Appendix A

Sample Forms

LABORATORY LOG BOOK FORMAT

Specimen/ Sample#	Log-in-Date	Log-out Date	Type	SUB SAMPLES				
				Muscle	Liver	Kidney	Adipose	Gut Contents

Laboratory Name
Laboratory Address
NYSDEC Bureau of Habitat
Hudson River Reptiles and Amphibians
Sample ID: HR-98-001
Analysis: Media: HERP
Preservative:
Date: Time: By:

Laboratory Name
Laboratory Address
NYSDEC Bureau of Habitat
Hudson River Reptiles and Amphibians
Sample ID: HR-98-002
Analysis: Media: HERP
Preservative:
Date: Time: By:

Laboratory Name
Laboratory Address
NYSDEC Bureau of Habitat
Hudson River Reptiles and Amphibians
Sample ID: HR-98-003
Analysis: Media: HERP
Preservative:
Date: Time: By:

Laboratory Name
Laboratory Address
NYSDEC Bureau of Habitat
Hudson River Reptiles and Amphibians
Sample ID: HR-98-004
Analysis: Media: HERP
Preservative:
Date: Time: By:

Laboratory Name
Laboratory Address
NYSDEC Bureau of Habitat
Hudson River Reptiles and Amphibians
Sample ID: HR-98-005
Analysis: Media: HERP
Preservative:
Date: Time: By:

Laboratory Name
Laboratory Address
NYSDEC Bureau of Habitat
Hudson River Reptiles and Amphibians
Sample ID: HR-98-006
Analysis: Media: HERP
Preservative:
Date: Time: By:

Laboratory Name
Laboratory Address
NYSDEC Bureau of Habitat
Hudson River Reptiles and Amphibians
Sample ID: HR-98-007
Analysis: Media: HERP
Preservative:
Date: Time: By:

Laboratory Name
Laboratory Address
NYSDEC Bureau of Habitat
Hudson River Reptiles and Amphibians
Sample ID: HR-98-008
Analysis: Media: HERP
Preservative:
Date: Time: By:

Laboratory Name
Laboratory Address
NYSDEC Bureau of Habitat
Hudson River Reptiles and Amphibians
Sample ID: HR-98-009
Analysis: Media: HERP
Preservative:
Date: Time: By:

Laboratory Name
Laboratory Address
NYSDEC Bureau of Habitat
Hudson River Reptiles and Amphibians
Sample ID: HR-98-010
Analysis: Media: HERP
Preservative:
Date: Time: By:

Appendix B

Standard Operating Procedures

Standard Operating Procedure
For
Chain-of-Custody Records

INTRODUCTION

The chain-of-custody record allows for the tracking of possession and handling of individual samples from the time of field collection through laboratory analysis. All samples released from field operations shall be accompanied by a Chain-of-Custody Form (Attachment 007-1). This is done to insure the legal integrity of the sample materials collected. Every effort shall be made to keep as few people as possible in the chain of sample possession.

PROCEDURE

1. A completed Chain-of-Custody Form shall accompany each set of samples released from the study site. The Chain-of-Custody Form for all samples shall include the following information:

    a. Signature of Sampler
    b. Client/Project name
    c. Project Location
    d. Field Logbook Number (e.g. page no. in field book)
    e. Sample Number, Identification
    f. Date and time of sample collection
    g. Type of Sample (Air, water, soil, etc.)
    h. Analysis requested
    i. Preservative Added (Remarks section)
    j. Source of the Sample (Remarks section)
    k. Chain-of-Custody Tape Number
    l. Inclusive Dates of Possession
    m. Signatures of persons involved in chain of possession
    n. Name of person the analytical results are to the attention of (in lower right corner of the form).

2. The Chain-of-Custody Form is designed in quadruplicate. Each of the individual four sheets is a different color. Along the bottom of each sheet are the instructions describing who gets which copy. These instructions are as follows:

White Copy:	Original sheet to accompany sample to the lab and return to the Project Manager
Yellow Copy:	Laboratory Copy
Pink Copy:	Transporter Copy (optional)
Orange Copy:	Sampler Copy

Therefore, after the Chain-of-Custody Form has been completely filled out, the sampler signs the initial "Relinquished by" along with date and time and obtains the signature of the next person (i.e., transporter) in the chain-of-custody (in the initial "Received by" box along with date and time. The sampler then tears off the back (orange) copy for his records. Then the transporter delivers the samples to the analytical lab, he signs the second "Relinquished by" box along with date and time. and a laboratory representative signs the second "Received by" box along with the date and time. At this point, the transporter has the option of retaining the pink copy for his records.

Instructions shall be given to the laboratory regarding their responsibilities in returning the top sheet (white copy) to the Project Manager with the lab results. This sheet contains all sample information and original signatures. The lab should retain the yellow copy for their records.

If the sampler his- or herself delivers the samples to the laboratory, then the sampler should make certain the receiving party at the lab signs in the proper space, i.e., "Received for Laboratory".

3. The Chain-of-Custody form shall be completed in legible hand writing with indelible ink, with all appropriate information completed. Once completed, the form is either:

   a. placed in a plastic-wrap and included with the samples in the cooler, or

   b. fixed in an envelope taped securely in top of the cooler or plastic packing slip container (if available). This method allows for signatures to be included with each transfer of custody. This method is mandatory in the event a non-commercial courier is utilized to transport samples.

4. The sample container shall be sealed with chain-of-custody tape, containing the designation, date, and sampler's signature. The custody tape is especially important when shipping the container via overnight courier such as Federal Express and United Parcel Service.

Revision
Author: _____ Date: 3/13/96
Scientist, Engineer or Technician

Reviewed: _____ Date: 3/13/96
Director, Quality Assurance Unit

Approved: _____ Date: 3/13/96
Vice President

ATTACHMENT SOP-007-1

Chain-of-Custody Form (following page)

CHAIN OF CUSTODY RECORD

Nº 00

Client/Project Name	Project Location	ANALYSES
Project No.	Field Logbook No.	
Sampler: (*Signature*)	Chain of Custody Tape No.	

Sample No./ Identification	Date	Time	Lab Sample Number	Type of Sample							REMARKS

Relinquished by: (*Signature*)	Date	Time	Received by: (*Signature*)	Date	Time
Relinquished by: (*Signature*)	Date	Time	Received by: (*Signature*)	Date	Time
Relinquished by: (*Signature*)	Date	Time	Received for Laboratory: (*Signature*)	Date	Time
Sample Disposal Method:	Disposed of by: (*Signature*)			Date	Time
SAMPLE COLLECTOR	ANALYTICAL LABORATORY				

1974-3-84

WHITE - To accompany sample to the lab and returned to the Poject Manager. YELLOW - Lab copy PINK - Transporter copy ORANGE - Sampler copy

Standard Operating Procedure For
Deviation from Protocols or Standard Operating Procedures
and for Notation, Correction and Documentation of
an Unforeseen Circumstance

INTRODUCTION:

The objective of this procedure is to ensure the study quality and integrity in the event of:

(a) deviations from approved protocols or standard operating procedures and
(b) unforeseen circumstances which may affect the quality of the study.

