UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

SUBJECT: Tissue Analysis for use in FY78 Regional Toxic Data Collection

DATE: 25 AUG 1977

FROM: Robert Crim, Chief
Monitoring Branch (WH-553)

TO Surveillance and Analysis
Division Directors

The methods included in the enclosed "Sampling and Analysis Procedures for Screening of Fish for Priority Pollutants" should be used for tissue analyses in your FY78 Toxic Data Collection Activity. This program is discussed in Eckardt C. Beck's memorandum of June 9, 1977, subject: FY78 Regional Toxic Data Collection.

Technical questions or comments on these methods should be addressed to the Cincinnati Laboratory.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

ENVIRONMENTAL MONITORING AND
SUPPORT LABORATORY - CINCINNATI

SUBJECT: Fish Tissue Analysis DATE: August 23, 1977

FROM : Dwight G. Ballinger, Director
Environmental Monitoring and Support
Laboratory - Cincinnati

TO : Surveillance and Analysis Division Directors

Attached is a copy of "Sampling and Analysis Procedures for Screening of Fish for Priority Pollutants" for your use in collecting and analyzing fish samples in response to the State/Regional monitoring operating guidance. As with the recent document sent to you on sediment analysis (see memo of August 12, 1977), this document should also be considered as interim guidance. While we consider it the Agency's best source for the analysis of fish samples for priority pollutants, we recognize that changes may be warranted as a result of your regional experiences on a variety of collected fish samples. As noted in the foreword, we encourage your careful review of this protocol and your identification of any problems that may arise.

Attachment
As stated above

cc: Charles Brunot (w/attachment)

SAMPLING AND ANALYSIS PROCEDURES FOR SCREENING OF FISH FOR PRIORITY POLLUTANTS

U. S. ENVIRONMENTAL PROTECTION AGENCY

Environmental Monitoring and Support Laboratory
Cincinnati, Ohio 45263

August 23, 1977

Foreword

These guidelines for sample preparation and analysis of fish have been prepared by the staff of the Environmental Monitoring and Support Laboratory - Cincinnati, at the request of the Monitoring and Data Support Division, Office of Water and Hazardous Wastes, with the cooperation of many EPA Regional Laboratories, the Food and Drug Administration, the Southeast Water Research Laboratory, the Environmental Research Laboratory - Duluth, and the National Institute for Occupational Safety and Health.

The procedures represent the current state-of-the-art, but improvements are anticipated as more experience is obtained. Users of these methods are encouraged to identify problems to assist in updating the test procedures by contacting the Environmental Monitoring and Support Laboratory, EPA, Cincinnati, Ohio 45268.

CONTENTS

LIST OF TABLES

Sampling and Analysis Procedures for Screening of Fish for Priority Pollutants

Sample Handling

1. Collection

Separate analyses for all priority pollutants are done on the same sample of fish. A minimum of 250 grams is required for the total protocol. Small fish must be combined by species to obtain this minimum weight.

2. Preservation

Field sampling requires an ice chest packed with dry ice. Collected samples are wrapped in aluminum foil, labeled with freezer tape, and transported in the chest. The dry ice must be replaced as needed until subsamples are obtained for purgeable organics.

3. Processing

To prepare samples for analytical pretreatments, wrap and weigh each fish. Combine small fish by site and species until a minimum combined weight of 250 grams is obtained. Chop the sample into one-inch chunks using a sharp knife and mallet. Grind the sample with 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. Weigh five 10.0 gram portions of the sample into separate 125 vials. Using a crimper, quickly

U. S. Environmental Protection Agency
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and tightly secure a septum to each bottle with a seal. Store these sample aliquots in a freezer until ready for Volatile Organics analyses. Transfer remaining fish samples to a glass container and store in a freezer for later subsampling and analysis.

4. - Special Equipment and Materials

4.1 Knife, heavy blade (or meat cleaver)

4.2 Mallet, plastic faces, 2-3 pounds.

4.3 Electric meat grinder, 1/2 HP.

4.4 Ice chest.

4.5 Dry ice.

4.6 Aluminum foil.

4.7 Freezer tape, for labels.

4.8 Freezer

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

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

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

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

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 with either of the two procedures described below. Method A employs a blender, while a Tissumizer or equivalent is required for Method B. Both procedures result in an extract that can be incorporated directly into current EPA test procedures for pesticides[1] or PCBs[2] as cited in the Federal Register[3].

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 Büchner 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 37202, 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: Weigh a 25-50 g portion of frozen, ground fish and add to a high speed blender. Add 100 g 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.

