection (FI) valve is electronically switched, moving the sample loop into the Fill or Inject positions. In the Fill position the sample loop is filled by sample by Pump 1.

6.6.3 The FI valve then switches to the Inject position, moving the sample loop into the path of the HCl acid carrier stream. The HCl carrier stream pushes the sample to the mixing manifold.

6.6.4 The second channel of Pump 2 transports the reductant, $SnCl_2$, to the mixing manifold. The reductant stream and the sample merge at the mixing manifold reacting to form metallic mercury as shown below.

$$Hg^{+2} + Sn^{+2} \rightarrow Sn^{+4} + Hg^{0}$$

6.6.5 An argon carrier stream is merged with the reaction solution at the mixing manifold and the gas/liquid mixture is transported to a separator.

6.6.6 The gas/liquid mixture is passed through the separator and the liquid is drawn off by Pump 2. The metallic vapor is transported to the absorption cell.

6.6.7 For a single-pump Flow Injection system (Figure 3) all flows are controlled with one pump.

7.0 Reagents and Standards

7.1 Reagents may contain elemental impurities which bias analytical results. All reagents should be assayed by the chemical manufacturer for mercury and meet ACS specifications. The assayed mercury level of all solid reagents used in this method should not exceed 0.05 ppm. It is recommended that the laboratory analyst assay all reagents for mercury.

7.2 Reagent water, ASTM type II or better[8].

7.3 Nitric acid (HNO_3) (CAS RN 7697-37-2), conc. (specific gravity 1.41): Assayed mercury level is not to exceed 1 μg/L. If a high reagent blank is obtained, it may be necessary to distill the nitric acid.

7.3.1 Approximately 1:1 Nitric acid solution: Add 500 mL concentrated HNO_3 to 400 mL reagent water and dilute to 1 L with reagent water.

7.4 Sulfuric acid (H_2SO_4) (CAS RN 7664-93-9), Conc. (specific gravity 1.84): Reagent grade.

7.4.1 Sulfuric acid, 0.5 N: Slowly add 14.0 mL of conc. H_2SO_4 to 500 mL of reagent water and dilute to 1 L with reagent water.

7.5 Stock mercury standard - May be purchased from a reputable commercial source or prepared from high-purity grade chemicals. The stock standard should be stored in Teflon bottles. One of the following procedures may be used to prepare the stock standard:

7.5.1 Stock inorganic mercury standard: Dissolve 0.1354 g of mercuric chloride (CAS RN 7487-94-7) in 75 mL of distilled water. Add 10 mL of concentrated nitric acid and adjust the volume to 100.0 mL. 1 mL = 1 mg Hg.

7.5.2 Stock organic mercury standard: Dissolve 0.2018 g of 2-ethylmercurimercaptobenzoic acid, sodium salt (CAS RN 54-64-8) in 75 mL of distilled water. Add 10 mL of concentrated nitric acid and adjust the volume to 100.0 mL. 1 mL = 1 mg Hg.

7.6 Intermediate mercury stock standard: Make successive dilutions of the inorganic or organic (based on the expected sample characteristics) stock mercury standard to obtain an intermediate standard containing 0.1 μg Hg per mL. This intermediate standard should be prepared fresh daily. Acidity of the intermediate standard should be maintained at 0.15% nitric acid. This acid should be added to the flask as needed before the addition of the aliquot.

7.7 Potassium permanganate($KMNO_4$) (CAS RN 7722-64-7): 5% solution, w/v. Dissolve 25 g of potassium permanganate in 500 mL distilled water.

7.8 Potassium persulfate ($K_2S_2O_8$) (CAS RN 7727-21-1): 5% solution, w/v. Add 25 g of potassium persulfate to 500 mL of distilled water and heat at 40 ^oC for 20 minutes until dissolved.

7.9 Sodium chloride (NaCl) (CAS RN 7647-14-5) - hydroxylamine hydrochloride solution ($NH_2OH{\cdot}HCl$) (CAS RN 5470-11-1): Dissolve 60 g of sodium chloride and 120 g of hydroxylamine hydrochloride in distilled water and dilute to 500 mL.

7.10 Stannous chloride ($SnCl_2{\cdot}2H_2O$) (CAS RN 7772099-8): Dissolve 5.5 g of stannous chloride in 15 mL hydrochloric acid (7.14) and dilute to 500 mL with distilled water.

7.11 Blanks: Three types of blanks are required for the analysis The calibration blank is used in establishing the analytical curve, the laboratory reagent blank is used to assess possible contamination from the sample preparation procedure, and the laboratory fortified blank is used to assess routine laboratory performance.

7.11.1 The calibration blank must contain all reagents in the same concentration and in the same volume as used in preparing the calibration solutions and is carried through the sample preparation scheme.

7.11.2 The laboratory reagent blank (LRB) is prepared in the same manner as the calibration blank and must be carried through the entire sample preparation scheme.

