UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION 1 - ESD BIOLOGY SECTION
60 Westview Street, Lexington, Massachusetts 02173

MEMORANDUM

DATE: April 20, 1994

SUBJ: Region I ESD Fish Tissue Collection, Handling and Analysis SOP

FROM: Celeste Philbrick Barr CPB
Aquatic Biologist

TO: Nancy Barmakian/Vickie Maynard, ESD QA
Patti Tyler, ESD Biologist
Steve Mierzykowski, Ken Munney, Tim Pryor USFWS
Susan S virsky, SF Support Section
Ken Finkelstein, NOAA

THRU: Howard Davis, Biology Section Chief HSD

Attached is the final draft of the Region I Environmental Services Division Standard Operating Procedure for Fish Tissue Collection, Handling, and Analysis. This SOP is the result of input from all of you as well as verbal and published contributions from within EPA and from USFWS and NOAA. I apologize for the delay in sending this to you. I wanted to give John Moore of USFWS in Pawtuxet Maryland an opportunity to comment since he has worked in a parallel program for at least ten years. I called him after one month and he said he had read it and did have a few minor written comments to submit. I have not received them and so will send this out because I feel it has incorporated all major and minor comments from all reviewers to date. If you find any errors in it, please let me know so they can be corrected.

cc: Peter Nolan, ESD Biologist
Dave McDonald, Lockheed ESAT ESD

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TECHNICAL GUIDELINES FOR FISH TISSUE PREPARATION AND ANALYSES

INTRODUCTION

The objectives for sampling and analyzing biological tissue should be determined in a scoping meeting attended by EPA biologists, the project or site manager, consultants to the project, a state fisheries biologist, a member of EPA QA section and a chemist or representative from the EPA Contract Lab Program (CLP). The data quality objectives will dictate the detection limits needed in the chemical analyses and the numbers and species of fish to be collected (based on whether human health risk assessment is to be performed in addition to ecological risk assessment). Practical knowledge of the biota present at the site, the bioaccumulation potential of the contaminants of concern and of the limits of the analytical methods to be employed should be input at this point in the planning process.

I. SAMPLE COLLECTION

A. Time of Year

The most important factors to consider in scheduling a fish collection are the seasons of spawning, migration and highest potential for bioaccumulation of contaminants. The spawning and migration seasons of target fish should be confirmed for each specific site. Spawning movement may bring species away from their usual areas affecting their actual or supposed exposure.

B. Number, size, and species of fish

1. Species selection

Typically a minimum of two, or more preferably three species will be selected to represent different trophic levels in the food chain. The usual fish trophic levels are a bottom feeding, scavenging species (i.e. bullheads), an omnivorous species (i.e. perch), and a predatory species (i.e. largemouth bass).

The target species should be 1) harvested by exposed population or be representative of primary harvested species, 2) be representative of the study area, 3) be abundant and large enough to obtain adequate sample size for chemical analysis and 4) not be part of a "put and take" stocking program ie. brook or rainbow trout.

information in the risk assessment. The numbers of fish to be caught should be determined prior to the field sampling day.

It must be noted that because the samples will often be analyzed for the different analytical parameters by different labs, each set of analytical parameters (i.e. inorganics) will require 5 fish/species/size. To reduce the numbers of fish required, attempts to obtains bids for on total analyses could be made prior to the sampling event.

Measurement of lipid concentrations is required.

C. Collection methods

Fish collection gear will be selected as appropriate to capture the appropriate fish species, sizes, and numbers. Gear could include electrofishing equipment, gill nets, seine nets, or other alternatives as necessary. The gear will be properly handled to avoid any cross contamination or field contamination of samples.

For EPA-lead Superfund sites, USFWS, under an interagency agreement and subject to availability and funding, will usually provide the field support for sample collection. In lieu of USFWS personnel, only trained persons will operate electrofishing equipment.

Some states have restrictions on collection methods. The state fisheries agency must be contacted about this and for fish collection permits prior to designing the sampling plan. State size limits on fish should be observed during sampling.