The procedure set forth herein is appropriate and desirable in all studies. However, it is a requirement of regulatory law for studies conducted under 40 CFR Part 160, the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), Good Laboratory Practice Standards and 40 CFR Part 792, Toxic Substance Control Act (TSCA), Good Laboratory Practice Standards.

Specifically, FIFRA requires at 40 CFR § 160.81 (Standard Operating Procedures) that

"(a) A testing facility shall have standard operating procedures in writing setting forth study methods that management is satisfied are adequate to insure the quality and integrity of the data generated in the course of a study. All deviations in a study from standard operating procedures shall be authorized by the Study Director and shall be documented in the raw data. Significant changes in established standard operating procedures shall be properly authorized in writing by management."

At 40 CFR 160.33 (Study Director)... "The Study Director shall assure that:

(a) the protocol, including any change, is approved as provided by §160.120 and is followed. [Emphasis added]

...
(c) unforseen [sic] circumstances that may affect the quality and integrity of the study are noted when they occur, and corrective action is taken and documented. [Emphasis added]

...
"(e) all applicable good laboratory practice regulations are followed".

Further, at 40 CFR § 160.35 (Quality assurance unit)
...

"(b) The quality assurance unit shall:

...

(5) Determine that no deviations from approved protocols or standard operating procedures were made without proper authorization and documentation." [Emphasis added.]

PROCEDURE:

A. Record of Deviation from Protocol or Standard Operating Procedure

1. Record the facts of the deviation legibly in ink using Form QAU-005 or a field book, bench log, or other recognized and reliable record keeping device at the time of the deviation. Initial and date each page of entry at that time.

   The facts of the deviation should include but not necessarily be limited to:

   Date of occurrence:
   Time:
   File No.:
   Study designation:
   Test Substance:
   CAS No.:
   Study sponsor:
   Study phase or segment (Ref: Protocol):
   Weather:
   Temperature:
   Personnel on site:
   Visitors:
   Contractors:
   Equipment:
   Standard Operating Procedure (title and number):
   Protocol (title, date, section):
   Type of deviation (inadvertent or planned):
   Nature of deviation:
   Observations:
   Problems:

2. List anticipated consequences of deviation.

3. Determine correction action alternatives and rank with most desirable first.

4. Contact Study Director/Project Manager as soon as conveniently possible for authorization to proceed with corrective action.

5. Document the corrective action, e.g. change in procedure, materials, equipment, personnel, time.

6. Retain all notes and records of the deviation for archiving as raw data.

7. Prepare full documentation of the facts of deviation, authorization and corrective action on Form QAU-005.

8. Sign and date Form QAU-005.

9. Obtain authorj:zation signature of Study Director/Project Manager on Form QAU-005.

10. Obtain signature of Quality Assurance Unit Director indicating review of completed Form QAU-005.

B. Record of Unforeseen Circumstances which may Affect the Quality of the Study

1. Record the facts of the unforeseen circumstances legibly in ink using form QAU-005 or a field book, bench log, or other recognized and reliable record keeping device at the time of occurrence. Initial and date each page of entry at that time. The facts of the unforeseen circumstances should include but not necessarily be limited to those items listed under A. 1. above. A wide variety of unforeseen circumstances may impinge on the quality of a study. For example, a lightning strike induces a charge on a cable and distorts information in the data logger or destroys a sensing unit at the weather station; a hurricane flood submerges monitoring wells; a bee sting or threat of snake bite causes personnel to drop a box of water samples; or an accident delays delivery of refrigerated samples.

2. List the consequences of the unforeseen circumstance.

3. Determine if corrective action is possible.

4. List corrective action alternatives with most desirable first.

5. Notify Study Director/Project Manager as soon as conveniently possible of unforeseen circumstances.

6. Obtain authorization from Study Director/Project Manager to proceed with corrective action.

7. Document the corrective action, e.g. change in procedure, materials, equipment, personnel, time.

8. Retain all original notes and records of the unforeseen circumstances for archiving as raw data.

9. Prepare full documentation of the facts of the unforeseen circumstances, notification, authorization, and corrective action on Form QAU-005.

10. Sign and date form QAU-005

11. Obtain authorization signatures of Study Director/Project Manager of Form QAU-005

12. Obtain signature of Quality Assurance Unit Director indicating review of completed Form QAU-005.

Revision
Author: _____ Date: 7/12/94
Scientist, Engineer or Technician

Reviewed: _____ Date: 7/12/94
Director, Quality Assurance Unit

Approved: _____ Date: 7/12/94
Vice President

Quality Assurance Unit
Record of
(Check One)

❑ A. *Deviation from Protocol or Standard Operating Procedure*
or
❑ B. *Notation, Correction and Documentation of Unforeseen Circumstances*

Ref: 40 CFR § 160.81, § 160.33 and § 160.35 and SOP-018

Dates of Occurrence:

Study Designation:

Study Location:

Test substance: CAS No.:

Study Sponsor:

Study phase (or segment):

Site of problem:

Scientist, engineer or technician: Initials: _____Date: _____

Findings:

Weather: F; Sky: Clear, Partly Cloudy, Fair, Rain, Snow; Wind-

List: Personnel, Visitors, Contractors:

Equipment (e.g. Permeameter, Data Logger, Drill rig, Dozer):

Protocol title, date and section number:

Standard Operating Procedure title:

SOP No.:

Type of deviation (inadvertent or planned):

Nature of deviation or unforeseen circumstance:

Observations:

Problems:

Continuation Sheet

Initials: _____ Date:_____

(Instruction: Number paragraphs consistent with the numbers of items listed under Procedure A or B in SOP 118. Begin with No. 2. Sheet 1 of this report addresses Item No. 1.)

Continuation Sheet Initials: _____ Date:_____

Report Prepared by: _____ Date _____
Scientist, Engineer or Technician

Authorized by: _____ Date _____
Study Director/Project Manager

Reviewed by: _____ Date _____
Vice President

Appendix C

Laboratory Methods

Appendix C-1

PCB and Organochlorine Pesticides Methods

APPENDIX D

Analytical methods for analysis of PCB, organochlorine pesticides, lipid and moisture content used by the contract laboratory; the methods are those of the US Fish and Wildlife Service based on US Environmental Protection Agency methodology.

Methodology currently in Use

Code	Method Title
001	Analysis for Organochlorine Pesticides and PCBs in Animal and Plant Tissue.
002	Analysis for Organochlorine Pesticides and PCBs in Soil and Sediment.
005	Analysis for Organochlorine Pesticides and PCBs. Aliphatic and polynuclear Aromatic Hydrocarbons in Water.
006	Analysis for Chlorinated Hydrocarbon Pesticides and Related Compounds - Micro Method.
011	Analysis for Organochlorine Pesticides and PCBs in Blood .Serum, Plasma or Whole Blood.
015	Elution Profiles for Florisil, Silica Gel and Silicic Acid Column Separations.

Method 1. Analysis For Orqanochlorine Pesticides and PCBs in Animal and Plant Tissue.