7.11.3 The laboratory fortified blank (LFB) is prepared by fortifying 100 mL of laboratory reagent blank solution with mercury to a suitable concentration of >10X the MDL, but ≤the midpoint of the calibration curve. The LFB must be carried through the entire sample preparation scheme.

7.12 Instrument performance check (IPC) solution: The IPC solution is used to periodically verify instrument performance during analysis. It must contain all reagents in the same concentration as the calibration solutions and mercury at an appropriate concentration to approximate the midpoint of the calibration curve. The IPC solution should be prepared from the same intermediate mercury stock standard (Sect. 7.6) as used to prepare the calibration solutions.

7.13 Quality control check sample (QCS): For initial and periodic verification of calibration standards and instrument performance, analysis of a QCS is required. The QCS must be obtained from an outside source different from the standard stock solution, but prepared in the same manner as the calibration solutions. The concentration of the mercury in the QCS solution should be such that the resulting solution will provide an absorbance reading near the midpoint of the calibration curve. The QCS should be analyzed quarterly or more frequently as needed to meet data-quality needs.

7.14 Hydrochloric acid (HCl) (CAS RN 7647-01-0), Conc. (specific gravity 1.17): Assayed mercury level is not to exceed 1 μg/L.

7.14.1 Hydrochloric acid 3% v/v: Dilute 30.0 mL of concentrated hydrochloric acid to 1.0 liter.

8.0 Sample Collection, Preservation, and Storage

8.1 Because of the extreme sensitivity of the analytical procedure and the presence of mercury in a laboratory environment, care must be taken to avoid extraneous contamination. Sampling devices, sample containers, and plastic items should be determined to be free of mercury; the sample should not be exposed to any condition in the laboratory that may result in contamination from airborne mercury vapor.

8.2 Samples should be collected in a 1 L plastic container filled to the top, acidified with nitric acid to a pH less than 2. For the determination of total mercury (inorganic plus organic) in aqueous samples, samples are not filtered, but acidified with (approximately 1:1) nitric acid (Sect. 7.3.1) to pH < 2 (normally, 3 mL of (approximately 1:1) nitric acid per liter of sample is sufficient for most ambient and drinking water samples). Preservation may be done at the time of collection: however, to avoid the hazards of strong acids in the field, transport restrictions, and possible contamination, it is recommended that the samples be returned to the laboratory as soon as possible after collection and acid preserved upon receipt in the laboratory. Following acidification, the sample should be mixed, held for sixteen hours, and then verified to be pH < 2 just prior to withdrawing an aliquot for processing. If for some reason, such as high alkalinity, the sample pH is verified to be > 2, more acid must be added and the sample held for an additional sixteen hours until verified to be pH < 2. The preserved sample must be analyzed within 28 days of collection.

Note: When the nature of the sample is either unknown or is known to be hazardous, acidification should be done in a fume hood. See Section 5.3.

8.3 A field blank should be prepared and analyzed as required by the data user. Use the same container and acid as used in sample collection.

9.0 Quality Control

9.1 Each laboratory using this method in regulated environmental monitoring is required to operate a formal quality assurance/control program. The minimum initial requirements of this program consist of an initial demonstration of laboratory capability by analysis of laboratory reagent blanks, fortified blanks, and samples used for continuing check on method performance. Commercially available water

quality control samples are acceptable for routine laboratory use. The laboratory is required to maintain performance records that define the quality of the data generated.

9.2 Initial Demonstration of Performance (mandatory).

9.2.1 The initial demonstration of performance is used to characterize instrument performance (determination of linear dynamic ranges and analysis of quality control samples) and laboratory performance (determination of method detection limits) prior to analyses conducted by this method.

9.2.2 Linear dynamic range (LDR): The upper limit of the LDR must be established. It must be determined from a linear calibration prepared from a minimum of three different calibration standards, one of which is close to the upper limit of the linear range. The LDR should be determined by analyzing succeedingly higher standard concentrations until the observed analyte concentration is no more than 10% below the stated concentration of the standard. The determined LDR must be documented and kept on file. The LDR which may be used for the analysis of samples should be judged by the analyst from the resulting data. Determined sample analyte concentrations that are greater than 90% of the determined upper LDR limit must be diluted and reanalyzed. The LDR should be verified annually or whenever, in the judgment of the analyst, a change in analytical performance caused by either a change in instrument hardware or operating conditions would dictate they be redetermined.

9.2.3 The initial analysis of four aliquots of a mid range standard (i.e., digestion through atomic absorption measurement) is required. The average percent recovery and the standard deviation of the four percent recoveries must be calculated using the following equations:

$$\text{Ave. \%R} = \frac{\left(\sum_{i=1}^{n} x\right)}{n}$$

$$s_p = \sqrt{\frac{\sum_{i=1}^{n} x^2 - \frac{\left(\sum_{i=1}^{n} x\right)^2}{n}}{n-1}}$$

Where:

Ave. %R:	Average percent recovery
s_p:	Standard deviation of percent recoveries
n:	Number of samples
x:	Individual percent recovery

9.2.4 Quality control check sample (QCS): When beginning the use of this method, on a quarterly basis, after the preparation of stock or calibration standard solutions or as required to meet data quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analysis of a QCS (Sect. 7.13). To verify the calibration the QCS must be within $\pm$ 10% of the stated value. If the calibration standard cannot be verified, performance of the determinative step of the analysis is unacceptable. The source of the problem must be identified and corrected before either proceeding on with the initial determination of method detection limits or continuing with on-going analyses.