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

3.1.2 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 25-50 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.

3.1.3 Pour the combined extracts and rinses through a column of anhydrous Na_2SO_4, 20 mm x 100 mm long, 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: Weigh 20.0 grams of frozen, ground fish in 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 minute. Centrifuge and decant the solvent through a column of anhydrous Na_2SO_4, 20 mm x 100 mm long, and collect the eluate in a 500 ml Kuderna-Danish concentrator. Repeat the dispersion twice more 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: Unless prior experience would indicate the fish species fat content is low (less than 3g/extract), the hexane-acetonitrile clean-up 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.[1,2] An electron capture detector is used for final measurement, and results are calculated in micrograms per kilogram. Identifications

may be confirmed by GC/MS techniques as described in the analytical protocol for wastewaters[4].

3.4 Quality Control: Standard quality assurance protocols should be employed, including blanks, duplicates, and dosed samples as described in the "Analytical Quality Control Handbook"[6]. Dose fish sample aliquots by injecting minimum amounts (<20 µl total) of concentrated pesticide or PCB solutions into the solid sub-sample 10-15 minutes before extraction.

4. Reporting of Data

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

TABLE 1 PRIORITY POLLUTANTS ANALYZED BY PESTICIDE AND PCB PROCEDURES

Pesticides	PCBs
Aldrin	Aroclor 1016
a-BHC	Aroclor 1221
b-BHC	Aroclor 1232
d-BHC	Aroclor 1242
g-BHC	Aroclor 1248
Chlordane	Aroclor 1254
DDD	Aroclor 1260
DDE	
DDT	
Dieldrin	
a-Endosulfan	
b-Endosulfan	
Endosulfan sulfate	
Endrin	
Endrin aldehyde	
Heptachlor	
Heptachlor epoxide	
Toxaphene	

Analysis of Fish for General Organics by Solvent Extraction

1. Scope

1.1 This method is designed to determine those "unambiguous priority pollutants" associated with the Consent Decree[4] that are solvent extractable and amenable to gas chromatography. These compounds are listed in Tables II and III of this section. It is a gas chromatographic-mass spectrometric method intended for qualitative and semi-quantitative determination of these compounds. While this approach has not been sufficiently tested through extensive experimentation, it is based on laboratory experience and is presently our 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 20.0 grams of ground, homogenous fish in a 100 ml centrifuge tube. Add 20 ml acetonitrile and insert the Tissumizer into the sample. Turn on Tissumizer and disperse the fish into the solvent for 1 minute. Centrifuge and decant the solvent into

a 2 liter separatory funnel which contains 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 hexane. Shake the separatory funnel for two minutes. 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 5-10 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. 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 hexane each time. 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 may be employed. However, special expensive equipment is necessary for this procedure[5].

3.4 Quality Control: Standard quality assurance protocols should be employed, including blanks, duplicates and dosed samples as described in the "Analytical Quality Control Handbook"[6]. Dosing can be accomplished by injecting 1-20 µl of a standard solution, into the homogenized tissue contained in a centrifuge tube.

5. Reporting of Data

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

Table III Acid Extractables

Compound Name	RRT[1] (2-nitrophenol)	Limit of Detection (ng)	Characteristic EI ions (Rel. Int.)	CI ions (Methane)
-chlorophenol	0.63	100	128(100), 64(54), 130(31)	129, 131, 1
henol	0.66	100	94(100), 65(17), 66(19)	95, 123, 13
,4-dichlorophenol	0.96	100	162(100), 164(58), 98(61)	163, 165, 1
-nitrophenol	1.00	100	139(100), 65(35), 109(8)	140, 168, 1
-chloro-m-cresol	1.05	100	142(100), 107(80), 144(32)	143, 171, 1
,4,6-trichlorophenol	1.14	100	196(100), 198(92), 200(26)	197, 199, 2
,4-dimethylphenol	1.32	100	122(100), 107(90), 121(55)	123, 151, 1
,4-dinitrophenol	1.34	2 µg	184(100), 63(59), 154(53)	185, 213, 2
,6-dinitro-o-cresol	1.42	2 µg	198(100), 182(35), 77(28)	199, 227, 2
-nitrophenol	1.43	100	65(100), 139(45), 109(72)	140, 168, 1
entachlorophenol	1.64	100	266(100), 264(62), 268(63)	267, 265, 2
euterated anthracene (d10)	1.68	40	188(100), 94(19), 80(18)	189, 217

Column: 6' glass, 2 mm i.d.
Tenax GC - 60/80 mesh
180° - 300° @ 8°/min.
He @ 30 ml/min

