



State of West Virginia
 Department of Administration
 Purchasing Division
 2019 Washington Street East
 Post Office Box 50130
 Charleston, WV 25305-0130

Request for Quotation

RFQ NUMBER
DEP14746

PAGE
1

ADDRESS CORRESPONDENCE TO ATTENTION OF:
CHUCK BOWMAN
304-558-2157

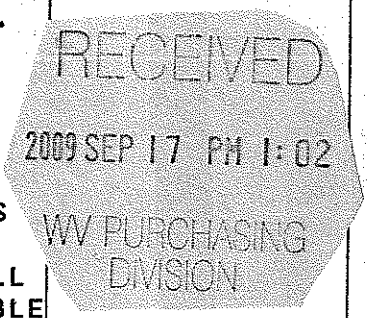
***626144827 304-757-8954**
BID CHEM TESTING INC
PO BOX 634
PUTNAM VILLAGE SHOPPING CTR
TEAYS WV 25569-0634

ENVIRONMENTAL PROTECTION
DEPARTMENT OF
OFFICE OF WATER RESOURCES
601 57TH STREET SE
CHARLESTON, WV
25304 304-926-0499

DATE PRINTED 08/31/2009	TERMS OF SALE Net 30	SHIP VIA Best Way	FOB	FREIGHT TERMS
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BID OPENING DATE: **09/17/2009** BID OPENING TIME **01:30PM**

LINE	QUANTITY	UOP	CAT NO	ITEM NUMBER	UNIT PRICE	AMOUNT
0001	1	LS		493-09		
<p>ANIMAL TISSUE SAMPLE ANALYSIS.</p> <p>THE WEST VIRGINIA PURCHASING DIVISION, FOR THE AGENCY, THE WEST VIRGINIA DEPARTMENT OF ENVIRONMENTAL PROTECTION, IS SOLICITING BIDS FOR THE HOMOGENIZATION AND ANALYSIS OF ANIMAL TISSUE SAMPLES FOR METALS AND POLYCHLORINATED BIPHENYLS PER THE FOLLOWING BID REQUIREMENTS AND ATTACHED SPECIFICATIONS.</p> <p>PLEASE PAY CLOSE ATTENTION TO THE REQUIRED INFORMATION SUBMISSIONS NOTED ON THE THREE (3) PAGE BID SCHEDULE.</p> <p>EXHIBIT 3</p> <p>LIFE OF CONTRACT: THIS CONTRACT BECOMES EFFECTIVE UPON AWARD AND EXTENDS FOR A PERIOD OF ONE (1) YEAR OR UNTIL SUCH "REASONABLE TIME" THEREAFTER AS IS NECESSARY TO OBTAIN A NEW CONTRACT OR RENEW THE ORIGINAL CONTRACT. THE "REASONABLE TIME" PERIOD SHALL NOT EXCEED TWELVE (12) MONTHS. DURING THIS "REASONABLE TIME" THE VENDOR MAY TERMINATE THIS CONTRACT FOR ANY REASON UPON GIVING THE DIRECTOR OF PURCHASING 30 DAYS WRITTEN NOTICE.</p> <p>UNLESS SPECIFIC PROVISIONS ARE STIPULATED ELSEWHERE IN THIS CONTRACT DOCUMENT, THE TERMS, CONDITIONS AND PRICING SET HEREIN ARE FIRM FOR THE LIFE OF THE CONTRACT.</p> <p>RENEWAL: THIS CONTRACT MAY BE RENEWED UPON THE MUTUAL</p>						



SEE REVERSE SIDE FOR TERMS AND CONDITIONS

SIGNATURE: *[Signature]* TELEPHONE: **304 757 8954** DATE: **09-16-09**
 TITLE: **President** FEIN: **550732395** ADDRESS CHANGES TO BE NOTED ABOVE

WHEN RESPONDING TO RFQ, INSERT NAME AND ADDRESS IN SPACE ABOVE LABELED 'VENDOR'

