EPA 600/4-81-055

United States
Environmental Protection
Agency

EPA

Research and Development

Interim Methods For The Sampling and Analysis of Priority Pollutants in Sediments and Fish Tissue

Prepared for

Regional Guidance

Prepared by

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DRAFT

Interim Methods for the Sampling and Analysis of Priority Pollutants in Sediments and Fish Tissue

U. S. Environmental Protection Agency
Environmental Monitoring and Support Laboratory
Cincinnati, Ohio 45268

August 1977
Revised October 1980

FOREWORD

This collection of draft methods for the analyses of fish and sediment samples for the priority pollutants was originally prepared as guidance to the Regional Laboratories. The intention was to update and revise the methods as necessary if and when shortcomings and/or problems were identified. Some problems such as the formation of soap in the "phenol in fish" method have been identified. Consequently, this method has been deleted. Additionally, both the sediment and fish methods for volatile organics by purge and trap analysis have been replaced. Other editorial and technical changes have also been made to the original methods.

It is the intention of the Environmental Monitoring and Support Laboratory - Cincinnati (EMSL-Cincinnati) to improve and correct methods as necessary. Consequently, the user of these methods would be providing EPA a service in calling our attention to any problems and in making suggestions to improve the methods. Comments should be directed to:

Director, Environmental Monitoring and Support Laboratory
26 W. St. Clair Street
Cincinnati, Ohio 42568

SAMPLE HANDLING

1. Collection

1.1 Samples shall be collected according to recognized procedures. Preferably, all analyses should be performed on the same sample. A minimum of 250 grams are required for the total protocol.

1.2 The recommended container for the sediment sample is a standard one-quart, wide-mouth, screw-cap, glass bottle with a Teflon lid liner. It is particularly important that glassware used in organic residue analyses be scrupulously cleaned before initial use. At the time of collection, the bottle should be filled nearly to the top with the sediment sample. If the sample is collected below a water column, the threads and sealing surfaces should be washed off with sample water. "Top off" the collected sediment sample with sample water and seal with the Teflon-lined screw cap. Maximum effort must be made to seal the sample with a minimum of gaseous headspace. The sample must remain sealed until the aliquots for volatile organics are taken for analyses.

1.3 In the case of small fish, a sufficient number should be combined by sampling site location and species to obtain the minimum weight. The collected samples are wrapped in aluminum foil, labeled with freezer tape, and placed in the freezer chest with dry ice.

2. Preservation

2.1 The sediment sample should be labeled with freezer tape and transferred to the laboratory in an ice chest maintained at or near $4^{o}C$. The samples should be processed as soon as possible.

2.2 Fish samples are to be frozen at the time of collection and must remain frozen until the subsamples are taken for purgeable organics.

3. Processing

3.1 Sediment

3.1.1 Decant the water from the top of the sediment. Transfer the sediment into a Pyrex tray and mix thoroughly with a Teflon spatula. Discard sticks, stones, and other foreign objects, if present. Weigh five 10.0-gram portions of the sample into separate 125-ml vials. Using a crimper, tightly secure a septum to each bottle with an aluminum seal. Store these sample aliquots in a freezer until ready for volatile organics analysis.

3.1.2 Determine the percent solids in the sediment by drying a 10-25g portion in a tared evaporating dish, overnight, at $103^{o}C$.

Calculate the % solids using the equation:

$$\% \text{ solids} = \frac{A}{B} \times 100$$

where: A = weight of dry residue in grams

B = weight of wet sample in grams

3.1.3 Transfer half of the remaining sediment sample back to the original sample bottle and store at $4^{o}C$. This portion will be used for those analyses requiring a wet sample.

Spread the other half of the sample uniformly in the tray and allow to dry at room temperature for four or five days in a contaminant-free environment. When dry - less than 10% water - grind the sample with a large mortar and pestle to a uniform particle size. Discard any foreign objects found during grinding and transfer the powdered sediment into a wide-mouthed glass jar and seal with a Teflon-lined lid. This air-dried sample will be used for those analyses requiring an air-dried sample.

3.2 Fish

3.2.1 To prepare the fish sample for analytical pretreatment, unwrap and weigh each fish. Combine small fish by site and species until a minimum combined weight of 250g is obtained. Chop the sample into 1-inch chunks using a sharp knife and mallet.

3.2.2 Grind the sample using a large commercial meat grinder that has been precooled by grinding dry ice. Thoroughly mix the ground material. Regrind and mix material two additional times. Clean out any material remaining in the grinder; add this to the sample and mix well.

3.2.3 Weigh five 10.0g portions of the sample into separate 125-ml vials. Using a crimper, tightly secure a septum to each bottle with a seal. Store these sample aliquots in a freezer until ready for volatile organics analysis.

3.2.4 Transfer the remaining fish sample to a glass container and store in a freezer for later subsampling and analysis.

4. Special Equipment and Materials

4.1 Ice chest.

4.2 Wide-mouth quart bottles with Teflon lid liners.

4.3 Teflon-coated or porcelain spatula.

4.4 Pyrex glass tray, 8x12x2-inch.

4.5 Mortar and pestle (large).

4.6 Knife, heavy blade (or meat cleaver).

4.7 Mallet, plastic faces, 2 to 3 lb.

4.8 Electric meat grinder, 1/2 HP.

4.9 Dry ice.

4.10 Aluminum foil

4.11 Freezer tape, for labels.

4.12 Freezer.

4.13 Vials, 125-ml Hypo-Vials (Pierce Chemical Co., #12995), or equivalent.

4.14 Septa, Tuf-Bond (Pierce #12720), or equivalent.

4.15 Seals, aluminum (Pierce #13214), or equivalent.

4.16 Crimper, hand (Pierce #13212), or equivalent.

Analysis of Sediments for Chlorinated Pesticides,
Polychlorinated Biphenyls and Non-polar Neutrals

1. Scope

1.1 The compounds listed in Table I are extracted from air-dried sediment by the Soxhlet extraction technique. The extract is subsequently analyzed for pesticides and PCBs using approved methods (1) as cited in the Federal Register (2). The remaining compounds are determined using the methods described in Appendix II of the Federal Register (3). While the above referenced methods have been proven for pesticides and PCBs, they have not been sufficiently tested through extensive experimentation for the non-polar neutral compounds in Table I.

2. Special Apparatus and Materials

2.1 Soxhlet extractor, 40-mm ID, with 500-ml round bottom flask.

2.2 Kuderna-Danish, 500-ml, with 10-ml graduated receiver and 3-ball Snyder column.

2.3 Chromatographic column - Pyrex, 20-mm ID x approximately 400-mm long, with coarse fritted plate on bottom.

3. Procedure

3.1 Extraction

3.1.1 Weigh 30.0 grams of the previously air-dried sample into a tared 200-ml beaker. Add 3 ml distilled water (10% of

sample weight), mix well and allow to stand for 2 hours while mixing occasionally.

3.1.2 Place about 1/2" of preextracted glass wool in the bottom of the Soxhlet extractor chamber and quantitatively transfer the contents of the beaker into the chamber. Place a second glass wool plug on top of the sample. Wash the 200-ml beaker and all mixing tools several times with a 1:1 hexane/acetone mixture. Cycle the wash mixture through the extractor using a total of 300 ml of the mixed solvent.

3.1.3 Attach the extractor to a 500-ml round bottom flask containing a boiling stone and extract the solids for 16 hours.

3.1.4 After extraction is complete, dry and filter the extract by passing it through a 4" column of hexane-washed sodium sulfate. Wash the 500-ml flask and the sodium sulfate with liberal amounts of hexane. Collect the eluate in a 500-ml K-D evaporative flask with a 10-ml ampul. Concentrate the sample extract to 6-10 ml.