Compound Name	RRT[1] (hexachloro-benzene)	Limit of Detection (ng)	Characteristic EI ions (Rel. Int.)	CI ions (Methane)
,2-dichlorobenzene	0.35	40	146(100), 148(64), 113(12)	146, 148, 15
,4-dichlorobenzene	0.36	40	146(100), 148(64), 113(11)	146, 148, 15
exachloroethane	0.38	40	117(100), 199(61), 201(99)	199, 201, 20
,2-dichlorobenzene	0.39	40	146(100), 148(64), 113(11)	146, 148, 15
is(2-chloroisopropyl) ether	0.47	40	45(100), 77(19), 79(12)	77, 135, 137
exachlorobutadiene	0.55	40	225(100, 223(63), 227(65)	223, 225, 22
,2,4-trichlorobenzene	0.55	40	74(100), 109(80), 145(52)	181, 183, 20
aphthalene	0.57	40	128(100), 127(10), 129(11)	129, 157, 16
is(2-chloroethyl)ether	0.61	40	93(100), 63(99), 95(31)	63, 107, 109
exachlorocyclopentadiene	0.64	40	237(100), 235(63), 272(12)	235, 237, 27
itrobenzene	0.64	40	77(100), 123(50), 65(15)	124, 152, 16
is(2-chloroethoxy)methane	0.68	40	93(100), 95(32), 123(21)	65, 107, 137
-chloronaphthalene	0.76	40	162(100), 164(32), 127(31)	163, 191, 20
cenaphthylene	0.83	40	152(100), 153(16), 151(17)	152, 153, 19
cenaphthene	0.86	40	154(100), 153(95), 152(53)	154, 155, 15
sophorone	0.87	40	82(100), 95(14), 138(18)	139, 167, 17
luorene	0.91	40	166(100), 165(80), 167(14)	166, 167, 19
,6-dinitrotoluene	0.93	40	165(100), 63(72), 121(23)	183, 211, 22
,2-diphenylhydrazine	0.96	40*	77(100), 93(58), 105(28)	185, 213, 21
,4-dinitrotoluene	0.98	40	165(100), 63(72), 121(23)	183, 211, 22
-nitrosodiphenylamine	0.99	40*	169(100), 168(71), 167(50)	169, 170, 19
exachlorobenzene	1.00	40	284(100), 142(30), 249(24)	284, 286, 29
-bromophenyl phenyl ether	1.01	40	248(100), 250(99), 141(45)	249, 251, 27
henanthrene	1.09	40	178(100), 179(16), 176(15)	178, 179, 20
nthracene	1.09	40	178(100), 179(16), 176(15)	178, 179, 20
imethylphthalate	1.10	40	163(100), 164(10), 194(11)	151, 163, 19
iethylphthalate	1.15	40	149(100), 178(25), 150(10)	177, 223, 25
luoranthene	1.23	40	202(100), 101(23), 100(14)	203, 231, 24
yrene	1.30	40	202(100), 101(26), 100(17)	203, 231, 24
i-n-butylphthalate	1.31	40	149(100), 150(27), 104(10)	149, 205, 27
enzidine	1.38	40*	184(100), 92(24), 185(13)	185, 213, 22
utyl benzylphthalate	1.46	40	149(100), 91)50)	149, 299, 32

Table II Base-neutral Extractables (Cont'd.)

Compound Name	RRT[1] (hexachloro-benzene)	Limit of Detection (ng)	Characteristic EI ions (Rel. Int.)	CI ions (Methan
chrysene	1.46	40	228(100), 229(19), 226(23)	228, 229, 1
bis(2-ethylhexyl)phthalate	1.50	40	149(100), 167(31), 279(26)	149
benzo(a)anthracene	1.54	40	228(100), 229(19), 226(19)	228, 229, 1
benzo(b)fluoranthene	1.66	40	252(100), 253(23), 125(15)	252, 253, 1
benzo(k)fluoranthene	1.66	40	252(100), 253(23), 125(16)	252, 253, 1
benzo(a)pyrene	1.73	40	252(100), 253(23), 125(21)	252, 253, 1
indeno(1,2,3-cd)pyrene	2.07	100	276(100), 138(28), 277(27)	276, 277, 1
dibenzo(a,h)anthracene	2.12	100	278(100), 139(24), 279(24)	278, 279, 1
benzo(g h i)perylene	2.18	100	276(100), 138(37), 277(25)	276, 277, 1
N-nitrosodimethylamine			42(100), 74(88), 44(21)	
N-nitrosodi-n-propylamine			130(22), 42(64), 101(12)	
4-chloro-phenyl phenyl ether			204(100), 206(34), 141(29)	
endrin aldehyde				
3,3'-dichlorobenzidine			252(100), 254(66), 126(16)	
2,3,7,8-tetrachlorodibenzo-p-dioxin			322(100), 320(90), 59(95)	
bis(chloromethyl)ether			45(100), 49(14), 51(5)	

1% SP-2250 on 100/120 mesh Supelcoport in a 6' x 2 mm id glass column; He @ 30 ml/min; Program: 50° for 4 min, then 8°/min to 260° and hold for 15 min.