D. Field measurements and methods

Field measurements should include total fish length (measured to the nearest millimeter) and fresh weight (measured to the nearest gram). If age is required, scales should be removed from just behind the dorsal fin above the lateral line. Fish that do not possess scales may be aged using spines or otoliths. Large fish that are being filleted should also be sexed. Fillets must then be weighed. Examine all fish and record the presence of any abnormalities, lesions or unusual growths. See Attachment A for a guide to fish examination (USF&W 1990).

If a site-specific food chain is being derived, stomachs should be preserved separately for content identification.

Fish will be filleted in the field. Filleting and skinning

II. CONTAMINANT-SPECIFIC ISSUES AND SAMPLE HANDLING

A. Volatile and Semi-volatile organics analysis

Most Volatile organic compounds do not bioaccumulate in fish due to chemical and physical properties (R. Pruell EPA ERL-Narragansett, personal communication).

The requirement for semi-volatile organic analyses should also be given careful consideration. Vertebrates metabolize PAHs easily while invertebrates metabolize PAHs very slowly (EPA 1993). This may require analysis of PAH metabolites.

In order to assess the potential or actual effects of PAHs, histopathological examinations of the fish by a certified histopathologist, may be considered.

The samples shall not come into contact with plastics. Handle samples with stainless steel, anodized aluminum forceps or borosilicate glass. Separate utensils should be used for removing outer tissue and for resection tissue for analysis to avoid cross contamination. A rough-surfaced glass cutting board is recommended or a cutting board of another material can be used if covered with heavy-duty aluminum foil. Decontaminate implements between samples with a detergent solution, a tap water rinse, distilled water, methanol or isopropanol. Dry the surfaces between samples to avoid using hexane or methylene chloride. All decontamination fluids should be containerized and disposed of in an EPA-approved manner.

Samples of specific organs (i.e. liver), should be taken immediately after sampling and frozen separately in certified, precleaned glass containers.

All other samples should be placed in the smallest, precleaned glass jar that will adequately hold the sample. The jars should have a teflon liner inside the lid. As alternative method of packaging is to wrap the samples in hexane or methanol-rinsed heavy-duty aluminum foil (dull side toward specimen). The samples should be double wrapped and sealed in double Ziploc bags (one inside the other). (TetraTech 1986) The sample tag should be placed inside the airtight Ziploc bag.

Samples should be dry iced for shipping and cooled to 4 degrees C immediately after collection and preparation. Samples must be frozen to -20 degrees C once received in laboratory. If analysis is to be conducted on selected tissues, especially internal

III. LABORATORY PREPARATION

The analytical laboratory should follow the procedures outlined in EPA 1993 under tissue preparation.

* Upon receipt of samples, they should be kept frozen at -20 degrees C until processing. Fillet and offal samples will be submitted and analyzed as separate samples.

* Samples should not be defrosted prior to homogenization.

* Weight of each sample to be processed in the lab should be recorded either as wet weight or dry weight to the nearest 0.1 gram. Percent moisture must be determined to convert dry weight measurements to wet weight.

* Blender blades should be of special material depending upon analyses. When chromium, nickel, aluminum or zinc are contaminants of concern, use titanium or carbon knives and blender blades rather than stainless steel. Blanks may be run through blenders to certify no contamination is being introduced.

IV. ANALYSES

A. Analytical methods

A performance-based prequalification process for biological tissue analysis has been established by EPA Region I Quality Assurance Section (ESD-QA) through the EPA Contract Lab Program (CLP). Four criteria will be used to determine acceptability during the prequalification process. 1) The laboratory must accurately and precisely analyze and report their results for a traceable standard reference material (SRM). 2) The laboratory must also demonstrate that they can successfully spike a fish sample, and recover the spiked material. It is recommended that cultured fish be used for the fish blank. This demonstrates the ability of the lab to extract/digest, clean-up, and analyze real tissue which may have interferences, 3) The laboratory must demonstrate their ability to successfully produce a homogenate for analysis, and 4) The laboratory must demonstrate to ESD-QA that they can report their data accurately and in a manner defensible in court.
The analytical methods of the labs may differ and the will be determined once a lab is chosen for the specific project.