3.2 Cleanup and Separation

3.2.1 Adjust the sample extract volume to 10 ml and clean up the extraction by Florisil column chromatography according to the 304(g) methodology for PCBs (1), part 10.3. For sulfur removal, continue with part 10.5.3.4 of that method. NOTE: If sulfur crystals are present in the extract, separate the crystals from the sample by decantation.

3.2.2 Analyze the Florisil eluates for the pesticides and PCBs appearing in Table I, according to the approved methods (1).

3.2.3 Analyze remaining compounds of Table I, Column C, using the methods described in Appendix II of the Federal Register (3).

3.3 Standard quality assurance protocols should be employed, including blanks, duplicates and dosed samples as described in the "Analytical Quality Control Handbook" (4). Dosing can be accomplished by injecting 1-20 µl of a standard into the homogenized sediment contained in the Soxhlet extractor chamber.

4. Reporting of Data

4.1 Report results in µg/kg on a dry weight basis using the percent moisture values determined earlier. Report all quality control data with the analytical results for the samples.

Analysis of Fish for Chlorinated Pesticides and Polychlorinated Biphenyls

1. Scope

1.1 The chlorinated pesticides and polychlorinated biphenyls (PCBs) listed in Table I are extracted from fish using either method A or B as described below. Method A employs a blender, whereas a Tissumizer or the equivalent is required for Method B. Either procedure results in an extract that can be incorporated directly into the approved procedures (1) for pesticides or PCBs as cited in the Federal Register (2).

2. Special Apparatus and Materials

2.1 Method A Only

2.1.1 Blender, high-speed - Waring Blender, Courdos, Omni-Mixer, or equivalent. Explosion proof model recommended. Quart container is suitable size for routine use.

2.1.2 Buchner funnel - porcelain, 12-cm.

2.1.3 Filter paper - 110 mm sharkskin circles.

2.1.4 Flask, vacuum filtration - 500 ml.

2.2 Method B Only

2.2.1 Tissumizer SDT-182EN (available from Tekmar Company, P. O. Box 37207, Cincinnati, Ohio, 45222), or equivalent.

2.2.2 Centrifuge - capable of handling 100 ml centrifuge tubes.

2.3 Method A & B

2.3.1 Kuderna-Danish concentrator - 500 ml, with 10-ml graduated receiver and 3-ball Snyder column.

2.3.2 Chromatographic column - pyrex, 20 mm ID x approximately 400 mm long, with coarse fritted plate on bottom.

3. Procedures

3.1 Method A

3.1.1 Weigh a 25 to 50g portion of frozen, ground fish and add to a high-speed blender. Add 100g anhydrous Na_2SO_4 to combine with the water present and to disintegrate the sample. Alternately, blend and mix with a spatula until the sample and sodium sulfate are well mixed. Scrape down the sides of the blender jar and break up the caked material with the spatula. Add 150 ml of hexane and blend at high speed for 2 min.

3.1.2 Decant the hexane supernatant through a 12-cm Buchner filter with two sharkskin papers into a 500-ml suction flask. Scrape down the sides of the blender jar and break up the caked material with the spatula. Reextract the residue in the blender jar with two 100 ml portions of hexane, blending 3 min. each time. (After one min. of blending, stop the blender, scrape the material from the sides of the blender jar, and break up the caked material between extractions.)

3.1.3 Decant the hexane supernatants through the Buchner and combine with the first extract. After the last blending, transfer the residue from the blender jar to the Buchner, rinsing the blender jar and material in the Buchner with three 25 to 50 ml portions of hexane. Immediately after the last rinse, press the residue in the Buchner with the bottom of a clean beaker to force out the remaining hexane.

3.1.4 Pour the combined extracts and rinses through a column of anhydrous Na_2SO_4, 20 mm x 100 mm, and collect the eluate in a 500 ml Kuderna-Danish concentrator. Wash the flask and then the column with small portions of hexane and concentrate the extract below 10 ml.

3.2 Method B

3.2.1 Weigh 20.0g of frozen, ground fish and add to a 100-ml centrifuge tube. Add 20 ml of hexane and insert the Tissumizer into the sample. Turn on the Tissumizer and disperse the fish in the solvent for 1 min. Centrifuge and decant the solvent through a column of anhydrous Na_2SO_4, 20 mm x 100 mm, and collect the eluate in a 500-ml Kuderna-Danish concentrator.

3.2.2 Repeat the dispersion twice using a 20-ml aliquot each time, combining all dried portions of solvent in the concentrator. Rinse the Tissumizer and the column with small portions of hexane and concentrate the extract below 10 ml.

3.3 Cleanup and Analysis

3.3.1 Unless prior experience would indicate the fish species fat content is low (less than 3g per extract), the hexane/acetonitrile cleanup procedures described in the reference methods should be followed. In all cases, Florisil column chromatography should be used to clean up the extracts before gas chromatography (1). An electron capture detector is used for final measurement, and results

are calculated in µg/kg. Identifications can be confirmed by GC/MS techniques as described in Appendix II of the Federal Register (3).

3.4 Quality Control

3.4.1 Standard quality assurance protocols should be employed, including blanks, duplicates, and dosed samples as described in the "Analytical Quality Control Handbook (4).

3.4.2 Dose fish sample aliquots by injecting minimum amounts (< 20 µl total) of concentrated pesticide or PCB solutions into the solid subsample 10 to 15 minutes before extraction.

4. Reporting of Data

4.1 Report results in µg/kg on a wet tissue basis. Report all quality control data with the analytical results for the samples.

Analysis of Sediment for General Organics

by Mechanical Dispersion Extraction

1. Scope

1.1 This method is designed to determine solvent extractable organic compounds amenable to gas chromatography. Tables I, II, and III are a summary of compounds that should be extracted at an 80-100% efficiency. It is a GC/MS method intended for qualitative and semi-quantitative determination of these compounds. Although this approach has not been sufficiently tested through extensive experimentation, it is based on laboratory experience and is presently considered to be a reasonable analytical approach for these organic materials in sediment.

1.2 This method is not applicable to those very volatile pollutants listed in Table IV.

2. Special Apparatus and Materials

2.1 Mechanical dispersion device - Tissumizer

2.2 Centrifuge - capable of handling 100-ml centrifuge tubes.

2.3 Separatory funnels - 2 liter with Teflon stopcock.

2.4 Sieve, 20 mesh.

3. Procedure

3.1 Weigh 20.0g of wet, well-mixed sediment into a 100 ml centrifuge tube. If the sediment contains grit larger than 20 mesh, it is necessary to extrude the sample through a 20-mesh sieve in order to prevent damage to the mechanical dispersion device. Add 20 ml

acetonitrile and insert the dispersion device into the sample. Disperse the sediment into the solvent for 1 min. Centrifuge and decant the solvent into a 2-liter separatory funnel containing 1300 ml of a 2 percent aqueous solution of sodium sulfate previously adjusted to pH 11 with 6N NaOH. Repeat the dispersion twice, using a 20-ml aliquot each time, and combine the acetonitrile washings in the separatory funnel. CAUTION: The dispersion should be carried out in a fume hood to avoid exposure to acetonitrile.

3.2 Extract the aqueous acetonitrile solution in the separatory funnel with 60 ml of hexane for 2 min. Drain the aqueous layer into a 2-liter Erlenmeyer flask and pour the hexane extract through a short column of anhydrous sodium sulfate prerinsed with hexane. Collect the dried extract in a 500-ml Kuderna-Danish (K-D) flask fitted with a 10 ml ampul. Repeat the extraction and drying steps twice, combining the extracts.

3.3 Evaporate the extract to 5 to 10 ml in a 500-ml K-D apparatus fitted with a 3-ball Snyder column and a 10-ml calibrated receiver tube. Allow the K-D to cool to room temperature. Remove the receiver and adjust the volume to 10 ml. Label this as the base neutral fraction. If additional sensitivity is required, add fresh boiling chips, attach a two-ball micro-Snyder column, and carefully evaporate to 1.0 ml or when active distillation ceases.