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14. **HIPAA BUSINESS ASSOCIATE ADDENDUM:** The West Virginia State Government HIPAA Business Associate Addendum (BAA), approved by the Attorney General, and available online at the Purchasing Division's web site (<http://www.state.wv.us/admin/purchase/vrc/hipaa.htm>) is hereby made part of the agreement. Provided that, the Agency meets the definition of a Cover Entity (45 CFR §160.103) and will be disclosing Protected Health Information (45 CFR §160.103) to the vendor.
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SHIP TO

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 DEPARTMENT OF
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 25304 304-926-0499

DATE PRINTED	TERMS OF SALE	SHIP VIA	F.O.B	FREIGHT TERMS
08/31/2009	Net 30	Best Way		
BID OPENING DATE: 09/17/2009		BID OPENING TIME 01:30PM		

LINE	QUANTITY	UOP	CAT NO	ITEM NUMBER	UNIT PRICE	AMOUNT
<p>WRITTEN CONSENT OF THE SPENDING UNIT AND VENDOR, SUBMITTED TO THE DIRECTOR OF PURCHASING THIRTY (30) DAYS PRIOR TO THE EXPIRATION DATE. SUCH RENEWAL SHALL BE IN ACCORDANCE WITH THE TERMS AND CONDITIONS OF THE ORIGINAL CONTRACT AND SHALL BE LIMITED TO TWO (2) ONE (1) YEAR PERIODS.</p> <p>CANCELLATION: THE DIRECTOR OF PURCHASING RESERVES THE RIGHT TO CANCEL THIS CONTRACT IMMEDIATELY UPON WRITTEN NOTICE TO THE VENDOR IF THE COMMODITIES AND/OR SERVICES SUPPLIED ARE OF AN INFERIOR QUALITY OR DO NOT CONFORM TO THE SPECIFICATIONS OF THE BID AND CONTRACT HEREIN.</p> <p>OPEN MARKET CLAUSE: THE DIRECTOR OF PURCHASING MAY AUTHORIZE A SPENDING UNIT TO PURCHASE ON THE OPEN MARKET, WITHOUT THE FILING OF A REQUISITION OR COST ESTIMATE, ITEMS SPECIFIED ON THIS CONTRACT FOR IMMEDIATE DELIVERY IN EMERGENCIES DUE TO UNFORESEEN CAUSES (INCLUDING BUT NOT LIMITED TO DELAYS IN TRANSPORTATION OR AN UNANTICIPATED INCREASE IN THE VOLUME OF WORK.)</p> <p>QUANTITIES: QUANTITIES LISTED IN THE REQUISITION ARE APPROXIMATIONS ONLY, BASED ON ESTIMATES SUPPLIED BY THE STATE SPENDING UNIT. IT IS UNDERSTOOD AND AGREED THAT THE CONTRACT SHALL COVER THE QUANTITIES ACTUALLY ORDERED FOR DELIVERY DURING THE TERM OF THE CONTRACT, WHETHER MORE OR LESS THAN THE QUANTITIES SHOWN.</p> <p>ORDERING PROCEDURE: SPENDING UNIT(S) SHALL ISSUE A WRITTEN STATE CONTRACT ORDER (FORM NUMBER WV-39) TO THE VENDOR FOR COMMODITIES COVERED BY THIS CONTRACT. THE ORIGINAL COPY OF THE WV-39 SHALL BE MAILED TO THE VENDOR AS AUTHORIZATION FOR SHIPMENT, A SECOND COPY MAILED TO THE PURCHASING DIVISION, AND A THIRD COPY RETAINED BY THE SPENDING UNIT.</p>						

SEE REVERSE SIDE FOR TERMS AND CONDITIONS

SIGNATURE	TELEPHONE	DATE
	304 757 8954	09-16-09
TITLE	FEIN	ADDRESS CHANGES TO BE NOTED ABOVE
President	550732395	

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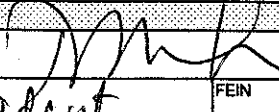
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25304 304-926-0499