9.2.5 Method detection limits (MDL): A mercury MDL must be established using an LRB solution fortified at a concentration of two to three times the estimated detection limit. To determine MDL values, take seven replicate aliquots of the fortified LRB and process through the entire analytical method. Perform all the calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows[9]:

MDL = (t) (s)

Where:

t : Students' t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates]
s : Standard deviation of the replicate analyses.

Note: If the relative standard deviation (RSD) from the analyses of the seven aliquots is < 10%, the concentration used to determine the mercury MDL may have been inappropriately high for the determination. If so, this could result in the calculation of an unrealistically low MDL. Concurrently, determination of MDL in a LRB solution represents a best-case situation and does not reflect possible matrix effects of real world samples. However, successful analyses of LFMs (Sect. 9.4) can give confidence to the MDL value determined in LRB solution.

The MDL must be sufficient to detect mercury at the required level according to compliance monitoring regulation (Sect. 1.2). The mercury MDL should be determined annually, when a new operator begins work or whenever, in the judgment of the analyst, a change in analytical performance caused by either a change in instrument hardware or operating conditions would dictate they be redetermined.

9.3 Assessing Laboratory Performance (mandatory).

9.3.1 Laboratory reagent blank (LRB): The laboratory must analyze at least one LRB (7.11.2) with each batch of 20 or fewer samples of the same matrix. LRB data are used to assess contamination from the laboratory environment. LRB values that exceed the MDL indicate laboratory or reagent contamination should be suspected. When an LRB value is equal to 10% or more of the analyte level determined for a sample or is 2.2 times the analyte MDL, whichever is greater, fresh aliquots of the samples must be prepared and analyzed again for the affected analytes after the source of contamination has been corrected and acceptable LRB values have been obtained.

9.3.2 Laboratory fortified blank (LFB): The laboratory must analyze at least one LFB (7.11.3) with each batch of samples. Calculate accuracy as percent recovery using the following equation:

$$R = \frac{(LFB-LRB)}{C}(100)$$

Where:

R: % Recovery
LFB: Mercury concentration observed in the laboratory fortified blank
LRB: Mercury concentration observed in the laboratory reagent blank
C: Mercury concentration added to the LFB

In drinking water monitoring, the control limits for the percent recovery of mercury from a laboratory reagent blank are 85-115%. The aforementioned control limits for the percent recovery of mercury from a laboratory reagent blank are also suggested for use in wastewater monitoring until the 1994 version of EPA Method 245.1 or other limits are promulgated in wastewater regulations.

9.3.3 The laboratory must use LFB analysis data to assess laboratory performance against the required control limits of 85-115% (Sect. 9.3.2). When sufficient internal performance data becomes available (usually a minimum of twenty to thirty analyses), optional control limits can be developed from the mean percent recovery (x) and the standard deviation (s_p) of the mean percent recovery. These data can be used to establish the upper and lower control limits as follows:

$$\text{Upper Control Limit} = x + 3s_p$$
$$\text{Lower Control Limit} = x - 3s_p$$

The optional control limits must be equal to or better than the required control limits of 85-115%. After each five to ten new recovery measurements, new control limits can be calculated using only the most recent twenty to thirty data points. Also, the standard deviation (s_p) data should be used to establish an on-going precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

9.3.4 Instrument performance check (IPC) solution: For all determinations the laboratory must analyze the IPC solution (7.12) and a calibration blank immediately following each calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. Analysis of the calibration blank should always be < the MDL. Analysis of the IPC solution immediately following calibration must verify that the instrument is within $\pm$ 5% of the calibration. Subsequent analyses of the IPC solution must be within $\pm$ 10% of calibration. If the calibration cannot be verified within the specified limits, analysis must be discontinued, the cause determined and/or in the case of drift the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution must be kept on file with the sample analyses data.

9.4 Assessing Analyte Recovery and Data Quality

9.4.1 Sample homogeneity and the chemical nature of the sample matrix can affect mercury recovery and the quality of the data. Taking separate aliquots from the sample for replicate and fortified analyses can in some cases assess the effect. Unless otherwise specified by the data user, laboratory or program, the following laboratory fortified matrix (LFM) procedure (9.4.2) is required.

9.4.2 The laboratory must add a known amount of mercury to a minimum of 10% of the samples or one sample per sample set, whichever is greater. In each case the LFM aliquot must be a duplicate of the aliquot used for sample analysis. Select a sample with a low mercury background that is representative of the type of water samples being analyzed. It is recommended that this sample be analyzed prior to fortification. The concentration of mercury added may vary based on the nature of the samples being analyzed. When possible, the concentration should be the same as that added to the LRB, but should not exceed the midpoint concentration of the calibration curve. It is suggested that one QC sample should contain an organic mercury spike to monitor the effectiveness of the sample digestion. Over time, samples from all routine sample sources should be fortified.