Ten gram tissue samples are thoroughly mixed with anhydrous sodium sulfate and soxhlet extracted with hexane for seven hours. The extract is concentrated by rotary evaporation; transferred to a tared test tube, and further concentrated to dryness for lipid determination. The weighed lipid sample is dissolved in petroleum ether and extracted four times with acetonitrile saturated with petroleum ether. Residues are partitioned into petroleum ether which is washed, concentrated; and transferred to a glass chromatographic column containing 20 grams of Florisil. The column is eluted with 200. ml 6% diethyl ether/94% petroleum ether (Fraction I) followed by 200 ml 15% diethyl ether/85% petroleum ether (Fraction II). Fraction II is concentrated to appropriate volume for quantification of residues by packed or capillary column electron capture gas chromatography. Fraction I is concentrated and transferred to a Silicic acid chromatographic column for additional cleanup required for separation of PCBs from other organochlorines. Three fractions are eluted from the silicic acid column. Each is concentrated to appropriate volume for quantification of residues by packed or megabore column, electron capture gas chromatography. PCBS are found in Fraction II.

Method 6. Analysis For Chlorinated Hydrocarbon Pesticides And Related Compounds - Micro Method

This method is necessary when sample size is limited (below 4 g. approximately) and in case of organ tissue as substrate and is a modified version of the method described In Manual of Analytical Methods for the Analysis of Pesticides in Humans and Environmental Samples, EPA-600/B-80-038; June 1980, Section 5, A (2). It is suitable for adipose, kidney, liver, muscle, brain, and other tissues:

1. Weigh 0.5 g or less of well-mixed tissue into a size 22 Duall tissue grinder.
2. Extract tissue by grinding three times with acetonitrile: the first time being with 4 ml followed by two 2.5 ml portions.
3. Remove the pestle after each grinding and centrifuge, decanting the extract into a 50 mm glass stoppered graduated mixing cylinder.
4. Combine all extracts and record the total volume of the three extracts.
5. Add a volume of PRQ water equivalent to 3.3 times the extract volume. Then add 2 ml saturated NaCl solution.
6. Extract the aqueous acetonitrile mixture with 5 ml. hexane by vigorous shaking for 1 minute.
7. Allow layers to separate and remove the hexane layer with a Pasteur pipet into a 15 ml screw-capped culture tube.
8. Re-extract twice with 2 ml hexane each time, combining the extracts into the culture tube.
9. Concentrate the combined hexane extracts under nitrogen to approximately 0.5 ml volume.

10. Clean-up on a florisil mini-column as described in Method 2, steps 8, 9, 10, and 11.

Note For brain tissue additional treatment is necessary before column clean-up:

11. Proceed through Steps 1-9 above, add 0.3 ml acetic anhydride and 0.3 ml pyridine, cap tightly and incubate for 30 minutes in a water bath at 60-65^{o}C.

12. Add 8 ml PRQ water and 1 ml saturated NaCl and extract three times with 2 ml hexane, combining the extracts into a clean tube.

13. Concentrate the combined extracts under nitrogen to about 0.3 ml and proceed with florisil mini-column clean-up. (Step 10)

Note The following changes in sample handling, particularly column clean-up, should be observed for Kepone analysis:

14. Maintain the integrity of the analyte in sample extracts by insuring that the samples are not allowed to reach dryness during concentration steps. Kepone easily adheres to glass, but the use of polar solvents, such as methanol and acetonitrile within the analysis will provide better recoveries of this analyte.

15. Modifications to florisil mini-column clean-up are as follows;

    * Following addition of sample to the column, apply a 1 ml rinse of 1% methanol in hexane to the sample tube. This rinse should be added after the first phase of the first fraction (12 mls hexane) and will insure removal of trace quantities of kepone adhered to glass. Decrease the total volume of the second phase of the first fraction (12 mls 1% methanol/hexane) to 11 mls.

    * Modify the total volume of the second fraction from 24 mls to 36 mls 1% methanol/hexane. This fraction contains Kepone.

* Concentrate column fractions on N-EVAP and transfer with 1% methanol/hexane to calibrated test tubes. Adjust sample volume to calibrated level and proceed to determination by gas chromatograph.

Elution Profiles for Florisil, Silica Gel and Silicic Acid Column Separations

A. Florisil Column:

1. Fraction I (6% ethyl ether containing 2% ethanol, 94% petroleum ether). HCB, alpha-BHC, beta-BHC, gamma-BHC, delta-BHC, oxychlordane, heptachlor epoxide, gamma-chlordane, trans-nonachlor, toxaphene, PCBs, o,p'-DDE, alpha-chlordane, p,p'-DDE, p,p'-DDT, cis-nonachlor, o,p'-DDT, p,p'-DDD, p,p'-DDT, mirex, dicofol, endosulfan I (Split with FII), octachlorostyrene, chlorpyrilfos, ,and .methyl chlorpyrifos.
2. Fraction II (15% ethyl ether containing 2% ethanol, 85% petroleum ether) dieldrin, endrin, dacthal, endosulfan I (split with FI), endosulfan II (split with FIII), endosulfan sulfate (split with FIII), diazinon, EPN, ethyl parathion, and methyl parathion.
3. Fraction III (50% ethyl ether containing 2% ethanol, 50% petroleum ether) endosulfan II (split with FII), endosulfan sulfate (split with FII), malathion.

B. Florisil Mini-Column:

1. Fraction I (12 ml hexane followed by 12 ml 1% methanol in hexane)

   HCB, gamma-BHC (2.5%),. alpha-BHC (splits with FII),

   trans-nonachlor, o,p'-DDE, p,p'-DDE, o,p'-DDD, p,p'-DDD (splits with FII), o,p'-DDT, p,p'-DDT, mirex, cis-nonachlor, cis-chlordane, trans-chlordane,PCBs, Photomirex and derivatives.

2. Fraction II (24 ml 1% methanol in hexane)

   gamma BHC (75%), beta-BHC, alpha-BHC (splits with FI), delta-BHC, oxychlordane, heptachlor epoxide, toxaphene, dicofol, dacthal, endosulfan I, endosulfan II, endosulfan sulfate, octachlorostyrene, Kepone (with additional 12mls 1% methnol in hexane), alachlor, atrazine, chlorpyrifos, diazinon, EPN, ethyl parathion, malathion, methyl chlorpyrifos, methyl parathion, metolachlor, metribuzin, propazine, simazine.

C. Silica Gel:

1. SG Fraction I (120 ml petroleum ether)

   n-decane, n-undecane, n-dodecane, n-tridecane, n-tetradecane, n-pentadecane, n-hexadecane, n-heptadecane, n-octadecane, n-nonadecane, n-eicosane, n-heneicosane, n-docosane, n-tricosane. n-tetracosane, n-pentacoscne, n-hexacosan, n-heptacosane, n-octacosane, n-nonacosane, n-tricontane, n-hentriacontane, n-dotriacontane,. n-tritriaconfane, n-teratriacontane, pristane,and phytane.

2. SG Fraction II (100 ml 40% methylene chloride in petroleum ether followed by 50 ml methylene chloride).

naphthalene, 2-methylnaphthalene, 1-methylnaphthalene, biphenyl, 2-6-dimethylnaphthalene, acenaphthalene, acenaphthene, 2,3,5-trimethylnapthalene, fluorene, dibenzothiophene, phenanthrene, anthracene, 1-methylphenanthrene, fluoranthene, pyrene, benzo[a]anthracene, chrystene, benzo[b]pyrene, perylene, indeno[1,3,3-cd]pyrene, dibenz[a,h]anthracene, benzo[g,h,i]perylene.