DATE PRINTED 08/31/2009	TERMS OF SALE Net 30	SHIP VIA	FOB	FREIGHT TERMS
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<p>BANKRUPTCY: IN THE EVENT THE VENDOR/CONTRACTOR FILES FOR BANKRUPTCY PROTECTION, THE STATE MAY DEEM THE CONTRACT NULL AND VOID, AND TERMINATE SUCH CONTRACT WITHOUT FURTHER ORDER.</p> <p>THE TERMS AND CONDITIONS CONTAINED IN THIS CONTRACT SHALL SUPERSEDE ANY AND ALL SUBSEQUENT TERMS AND CONDITIONS WHICH MAY APPEAR ON ANY ATTACHED PRINTED DOCUMENTS SUCH AS PRICE LISTS, ORDER FORMS, SALES AGREEMENTS OR MAINTENANCE AGREEMENTS, INCLUDING ANY ELECTRONIC MEDIUM SUCH AS CD-ROM.</p> <p>REV. 05/26/2009</p> <p>NOTICE</p> <p>A SIGNED BID MUST BE SUBMITTED TO:</p> <p>DEPARTMENT OF ADMINISTRATION PURCHASING DIVISION BUILDING 15 2019 WASHINGTON STREET, EAST CHARLESTON, WV 25305-0130</p> <p>THE BID SHOULD CONTAIN THIS INFORMATION ON THE FACE OF THE ENVELOPE OR THE BID MAY NOT BE CONSIDERED:</p> <p>SEALED BID</p> <p>BUYER: CB-23</p> <p>RFQ. NO.: DEP14746</p>						

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TITLE: **President** FEIN: **550732395** ADDRESS CHANGES TO BE NOTED ABOVE

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<p>BID OPENING DATE: 09/17/2009</p> <p>BID OPENING TIME: 1:30 PM</p> <p>PLEASE PROVIDE A FAX NUMBER IN CASE IT IS NECESSARY TO CONTACT YOU REGARDING YOUR BID: ----- 304-757-9676 -----</p> <p>CONTACT PERSON (PLEASE PRINT CLEARLY): ----- Mukesh Shah -----</p> <p>***** THIS IS THE END OF RFQ DEP14746 ***** TOTAL: _____</p>						

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SIGNATURE: TELEPHONE: 304 757 8954 DATE: 09-16-09

TITLE: President FEIN: 550732395 ADDRESS CHANGES TO BE NOTED ABOVE

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Contract Specifications for the Homogenization and Analysis of Animal Tissue Samples for Metals and Polychlorinated Biphenyls

Area of Work/Bid Award

The Department of Environmental Protection (DEP), Division of Water and Waste Management is seeking bids for the processing and analysis of tissue samples for total mercury, total PCBs, and/or total selenium.

The DEP collects animal tissues to support the development of consumption advisories and for body burden and bioaccumulation studies. Typically, samples consist of whole fish or fillets (edible portions). However, DEP may also submit samples consisting of fish organs, aquatic insects, bird eggs¹, or other aquatic species. The vendor must be prepared to receive and process frozen fillets, whole fish, organs, egg and/or insects. Rarely, fresh-fish samples will be submitted for the laboratory to process into fillets.

The volume of work entailed by this program can vary greatly from year-to-year:

2005 – 236 samples

2006 – 375 samples

2007 – 283 samples

2008 – 143 samples

The estimated number of samples for 2009 is 200 samples for total mercury, total selenium, and total PCBs.

Legal action based upon analytic results is possible. Therefore, the vendor or vendors selected must have a quality control program in place and meet the following qualifications.

- The laboratory and any subcontractors must be certified by DEP's laboratory Quality Assurance Program for the analytes listed above at the time of bid submission.
- Capable of attending and providing expert testimony in legal proceedings, upon request.

¹ Note: Egg samples may contain mature embryos, which will contain bones, beaks, and claws. Egg shells may also be included in the sample. These materials may be difficult to homogenize without a grinder.