3.4 Return the aqueous acetonitrile solution to the separatory funnel and adjust the pH with 6N HCl to pH 2 or less. Extract three times with 60 ml of hexane each time. Combine the extracts, dry, and concentrate as described above. Label this as the acid fraction.

3.5 Analyze both extracts according to the methods described in Appendix II of the Federal Register (3). Should the aceonitrile partition used in this procedure not sufficiently remove interferences, florisil (1), alumina (5), and silica gel (1), column chromatographic cleanup and separation techniques can be employed. Sulfur can be removed by treatment with mercury (1).

3.6 Standard quality control assurance protocols should be employed, including blanks, duplicates and dosed samples, as described in the "Analytical Quality Control Handbook" (4). Dosing can be accomplished by injecting 1-20 µl of a standard solution into the homogenized sediment contained in the centrifuge tube.

4. Reporting of Data

4.1 Report results in µg/kg on a dry weight basis using the percent moisture values determined earlier. Report all quality control data with the analytical results for the samples.

Analysis of Fish for General Organics by Solvent Extraction

1. Scope

1.1 This method is designed to determine solvent extractable organic compounds amenable to gas chromatography. These compounds are listed in Tables II and III. It is a GC/MS method intended for qualitative and semi-quantitative determination of these compounds. Although this approach has not been sufficiently tested through extensive experimentation, it is based on laboratory experience and is presently the best analytical approach for these organic materials in fish.

2. Special Apparatus and Materials

2.1 Tissumizer SDT-182EN (available from Tekmar Company, P. O. Box 37202, Cincinnati, Ohio 45222), or equivalent.

2.2 Centrifuge - capable of handling 100-ml centrifuge tubes.

2.3 Separatory funnels - 2-liter with Teflon stopcock.

2.4 Organic-free water - prepared by passing distilled water through an activated carbon column.

3. Procedure

3.1 Weigh 20.0g of ground, homogeneous fish and add to a 100-ml centrifuge tube. Add 20 ml of acetonitrile and insert the Tissumizer into the sample. Turn on the Tissumizer and disperse the fish into the solvent for 1 min. Centrifuge and decant the solvent into a 2-liter separatory funnel containing 1300 ml of a 2 percent aqueous solution of sodium sulfate. Repeat the dispersion twice, using a 20-ml aliquot each time, and combine the acetonitrile in the separatory funnel.

CAUTION: The dispersion should be carried out in a fume hood to avoid exposure to acetonitrile.

3.2 Adjust the pH of the sodium sulfate/acetonitrile solution with 6N NaOH to pH 11 or greater. Use multirange pH paper for the measurement. Extract the aqueous acetonitrile solution with 60 ml of hexane. Shake the separatory funnel for 2 min. Drain the aqueous layer into a 2-liter Erlenmeyer flask and pour the hexane extract through a short column of prerinsed anhydrous sodium sulfate. Collect the dried extract in a 500-ml Kuderna-Danish (K-D) flask fitted with a 10-ml ampul. Repeat the extraction and drying steps twice, combining the extracts. Evaporate the extract to 5 to 10 ml in a 500-ml K-D apparatus fitted with a 2-ball Snyder column and a 10 ml. Analyze by GC/MS. If additional sensitivity is required, add fresh boiling chips, attach a two-ball micro-Snyder column, and carefully evaporate to 1.0 ml or when active distillation ceases.

3.3 Return the aqueous acetonitrile solution to the separatory funnel and adjust the pH with 6N HCl to pH 2 or less. Extract three times with 60 ml of hexane each time. Combine the extracts, dry, and concentrate as described above. Analyze by GC/MS.

NOTE: Should the partition used in this procedure not sufficiently remove the lipid material, gel permeation can be employed. (However, special expensive equipment is necessary for this procedure (6)).

3.4 Standard quality assurance prptocols should be employed, including blanks, duplicates, and dosed samples, as described in the

"Analytical Quality Control Handbook" (4). Dosing can be accomplished by injecting 1 to 20 µl of a standard solutuon into the homogenized tissue contained in a centrifuge tube.

4. Reporting of Data

4.1 Report results in µg/kg on a wet tissue basis. Report all quality control data with the analytical results for the samples.

Determination of Purgeable Organics in Sediment

1. Scope

1.1 This procedure is intended for use in the analysis of volatile organic compounds found in sediment samples (Table IV). The procedure applies a modified purge/trap technique in the direct analysis of an undiluted sediment sample. The method relies on the use of a mass spectrometer detection system, although other selective detectors may be used for specific compound types.

1.2 Under ideal conditions, the minimum detectable limit has been determined to be 0.5 ppb. Actual detection limits will vary due to sediment surfaces, water content and nonvolatile organic loading factors which will influence the partition coefficients of the volatile organics.

2. Special Apparatus and Materials

2.1 Tekmar LSC-1 or equivalent purge/trap apparatus.

2.2 Septum - Teflon-faced silicone (Pierce 12722).

2.3 Vial with sealable cap - 20 ml (Pierce-Hypovial).

2.4 Heating tape with temperature control.

2.5 Hand crimper

2.6 Standard solutions of compounds of interest - two concentration levels (10 µg/l and 100 µg/l).

3. Procedure

3.1 Allow samples to equilibrate to room temperature for weighing and analysis. Samples may be shipped and stored at wet ice temperatures; freezing is not necessary.

3.2 Drill two holes into the septum to allow for the snug insertion of two 1/8" glass tubes to be used as purge gas inlet and outlet.

3.3 The purge gas inlet should be extended to the bottom of the septum vial. The purge gas outlet should extend 1/2" below the septum.

3.4 Wrap the vial in heating tape and connect the glass tubes to the appropriate gas lines.

3.5 Heat the sample at 80°C for 5 minutes.

3.6 Withdraw the source of heat and purge the sample chamber with helium gas for 4 minutes at a rate of 60 ml/min.

3.7 Desorb the trapped organics from the trap tube onto the chromatographic column for analysis.

3.8 Standard quality assurance protocols should be employed, including blanks, duplicates and dosed samples as described in the "Analytical Quality Control Handbook" (4).

4. Calibration

4.1 For purposes of this procedure, five sample vials of each sediment type must be available.

4.2 Dose one sample vial through the septum with 10 µl of one standard solution. Dose a second vial with 10 µl of the other standard solution.

4.3 Proceed with the analysis of the two dosed samples and one non-dosed sample, using the procedure described in Section 3. Store the two remaining vials at 4°C for possible future analysis.

4.4 After each sample has been analyzed, proceed with data analysis. Subtract the peak areas of compounds found in the undosed sample from the corresponding compounds contained in the dosed sample. Construct a calibration curve from the corrected dosed data; quantify the unknown.

NOTE: If the calculated sample concentration is greater than the concentration of the dosed standard used in the dosing step, it will be necessary to prepare additional standards to bracket the unknown.

4.5 Dry weight of the sediment is obtained after the analysis, by first removing the Teflon seal and drying the sample vial at 103-105^{o}C overnight. The gross dry weight is obtained after reequilibrating the sample vial to room temperature. The tare weight of the vial is then determined after removal of the dried sediment.

5. Reporting of Data

5.1 Report all results in µg/kg on a dry weight basis. Report all quality control data with the analytical results for the sample.

Analysis of Fish for Volatile Organics by Purge and Trap Analyses

1. Scope

1.1 This method is designed to determine volatile organic compounds amenable to purge and trap analyses. These compounds are listed in Table IV. It is a GC/MS method intended for qualitative and semi-quantitative determination of these compounds.