Conditioning of column with base is required.

Table II Base-neutral Extractables

pound Name	RRT[1] (hexachloro-benzene)	Limit of Detection (ng)	Characteristic EI ions (Rel. Int.)	CI ions (Methane)
chlorobenzene	0.35	40	146(100), 148(64), 113(12)	146, 148, 150
chlorobenzene	0.36	40	146(100), 148(64), 113(11)	146, 148, 150
oroethane	0.38	40	117(100), 199(61), 201(99)	199, 201, 203
chlorobenzene	0.39	40	146(100), 148(64), 113(11)	146, 148, 150
chloroisopropyl)				
	0.47	40	45(100), 77(19), 79(12)	77, 135, 137
lorobutadiene	0.55	40	225(100, 223(63), 227(65)	223, 225, 227
trichlorobenzene	0.55	40	74(100), 109(80), 145(52)	181, 183, 209
ylene	0.57	40	128(100), 127(10), 129(11)	129, 157, 169
chloroethyl)ether	0.61	40	93(100), 63(99), 95(31)	63, 107, 109
lorocyclopentadiene	0.64	40	237(100), 235(63), 272(12)	235, 237, 239
ene	0.64	40	77(100), 123(50), 65(15)	124, 152, 164
chloroethoxy)methane	0.68	40	93(100), 95(32), 123(21)	65, 107, 137
naphthalene	0.76	40	162(100), 164(32), 127(31)	163, 191, 203
thylene	0.83	40	152(100), 153(16), 151(17)	152, 153, 181
thene	0.86	40	154(100), 153(95), 152(53)	154, 155, 183
one	0.87	40	82(100), 95(14), 138(18)	139, 167, 178
ne	0.91	40	166(100), 165(80), 167(14)	166, 167, 195
nitrotoluene	0.93	40	165(100), 63(72), 121(23)	183, 211, 223
phenylhydrazine	0.96	40*	77(100), 93(58), 105(28)	185, 213, 225
nitrotoluene	0.98	40	165(100), 63(72), 121(23)	183, 211, 223
osodiphenylamine	0.99	40*	169(100), 168(71), 167(50)	169, 170, 198
lorobenzene	1.00	40	284(100), 142(30), 249(24)	284, 286, 288
phenyl phenyl ether	1.01	40	248(100), 250(99), 141(45)	249, 251, 277
threne	1.09	40	178(100), 179(16), 176(15)	178, 179, 207
zene	1.09	40	178(100), 179(16), 176(15)	178, 179, 207
ylphthalate	1.10	40	163(100), 164(10), 194(11)	151, 163, 164
lphthalate	1.15	40	149(100), 178(25), 150(10)	177, 223, 251
nthene	1.23	40	202(100), 101(23), 100(14)	203, 231, 243
	1.30	40	202(100), 101(26), 100(17)	203, 231, 243
utylphthalate	1.31	40	149(100), 150(27), 104(10)	149, 205, 279
ine	1.38	40*	184(100), 92(24), 185(13)	185, 213, 225
benzylphthalate	1.46	40	149(100), 91)50)	149, 299, 327

Table II Base-neutral Extractables (Cont'd.)

ound Name	RRT[1] (hexachloro-benzene)	Limit of Detection (ng)	Characteristic EI ions (Rel. Int.)	CI ions (Methane)
ne	1.46	40	228(100), 229(19), 226(23)	228, 229, 257
-ethylhexyl)phthalate	1.50	40	149(100), 167(31), 279(26)	149
(a)anthracene	1.54	40	228(100), 229(19), 226(19)	228, 229, 257
(b)fluoranthene	1.66	40	252(100), 253(23), 125(15)	252, 253, 281
(k)fluoranthene	1.66	40	252(100), 253(23), 125(16)	252, 253, 281
(a)pyrene	1.73	40	252(100), 253(23), 125(21)	252, 253, 281
(1,2,3-cd)pyrene	2.07	100	276(100), 138(28), 277(27)	276, 277, 305
o(a,h)anthracene	2.12	100	278(100), 139(24), 279(24)	278, 279, 307
(g h i)perylene	2.18	100	276(100), 138(37), 277(25)	276, 277, 305
rosodimethylamine			42(100), 74(88), 44(21)	
rosodi-n-propylamine			130(22), 42(64), 101(12)	
ro-phenyl phenyl ether			204(100), 206(34), 141(29)	
aldehyde				
ichlorobenzidine			252(100), 254(66), 126(16)	
,8-tetrachlorodibenzo-dioxin			322(100), 320(90), 59(95)	
loromethyl)ether			45(100), 49(14), 51(5)	