Scope

In administering and enforcing most of the pollution control laws of the state, the importance of quality control cannot be overstated. Quality control measures must be strictly adhered to in all phases of sample collection, preservation, transportation, and analysis. The quality control and analytical work, as they relate to the contractor's responsibility, is divided into four (4) major steps:

- STEP 1 – Collection of sample from specified office
- STEP 2 – Preparation of homogenized sample
- STEP 3 – Conduct specified analysis on sample in a timely and professional manner.
- STEP 4 – Establishment of continuing program to ensure the reliability of analytical data.
(Quality Assurance/Quality Control)
- STEP 5 – Legal testimony.

Step 1 – Collection of Samples from Specified Locations

Sample collection for DEP shall be conducted by DEP and Division of Natural Resources (DNR) scientists. The vendor may be required to pick up fish samples from the following locations:

DEP Headquarters
601 57th Street SE
Charleston, WV 25304
Primary Contact: Janice Smithson, (304) 926-0499 ext 1051

DNR Main Office
State Capitol Complex, Building 3
Charleston, WV
Primary Contact: Bret Preston, (304) 558-2771

DNR District 1 – Wildlife Resources
1110 Railroad Street
PO Box 99
Farmington, WV 26571
Primary Contact: Frank Jernejcic, (304) 825-6787

DNR District 2 – Wildlife Resources
#1 Depot Street
Romney, WV 26757
Primary Contact: Jim Hedrick, (304) 822-3551

DNR District 3 – Wildlife Resources
 WV State Wildlife Center
 Box 38
 French Creek, WV 26218
 Primary Contact: Kevin Yokum, (304) 924-6211

DNR District 4 – Wildlife Resources
 2006 Robert C. Byrd Drive
 Beckley, WV 25801
 Primary Contact: Mark Scott, (304) 256-6947

DNR District 5 – Wildlife Resources
 Rt. 1 Box 484
 Point Pleasant, WV 25550
 Primary Contact: Zack Brown, (304) 675-0871

DNR District 6 – Wildlife Resources
 2311 Ohio Avenue
 Parkersburg, WV 26101
 Primary Contact: Scott Morrison, (304) 420-4550

DNR – Elkins Operations Center
 Ward Road, PO Box 67
 Elkins, WV 26241
 Primary Contacts: Mike Shingleton or Dan Cincotta, (304) 637-0245

DEP and DNR personnel will initiate a chain-of-custody (COC) report for the sample(s). The COC will indicate the date(s) of sample collection, species, tissue type, number of containers and desired analyses. The COC will also indicate the project objectives (i.e., consumption advisory vs. whole-fish studies) to assure that samples are processed to meet the needs of DEP.

The vendor shall document the date/time the samples were obtained from DEP/DNR, condition of the sample, and date/time the samples were received at the vendor's facility. The vendor shall be responsible for holding times, preservation of the samples and maintaining an internal COC from the time the vendor obtained the sample until the time the analysis is accepted by DEP. The vendor shall also maintain records of the results of analyses for a minimum of five (5) years.

Step 2 – Sample Homogenization

Contaminants are not distributed evenly throughout biological tissue. Therefore, it is crucial to obtain a homogenous sample by grinding or processing the tissue to an even, thoroughly-mixed consistency.

A. Consumption Advisory Samples

Typically, these samples will be processed into fillets by the DEP/DNR sample collectors. However, there may be occasion for the vendor to perform this activity. Fillets shall be obtained in accordance with procedures presented in *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1: Fish Sampling and Analysis*, Third Edition², Sections 7.2.2.6 and 7.2.2.7. (See attachment A).

Catfish fillets shall be "skin-off"; that is, the skin shall be removed from all fillets. Scaled fish fillets shall be "skin-on, scales-off".

Most fish consumption samples will consist of composites of three to five fish. DEP will clearly identify which fish comprise each composite sample. These sets of fish will be processed as a unit, as though they are a single sample.

Homogenates shall be prepared in accordance with procedures presented in *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1: Fish Sampling and Analysis*, Third Edition. Section 7.2.2.8 presents guidance for homogenization of individual fish samples. Section 7.2.2.9 presents homogenization procedures for multi-fish composites. (See Attachment A).