1.2 Although the above approach has not been sufficiently tested through extensive experimentation, it is based on laboratory experience and is presently considered to be the best analytical approach for volatile organic materials in fish.

2. Special Apparatus and Materials

2.1 Sonifier Cell Disrupter W-350 with microprobe (manufactured by Brawson Sonic Power Co., Danbury, Connecticut), or equivalent.

2.2 Gas-tight syringe - 5 cc.

2.3 Organic-free water - Prepared by passing distilled water through an activated carbon column.

2.4 Standard solutions - Prepare three standard methanol solutions of the compounds listed in Table IV at the 50 ng/µl, 150 ng/µl, and 300 ng/µl concentrations. The standard solutions should be stored at less than $0^{o}C$. Solutions should be allowed to warm to room temperature before dosing. Fresh standards should be prepared weekly.

NOTE: Specific GC detectors can be substituted for the MS.

2.5 Tekmar LSC-1 or equivalent purge/trap apparatus.

2.6 Septum - Teflon-faced silicone (Pierce 12722).

2.7 Vial with sealable cap - 20 ml (Pierce-Hypovial).

2.8 Heating tape with temperature control unit.

3. Procedure

3.1 Remove four of the sample vials containing 10.0g of homogenized fish from the freezer. Open the vials and add 10 ml of organic-free water to each while the fish is still frozen. Sonify the fish for 30 sec. at maximum probe power. Immediately reseal the vials.

3.2 Dose one sample vial through the septum below the water level with 10 µl of the 50 ng/µl standard methanol solution. Dose a second vial with 10 µl of the 150 ng/µl standard and a third vial with 10 µl of the 300 ng/µl standard.

3.3 Allow samples to equilibrate to room temperature for weighing and analysis.

3.4 Drill two holes into the septum to allow for the snug insertion of two 1/8" glass tubes to be used as purge gas inlet and outlet. The purge gas inlet should be extended to the bottom of the system vial. The purge gas outlet should extend 1/2" below the septum cap.

3.5 Wrap each vial with heating tape and connect the glass tubes to the appropriate gas line.

3.6 Heat each sample at 80°C for 5 minutes.

3.7 Withdraw the source of heat and purge the sample chamber with helium gas for 4 minutes at a rate of 60 ml/min.

3.8 After purging is complete, desorb the trap tube into the gas chromatograph for analysis. The trap column should be rapidly heated to 180°C and backflush with an inert gas at 20 to 60 ml/min. for 4 minutes during this procedure.

3.9 Analyze the undosed sample first, followed by the 50 ng/µl dosed sample. If no compounds of interest are found in the undosed sample and the dosed sample produces peaks to indicate recovery of the protocol compounds, do not analyze the remaining samples. Calculate lower limits of detection based on the response obtained from the dosed sample. If compounds are observed in the undosed sample, analyze the two remaining dosed samples in exactly the same manner. Subtract the peak areas of compounds found in the undosed sample from the corresponding compounds contained in the dosed data; quantify the unknown.

NOTE: If the calculated sample concentration is greater than the concentration of the dosed standard used in the dosing step, it is necessary to prepare additional standards in order to bracket the unknown. Utilize the remaining sample in the freezer for this purpose.

3.10 Standard quality assurance protocols should be employed, including blanks, duplicates, and dosed samples, as described in the "Analytical Quality Control Handbook" (4).

4. Reporting of Data

4.1 Report all results in µg/kg on a wet tissue basis. Report all quality control data with the analytical results for the samples.

Analysis of Sediment for Cyanide

1. Scope and Application

   1.1 This method is used for the determination of cyanide in sediments. Insoluble cyanide complexes are dissolved in 10% sodium hydroxide. The cyanide, as hydrocyanic acid (HCN), is released from the sample by means of a reflux-distillation and absorbed in a scrubber containing sodium hydroxide solution. The cyanide in the absorbing solution is then determined colorimetrically or potentiometrically or by titration.

   1.2 For cyanide levels exceeding 0.2 mg per 200 ml of absorbing liquid, the silver nitrate titrimetric method is used. For cyanide levels below this value, the colorimetric procedure is used. The probe method may be used for concentrations of 0.001 to 200 mg per 200 ml absorbing liquid.

2. Sample Preparation

   2.1 Although a dry sample is preferred, a wet sample may also be taken for analysis. In either case, the sediment samples must be well mixed to ensure a representative aliquot.

3. Interferences

   3.1 Interferences are eliminated or lessened by using the distillation procedure.

   3.2 Fatty acids will distill and form soap under the alkaline titration conditions. Therefore, acidification and extraction with isooctane, hexane, or chloroform is recommended.

3.3 Ammonia and thiosulfate interfere with the electrode method yielding higher measurements of cyanide ion activity than are actually present.

4. Preparation of Calibration Curve

4.1 The calibration curve is prepared as described in step 8.7, (Method 335.2), Reference 7.

4.2 The standards must contain the same concentration of NaOH (7.1) as the sample.

4.3 At least one standard should be treated as outlined below.

4.4 The calibration curve is prepared by plotting the absorbance or the mv reading versus the cyanide concentration.

5. Sample Procedure

5.1 Place a weighed portion of the well-mixed sediment (1 to 10g) in an 800 ml beaker with 500 ml of 10% NaOH solution and stir for 1 hour.

5.2 Transfer the mixture to a 1-liter boiling flask. Rinse the beaker with several portions of deionized distilled water and add to the boiling flask.

5.3 Add 50 ml of 5% NaOH solution to the absorbing tube and dilute if necessary with deionized, distilled water to obtain an adequate depth of liquid in the absorber. Connect the boiling flask, condenser, absorber, and trap in the distillation train as shown in Figure 1 (Method 335.2), Reference 7.

5.4 Add 50 ml of conc. H_2SO_4 slowly through the air inlet tube. Rinse with distilled water. Add 20 ml of $MgCl_2.6H_2O$ (510 g/l) solution through the air inlet tube and again rinse with distilled water. Continue with steps 8.4 through 8.6 (Method 335.2), Reference 7.

5.5 Record the absorbance or my reading and determine the cyanide concentration from the calibration curve.

6. Quality Assurance

6.1 Initially demonstrate quantitative recovery with each distillation digestion apparatus by comparing distilled aqueous standards to non-distilled aqueous standards. Each day, distill at least one standard to confirm distillation efficiency and purity of reagents.

6.2 At least 15% of the cyanide analyses should consist of duplicate and spiked samples. Quality control limits should be established and confirmed as described in Chapter 6 of the "Analytical Quality Control Handbook," Reference 4.

7. Reporting of Data

7.1 Report cyanide concentrations on a dry weight basis as follows: less than 1.0 mg/kg, to the nearest 0.01 mg/kg; 1.0 mg/kg and above, to two significant figures.

7.2 Report all quality control data with the analytical results for the samples.

Analysis of Fish for Cyanide

1. Scope and Application

   1.1 This method is used for the determination of cyanide in fish. All samples must be distilled prior to the analytical determination. For cyanide levels exceeding 0.2 mg/200 ml of absorbing liquid, the silver nitrate titrimetric method is used. For cyanide levels below this value, the colorimetric procedure is used.

2. Sample Preparation

   2.1 A 5g portion of the frozen, ground fish (see "Sample Handling") is used for the analysis. The sample should be thawed before the analysis begins.

3. Preparation of Calibration Curve

   3.1 The calibration curve is prepared from values for portions of spiked fish tissue distilled in the manner used for the tissue sample being analyzed. For preparation of the calibration standards, choose and weigh a 50g portion of fish and blend in a Waring blender (or equivalent) with 10 ml of 10% NaOH and sufficient deionized, distilled water to bring the volume of the mixture to 500 ml.