D. Silicic Acid:

1. SA Fraction I (20 ml petroleum ether)

   HCB, mirex, octachlorstyrene

2. SA Fraction II (100 ml petroleum ether)

   PCBs, photomirex derivatives

3. SA Fraction III (20 ml mixed solvent: 1% acetonitrile, 80% methylene chloride, 19% hexane)

   alpha-BHC, beta-BHC, gamma-BHC, delta-BHC, oxychlordane, heptachlor epoxide, gamma-chlordane, trans-chlordane, toxaphene, o,p'-DDE, alpha-chlordane, p,p'-DDE, o,p'-DDT, cis-nonachlor, o,p'-DDT, p,p'-DDD, p,p'-DDT, dicofol.

Procedure for the Quantification of a Multi-Residue Sample in the Presence of Interferences.

If preliminary examination of a sample by EC/GC indicates the presence of PCBs, further fractionation of the analytes in the sample extract is performed using a silicic acid column. The result is three fractions, one of which contains PCBs separate, from other analytes. This step effectively eliminates PCB interferences in quantitation of other analytes present in the extract.

All samples for multi-residue organochlorine analyses are determined by a single injection split onto two different megabore capillary columns enabling closely eluting pairs from one column to be, completely resolved on the other column. In such cases, the value from the. column giving complete resolution is reported; otherwise when a value is obtainable from each column, the mean value is normally reported.

If toxaphene is encountered at concentrations greater than 50 times that of the other OC analytes, an interfering toxaphene component can be quantified from a toxaphene standard and an appropriate correction factor applied; thereby giving a net value for calculating the resultant value for the analyte. Again, two column analysis often results in a completely resolved analyte from which quantification can be made. Historically, toxaphene, when encountered, is less than 50 times the concentration of the other OC analytes, and because its response factor is so much less than that of the other analytes, interference from toxaphene can be diluted out so that its interference with the other analytes is negligible.

Appendix C-2

Metals, Hg, Cd, Pb Methods

STANDARD OPERATING PROCEDURE
HG.1998.FISH.1
MERCURY IN FISH TISSUES

ABSTRACT: Samples are digested and analyzed for total mercury by cold vapor atomic absorption spectrometry (CVAAS), using a Leeman Labs AP/PS200II Mercury Analysis System. A tissue sample is digested in concentrated nitric acid and sulfuric acid, then oxidized with potassium permanganate and potassium persulfate. Sodium chloride-hydroxlamine sulfate is added to reduce excess potassium permanganate. The sample is reduced with stannous chloride and mercury vapor is carried by argon gas to an optical cell with a mercury lamp (254nm). Concentration is determined directly from a calibration curve.
BASIS: The method is based on EPA Method 245.6 - Determination of Mercury in Tissues by Cold Vapor Atomic Absorption Spectrometry, Revision 2.3 (April 1991).

Introduction. This is a standard operating procedure in the Analytical Services Unit of Hale Creek Field Station, NYSDEC, Gloversville, NY for the analysis of total mercury in fish. **WARNING: MERCURY IS EXTREMELY TOXIC AND SHOULD BE HANDLED WITH EXTRA CARE. ACIDS, OXIDIZERS AND REDUCING AGENTS, USED IN THIS PROCEDURE, ARE EXTREMELY CORROSIVE. INHALATION AND CONTACT WITH EYES AND SKIN SHOULD BE AVOIDED.**

Digestion. Digest samples using a Leeman Labs AP200II (Ref: LEEMAN 1997).

PROTECTIVE CLOTHING REQUIRED: (1) Lab coat and apron, (2) vinyl, latex or neoprene gloves, and (3) protective eyewear for all work involving handling of chemicals.

MATERIALS NEEDED: (1) 50 ml glass, polyethylene or polypropylene tubes - 28.7mm x 103mm (Leeman or Nalge). (2) tracemetal nitric acid (Leeman or Fisher). (3) tracemetal sulfuric acid (Leeman or Fisher). (4). certified ACS nitric acid (Fisher). (5) potassium permanganate 5% (Leeman or LabChem). (6) potassium persulfate 5% (Leeman or LabChem). (7) Sodium chloride-hydroxylamine sulfate 12% (Leeman or GFS Chemicals). (8) deionized water (dH20). (9) reference material, if available, or matrix spikes at 0.5 to 5x the expected concentration in the samples. (10) Mercury Reference Standard Solution (Leeman or Fisher - 1000 ppm). (11) Saran Wrap - 11.5 in. width. (12) Leeman Labs AP/PS200II Mercury Analysis System.

PROCEDURE: (1) Prep the AP200II: Turn on the argon supply and the hood over the AP200II; Perform scheduled maintenance, as required; Vent rinse bottles; Fill reagent bottles, if necessary and enter volumes in Reagent Selection; Replace rinse with reagent bottles; Pressurize and run the Change Reagents Macro; Empty and rinse the waste tube; Change dH20 in the rinse tank; Empty water bath drain pan; Fill water bath supply carboy.

(2) Weigh samples or reference material into tared 50 ml tubes; enter weights and sample rack data. Use approximately 0.5 g (0.4-0.6 g) of fish tissue or 0.08 g (0.06 -1.0 g) of dried reference material. Rinse spatula with 20% HNO3 (reagent grade),-tap water and dH20 and dry with acetone prior to using for reference material. Rinse spatula with 20% HNO3 (reagent grade), tap water and dH20 prior to using for each sample. Include one procedure blank, one reference sample or matrix spike, and one duplicate sample per 20-sample batch. Add 500ul dH20 to the procedure blank and 420ul dH20 to the dried reference material to make these volumes equivalent to the samples.

(3) Prepare calibration standards in 50 ml tubes.

For Std. Conc.	Use 500ul of	
0 ppb	dH20	
4 ppb	8 ppb	(F)
25 ppb	50 ppb	(E)
100 ppb	200 ppb	(D)
300 ppb	600 ppb	(C)
600 ppb	1200 ppb	(B)

Preparation Method*		Final Conc.
A.	1ml (1000ppm) to 100ml	10 ppm
B.	12ml (A) to 100ml	1200 ppb
C.	6ml (A) to 100ml	600 ppb
D.	2ml (A) to 100ml	200 ppb
A1.	5ml (A) to 50ml	1000 ppb
E.	5ml (A1) to 100ml	50 ppb
F.	4m1 (D) to 100ml	8 ppb

* using 1.8% tracemetal HNO3 as diluent

Using the above calibration standards, the equivalent mercury concentration in samples (when 0.5 g of sample is used) and the approximate percent of samples that will be below that concentration (when 0.5 g of sample is used) are as follows:

Std. Conc.	Sample Conc.	% Below Conc.*
4 ppb	8 ppb	4%
25 ppb	50 ppb	12%
100 ppb	200 ppb	51%
300 ppb	600 ppb	92%
600 ppb	1200 ppb	99%

* based on 2113 samples analyzed prior to 1997

Other standard concentrations can be used, depending on the expected concentration range of the samples.

(4) Prepare 2 calibration check standards per 20 sample- batch at standard concentration = 100 ppb.

(5) Add 5 ml dH20 to each standard. Place a vapor barrier and frame on each sample and standard rack.