TABLE 1. INORGANIC PRACTICAL DETECTION LIMITS

COMPOUND	DETECTION LIMIT (mg/kg)
Aluminum	3.0
Arsenic	0.5
Barium	0.2
Beryllium	0.1
Cadmium	0.1
Chromium	0.1-0.2
Cobalt	0.2
Copper	0.5
Lead	0.5
Mercury	0.2
Nickel	0.5
Selenium	1.0
Silver	0.2
Vanadium	0.3
Zinc	0.5

Detection limits are based on those of USFWS.

*Practical Detection Limits are based on 10 grams wet weight with a final volume of 100 ml.

TABLE 2. ORGANICS PRACTICAL DETECTION LIMITS

COMPOUND	DETECTION LIMIT (mg/kg)
Alpha-BHC	0.01
Chlordane	0.01
Chloropyrifos	0.01
Dieldrin	0.01
DDT,DDTR	0.01
Endrin	0.01
Gamma-BHC	0.01
Heptachlor Epoxide	0.01
Methoxychlor	0.01
Oxychlordane	0.01
Total PCBs	0.01
Toxaphene	0.01

Arochlor 1242, 1248, 1254, 1260 = 0.01

Detection limits are those determined achievable and ecologically useful by USFWS.

U.S. EPA 1993. Appendix I. Assessment and Control of Bioconcentratable Contaminants in Surface Waters: Field Evaluation of Residue Prediction Procedures. U.S. EPA, ORD, Duluth, MN. 143 pp.

U.S. EPA 1989. Assessing Human Health Risks from Chemically Contaminated Fish and Shellfish: A Guidance Manual. Office of Water Regulations and Standards (WH-552), EPA-503/8-89-002.

U.S. EPA 1987. A Compendium of Superfund Field Operations Methods OSWER Directive 9355.0-14. EPA/540/p-87/001. p. 12A-25.

U.S. EPA 1991. Methods for the Determination of Metals in Environmental Samples. Office of Research and Development. EPA/600/4-91/010.292 pp.

U.S EPA. 1992. National Study of Chemical Residues in Fish. EPA 823-R-92-008b. Washington, D.C.

U.S. EPA. 1991. Technical Support Document for Water Quality-based Toxics Control. EPA/505/2-90-001. Washington, D.C.

U.S. EPA. 1992. National Study on Chemical Residues in Fish. Volume II. Office of Science and Technology.

U.S. Fish and Wildlife Service. 1990. Chemical Analysis of Environmental Materials for Residues of Organic Contaminants. RFP-FWS-9-OAS-90-151. Division of Contracting and General Services, Arlington, VA.

U.S. Fish and Wildlife Service. 1991. Chemical Analysis of Environmental Materials for Residues of Inorganic Contaminants. RFP-FWS-9-OAS-91-111. Division of Contracting and General Services, Arlington, VA.

U.S. Fish and Wildlife Service, 1993. Standard Operating Procedures: Collection of Fish Tissue Samples. PCFO-EC-SOP-001. Panama City Field Office, Panama City, FL.

Verschueren, K. 1983. Handbook of Environmental Data on Organic Chemicals. Van Nostrand Reinhold, New York, NY. 1310 pp.

Accession no._____

Submitted by _____ Date _____

Submitter's code _____ Chain of custody: yes _____ no _____

Species _____ Length _____ Weight _____

GROSS INTERNAL EXAMINATION

Skin ( ) Normal ( ) Excessive mucus ( ) Abnormal color _____

( ) Lesions: ( ) Single ( ) Multiple ( ) Closed ( ) Open
( ) Hemorrhagic ( ) Necrotic ( ) Ulcer ( ) Blister ( )Tumor
( ) Lost scales ( )Abrasions
Location: _____

Wet mount/smear: _____

Eyes ( ) Normal ( ) Exophthalmia ( ) Cataract ( ) Hemorrhagic
( ) Opaque Cornea ( ) Lens lost ( ) Parasites ( ) Bilateral