   3.2 Using a volumetric pipet which has had the tip removed, withdraw eight 50 ml portions and place in a series of 1 liter boiling flasks. Seven of the flasks should be spiked with progressively larger volumes of the cyanide standard as given in 8.7 (Method 335.2), Reference 7. Adjust the final volume of each flask to 500 ml with deionized, distilled water.

3.3 Add 50 ml of 5% NaOH solution to the absorbing tube and dilute, if necessary, with deionized distilled water to obtain an adequate depth of liquid in the absorber. Connect the boiling flask, condenser, absorber, and trap in the train as shown in Figure 1 (Method 335.2), Reference 7.

3.4 Continue with step 8.2 through 8.6 (Method 335.2), Reference 7.

3.5 The calibration curve is prepared by plotting the absorbance versus the cyanide concentration. The blank absorbance value must be subtracted from each value before plotting the curve.

4. Sample Procedure

4.1 Place a weighed portion of the ground fish (approximately 5g) in a blender with 100 ml of deionzized, distilled water and 1 ml of 5% NaOH solution.

4.2 Blend until a homogeneous mixture is obtained and transfer to a 1-liter boiling flask. Rinse the blender with several portions of deionized, distilled water totaling 400 ml and add to the boiling flask.

4.3 Add 50 ml of 5% NaOH solutuion to the absorbing tube and dilute if necessary with deionized, distilled water to obtain an adequate depth of liquid in the absorber. Connect the boiling flask, condenser, absorber, and trap in the distillation train as shown in Figure 1 (Method 335.2) and continue with step 8.2 through 8.6, Reference 7.

4.4 Read the absorbance and determine the cyanide concentratjion from the calibration curve.

5. Quality Assurance

5.1 Initially, demonstrate quantitative recovery with each distillation digestion apparatus by comparing distilled aqueous standards to non-distilled aqueous standards. Each day, distill at least one standard to confirm distillation efficiency and purity of reagents.

5.2 At least 15% of the cyanide analyses should consist of duplicate and spiked samples. Quality control limits should be established and confirmed as described in Chapter 6 of the "Analytical Quality Control Handbook," Reference 4.

6. Reporting of Data

6.1 Report cyanide concentrations as follows: less than 1.0 mg/kg, to the nearest 0.01 mg; 1.0 mg/kg and above, to two significant figures.

6.2 Report all quality control data with the analytical results for the samples.

Analysis of Sediment for Phenols

1. Scope and Application

   1.1 This method is used for the determination of phenolics in sediments. All samples must be distilled prior to the determination of phenols, using the procedure given on page 576, Reference 8. Use Method 510B for samples that contain less than 1 mg phenol/kg and method 510C for samples that contain more than 1 mg phenol/kg.

2. Sample Preservation and Preparation

   2.1 Biological degradation is inhibited by cooling the sample to $4^{o}C$. If the sample cannot be analyzed within 24 hours, it should be frozen.

   2.2 A 5g portion of the wet, or air-dried sediment is used for the analysis. If the sample has been frozen, it should be thawed before the analysis begins.

3. Preparation of Calibration Curve

   3.1 The calibration curve is prepared as described on p. 579, 4.a.3 (Ref. 8) for samples containing less than 1 mg/kg and p. 581 for samples above 1 mg/kg.

   3.2 Record the absorbance of the standards and plot the values against micrograms of phenol.

4. Sample Procedure

   4.1 Place a 5g portion of the wet, or air-dried sediment into a 200 ml beaker with 100 ml of distilled water. Mix well and lower the pH to 4.0 with (1 + 0) H_3PO_4 using a pH meter.

4.2 Add 5 ml of 10% $CuSO_4$ solution, mix and transfer to a 1-liter distilling flask.

4.3 Rinse the beaker with several portions of distilled water and add to the distilling flask. Adjust the volume in the flask to 500 ml.

4.4 Using a 500-ml graduated cylinder as a receiver, begin the distillation as described on p. 577, Method 510A; 4b, Reference 8.

4.5 Continue with the procedure using either the Chloroform Extraction Method 510B, p. 577, Reference 8, or the Direct Photometric Method 510C, p. 580, Reference 8.

4.6 Record the absorbance and determine the micrograms of phenol from the appropriate calibration curve.

5. Quality Assurance

5.1 Demonstrate quantitative recovery with each distillation apparatus by comparing aqueous distilled standards to non-distilled standards. Each day, distill at least one standard to confirm the distillation efficiency and purity of reagents.

5.2 At least 15% of the phenol analyses should consist of duplicate and spiked samples. Quality control limits should be established and confirmed as described in Reference 4.

6. Reporting of Data

6.1 Report phenol concentrations on a dry weight basis as follows:

Method 510B, to the nearest µg/kg

Method 510C, for less than 1.0 µg/kg to the nearest 0.01 µg and for 1.0 mg/kg and above to two significant figures.

6.2 Report all quality control data when reporting results of sample analysis.

Analysis of Sediment for Mercury

1. Scope and Application

   1.1 This method is used for the determination of total mercury (organic and inorganic) in sediment. A weighed portion of the sample is digested with aqua regia for 2 minutes at 95°C followed by oxidation with potassium permanganate. Mercury is subsequently measured by the cold vapor technique.

   1.2 The range of the method is 0.2 to 5 µg/g but may be extended above or below the normal range by increasing or decreasing sample size or through instrument and recorder control.

   1.3 For a complete description of the method, the reader is referred to "Methods for Chemical Analysis of Water and Waste" (7), Method 245.5.

2. Sample Preparation

   2.1 Although a wet sample may be taken for analysis, a dry sample provides for ease of handling, better homogeniety, and better storage.

3. Preparation of Calibration Curve

   3.1 The calibration curve is prepared using distilled water standards, treated in the same manner as the sediment samples being analyzed. Plot peak height versus the mercury concentration. The peak height of the blank is subtracted from each of the other values.

4. Sample Procedure

   4.1 Weigh 0.2 to 0.3g portions of the dry sample and place in the bottom of a BOD bottle. (If a wet sample is to be analyzed, a

proportionately larger sample must be taken.) Add 5 ml of distilled water and 5 ml of aqua regia and place the bottle in a water bath maintained at 95°C for 2 minutes.

4.2 Cool, add 50 ml distilled water, 15 ml of potassium permanganate solution and return the bottle to the water bath for an additional 30 minutes. Add additional $KMnO_4$, as necessary, to maintain oxidizing conditions.

4.3 Continue with the procedure as described.

5. Calibration

5.1 Measure the peak height of the unknown from the chart and read the mercury value from the standrd curve.

5.2 Calculate the mercury concentration in the sample by the formula:

$$\mu g\ Hg/gram = \frac{\mu g\ Hg\ in\ aliquot}{wt.\ of\ aliquot\ in\ gms}$$

5.3 Report mercury concentrations on a dry weight basis as follows: Below 0.1 µg/gm, < 0.1 µg; between 0.1 and 10 µg/gm, to nearest 0.01 µg; above 10 µg/gm, to nearest µg.

6. Quality Assurance

6.1 Standard quality assurance protocols should be employed, including blanks, duplicates, and spiked samples, as described in the "Analytical Quality Control Handbook" (4).

7. Precision and Accuracy

7.1 The following standard deviations on replicate sediment samples were recorded by a single operator at the indicated levels: 0.29 µg/gm±0.02 and 0.82 µg/gm±0.03. Recovery of mercury at these levels, added as methyl mercuric chloride, was 97% and 94%, respectively.

Analysis of Fish for Mercury

1. Scope and Application

   1.1 This method is used for determination of total mercury (organic and inorganic) in fish. A weighed portion of the sample is digested with sulfuric and nitric acid at 58°C followed by overnight oxidation with potassium permanganate at room temperature. Mercury is subsequently measured by the conventional cold vapor technique.