PRIOR TO STEP 6 THE VERTICAL SASH HOOD IN THE METALS LAB SHOULD BE TURNED ON AND SHOULD REMAIN ON FOR THE DURATION OF THE DIGESTION AND ANALYSIS.

(6) **CAREFULLY**, add 3 ml tracemetal H2SO4 to each sample, standard and QC sample. [If the automated dispenser on the AP200II adds H2SO4 (see step 7), omit step 6]

(7) Place racks in water bath and start digestion (F9). The program will add reagents according to the entries in Reagent Additions. 3 ml H2SO4 and 0.8 ml HNO3 are added to each cup. The water bath fills and heats for 40 minutes to 80C. The heater turns off and the bath is drained. The bath is refilled with water to cool the samples. 10 ml 5% potassium permanganate and 6 ml 5% potassium persulfate are added to each cup. The water bath fills and heats for 90 minutes at 30C. The water bath drains and refills, then pauses.

(8) Add 5 ml dH20 to each sample and QC sample. Empty the waste tube (0-7).

(9) Restart the program. 8 ml NaCl-hydroxylamine sulfate 12% is added to each cup. After 15 minutes, there is a final mixing step.

(10) At the end of the digestion, cover the racks with Saran Wrap and allow to stand overnight.

(11) Shut down the AP200II: Vent the reagent bottles; Replace the reagent bottles with rinse bottles; Pressurize and run the Change Reagents Macro; Empty and rinse the waste tube (0-7).

Analysis. Determine total mercury by atomic absorption spectroscopy, using a Leeman Labs PS200II (Ref: LEEMAN 1997).

PROTECTIVE CLOTHING REQUIRED: For preparation of standards, handling digested samples and cleaning glassware: wear lab coat, gloves and protective eyewear.

MATERIALS NEEDED: (1) tracemetal HNO3 (Leeman or Fisher). (2) certified ACS pitric acid (Fisher). (3) Stannous Chloride 10% (Leeman, Fisher or GFS Chemicals). (4) Leeman Labs AP/PS200II Mercury Analysis System.

PROCEDURE: (1) Prep the PS200II: Turn on the argon supply; Perform scheduled maintenance, as required; Fill rinse tank with 5% tracemetal nitric acid in dH20; Place reductant tube in reductant and sample tube in rinse tank; Secure PS pump tubing; Start argon, pump, HG lamp.

(2) Mix samples and standards.

(3) Set up the run: Autosampler setup; Open new folder; Reset for new calibration. Program at least two replicate readings for each sample, standard or blank.

(4) Analyze standards (F6) - calibration is performed by direct determination of concentration from a calibration curve. [The standard enters the system an is mixed with stannous chloride to form mercury vapor. Argon carries the vapor to an optical cell. A mercury lamp delivers a source of emission at 254nm. Absorbance is measured by a detector.] Check and accept calibration.

(5) Analyze samples (F8) and check standards (F7); print report (F4).

(6) Place reductant tube in rinse tank for 10 minutes. Remove reductant tube and sample tube from rinse tank. Empty rinse tank.

(7) Turn off lamp, pump, argon gas. Loosen pump tubing.

(8) Clean glass tubes: soak and brush with solution of PEX and warm water; rinse 2x with tap water, 2x with 20% HNO3 (certified ACS), 2x with tap water and 3x with dH20; dry in drying oven at 120C overnight.

Quality Control. The following QC should be a part of every analysis:

(1) Reference Material (e.g. DORM-2 dogfish muscle, NRC, Canada) should be analyzed once per 20 samples or once per batch, whichever is more frequent. Percent recovery should be 85%-115% (Ref: USEPA 1995).

(2) Check Standards should be analyzed once per 10 samples. Percent recovery should be 80% -120% (Ref: USEPA 1995).

(3) Procedure Blanks should be analyzed once per 20 samples or once per batch, whichever is more frequent. Mercury concentration should be less than the MOL (Ref: USEPA 1995).

(4) Laboratory Duplicates should be analyzed once per 20 samples or once per batch, whichever is more frequent. [RPD] should be less than or equal to 20% (Ref: USEPA 1995).

(5) Analytical Replicates (replicate analyses of the same subsample digest) should be routine. Each subsample should be analyzed at least in duplicate (Ref: USEPA 1995). The RSD should be. less than or equal to 15% (this a laboratory-determined limit).

(6) Calculated recovery from calibration standards should be 95%-105%, if using a 3-point calibration (Ref: USEPA 1995). If more than 3 points are used, a correlation coefficient r >= .9995 is also acceptable (this is a laboratory-determined limit).

Results.

CALCULATIONS: PPM = calculated ppb concentration
X dilution factor X 0.001
/ weight of subsample in g

METHOD DETECTION LIMIT (MDL) = $t_{(0.9.9)}$ **X** S, where t is the appropriate (i.e n-1 degrees of freedom) one-sided 99% Student's t-statistic and S is the standard deviation from a minimum of seven replicate analyses of a fish sample with an Hg concentration 3 to 5x the MDL.

Conclusions. I am confident that the method described above is technically justified to address issues relating to total mercury contamination in fish in New York State.

3/11/98

References.

Leeman Labs, 1997. AP/PS200II Mercury Analysis Systems Manual #150-00103. Leeman Labs Inc., Hudson, NH.

USEPA, 1995. Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories. Volume 1: Fish Sampling and Analysis. 2nd Edition. U.S. Environmental Protection Agency, Washington, DC.

STANDARD OPERATING PROCEDURE
CD-AT-3

CADMIUM IN ANIMAL TISSUE - 3

ABSTRACT: Samples are digested in concentrated nitric acid in a CEM Model MDS 81-D microwave oven. The digestions are performed in closed CEM 120 ml PFA teflon vessels. Cadmium is analyzed on a Perkin-Elmer Model 5100 Atomic Absorption Spectrophotometer (Zeeman), equipped with an HGA-600 Graphite Furnace, AS-60 Autosampler, and AA WinLab software. An electrodeless discharge lamp, graphite tubes fitted with L'vov platforms and an ammonium phosphate/magnesium nitrate matrix modifier are used. Wavelength, slit width, lamp current and furnace temperature are set according to the manufacturer's recommendations. Concentration is determined directly from a calibration curve.

Introduction. This is a standard operating procedure in the Analytical Services Unit of Hale Creek Field Station, NYSDEC, Gloversville, NY for the analysis of total cadmium in animal tissues. **WARNING: CADMIUM IS TOXIC AND SHOULD BE HANDLED WITH EXTRA CARE. NITRIC ACID, USED IN THIS PROCEDURE, IS CORROSIVE AND CONTACT WITH EYES AND SKIN SHOULD BE AVOIDED.**

Digestion. Digest samples in concentrated nitric acid in a CEM Model MDS 81-D microwave oven (CEM Corp., 1985).

PROTECTIVE CLOTHING REQUIRED: (1) Lab coat and apron. (2) vinyl, latex or neoprene gloves for all procedures. (3) protective eyewear for all work involving handling of concentrated acid.