9.4.3 Calculate the percent recovery, corrected for background concentration measured in the unfortified sample aliquot, and compare these values to the control limits to the designated LFM recovery range of 70-130% for drinking water samples. Percent recovery may be calculated using the following equation:

$$R = \frac{(LFM - LSM)}{C} (100)$$

Where:

R: % Recovery
LFM: Mercury concentration observed in the laboratory fortified matrix sample
LSM: Mercury concentration observed in the laboratory sample matrix
C: Mercury concentration added to the LFM

The aforementioned control limits for the percent recovery of mercury from a fortified sample matrix are also suggested for use in wastewater monitoring until the 1994 version of EPA Method 245.1 or other limits are promulgated in wastewater regulations.

9.4.4 If mercury recovery falls outside the designated range, and the laboratory performance is shown to be in control (9.3), the sample recovery problem encountered with the fortified drinking water or wastewater sample is judged to be matrix related, not system related. The result for mercury in the unfortified sample must be labeled to inform the data user that the results are suspect due to matrix effects.

9.4.5 Compute the relative percent difference (RPD) between two duplicate spiked sample results using the following formula:

$$RPD = \frac{100(LD_2 - LD_1)}{(LD_1 + LD_2)/2}$$

Where:

RPD: Relative percent difference
LD_1: The first duplicate spiked sample result
LD_2: The second spiked sample (duplicate) result

10.0 Calibration and Standardization

10.1 Set up FIAS (used in conjunction with an atomic absorption spectrophotometer) or FIMS stand-alone system.

10.1.1 Assemble the Flow Injection System as shown in *Figure 2 or 3*. Set up the operating conditions as specified in Table III. For more detailed information refer to instrument manual provided with the Flow Injection system. Turn on the autosampler and atomic absorption spectrophotometer. All instrument parameters recommended by the manufacturer should be used.

10.1.2 Align the FIAS quartz cell in the optical path.

10.1.3 Allow the system to warm up for 30 minutes.

10.1.4 Feed all reagents through the system (see *Figures 1,2, and 3).* Refer to the manual supplied with the Flow Injection for more detailed information.

10.2 Mercury calibration standards: Transfer 0, 0.5, 1.0, 5.0, 10.0, and 20.0 mL aliquots of the intermediate stock mercury solution containing 0.1 μg Hg (7.6) to a series of sample containers containing 40 mL of reagent water. Add enough reagent water to make a total volume of 100 mL and mix thoroughly. Prepare calibration standards daily. Process as described in Sections 11.1.2 and 11.1.3.

10.3 Cool the solution and add 6 mL of sodium chloride-hydroxylamine hydrochloride solution (7.6) to each sample container to reduce the excess permanganate. When the solution becomes decolorized transfer to the autosampler

10.4 Prepare the calibration curve by plotting peak absorbance of the known standards versus concentration. The standard curve must comply with Sect. 9.2.2. Figure 5 shows a typical FIAS calibration curve and Figure 6 shows a typical FIMS calibration curve.

11.0 Procedure

11.1 Sample Digestion

11.1.1 Transfer 100 mL of sample [or an aliquot of the sample diluted with reagent water (7.2) to 100 mL], into a sample digestion bottle (6.5.2).

11.1.2 Add 5 mL of sulfuric acid (7.2) and 2.5 mL of nitric acid (7.3) to the container.

11.1.3 Next add 15 mL of potassium permanganate solution (7.7) to each sample. Sewage samples and samples containing high salts may require additional permanganate. If necessary, add 15 mL portions of the potassium permanganate solution until the purple color persists for at least 15 minutes. Be sure to mix sample after each addition. The same amount of potassium permanganate solution should be added to the standards and blanks as that added to the samples.

Add 8 mL of potassium persulfate (7.8) to each bottle, lay the stopper across the neck, and heat for 2 hours in a covered water bath at 95 ^{o}C.

11.1.4 Remove the sample containers from the water bath and cool to room temperature.

11.1.5 When the samples are at room temperature. to each container add 6 mL of sodium chloride-hydroxylamine hydrochloride (7.9) to reduce the excess permanganate. For samples containing high chlorides, continue to add the sodium chloride-hydroxylamine hydrochloride (up to 25 mLs) until the potassium permanganate is completely reduced. Be certain to mix the sample after each addition.

11.1.6 Transfer the samples to an autosampler.

11.2 Sample Analysis

11.2.1 Before beginning daily calibration, the instrument should be reconfigured to the optimized conditions. Allow the system to stabilize.

11.2.2 Following calibration, the digested samples are analyzed in the same manner as the standard solutions.

11.2.3 At the completion of the analysis all tubing should be flushed with deionized water and then air. Release the pump tension on all tubing.