   1.2 The range of the method is 0.2 to 5 µg/g but may be extended above or below the normal instrument and recorder control.

2. Sample Preparation

   2.1 The sample may be prepared as described under "Sample Handling" or the special metal procedure may be used. A 0.2 to 0.3g portion should be taken for each analysis. The sample should not be allowed to thaw before weighing.

3. Preparation of Calibration Curve

   3.1 The calibration curve is prepared from values for portions of spiked fish tissue treated in the manner used for the tissue samples being analyzed. For preparation of the calibration standards, choose a 5g portion of fish and blend in a Waring blender.

   3.2 Transfer accurately weighed portions to each of six dry BOD bottles. Each sample should weigh about 0.2 grams. Add 4 ml of conc. H_2SO_4 and 1 ml of conc. HNO_3 to each bottle and place in water bath at 58°C until the tissue is completely dissolved (30 to 60 min.).

3.3 Cool and transfer 0-, 0.5- 1.0-, 2.0-, 5.0- and 10.0- ml aliquots of the working mercury solution containing 0 to 1.0 µg of mercury to the BOD bottles. Cool to 4°C in an ice bath and cautiously add 15 ml of potassium permanganate solution. Allow to stand overnight at room temperature under oxidizing conditions.

3.4 Add enough distilled water to bring the total volume to approximately 125 ml. Add 6 ml of sodium chloride-hydroxylamine sulfate solution to reduce the excess permangante.

3.5 Wait at least 30 sec. after the addition of hydroxylamine. Treating each bottle individually, add 5 ml of the stannous sulfate solution and immediately attach the bottle to the aeration apparatus.

3.6 Continue with the procedure as given in Method 245.1 for water (7). The calibration curve is prepared by plotting the peak height versus the mercury concentration. The peak height of the blank is subtracted from each of the other values.

4. Sample Procedure

4.1 Weigh 0.2 to 0.3g portions of the sample and place in the bottom of a dry BOD bottle. Care must be taken that none of the sample adheres to the side of the bottle. Add 4 ml of conc. H_2SO_4 and 1 ml of conc. HNO_3 to each bottle and place in a water bath maintained at 58°C until the tissue is completely dissolved (30 to 60 minutes).

4.2 Cool to 4°C in an ice bath and cautiously add 5 ml of potassium permanganate solution in 1 ml increments. Add an additional 10 ml of more of permangante, as necessary to maintain oxidizing

conditions. Allow to stand overnight at room temperature (see NOTE). Continue as described under 3.4.

NOTE: As an alternate to the overnight digestion, the solubilization of the tissue may be carried out in a water bath at 80°C for 30 min. The sample is then cooled and 15 ml of potassium permanganate solution added cautiously. At this point, the sample is returned to the water bath and digested for an additional 90 min. at 30°C (9). If this method is followed, the calibration standards must also be treated in this manner. Continue as described under 3.4.

5. Calculation

5.1 Measure the peak height of the unknown from the chart and read the mercury value from the standard curve.

5.2 Calculate the mercury concentration in the sample by the formula:

$$\mu g\ Hg/gram = \frac{\mu g\ Hg\ in\ aliquot}{wt.\ of\ aliquot\ in\ gms}$$

5.3 Report mercury concentrations as follows:

Below 0.1 µg/gm, < 0.1 ug; between 0.1 and 1 µg/gm, to nearest 0.01 µg; between 1 and 10 µg/gm, to nearest 0.1 µg; above 10 µg/gm, to nearest µg.

6. Quality Assurance

6.1 Standard quality assurance protocols should be employed, including blanks, duplicates, and spiked samples as described in the "Analytical Quality Control Handbook" (4).

6.2 Report all quality control data when reporting resaults of sample analyses.

7. Precision and Accuracy

7.1 The following standard deviations on replicate fish samples were recorded at the indicated levels: 0.19 µg/gm±0.02, 0.74 µg/gm±0.05, and 2.1 µg/gm±0.06. The coefficients of variation at these levels were 11.9%, 7.0%, and 3.6%, respectively. Recovery of mercury at these levels, added as methyl mercuric chloride, was 112%, 93%, and 86%, respectively.

Analysis of Sediments for Metals

1. Scope and Application

   1.1 This method is used for the determination of antimony, beryllium, cadmium, chromium, copper, lead, nickel, silver, thallium, and zinc in sediments.

2. Summary of Method

   2.1 The sediment is prepared for analysis by drying and grinding the sample. A representative portion is subjected to wet oxidation-digestion prior to analysis by atomic absorption.

   2.2 For a discussion of basic principles, general operating parameters, preparation of standards and calibration, and the method of standard addition, the reader is referred to "Methods for Chemical Analysis of Water and Wastes" (7) and the individual methods as follow:

Page References to "Methods for Chemical Analysis of Water and Wastes, 1979"[7]

ELEMENT	Ag	Be	Cd	Cr	Cu	Ni	Pb	Sb	Tl	Zn
METHOD	272.1	210.1	213.1	218.1	220.1	249.1	239.1	204.1	279.1	289.1

3. Preservation and Handling

3.1 The sample should be stored at 4°C if the analysis can be carried out within 7 days of collection. For longer periods, the samples should be frozen. An alternative is to dry the sample as soon as possible, grind it with a mortar and pestle removing rocks, sticks, and other foreign objects and store the sediment in a vial or other suitable container.

3.2 Dust in the laboratory environment, impurities in reagents, and impurities on laboratory apparatus, which the sample contacts, are all sources of potential contamination. All glassware should be thoroughly washed with detergent and tap water, rinsed with 1:1 nitric acid, tap water, and finally deionized, distilled water in that order. NOTE: Chromic acid may be useful to remove organic deposits from glassware; however, the analyst should be cautioned that the glassware must be thoroughly rinsed with water to remove the last trace of chromium. This is especially important if chromium is to be included in the analytical scheme. A commercial product - NOCHROMIX - available from Godax Laboratories, 6 Varick, New York, NY 10013, can be used in place of chromic acid.

4. Sample Preparation

4.1 Dry a representative portion of the well-mixed sample (10 to 25g) at 60°C until all moisture has been removed.

4.2 Grind the dry sample with a mortar and pestle, removing sticks, stones, and other foreign material. Store the sample in glass or plastic vials removing aliquots as needed.

5. Procedure

5.1 Weigh 1.00g of the well-mixed sediment into a 250 ml Erlenmeyer flask and add 50 ml deionized water, 0.5 ml HNO_3 (sp. gr. 1.42) and 5 ml of HCl (sp. gr. 1.10) to each flask.

5.2 Heat the samples, blanks and standards on a hotplate maintained at approximately 95°C until the volume has been reduced to 15 to 20 ml, making certain that the samples do not boil.

5.3 Cool and clarify the sample by centrifugation or by filtration through Whatman No. 42 filter paper or equivalent.

5.4 Dilute the sample to 100 ml or some appropriate volume based on the concentration present.

5.5 Proceed with the appropriate method for the atomic absorption analysis of the metals of interest.

6. Calibration

6.1 From the values read off the appropriate calibration curve, calculate the concentration of each metal pollutant in the sediment as follows:

$$\text{mg/kg} = \frac{\text{mg/l of constituent prepared sample} \times \text{volume of prepared sample in ml}}{\text{weight of dry sample in g}}$$

7. Quality Assurance

7.1 Standard quality assurance protocols should be employed, including blanks, duplicates, spiked and samples as described in the "Analytical Quality Control Handbook" (4).

7.2 Report all quality control data when reporting results of sample analyses.

Analysis of Fish for Metals

1. Scope

   1.1 This method is used for the determination of antimony, arsenic, beryllium, cadmium, chromium, copper, lead, nickel, selenium, silver, thallium, and zinc in fish tissue.