SP-2250 on 100/120 mesh Supelcoport in a 6' x 2 mm id glass column; He @ 30 ml/min; ogram: 50° for 4 min, then 8°/min to 260° and hold for 15 min.

nditioning of column with base is required.

Table III Acid Extractables

ound Name	RRT[1] (2-nitrophenol)	Limit of Detection (ng)	Characteristic EI ions (Rel. Int.)	CI ions (Methane)
rophenol	0.63	100	128(100), 64(54), 130(31)	129, 131, 157
	0.66	100	94(100), 65(17), 66(19)	95, 123, 135
chlorophenol	0.96	100	162(100), 164(58), 98(61)	163, 165, 167
ophenol	1.00	100	139(100), 65(35), 109(8)	140, 168, 122
ro-m-cresol	1.05	100	142(100), 107(80), 144(32)	143, 171, 183
trichlorophenol	1.14	100	196(100), 198(92), 200(26)	197, 199, 201
methylphenol	1.32	100	122(100), 107(90), 121(55)	123, 151, 163
nitrophenol	1.34	2 µg	184(100), 63(59), 154(53)	185, 213, 225
nitro-o-cresol	1.42	2 µg	198(100), 182(35), 77(28)	199, 227, 239
ophenol	1.43	100	65(100), 139(45), 109(72)	140, 168, 122
chlorophenol	1.64	100	266(100), 264(62), 268(63)	267, 265, 269
ated anthracene (d10)	1.68	40	188(100), 94(19), 80(18)	189, 217

umn: 6' glass, 2 mm i.d.
Tenax GC - 60/80 mesh
190° - 300° @ 8°/min.
He @ 30 ml/min

Analysis of Fish for Volatile Organics by Head Space Analyses

1. Scope

1.1 This method is designed to determine those "unambiguous priority pollutants" associated with the Consent Decree[4] that are amenable to head space analyses. These compounds are listed in Table IV of this section. It is a gas chromatographic-mass spectrometric (GC-MS) method intended for qualitative and semi-quantitative determination of these compounds.

The head space analyses and the liquid-liquid extraction methods are complementary to one another. There is an area of overlap between the two and some compounds may be recovered by either method. The efficiency of recovery depends on the vapor pressure and water solubility of the compounds involved. Generally, the area of overlap may be identified by compounds boiling between 130°C and 150°C with a water solubility of approximately two percent. When compounds are efficiently recovered by both methods, the chromatography determines the method of choice. The gas chromatographic conditions selected for the head space method are, generally, not suitable for the determination of compounds eluting later than chlorobenzene.

1.2 While the above approach has not been sufficiently tested through extensive experimentation, it is based on laboratory experience and is presently our 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 - 5cc.

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

2.4 Head Space Standard Solutions - Prepare three standard methanol solutions of the compounds listed in Table IV at the following concentrations: 50 ng/µg, 150 ng/µl and 300 ng/µl. The standard solutions should be stored in the freezer at less than 0°C. Solutions should be allowed to warm to room temperature before dosing. Fresh standards should be prepared weekly. Procedures for preparing standards are outlined in the purge and trap section of ref. 4.

3. Procedure

3.1 Remove four of the sample vials containing 10.0 grams 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 seconds 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 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 Place all four sample vials into a 90°C water bath for 1 hour.

3.4 While maintaining the sample at 90°C, withdraw 2.0 ml of the head gas with a gas tight syringe and analyze by injecting into a GC, operating under the conditions recommended in ref. 4.

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

3.5 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 from 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.)

3.6 Quality Control: Standard quality assurance protocols should be employed, including blanks, duplicates, and dosed samples as described in the "Analytical Quality Control Handbook"[6].