11.2.4 During the analysis of samples, the laboratory must comply with the required quality control described in Sections 9.3 and 9.4.

12.0 Data Analysis and Calculations

12.1 From the prepared calibration curve (10.5) compute sample concentration by comparing response with the standard curve. The FIAS and FIMS systems will report the sample concentration directly.

12.2 The concentration of mercury in the sample is that derived from either a calibration graph or read directly from the instrument and must be corrected for any dilution performed before the digestion. Thus the observed concentration is multiplied by the dilution factor as follows:

Dilution Factor = T/A

Where:

A: Volume of aliquot taken for dilution (mL)
T: Total volume to which the sample aliquot is diluted (mL)

12.3 Report mercury concentrations to the proper significant figures in mg/L, μg/L, or ng/L as required.

13.0 Method Performance

13.1 The performance of the method in a single laboratory is shown in Table IV for drinking and wastewater spiked with organic or inorganic mercury. Table V shows the recoveries of mercury spikes in drinking water, groundwater, and an National Institute of Standards and Technology (NIST) reference material.

14.0 Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

Flow injection techniques reduce the volume of waste by requiring smaller amounts of reagents during the determinative step, because of the smaller volume of sample used. If it is possible to reduce the volume of sample digested, the waste generated will be further reduced.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions, consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's Department of Government Relations and Science Policy, 115 16th Street N.W., Washington, D.C. 20036, (202) 872-4477.

15.0 Waste Management

15.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hood and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult The Waste Management Manual for Laboratory Personnel, available from the American Chemical Society at the address listed in Section 14.2.

16.0 References

16.1. EPA Method 245.1, In: Methods for the Determination of Metals in Environmental Samples, Supplement 1, May 1994, EPA/600/R-94/111, Cincinnati, OH.

16.2 Perkin-Elmer FIAS Installation and System Description, Part Number B050-8399 ,The Perkin-Elmer Corporation, 761 Main Ave. Norwalk, CT, 06859 (1992).

16.3 Perkin-Elmer FIAS Setting Up and Performing Analyses, Part Number B050-8400, The Perkin-Elmer Corporation, 761 Main Ave., Norwalk, CT 06859 (1992).

16.4 Perkin-Elmer FIAS Recommended Analytical Conditions and General Information, Part Number B050-1820, The Perkin-Elmer Corporation, 761 Main Ave, Norwalk, CT 06859 (1994).

16.5 Perkin-Elmer FIMS Installation, Maintenance, and System Description, Part Number 0993-5202, The Perkin-Elmer Corporation, 761 Main Ave, Norwalk, CT 06859 (1994).

16.6 Perkin-Elmer FIMS Software Guide, Part Number 0993-5215, The Perkin-Elmer Corporation, 761 Main Ave, Norwalk, CT 06859 (1994).

16.7 Perkin-Elmer FIMS Setting Up and Performing Analyses, Part Number 0993-5203, The Perkin-Elmer Corporation, 761 Main Ave, Norwalk, CT 06859 (1994).

16.8 "Specification for Reagent Water", D1193, Annual Book of ASTM Standards, Vol. 11.01 (1990).

16.9 Code of Federal Regulations 40, Pt. 136, Appendix B (July 1, 1996).

17.0 Tables and Figures

Table I
Comparison of Method Detection Limits

Instrument Configuration	Method Detection Limit (MDL)
Flow Injection Analysis System (FIAS)/Hollow Cathode Lamp (HCL)	0.2 μg/L
Flow Injection Analysis System (FIAS)/Electrodeless Discharge Lamp (EDL)	0.06 μg/L
Flow Injection Mercury System (FIMS)	0.01 μg/L

Table II
Sample Volume Effects on FIMS Linearity and Instrument Detection Limit (IDL)

Sample Loop (μL)	IDL (ppt)	Linear Range (μg/L)
500	6	20
200	12	30
100	25	50

Table III
Recommended FIAS Operating Conditions

Wavelength	253.7 nm
Slit	0.7 nm
Cell Temperature	100°C
Sample Volume	500 μL
Notes: Argon carrier gas flow = 60-100 mL/min Electrodeless Discharge lamp (EDL) used 3% HCl carrier flow rate = 8 mL/min 1.1% $SnCl_2$ reductant flow rate = 4 mL/min	

Table IV
Precision and Recovery for Mercury-Spiked Waters using the FIAS

Sample	Hg Spike (μg/L)	Mean (μg/L)	SD	% RSD	% Recovery
Wastewater #1	1.00	0.96	0.04	4.3	96
Wastewater #2	1.00	1.04	0.06	5.4	104
Drinking Water #1	1.00	0.99	0.04	4.4	99
Drinking Water #2	1.00	1.08	0.05	4.4	108

Notes:
SD: Standard deviation
% RSD where N = 20
Spiking solutions:
For samples #1 = 1.00 μg/L Hg ($HgCl_2$)
For samples #2 = 1.00 μg/L Hg (2-Ethylmercurimercaptobenzoic acid, sodium salt)