2. Summary of Method

   2.1 The fish is prepared for analysis by being chopped into small pieces, homogenized in a blender with dry ice, and solubilized by either dissolution after dry ashing or a wet oxidation digestion. After sample preparation, atomic absorption - either direct aspiration, gaseous hydride, or a flameless technique - is used to measure the concentration of the pollutant.

3. Preservation and Handling

   3.1 Although an aliquot of the ground fish as prepared under "Sample Handling" may be used for the metals determination, it may be more desirable to prepare an individual fish to avoid possible metal contamination from the grinder. Dust in the laboratory environment, impurities in reagents, and impurities on laboratory apparatus that the sample contacts are all sources of potential contamination. All glassware should be thoroughly washed with detergent and tap water, rinsed with 1:1 nitric acid, then tap water, and finally deionized, distilled water.

   NOTE: Chronic acid may be useful to remove organic deposits from glasware; however, the analyst should be cautiuoned that the glassware must be thoroughly rinsed with water to remove the last

trace of chromium. This is especially important if chromium is to be included in the analytical scheme. A commercial product - NOCHROMIX - available from Godax Laboratories, 6 Varick Street, New York, NY, 10013, can be used in place of chromic acid.

4. Sample Homogenization

4.1 If a fish sample other than that prepared under "Sample Handling" is to be used for metals analyses, unwrap and weigh the frozen fish at the time of processing. Select a fish that weighs between 50 and 300g. If an analysis is required for a fish, < 300g, a 50g representative portion must be taken from the sample after it has been pretreated as described in "Sample Handling" on page 1 of this document.

4.2 After weighing, the fish should be chopped into approximately 1-in. or smaller chunks with a meat cleaver or a knife and mallet (2 to 3-lb). Smaller pieces ensure efficient grinding.

4.3 Place crushed or pelleted dry ice into the blender container. The weight of dry ice should be equal to, or greater than, the weight of the fish.

4.4 Turn on the blender for 10 sec. to pulverize the ice and chill the blender.

4.5 Add the pieces of fish and blend at high speed until the mixture is homogeneous. This usually requires 2 to 5 minutes. Add more dry ice if needed to keep the fish frozen.

4.6 Pour the homogenate into a plastic bag and close the bag with a rubber band. Do not seal the bag tightly to allow CO_2 to escape.

4.7 Place the bag in the freezer (-12°C for at least 16 hr.) until ready to proceed with the digestion step.

NOTE: If desired, the blender blades can be modified in order to have the leading edge of the blades (the sharpened edge) turned down so that, as it rotates, the blade will throw the material upwards. Stainless steel blades may be a possible source of nickel and chromium contamination and should be noted if detected. If a tantalum blade is available, it should be substituted for the stainless steel.

The hole in the blender lid should be enlarged sufficiently to allow the evolved gas to escape. Hold a cloth or labwipe over this hole when blending to prevent loss of the sample material. A glove should be worn to prevent possible freezing of the skin by escaping gas.

5. Reagents

5.1 Deionized, distilled water: Prepare by passing distilled water through a mixed bed of cation and anion exchange resins. Use deionized, distilled water for the preparation of all reagents and calibration standards and as dilution water.

5.2 Nitric acid (conc.): If metal impurities are present, distill reagent grade nitric acid in a borosilicate glass distillation apparatus.

5.3 Sulfuric acid, ACS grade (95.5% to 96.5%).

5.4 Sulfuric acid - 20% V/V solution. Carefully add 200 ml of concentrated H_2SO_4 to 500 ml of water. Cool and dilute to 1 liter with water.

5.5 Hydrochloric acid, ACS grade.

5.6 Hydrogen Peroxide, 50% stabilized ACS grade.

5.7 Dry ice (frozen carbon dioxide), pellet form preferred.

6. Apparatus

6.1 Blender, Waring, two-speed, stainless steel blade or tantalum blade, if available, glass container capacity 1000 ml, or equivalent equipment.

6.2 Drying oven - Controllable with the range of 100° to 150°C with less than ±5°C variation. Check calibration of oven temperature control to ensure accurate ashing temperatures. Furnace must be operated in suitable fume hood.

6.3 Hot plate, controllable within the range of 80°C to 400°C. Hot plate must be operated in fume hood.

7. Procedure

Except for mercury, which requires a cold vapor technique, the metals can be divided into two groups for continued processing.

Group I: Be, Cd, Cr, Cu, Pb, Ni, Ag, Tl, and Zn.

Group II: As and Se.

Group I is digested by a dry ashing process (11) with the use of an ashing aid; Group II is prepared utilizing a wet ashing process.

7.1 Group I - Metals

7.1.1 Remove the homogenized sample from the freezer and weigh approximately 10g into a tared, 100-ml tall form, Pyrex beaker. Subtract the beaker weight from the total and record the wet sample weight.

7.1.2 Add 25 ml of 20% sulfuric acid. Mix each sample thoroughly with a glass stirring rod ensuring all sample material is wetted by the acid. Rinse the stirring rod with water into the ashing vessel and cover the sample with a ribbed watch glass.

7.1.3 Heat the samples in an oven or furnace at 110±5°C until a charred viscous sulfuric acid/sample residue remains. Usually 12 to 16 hrs. (overnight) is sufficient. Transfer the ashing vessels containing the samples to a cold, clean muffle furnace which is provided with good external ventilation (fume hood), ensuring that the sample remains covered during the transfer. Initially set the furnace at 125°C and increase the temperature approximately every hour in 50° increments up to 275°C. Hold the temperature at 275°C for 3 hrs. Finally, increase the temperature to 450°C (at 50° per hour) and hold for 12 to 16 hrs. (overnight). Remove the covered ashing vessels from the furnace and allow to cool to room temperature in a clean, draft-free area.

7.1.4 After initial overnight ashing, some residual carbon may remain in the samples. Treat each sample ash with 0.5 ml of water and 1 ml of concentrated nitric acid (whether or not they are already white). Evaporate carefully just to dryness on a warm hotplate (in a fume hood). Place the ashing vessels (covered with watch glasses) in a cool muffle furnace and raise the temperature to 300°C and hold for

exactly 30 min. Remove each covered sample ash from the furnace and allow to cool as before. If residual carbon remains, repeat the nitric acid treatment until a carbon-free white ash is obtained. The covered ashing vessels containing the ash may be stored in a dessicator or in a laminar flow clean hood.

NOTE: Copious carbon residues (i.e., black ashes) after overnight ashing may indicate inefficient or uneven heating within the furnce. Routine calibration of the furnace is advised.

7.1.5 Add 0.5 ml of nitric acid and 10 ml of water to each cool ashing vessel, then warm gently on a hotplate at 80 to 90°C for 5 to 10 min. to effect dissolution of the ash. A small amount of insoluble white siliceous-like residue may remain undissolved; do not filter the residue because of the possibility of contamination. Quantitatively transfer the contents of each ashing vessel into a 100 ml volumetric flask, dilute to volume with water, and shake thoroughly. Allow any residue to settle to the bottom of the flask (about 2 hr). Do not shake the sample further before taking an aliquot. The sample is now ready for analysis.

NOTE: The presence of a precipitate other than the insoluble siliceous-like material may result in low or eratic results for Pb. Precipitate formation can result from heating the samples too long or at too high a temperature after nitric acid treatment of the ash.

Precipitate formation must be avoided by maintenance of appropriate ashing temperatures.