MATERIALS NEEDED: (1) CEM 120 ml PFA teflon vessels. (2) high purity concentrated HN03 (Ultrex .II, J.T. Baker). (3) Fisher tracemetal nitric acid. (4) Fisher certified ACS nitric acid. (5) deionized water (dH20). (6) reference material (e.g. DORM-1 or DORM-2 (NRC dogfish muscle), SRM 1566a (NIST oyster tissue), SRM 1577b (NIST bovine liver) or TORT-2 (NRC lobster hepatopancrea)). (7) 25 ml volumetric flasks. (8) Whatman 20 cc syringes. (9) 50 ml VWR polypropylene centrifuge tubes. (10) CEM Model MDS-81D Microwave Oven.

PROCEDURE: (1) Weigh sample or reference material into tared CEM vessel and record weight and vessel number - use approximately 1.0 g of wet sample or 0.2 g of dried reference material. Rinse spatula with 20% HNO3 (reagent grade), tap water and dH20 and dry with acetone, if necessary, prior to using for reference material. Rinse spatula with 20% HNO3 (reagent grade), tap water and dH20 prior to using for each sample. Include one procedure blank, one reference sample and one duplicate sample per 10-vessel batch.

(2) In the hood, add 9 ml Fisher tracemetal nitric acid to each vessel. If fewer than ten vessels are used for digestion, add 9.0 ml dH20 to each of the remaining vessels (to make a total of ten) or use sufficient additional "ballast" in Procedure Step 3 to avoid overheating.

(3) Still in the hood, place the relief valve on top of the vessel with the flat side down and cap the vessel hand-tight. Torque the vessel cap in the capping station. Connect teflon tubing between each vessel and the center container of the carousel and tighten finger-tight. Put 60 ml dH20 in each of two beakers and place them in the carousel opposite each other as "ballast" to avoid overheating.

(4) Place the carousel in the oven and digest the samples at the following power: 15 min at 30%, 10 min at 0%, 7 min at 60%,40 min at 0%.

WARNING: During operation of the microwave oven, the turntable must be on, the fan in the oven must be ON THE HIGHEST SETTING, the hood in the laboratory must be ON, and the window in the pesticide prep lab must be open a crack. During operation of the oven, THE OVEN MUST NOT BE LEFT UNATTENDED. IF POPPING OR RELEASE SOUNDS ARE HEARD FROM THE VESSELS, THE OVEN ENERGY MUST BE STOPPED IMMEDIATELY. The fan, however, shou1d remain on to expel any released gases to the hood. In addition, care must be taken to PROTECT PERSONS PASSING BY THE OVEN FROM POSSIBLE INJURY (e.g. don't 1et persons stand in front of the oven while it is in operation).

(5) When the cycle is completed, release the pressure on the vessel in the hood, loosen the cap using the capping station and uncap the vessel. Transfer the digestate to a labeled 25 ml volumetric flask, using a Whatman 20 cc syringe. Rinse the inside of the vessel 3x with dH20 and transfer the rinsate to the flask. Dilute to volume with dH20. Transfer the digestate from the volumetric flask to a 50 ml VWR polypropylene centrifuge tube.

(6) Clean all glassware and digestion vessels thoroughly. A procedure to clean digestion vessels is as follows:

Scrub each vessel out with soapy water (PEX). Add 50 ml 50% HNO3 (certified ACS grade), place the relief valve (flat side down) on each vessel, cap and torque. Connect a teflon tube between each vessel and the center container. Heat at 100% power for 10 minutes (See **WARNING** above; in addition, if fewer than 10 vessels are used, sufficient "ballast" must be present to avoid, overheating). Cool at 0% for 10 minutes. In the hood, release the pressure. Uncap and empty each vessel to waste. Rinse 2X with cold tap water and 3X with dH20. Dry in the hood.

Analysis. Determine elemental cadmium by atomic absorption spectroscopy (Perkin-Elmer, 1986 and Perkin-Elmer, 1996)

PROTECTIVE CLOTHING -REQUIRED: For work involving preparation of standards, chemical solutions or sample dilutions and for cleaning glassware: wear lab coat, gloves and protective eyewear.

MATERIALS NEEDED: (1) 0.2% solution of Ultrex II HNO3 in dH20 for diluent and blanks. (2) Fisher Chemical Cadmium Reference Solution SC1l8-l00 for 0.5, l.0 and 2.0 ppb standards in 0.2% HNO3. (3) 10% ammonium phosphate, monobasic: NH4H2P04 (Perkin-Elmer PIN N930-3445). (4) magnesium nitrate: Mg(NO3) 2*6H20 (Johnson Matthey puratronic 10799). (5) Perkin Elmer Model 5100 Atomic Absorption Spectrophotometer.

PROCEDURE: Determine cadmium by atomic absorption spectroscopy using a Perkin-Elmer Model 5100 Atomic Absorption Spectrophotometer, equipped with an HGA-600 Graphite Furnace, Zeeman Furnace Module, an electrodeless discharge lamp (Perkin- Elmer P/N 305-0615), AS-60 Autosampler, and AA WinLab software. Use graphite tubes with L'vov platforms (CPI or Perkin-Elmer) in the furnace. The conditions of operation are described in the method file 'Cd228' (attached). Wavelength, slit width and lamp current are set according to the manufacturer's (Perkin-Elmer) recommended conditions. A one percent solution of Ultrex II HNO3 is used as flushing liquid between sample analyses.

The solutions in the autosampler cups contain 1000 ul sample, standard or diluent and 125 ul matrix modifier. Five ul of the matrix modifier contains 200 ug NH4H2PO4 and 10 ug Mg (NO3)2. The matrix modifier is prepared by combining 60 ml of magnesium nitrate solution (0.576 g Mg(NO3)2*6H2O in 100 ml dH2O) with 40 ml of 10% NH4H2PO4.

Calibration is performed by direct determination of concentration from a calibration curve. A calibration curve is plotted using 0.5, 1.0 and 2.0 ppb standards.

Quality Control. The following QC should be a part of every analysis:

(1) Reference Material (e.g. SRM 1577b, NIST bovine liver) should be analyzed once per 20 samples or once per batch, whichever is more frequent. Percent recovery should be 85%-115% (Ref: USEPA 1995).

(2) Check Standards should be analyzed once per 10 samples. Percent recovery should be 80%-120% (Ref: USEPA 1995) .

(3) Procedure Blanks should be analyzed once per 20 samples or once per batch, whichever is more frequent. Cadmium concentration should be less than the MDL (Ref: USEPA 1995).

(4) Laboratory Duplicates should be analyzed once per 20 samples or once per batch, whichever is more frequent. IRPDI should be less than or equal to 20% (Ref: USEPA 1995).

(5) Analytical Replicates (replicate analyses of the same subsample digest) should be routine. Each subsample should be analyzed at least in duplicate (Ref: USEPA 1995). The RSD should be less than or equal to 15% (this a laboratory-determined limit).

(6) Correlation coefficient (r) for the calibration curve should be , at a minimum, >= 0.995. Preferably, r should be >= 0.998 (this is a laboratory-determined limit).

(7) Method Detection Limit (MDL) should be determined. MDL = $t_{(0.99)}$ **X** S, where t is the appropriate (i.e n-1 degrees of freedom) one-sided 99% Student's t-statistic and S is the standard deviation from a minimum of seven replicate analyses of a fish sample with a Cd concentration 3 to 5x the MDL.