4. Reporting of Data

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

Table IV

Characteristic Ions of Volatile Organics

Compound	EI Ions (Relative intensity)	Ion used to quantify
chloromethane	50(100); 52(33)	50
dichlorodifluoromethane	85(100); 87(33); 101(13); 103(9)	101
bromomethane	94(100); 96(94)	94
vinyl chloride	62(100); 64(33)	62
chloroethane	64(100); 66(33)	64
methylene chloride	49(100); 51(33); 84(86); 86(55)	84
trichlorofluoromethane	101(100); 103(66)	101
1,1-dichloroethylene	61(100); 96(80); 98(53)	96
bromochloromethane(IS)	49(100); 130(88); 128(70); 51(33)	128
1,1-dichloroethane	63(100); 65(33); 83(13); 85(8); 98(7); 100(4)	63
trans-1,2-dichloroethylene	61(100); 96(90); 98(57)	96
chloroform	83(100); 85(66)	83
1,2-dichloroethane	62(100); 64(33); 98(23); 100(15)	98
1,1,1-trichloroethane	98(100); 99(66); 117(17); 119(16)	97
carbon tetrachloride	117(100); 119(96); 121(30)	117
bromodichloromethane	83(100); 85(66); 127(13); 129(17)	127
bis-chloromethyl ether	79(100); 81(33)	79
1,2-dichloropropane	63(100); 65(33); 112(4); 114(3)	112
trans-1,3-dichloropropene	75(100): 77(33)	75
trichloroethylene	95(100); 97(66); 130(90); 132(85)	130
dibromochloromethane	129(100); 127(78); 208(13); 206(10)	127
cis-1,3-dichloropropene	75(100); 77(33)	75

TABLE IV Continued

Compound	EI Ions (Relative intensity)	Ion used to quantify
1,1,2-trichloroethane	83(95); 85(60); 97(100); 99(63); 132(9); 134(8)	97
benzene	78(100)	78
2-chloroethylvinyl ether	63(95); 65(32); 106(18)	106
2-bromo-1-chloropropane(IS)	77(100); 79(33);156(5)	77
bromoform	171(50);173(100); 175(50); 250(4); 252(11); 254(11); 256(4)	173
1,1,2,2-tetrachloroethene	129(64); 131(62); 164(78); 166(100)	164
1,1,2,2-tetrachloroethane	83(100); 85(66); 131(7); 133(7); 166(5); 168(6)	168
1,4-dichlorobutane(IS)	55(100); 90(30); 92(10)	55
toluene	91(100); 92(78)	92
chlorobenzene	112(100); 114(33)	112
ethylbenzene	91(100); 106(33)	106
acrolein	26(49); 27(100); 55(64); 56(83)	56
acrylonitrile	26(100); 51(32); 52(75); 53(99)	53

Analysis of Fish for Cyanide

1. Scope and Application

   1.1 This method is to be 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 to be used. For cyanide levels below this value, the colorimetric procedure is to be used.

2. Sample Preparation

   2.1 A 5-gram portion of the frozen ground fish as described under "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 by using portions of "spiked" fish tissue, distilled in the same manner as the tissue sample being analyzed. For preparation of the calibration standards, choose a 50 g portion of fish, weigh and blend in a Waring blender (or equivalent) with 10 ml of 10% NaOH and sufficient deionized distilled water so that the volume of the mixture will be 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 increasing volumes of the cyanide standard as given in 8.8 (Ref. 7). Adjust the final volume of each flask to 500 ml with deionized distilled water.

   3.3 Add 50 ml of a 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 Fig. 1 (Ref. 7).

3.4 Continue with step 8.2 through 8.7 (Ref. 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 5 g) in a blender along with 100 ml of deionized distilled water and 1 ml of a 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 a 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 and continue with step 8.2 through 8.7 (Ref. 7).

4.4 Read the absorbance and determine the cyanide concentration 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 analysis 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" (Ref. 6).

6. Reporting of Data

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

6.2 Report all quality control (QC) data along with the analytical results for the samples.

Analysis of Fish for Phenol

1. Scope and Application

1.1 This method is to be used for the determination of phenol in fish. Phenols are defined as hydroxy derivatives of benzene and its condensed nuclei. The 4-amino-antipyrine colorimetric method given determines phenol, the ortho- and meta-substituted phenols, and under proper pH conditions, those para-substituted phenols in which the substitution is a carboxyl, halogen, methoxyl or sulfonic acid group. Presumably, the 4-amino-antipyrine method does not determine those para-substituted phenols in which the substitution is an alkyl, aryl, nitro, benzoyl, nitroso, or aldehyde group.