7.1.6 The prepared sample should be analyzed by atomic absorption. For a discussion of basic principles, the method of standard addition, the chelation/solvent extraction procedures, general instrumental operating parameters, and preparation of standards and calibration see the section on "Atomic Absorption Methods" (7), and the individual analyses sheets as follow:

ELEMENT	Ag	Be	Cd	Cr	Cu	Ni	Pb	Sb	Tl	Zn
METHOD	272.1	210.1	213.1	218.1	220.1	249.1	239.1	204.1	279.1	289.1

7.1.7 Because of the adequate sensitivity by conventional flame AA and the expected concentration levels of cadmium, copper, and zinc in the sample, these three elements should be analyzed by direct aspiration. The furnace technique is preferred for the analysis of the other Group I metals because of their expected low concentrations. When using the furnace technique, the operating parameters and instructions as specified by the particular instrument manufacturer should be followed. If the concentration detected by the furnace procedure is beyond the working range of the standard curve, the sample should be either diluted and reanalyzed or analyzed by direct aspiration. The method of standard additions should be employed when needed. If the sample matrix is so complex that sample dilution followed by furnace analysis cannot be used, or if the use of the chelation/solvent extraction techniqe for

concentration of Ag, Ni, Pb, and Tl is preferred, the procedure as described in Methods for Chemical Analysis of Water and Wastes, Reference 7, should be utilized.

7.2 Group II - Metals

7.2.1 Remove the homogenized sample from the freezer and weigh approximately 5g into a tared, 120-ml conical beaker. Subtract the beaker weight from the total and record the wet sample weight.

7.2.2 Add 5 ml of conc. HNO_3. Then slowly add 6 ml of conc. H_2SO_4 and cover with a watch glass.

7.2.3 Place beaker on hot plate and warm slightly. Continue heating until the mixture becomes dark or a possible reducing condition is evident. Do not allow the mixture to char. Remove beaker from hotplate and allow to cool.
NOTE: Remove beaker if foaming becomes excessive.

7.2.4 Add an additional 5 ml of conc. HNO_3, cover with a watch glass, and return beaker to hotplate. Repeat step 7.2.3.

7.2.5 When mixture again turns brown, cool, and slowly add 5 ml of 50% hydrogen peroxide. Cover with watch glass and heat gently until the initial reaction has ceased. If the solution becomes dark, repeat the peroxide addition, several times if necessary, and heat to SO_3 fumes. If charring occurs, add further 1 ml portions of hydrogen peroxide until the fuming sulfuric acid remains colorless or very light yellow. (If at any stage it appears that the sulfuric acid may apprpach dryness, cool, add 2 to 3 ml of sulfuric acid, and continue.)

7.2.6 Cool, add 40 ml of conc. HCl and dilute to 100 ml with deionized, distilled water. The sample is now ready for analysis.

7.2.7 The Group II metals should be analyzed by atomic absorption using the gaseous hydride technique. The apparatus setup, standard preparation and calibration, and analysis procedure that is to be followed is given, starting on Page 159, Reference 8. From the prepared sample, a 25-ml aliquot should be withdrawn and the analysis continued as described in Section 3.d, Page 162, Reference 8.

8. Calculation

8.1 Using the values from the appropriate calibration curve, calculate the concentration of each metal pollutant in the fish as follows: If the concentration of standards in the calibration curve is plotted as mg/l,

$$\text{ug/gram} = \frac{\text{mg/l of constituent in prepared sample} \times \text{volume of prepared sample in ml}}{\text{weight of wet sample in g}}$$

If the concentration of standards in the calibration curve is plotted as µg/l,

$$\text{µg/gram} = \frac{\text{µg/l of constituent in prepared sample} \times \text{volume of prepared sample in ml}}{\text{weight of wet sample in g}}$$

9. Quality Assurance

9.1 Standard quality assurance protocols should be employed, including blanks, duplicates, and spiked samples, as described in the "Analytical Quality Control Handbook (4).

9.2 Report all quality control data when reporting results of sample analyses.

Analysis of Sediment for Arsenic and Selenium

1. Scope and Application

   1.1 This method is to be used for the determination of arsenic and selenium in sediment. A weighed portion of the wet, well-mixed sediment is digested with HNO_3 and H_2SO_4 followed by treatment with H_2O_2. Arsenic and selenium are subsequently determined by the gaseous hydride technique.

   1.2 The range of the method is _____ to _____ µg/g but may be extended by varying the sample size.

2. Sample Preparation

   2.1 The analysis should be performed on a wet, well-mixed sample.

3. Preparation of Calibration Curve

   3.1 The calibration curve is prepared using distilled water standards, treated in the same manner as the samples being analyzed.

4. Procedures

   4.1 Weigh approximately 5 grams of the wet, well-mixed, sediment into a tared 125-ml conical beaker.

   4.2 Add 5 ml of conc. HNO_3. Then slowly add 6 ml conc. H_2SO_4 and cover with a watch glass.

   4.3 Place the beaker on hot plate and warm slightly. (NOTE: Remove beaker if foraming becomes excessive.) Continue heating until the mixture becomes dark or a possible reducing condition is evident. Do not allow the mixture to char. Remove the beaker from the hotplate and allow to cool.

   4.4 Add an additional 5 ml of conc. HNO_3, cover with a watch glass, and return beaker to hot plate. Repeat step 4.3.

4.5 When mixture again turns brown, cool, and slowly add 5 ml of 50% hydrogen peroxide. Cover with watch glass and heat gently until the initial reaction has ceased. If the solution becomes dark, repeat the peroxide addition, several times if necessary, and heat to SO_3 fumes. If charring occurs, add additional 1 ml portions of hydrogen peroxide until the fuming sulfuric acid remains colorless or very light yellow. (If at any stage the sulfuric acid approaches dryness, cool, add 2 to 3 ml of additional sulfuric acid, and continue.)

4.6 Cool, add 40 ml of conc. HCl and dilute to 100 ml with deionized, distilled water. The sample is now ready for analysis by the gaseous hydride technique.

4.7 The apparatus setup, standard preparation and calibration, and analytical procedure to be followed is given beginning on page 159, Reference 8. A 25-ml aliquot should be withdrawn from the prepared sample and the analysis continued as described in Section 3.d, page 162, Reference 8.

5. Calibration

5.1 Calculate the concentration of arsenic and selenium present in mg/kg on a dry weight basis.

6. Quality Assurance

Standard quality assurance protocols should be employed, including blanks, duplicates, and spiked samples as described in the "Analytical Quality Control Handbook" (4).

REFERENCES

1. "Methods for Benzidine, Chlorinated Organic Compounds, Pentachlorophenol and Pesticides in Water and Wastewater," U. S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio, 45268, 1978.
2. Federal Register, Volume 41, No. 232, p. 52780, Wednesday, December 1976.
3. Federal Register, Volume 44, No. 233, p. 69464, Monday, December 3, 1979.
4. Handbook for Analytical Quality Control in Water and Wastewater Laboratories. U. S. Environmental Protection Agency, Technology Transfer, 1979.
5. Boyle, H. W., et al., Adv. Chem. Serv., 60, 207 (1966).
6. Stalling, D. L.; R. C. Tindle; J. L. Johnson; "Cleanup of Pesticide and Polychlorinated Biphenyl Residues in Fish Extracts by Gel Permeation Chromatography." JOAC, 55, 32-38 (1972).
7. "Methods for Chemical Analysis of Water and Wastes," U. S. Environmental Protection Agency, Technology Transfer. (1979).
8. "Standard Methods for the Examination of Water and Wastewater," 14th edition (1975).
9. Bishop, J. N., "Mercury in Fish," Ontario Water Resources Comm., Toronto, Ontario, Canada, 1971.
10. Jones, J. W.; R. J. Gajan; K. W. Boyer; J. A. Fiorino; "Dry Ash - Voltammetric Determination of Cadmium, Copper, Lead, and Zinc in Foods." JOAC, 60, 826. (1977).
11. Jones, J. W.; R. J. Gajan, K. W. Boyer; J. A. Fiorino; "Dry Ash - Voltammetric Determination of Cadmium, Copper, Lead, and Zinc in Foods." JOAC, 60, 826. (1977).