Table V
Recovery from Inorganic Mercury-Spiked Water Samples Using the FIMS

Sample	Hg Spike (μg/L)	Mean (μg/L)	SD	% RSD	% Rec
Ground water	1.4	1.41	0.003	0.2	101
Drinking water	1.4	1.38	0.02	1.4	99
NIST 1641c diluted to 0.2 μg/L	--	0.184	0.004	2.2	92
Wastewater	1.4	1.36	0.006	0.4	97

Notes:
SD: Standard Deviation
% RSD where N = 3

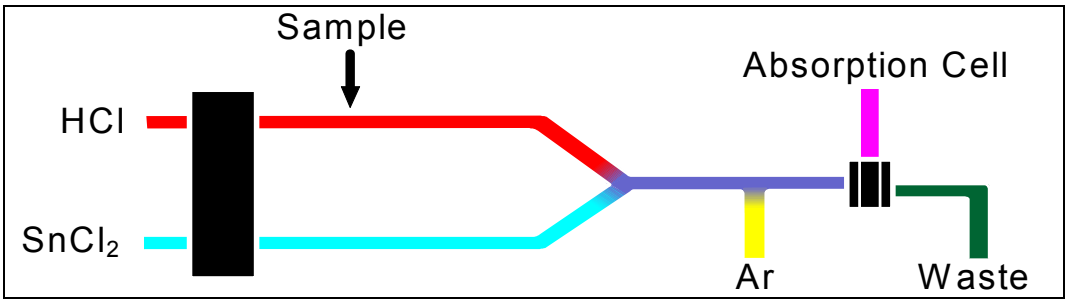

Figure 1. General schematic of flow injection.

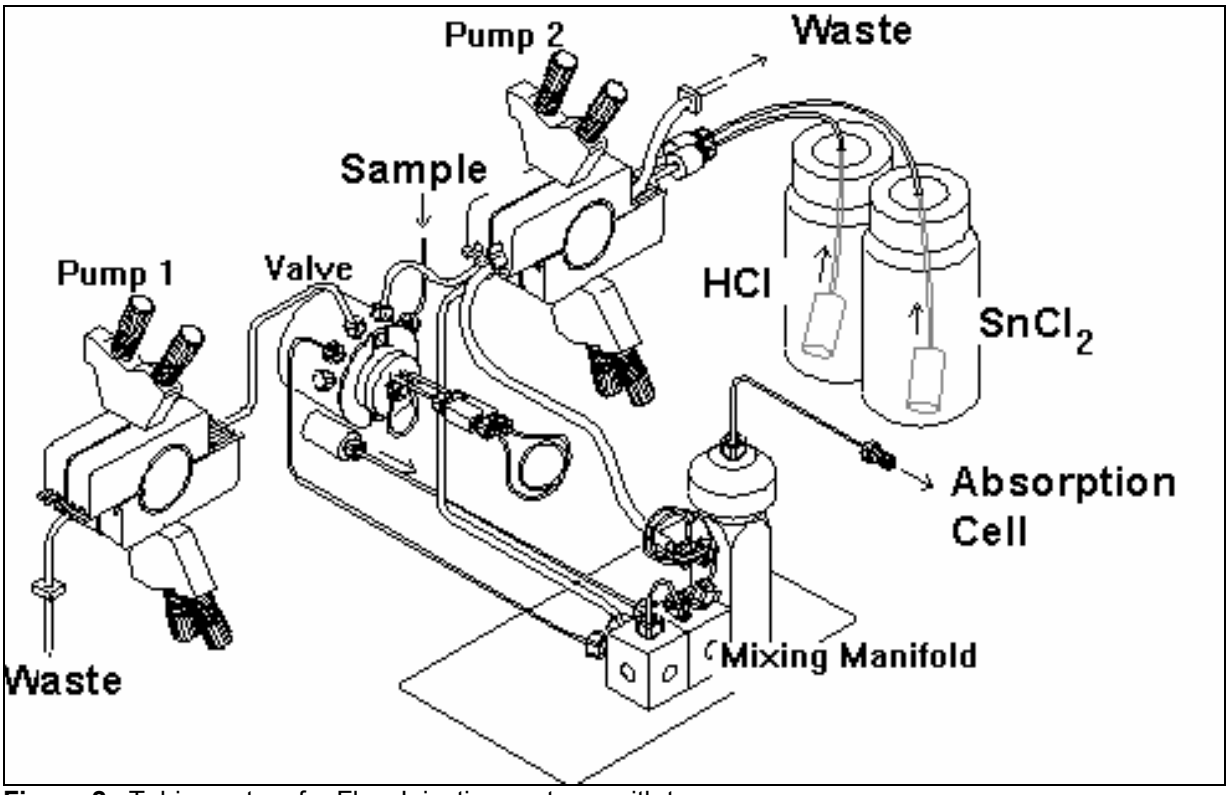

Figure 2. Tubing set up for Flow Injection systems with two pumps.