1.2 All samples must be distilled prior to the determination of phenols using the procedure given on page 576 (Ref. 8). Use method 510 B for samples that contain less than 1 mg/kg and method 510 C for samples that contain more than 1 mg/kg.

2. Sample Preparation

2.1 A 5-gram portion of the frozen, ground fish as described under "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 by using portions of "spiked" fish tissue, distilled in the same manner as the tissue samples being analyzed. For preparation of the calibration standards, choose a 50 g portion of fish, weigh, and blend in a Waring blender

(or equivalent) with sufficient deionized distilled water so that the total volume of the mixture will be 500 ml.

3.2 Transfer a 50 ml portion of mixture to a beaker using a volumetric pipet which has had the tip removed and determine the volume of (1 + 9) H_3PO_4 required to lower the pH to 4.0 using either methyl orange indicator or a pH meter. This volume of (1 + 9) H_3PO_4 is to be added to each 50 ml portion of fish mixture prior to the distillation step which follows.

3.3 Transfer 50 ml portions of the blended fish mixture to the distillation apparatus as shown in Fig. 318:1, p. 241 (Ref. 8), adding the volume of H_3PO_4 (determined above) to lower the pH to 4.0. Add 5 ml of a 10% $CuSO_4$ solution to each distillation flask along with appropriate volumes of the standard phenol solution (Ref. 8, p. 579, 3C). A blank and 7 standards should be distilled for preparation of the calibration curve. [NOTE: The minimum detectable quantity is 1 µg/l phenol in a 500 ml distillate.] Adjust the volume in the distillation flask to 500 ml. Use a 500 ml graduated cylinder as a receiver.

3.4 Begin the distillation and continue until a distillate volume of 450 ml is obtained. Stop the distillation and add 50 ml deionized distilled water to the distillation flask after boiling has ceased. Continue the distillation until a total of 500 ml has been collected. If the distillate is turbid, acidify with (1 + 9) H_3PO_4 and repeat the distillation as described.

3.5 Continue with the procedure as given in the chloroform extraction method 510 B, p. 577 (Ref. 8). Read the absorbance of the standards against a reagent blank at a wavelength of 460 nm. Plot absorbance against micrograms of phenol for the calibration curve.

3.6 Alternatively, follow the direct photometric method (510 C, p. 580, Ref. 8), for those samples in which the phenol concentration exceeds 1 mg/kg.

4. Sample Procedure

4.1 Place a weighed portion of the ground fish (approximately 5 g) in a blender along with 100 ml of deionized distilled water. Blend until a homogeneous mixture is obtained and transfer to a 1 liter boiling flask.

4.2 Rinse the blender with several portions of deionized distilled water and add to the distilling flask. Add a volume of (1 + 9) H_3PO_4 to bring the pH of the mixture to 4.0 (the same volume as determined for preparation of the calibration standards may be used). Add 5 ml of a 10% $CuSO_4$ solution and adjust the total volume to approximately 500 ml. Use a 500 ml graduated cylinder as a receiver.

4.3 Begin the distillation and continue as described in 3.4 - 3.6 above. Read the absorbance and determine the µg of phenol from the 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 Ref. 6.

6. Reporting of Data

6.1 Report phenol concentrations as follows:

Method 510 B to the nearest µg/kg

Method 510 C - when less than 1.0 µg/kg to the nearest 0.01 µg; 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 Fish for Mercury

1. Scope and Application

1.1 This method is to be used for the 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 (Ref. 7, page 118).

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.

2. Sample Preparation

2.1 The sample may be prepared as described under "Sample Handling" or the special metal procedure may be used. A 0.2-0.3 g 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 by using portions of "spiked" fish tissue, treated in the same manner as the tissue samples being analyzed. For preparation of the calibration standards choose a 5 g portion of fish, and blend in a Waring blender.

3.2 Remove six equal and accurately weighed portions (0.2 g) with a spatula and transfer to each of six dry BOD bottles. Add 4 ml of conc. H_2SO_4 and 1 ml of conc. HNO_3 and place in a water bath at 58°C until the tissue is completely dissolved (30-60 minutes).

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 so that the total volume is approximately 125 ml. Add 6 ml of sodium chloride-hydroxylamine sulfate solution to reduce the excess permanganate.

3.5 Wait at least 30 seconds after the addition of the 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 on page 121 (Ref. 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 0.2-0.3 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 and place in water bath at 58°C until the tissue is completely dissolved (30-60 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 10 ml additional permanganate, or more if necessary to maintain oxidizing conditions. Allow to stand overnight at room temperature (see NOTE 1). Continue

as described under (3.4). NOTE 1: 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 minutes. 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 minutes at 80°C (Ref. 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; 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" (Ref. 6).