An analysis is rejected if the QC is not within acceptable limits or if any of the following conditions exist:

1) the matrix has a significant effect on the analyte present in the sample. This is visually checked by looking at atomic peak profiles of the readings. If there is an obvious difference between the sample and the standards, the sample can be diluted and retested or the method of additions can be tried (Method CD-AT-1); or

2) the calculated characteristic mass at the beginning or end of a run is too high or low (the characteristic mass for these conditions is approximately 0.6-0.7 pg/0.0044 A-s); or

3) a determined value grossly deviates. on the basis of an outlier test from other values determined for the same sample digest.

Results.

CALCULATIONS: PPM = (mg/l concentration of analyte in injected solution) X (dilution factor) X (final volume of digestate in ml) / (weight of digested sample in g).

Conclusions. I am confident that the method used for digestion and analysis of cadmium described above is technically justified to address the issues of chemical contaminants in animal tissues in New York State.

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References.

CEM Corporation. 1985. Microwave Digestion System Model MDS81-D. CEM Corporation, Indian Trail, NC.

Perkin-Elmer. 1986. Reference Manual - Model 5100 Atomic Absorption Spectrophotometer. Vols. 1 and 2. Perkin-Elmer Corp., Norwalk, CT.

Perkin-Elmer. 1996. AA WinLab Software Guide. Perkin-Elmer Corp., Norwalk, CT.

Attachments:

- Method File Cd228 (2 pages)

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July 1, 1998

Identification
Method Name: Cd228
Technique: Furnace
Method Desc: Cdtest

Element: Cd
Instrument Model: 5100

Remarks

Instrument
Wavelength (nm): 228.8
Slit Width: 0.7

Read Time (sec): 5.0
Read Delay (sec): 0.0
BOC Time (sec): 2

Signal Type: Zeeman AA
Signal Measurement: Peak Area

Modified Spectrometer Settings: No

Calibration

Equations, Units, and Replicates: Element Calibration Equation	Calibration Units	Sample Units	Max Decimal Places	Max Significant Figures
Cd Non-linear	µg/L	µg/L	3	4

Replicates: Variable
Variable Replicate Parameters
Samples
Measure 1 replicates from the same cup unless concentration less than or equal to 2 use 3, concentration greater than – use 1.
Recovery Measurements: Same as sample
Calibration Solutions: Measure 3 replicates for all standards and blanks.
QC Samples: Use the number of replicates specified for the samples.

Sample
Sample Volume (µL): 20 Diluent Volume (µL): 0 Diluent Location: 36

Matrix Modifiers:	Modifier 1	Modifier 2
Volume (µL):	0	0
Location:	15	0
Add to blank and standards:	Yes	No
Add to reagent blank and samples:	Yes	No

	ID	Conc. (µL/L)	A./S Loc	Stock (µL)	Diluent (µL)
Calib. Blank	Calib Blank		36	20	0
Calib. Std. 1		0.50	37	20	0
Calif. Std. 2		1.00	38	20	0
Calif. Std. 3		2.00	39	20	0

Furnace Conditions

Step #	Temp (°C)	Ramp Time	Hold Time	Internal Flow	Gas Type	Read Step
1	120	10	50	300	Normal	
2	850	1	30	300	Normal	
3	20	1	15	300	Normal	
4	1650	0	5	0	Normal	X
5	2600	1	5	300	Normal	

Extraction System: No Injection Temp. (°C): 20

Furnace Clean-out? No

Sequence

Step	Action and Parameters
A	Piplet sample/std
B	Run furnace steps 1 to end

Pipet Speed (%): 100

Checks

Check correlation coefficient?	No
Recalibrate instrument periodically?	No
Analyze standards at end of run?	No
Check Precision?	No
Reanalyze samples beyond calibration range?	No
Perform matrix check calculations?	No
Perform recovery measurements?	No

Quality Control
Concentration Units: Calibration (µg/L)

Periodic Timing of Analyses
Count: Samples Interrupt sample to analyze QC: No
Frequency: Same for all QC's, 1

Maximum Retries After QC Failure
Maximum allowed reanalyses: 1 Action when maximum exceeded: Continue

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Options
Results Display/Printed Log - Modified Settings: No

Peaks to Save: Last Replicate for Each Sample

STANDARD OPERATING PROCEDURE
PB-AT-2

LEAD IN ANIMAL TISSUE - 2

ABSTRACT: Samples are digested in concentrated nitric acid in a CEM Model MDS 81-D microwave oven. The digestions are performed in closed CEM 120 ml PFA teflon vessels. Lead is analyzed on a Perkin-Elmer Model 5100 Atomic Absorption Spectrophotometer (Zeeman), equipped with an HGA-600 Graphite Furnace, AS-60 Autosampler, and AA WinLab software. An electrode less discharge lamp, graphite tubes fitted with L'vov platforms and an ammonium phosphate/magnesium nitrate matrix modifier are used. Wavelength, slit width, lamp current and furnace temperature are set according to the manufacturer's recommendations. Concentration is determined directly from a calibratiqn curve.

Introduction. This is a standard operating procedure in the Analytical Services Unit of Hale Creek Field Station, NYSDEC, Gloversville, NY for the analysis of total lead in animal tissues. **WARNING: LEAD IS TOXIC AND SHOULD BE HANDLED WITH EXTRA CARE. NITRIC ACID, USED IN THIS PROCEDURE, IS CORROSIVE AND CONTACT WITH EYES AND SKIN SHOULD BE AVOIDED.**

Digestion. Digest samples in concentrated nitric acid in a CEM Model MDS 81-D microwave oven (CKM Corp., 1985).

PROTECTIVE CLOTHING REQUIRED: (1) Lab coat and apron. (2) vinyl, latex or neoprene gloves for all procedures. (3) protective eyewear for all work involving handling of concentrated acid.

MATERIALS NEEDED: (1) CEM 120 ml PFA teflon vessels. (2) high purity concentrated HNO3 (Ultrex II, J.T.. Baker). (3) Fisher tracemetal nitric acid. (4) Fisher certified ACS nitric acid. (5) deionized water (dH20). (6) reference material (e.g. NRC or NIST). (7) 25 ml volumetric flasks. (8) Whatman 20 cc syringes. (9) 50 m1 VWR polypropylene centrifuge tubes. (10) CEM Model MDS-81D Microwave Oven.

PROCEDURE: (1) Weigh sample or reference material into tared CEM vessel and record weight and vessel number -use approximately 1.0 g of wet sample or 0.2 g of dried reference material. Rinse spatula with 20% HNO3 (reagent grade), tap water and dH2O and dry with acetone, if necessary, prior to using for reference material. Rinse spatula with 20% HNO3 (reagent grade), tap water and dH2O prior to using for each sample. Include one procedure blank, one reference sample and one duplicate sample per 10-vessel batch.

(2) In the hood add 9 ml Fisher tracemetal nitric acid to each vessel. If fewer than ten vessels are used for digestion, add 9.0 ml dH2O to each of the remaining vessels (to make a total of ten) or use sufficient additional "ballast" in Procedure Step 3 to avoid overheating.