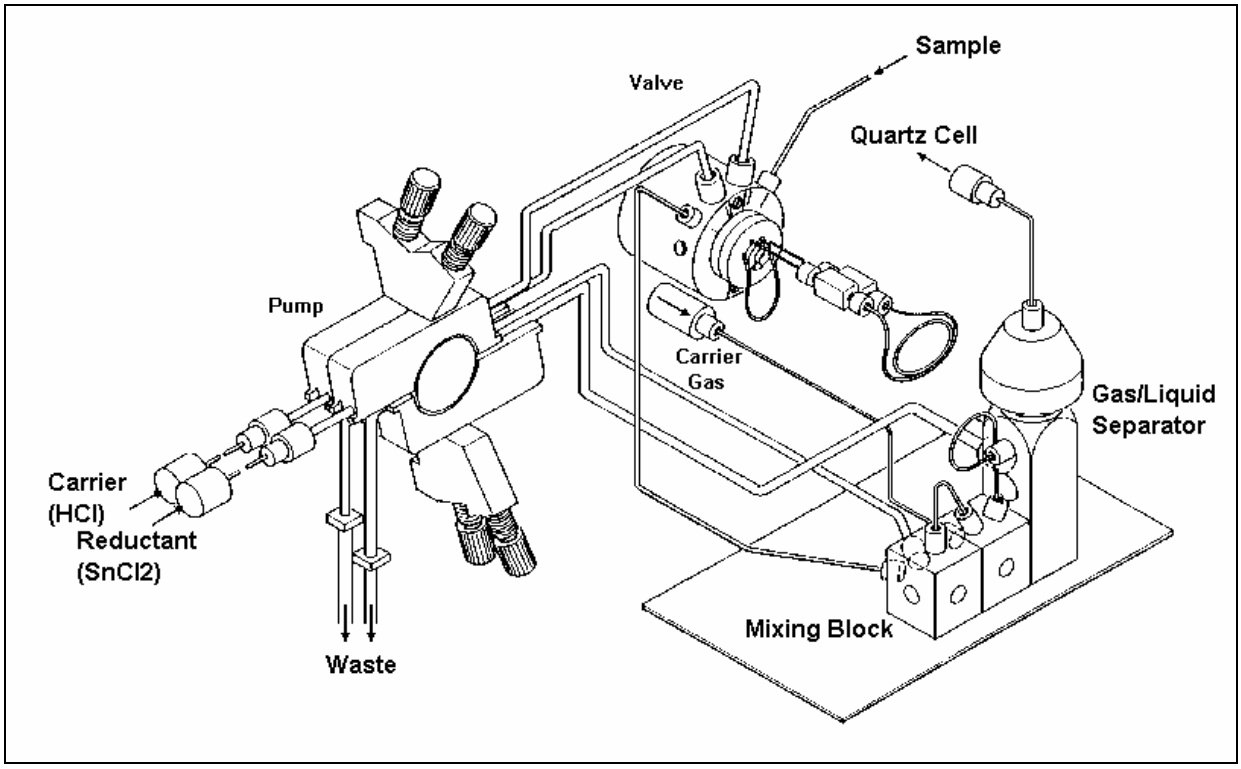

Figure 3. Tubing set up for Flow Injection Systems with one pump.