Fins ( ) Normal ( ) Frayed _____ ( ) Hemorrhagic _____
( ) Eroded _____ ( ) Deformed _____

Wet mount/smear: _____

Gills ( ) Normal ( ) Pale ( ) Mottled ( )Hemorrhagic ( ) Necrotic
( ) Excessive Mucus ( ) Hyperplasia ( ) Telangiectasia
( ) Gas emboli ( ) Cysts ( ) Large Parasites _____

( ) Fungus visible
Wet Mount/smear: _____

GROSS INTERNAL EXAMINATION

Adipose tissue ( ) Normal ( ) Excessive ( ) Reduced ( ) Petechial hemorrhage ( ) Color _____ ( ) Cysts

Liver ( ) Normal ( ) Enlarged ( ) Reduced Color: ( ) Pale ( ) Mottled
( ) Other _____ ( ) Texture _____
( ) Lesions: ( ) Single ( ) Multiple ( ) Tumor ( ) Necrotic
( ) Hemorrhage ( ) Cyst (parasite) ( ) Cyst (fluid)

Spleen ( ) Normal ( ) Enlarged ( ) Reduced ( ) Raspberry surface

ATTACHMENT B

ESD BIOLOGY SOP #23

Glassware cleaning methods for use in fish collection and analysis.

Glassware are cleaned using the following procedures:

1. Use a scrub brush and wash in hot tap water and non-phosphate detergent.

2. Rinse thoroughly with hot tap water.

3. Bathe glasswater briefly in 10% reagent-grade HCl.

4. Rinse five (5) times with deionized (MILLI-Q) water.

5. Using a squeeze bottle labeled appropriately rinse two (2) times with full-strength pesticide-grade acetone in a fume hood.

6. The glassware should be placed or held over a stainless steel tray during rinsing.

7. The waste acetone should be collected from the tray and stored in a waste bootle appropriately labeled and closed.

8. Using a squeeze bottle labeled appropriately rinse two (2) times with full-strength pesticide-grade methanol.

9. The glassware should be placed or held over a stainless steel tray during rinsing.

10. The waste methanol should be collected from the tray and stored in a waste bottle appropriately labeled and closed.

11. Dry glassware for at least 10 minutes in a drying oven set at 180°C.

12. Allow glassware to cool before using.

13. Glassware should be capped or covered with clean foil until needed.

REFERENCE:

USEPA REGION 10 LAB
BIOLOGY DRAFT PROTOCOLS
UPDATE: JUNE 3, 1993

* Some of the blender and grinder assembly parts are made of teflon material or hard plastic.

ATTACHMENT C

0.025mg/Kg/2 = 0.012 mg/Kg ~ 0.01 mg/Kg

0.4 mg/Kg

gamma-BHC,Lindane

AWQC Acute 100 ug/L
130 BCF (National Fish Study)

(100 ug/L)(0.3 ATU)[b](.05 A/C)[d] = 1.5 ug/L

(1.5 ug/L)(130)[a] = 0.195 mg/kg **0.1 mg/kg**

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alpha-BHC

see gamma-BHC **0.1 mg/Kg**

Heptachlor Epoxide

0.2 mg/Kg Piscivore NOEL (National Fish Study)
2, FIFRA rationale factor

0.2 mg/Kg/2[c] = 0.1 mg/Kg **0.1 mg/Kg**

Methoxychlor

AWQC Chronic 0.03 ug/L
8300 BCF, (National Fish Study)

(0.03 ug/L)(8300)[a] = .249 mg/Kg **0.1 mg/Kg**

Oxychlordane

more toxic than chlordane (p C-78 National Fish Study)

0.025 mg/Kg

Total PCBs

0.13 mg/Kg Piscivore NOEL (National Fish Study)
2, FIFRA rationale factor

0.13 mg/Kg/2[c] = 0.06 mg/Kg

detection problem **0.4 mg/Kg**

Toxaphene

AWQC Chronic 0.0002 ug/L
31,000 (Eisler, Table 6, Freshwater whole body)

(0.0002 ug/L)(31,000)[a] = 0.006 mg/Kg