6.2 Report all quality control data when reporting results 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

at these levels, added as methyl mercuric chloride, were 112, 93 and 86%, respectively.

Analysis of Fish for Metals

1. Scope

1.1 This method is designed to determine in whole fish those priority pollutants listed in the Consent Decree that are classified as heavy metals or considered toxic as they exist in their elemental form and associated compounds. Those pollutants are the following: antimony, arsenic, beryllium, cadmium, chromium, copper, lead, nickel, selenium, silver, thallium, and zinc.

2. Summary of Method

2.1 The fish is prepared for analysis by first being chopped into small pieces, homogenized in a blender with dry ice, and then 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 in the fish.

3. Preservation and Handling

3.1 While an aliquot of the ground fish as prepared under "Sample Handling" may be taken 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 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 Yarick Street, New York, NY 10013, may be used in place of chromic acid.

4. Sample Homogenization

4.1 If a separate 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 300 grams. If an analysis is required on a larger (>300 grams) a 50 gram representative portion must be taken from the sample after it has been pretreated as described in "Sample Handling" on page 1 of this document and proceed to step 4.3.

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

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

4.4 Turn on the blender for 10 seconds 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 2-5 minutes. Add more dry ice if needed.

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

4.7 Place the bag in the freezer (-12°C for at least 16 hours) 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 (1/2 inch - quartsize, 1 inch - gallonsize). 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, calibration standards, and as dilution water.

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

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

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

5.5 Hydrochloric acid, ACS grade (37-38%).

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°C 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 employs a cold vapor technique, the other pollutants 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 (10) with the use of an ashing aid, while Group II is prepared utilizing a wet ashing procedure.

7.1 Group I - Metals

7.1.1 Remove the homogenized sample from the freezer and weigh approximately 10 grams 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 Dry the samples in an oven or furnace at 110+ 5°C until a charred viscous sulfuric acid/sample residue remains. Usually 12 to 16 hours (overnight) is sufficient. Transfer the ashing vessels containing the dried samples to a cold, clean muffle furnace which is provided with good external ventilation (fume hood), ensuring that the sample remain covered during the transfer. Initially set the furnace at 125°C and increase the temperature approximately every hour in 50°C increments up to 275°C. Hold the temperature at 275°C for 3 hours. Finally, increase the temperature to 450°C (at 50°C per hour) and hold for 12 to 16 hours (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 minutes. 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 furnace. 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 80-90°C for 5 to 10 minutes 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 hrs.). Do not shake the sample further before taking an aliquot for analysis. 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 using either direct aspiration or furnace techniques. 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," pages 78-91 (Ref. 7) and the individual analyses sheets (page numbers listed in Table V).

Table V

Element	Methods for Chemical Analysis of Water and Wastes, 1974
Ag	p. 146
Be	p. 99
Cd	p. 101
Cr	p. 105
Cu	p. 108
Ni	p. 141
Pb	p. 112
Sb	p. 94
Tl	p. 149
Zn	p. 155

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 concentration. When using the furnace technique, the operating parameters and instructions as-specified by the particular instrument manufacturer should be followed. If the detected concentration by the furnace procedure is beyond the working range of the standard curve, the sample should either be 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 chelation/solvent extraction technique for concentration Ag, Ni, Pb and Tl is preferred, the procedure as described starting on page 89 (Ref. 7). should be utilized.

7.2 Group II - Metals

7.2.1 Remove the homogenized sample from the freezer and weigh approximately 5 grams into a tared 125 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 conc. H_2SO_4 and cover with a watch glass.

7.2.3 Place beaker on hot plate and warm slightly. (NOTE: Remove beaker if foaming becomes excessive.) 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.

7.2.4 Add an additional 5 ml of conc. HNO_3, cover with a watch glass, and return beaker to hot plate. 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 seems that the sulfuric acid may approach 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 (Ref. 8). From the prepared sample a 25 ml aliquot should be withdrawn and the analysis continued as described in section 3.d, page 162 (Ref. 8).

8. Calculation

8.1 From the values read off 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,

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

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

$$\mu g/gram = \frac{\frac{\text{µg/l of constituent in prepared sample}}{1000} \times \text{volume of prepared sample in ml}}{\text{weight of wet sample in g}}$$

9. Quality Assurance

9.1 Standard quality assurance protocols should be employed, including blanks, duplicates and dosed samples as described in the "Analytical Quality Control Handbook" (6).

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

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