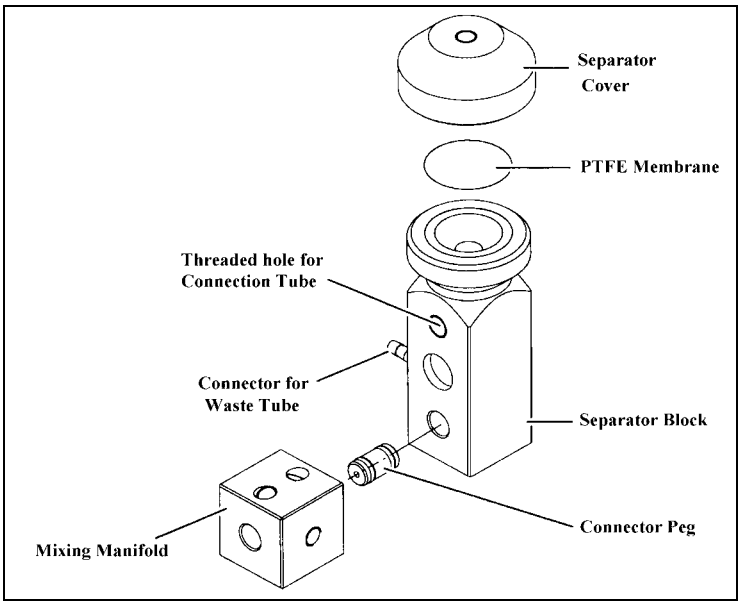

Figure 4. Mixing Manifold and Gas/Liquid Separator

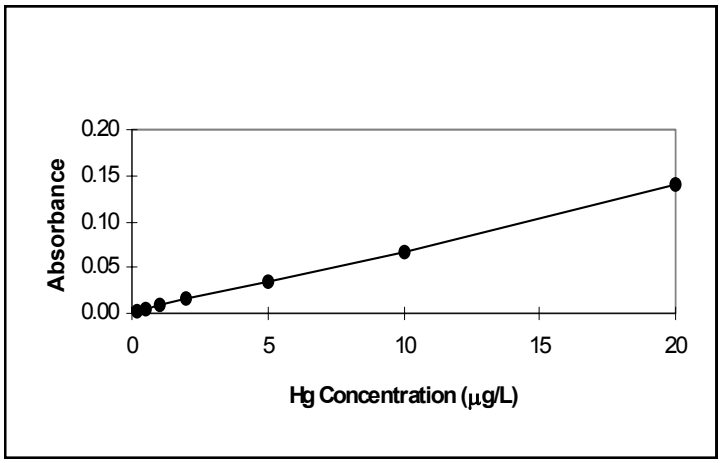

Figure 5. Typical FIAS calibration curve using 500 μL of sample.

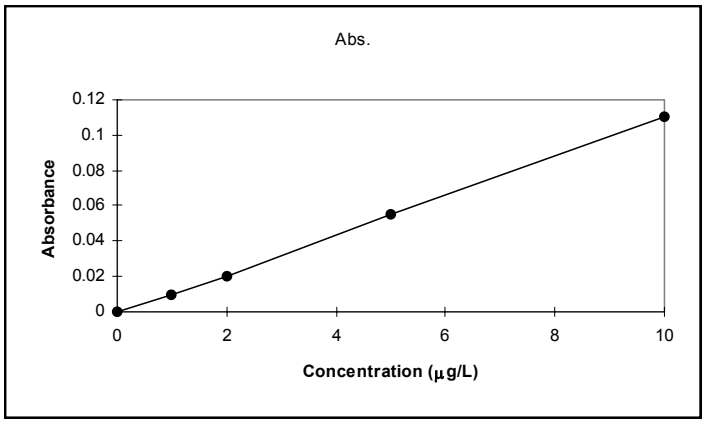

Figure 6. Typical FIMS calibration curve using 500 μL of sample.

Appendix 1

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL EXPOSURE RESEARCH LABORATORY
CINCINNATI, OH 45268

January 13, 1997

OFFICE OF
RESEARCH AND DEVELOPMENT

Ms Susan McIntosh
Ms Zoe Grosser
Perkin Elmer Corporation
761 Main Street
Norwalk, CT 06859-0001

Dear Ms McIntosh and Ms Grosser:

We are pleased to inform you that in the judgment of our technical staff, proposed Perkin Elmer Method ENVA-100, "The Application of Flow Injection Technology to Automating Cold Vapor Mercury Analyses", dated January 9, 1997, is an acceptable version of the 1994 version of Method 245.1, approved by EPA for determining mercury in drinking water. Therefore, fulfillment of the alternate test procedure study requirements is not necessary.

The Alternate Test Procedure Program Staff appreciates your interest and endeavors in the development of environmental methods.

Sincerely yours,

M. Kate Smith

M. Kate Smith, Ph.D.
Director
Ecological Exposure Research Division

cc: Office of Ground Water and Drinking Water (4601), Office of Water, U.S. Environmental Protection Agency, 401 M Street, S.W., Washington, D.C. 20460
304(h) Committee Chairman
USEPA Regional Administrators (All regions)
Quality Assurance Managers (All regions)
Environmental Services Division Chiefs (All regions)
Director, Water Management Division (All regions)

Appendix 2

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

FEB 26 1997

OFFICE OF
WATER

Ms. Susan McIntosh
Ms. Zoe Grosser
Perkin Elmer Corporation
761 Main Street
Norwalk, CT 06859-0001

Dear Ms. McIntosh and Ms. Grosser:

I am pleased to inform you that in the judgement of the technical staff at EPA's National Exposure Research Laboratory in Cincinnati, Ohio (NERL-Ci), Perkin Elmer Method ENVA-100, "The Application of Flow Injection Technology to Automating Cold Vapor Mercury Analyses," dated January 9, 1997, is an acceptable version of the 1994 version of Method 245.1, approved at 40 Code of Federal Regulations Part 136 for use in National Pollutant Discharge Elimination System compliance monitoring. Therefore, fulfillment of the alternate test procedure study requirements is not necessary.

Thank for your efforts in the development of environmental methods.

Sincerely,

William A. Telliard, Director
Analytical Methods Staff
Engineering and Analysis Division (4303)

cc: James O'Dell, ATP Coordinator, Ecological Exposure Research Division
M. Kate Smith, Ph. D., Director, Ecological Exposure Research Division
Office of Ground Water and Drinking Water (4601)
304(h) Committee Chairman
USEPA Regional Administrators (all Regions)
Quality Assurance Managers (all Regions)
Environmental Services Division Chiefs (all Regions)
Water Management Division Directors (all Regions)

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