(3) Still in the hood, place the relief valve on top of the vessel with the flat side down and cap the vessel hand-tight. Torque the vessel cap in the capping station. Connect teflon tubing between each vessel and the center container of the carousel and tighten finger-tight. Put 60 ml dH2O in each of two beakers and place them in the carousel opposite each other as "ballast" to avoid overheating.

(4) Place the carousel in the oven and digest the samples at the following power: 15 min at 30%, 10 min at 0%, 7 min at 60%, 40 min at 0%.

WARNING: During operation of the microwave oven, the turntable must be ON, the fan in the oven must be ON THE HIGHEST SETTING, the hood in the laboratory must be ON, and the window in the pesticide prep lab must be open a crack. During operation of the oven, THE OVEN MUST NOT BE LEFT UNATTENDED. IF POPPING OR RELEASE SOUNDS ARE HEARD FROM THE VESSELS, THE OVEN ENERGY MUST BE STOPPED IMMEDIATELY. The fan, however, shou1d remain on to expel any released gases to the hood. In addition, care must be taken to PROTECT PERSONS PASSING BY THE OVEN FROM POSSIBLE INJURY (e.g. don't let persons stand in front of the oven while it is in operation).

(5) When the cycle is completed, release the pressure on the vessel in the hood, loosen the cap using the capping station and uncap the vessel. Transfer the digestate to a labeled 25 ml volumetric flask, using a Whatman 20 cc syringe. Rinse the inside of the vessel 3x with dH2O and transfer the rinsate to the flask. Dilute to volume with dH2O. Transfer the digestate from the volumetric flask to a 50 ml VWR polypropylene centrifuge tube.

(6) Clean all glassware and digestion vessels thoroughly. A procedure to clean digestion vessels is as follows:

Scrub each vessel out with soapy water (PEX). Add 50 ml 50% HNO3 (certified ACS grade), place the relief valve (flat side down) on each vessel, cap and torque. Connect a teflon tube between each vessel and the center container. Heat at 100% power for 10 minutes (See **WARNING** above; in addition, if fewer than 10 vessels are used, sufficient "ballast" must be present to avoid overheating). Cool at 0% for 10 minutes. In the hood, release the pressure. Uncap and empty each vessel to waste. Rinse 2X with cold tap water and 3X with dH20. Dry in the hood.

Analysis. Determine elemental lead by atomic absorption spectroscopy (Perkin-Elmer, 1986 and Perkin-Elmer, 1996).

PROTECTIVE CLOTHING REQUIRED: For work involving preparation of standards, chemical solutions or sample dilutions and for cleaning glassware: wear lab coat, gloves and protective eyewear.

MATERIALS NEEDED: (1) 0.2% solution of Ultrex II HNO3 in dH2O for diluent and blanks. (2) Fisher Chemical Lead Reference Solution SL21 -100 for 25, 50 and 100 ppb standards in 5.0% HNO3. (3) 10% ammonium phosphate, monobasic: NH4H2PO4 (Perkin-Elmer PIN N930-3445). (4) magnesium nitrate: Mg(NO3)2*6H20 (Johnson Matthey puratronic 10799). (5) Perkin-Elmer Model 5100 Atomic Absorption Spectrophotometer.

PROCEDURE: Determine lead by atomic absorption spectroscopy using a Perkin-Elmer Model 5100 Atomic Absorption Spectrophotometer, equipped with an HGA-600 Graphite Furnace, Zeeman Furnace Module, an electrodeless discharge lamp, AS-60 Autosampler, and AA WinLab software. Use graphite tubes with L'vov platforms (CPI or Perkin-Elmer) in the furnace. Wavelength, slit width and lamp current are set according to the. manufacturer's (Perkin-Elmer) recommended conditions. A one percent solution of Ultrex II HNO3 is used as flushing liquid between sample analyses.

The solutions in the autosampler cups contain 1000 ul sample, standard or diluent and 125 ul matrix modifier. Five ul of the matrix modifier contains 200 ug NH4H2PO4 and 10 ug Mg(NO3)2. The matrix modifier is prepared by combining 60 ml of magnesium nitrate solution (0.576 g Mg(NO3)2*6H2O in 100 ml dH2O) with 40 ml of 10% NH4H2PO4.

Calibration is performed by direct determination of concentration from a calibration curve. A calibration curve is plotted using 25, 50 and 100 ppb standards.

Quality Control. The following QC~should be a part of every analysis:

(1) Reference Material (e.g. SRM 1577b, NIST bovine liver) should be analyzed once per 20 samples or once per batch, whichever is more frequent. Percent recovery should be 85%-115% (Ref: USEPA 1995).

(2) Check Standards should be analyzed once per 10 samples. Percent recovery should be 80%-120% (Ref: USEPA 1995).

(3) Procedure Blanks should be analyzed once per 20 samples or once per batch, whichever is more frequent. Cadmium concentration should be less than the MDL (Ref: USEPA 1995).

(4) Laboratory Duplicates should be analyzed once per 20 samples or once per batch, whichever is more frequent. IRPDI should be less than or equal to 20% (Ref: USEPA 1995).

(5) Analytical Replicates (replicate analyses of the same subsample digest) should be routine. Each subsample should be analyzed at least in duplicate (Ref: USEPA 1995). The RSD should be less than or equal to 15% (this a laboratory-determined limit).

(6) Correlation coefficient (r) for the calibration curve should be , at a minimum, >= 0.995. Preferably, r should be >= 0.998 (this is a laboratory-determined limit).

(7) Method Detection Limit (MDL) should be determined.
MDL = $t_{(0.99)}$ **X** S, where t is the appropriate (i.e n-l degrees of freedom) one-sided 99% Student's t-statistic and S is the standard deviation from a minimum of seven replicate analyses of a fish sample with a Ph concentration 3 to 5x the MDL.

An analysis is rejected if the QC is not within acceptable limits or if any of the following conditions exist:

1) the matrix has a significant effect on the analyte present in the sample. This is usually checked by looking at atomic peak profiles of the readings. If there is an obvious difference between the sample and the standards, the sample can be diluted and retested or the method of additions can be tried;
or

2) the calculated characteristic mass at the beginning or end of a run is too high or low; or

3) a determined value grossly deviates, on the basis of an outlier test, from other values determined for the same sample digest.

Results.

CALCULATIONS: PPM = (mg/l concentration of analyte in injected solution) X (dilution factor) X (final volume of digestate in ml) / (weight of digested sample in g).

Conclusions. I am confident that the method used for digestion and analysis of lead described above is technically justified to address the issues of chemical contaminants in animal tissues in New York State.

References.

CEM Corporation. 1985. Microwave Digestion System Model MDS81-D. CEM Corporation, Indian Trail, NC.

Perkin-Elmer. 1986. Reference Manual - Model 5100 Atomic Absorption Spectrophotometer, Vols. 1 and 2. Perkin-Elmer Corp., Norwalk, CT.

Perkin-Elmer. 1996. AA WinLab Software Guide. Perkin-Elmer Corp., Norwalk, CT.

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August 17, 1998


U.S. DEPARTMENT OF THE INTERIOR
MARCH 3, 1849


NOAA
NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION
U.S. DEPARTMENT OF COMMERCE


DEPARTMENT OF ENVIRONMENTAL CONSERVATION
NEW YORK STATE