B. Sample Preparation – Whole Fish Samples

Whole-fish samples may be processed as individual fish or as multi-fish composites. Multi-fish composites will generally be necessary due to small fish size. Whole fish homogenization shall be conducted in accordance with procedures presented in *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1: Fish Sampling and Analysis*, Third Edition, Appendix J. (See Attachment A). **Please note the following exception: All catfish samples designated as whole-fish samples are to be processed with skin on. This conflicts with the illustration in Figure J-1.**

Vendor will not be required to document abnormalities or determine age or sex of these fish.

C. Sample Preparation – Fish Eggs, Bird Eggs, Benthic Macroinvertebrates

Bird eggs may be processed as individual contents or as multi-egg composites, depending on sample volume and agency objectives. Embryonic egg contents, which include embryos as well as extra-organism material, shall be homogenized in accordance with the aforementioned procedures regarding whole-fish

² Environmental Protection Agency Document EPA 823-B-00-007, November 2000. Available on-line at www.epa.gov/waterscience/fishadvice

homogenization. A heavy-duty grinder may be required to thoroughly homogenize more mature bird embryos (i.e. those having developed beaks, legs/feet, and claws). Early stage embryos (i.e. those consisting of mostly yolk) may require mortar and pestle grinding in order to adequately combine tissue liquids and solids.

Fish eggs shall be homogenized via small blender devices or by mortar and pestle grinding. In general, methods are consistent with whole-fish homogenization procedures; however, all fish egg samples will represent composites of multiple eggs. Since fish eggs are extremely small and low sample volumes may be regularly submitted for analysis, homogenization equipment should be of an appropriate (small) size to ensure thorough mixing of tissues.

Macroinvertebrates may be submitted as individuals or composite samples for homogenization and analysis. Larger organisms and voluminous composites may be homogenized with standard equipment and techniques used to homogenize whole-fish samples. Smaller samples of benthic macroinvertebrates should be homogenized via mortar and pestle grinding. Note: special care should be taken to pulverize the exoskeletons of insects and crustaceans, which will be mixed with internal tissue as a complete homogenate.

Step 3 – Conduct Specified Analyses on Samples

In order for the laboratory to demonstrate the ability to produce acceptable results an external Quality Control Sample (QCS) must be analyzed. **This QCS must be a standard reference material consisting of biological tissue similar to fish and provide specified acceptance limits for the analytes of interest in this study.** This QCS must be analyzed with each batch of samples. A batch is any group of twenty (20) or fewer samples processed at the same time. In addition to the QCS, a method blank (reagent blank), a matrix spike (MS), and a matrix spike duplicate (MSD) must be digested with each batch. In any case where there is not sufficient tissue to analyze a MS/MSD pair, then a QCS duplicate must be analyzed with that batch in order to demonstrate acceptable precision.

Sample preparation will follow EPA Method 200.3 for digestate analysis by EPA Method 200.7 or EPA Method 200.8. If graphite furnace AA is to be used (SM3113B or EPA 200.9) hydrochloric acid (HCl) must be omitted from the digestion. Regardless of the digestion technique used, the pH of the digestate must be verified as <2 SU and the digestate must be properly identified and stored until the laboratory is instructed as to the disposition of the digestate or for a period of one (1) year. Tissue (sample) in excess of that necessary for analysis must be stored frozen at or less than -20.0° C until the laboratory is instructed as to the disposition of the sample or for a period of one (1) year.

Dry weight values may be requested for certain analytes. If such a request is made the lab is to report both wet and dry weight values in addition to percent moisture. Each reported value must be clearly identified as wet weight or dry weight.

Selenium (Se) may be analyzed by EPA 200.9 (Rev. 2.2 1994), EPA 200.8, EPA 270.2, SM 6020 or SM 3113 B. The laboratory must submit documentation to demonstrate the ability to achieve a wet weight Method Detection Limit of 1.0 ppm (mg/kg).

Mercury (Hg) must be analyzed by EPA 245.5, EPA 245.7, SW-846 Method 7471A, or EPA 200.8 (Rev. 5.4 1998). Appropriate digestion for the method of choice must be employed. The laboratory must submit documentation to demonstrate the ability to achieve a wet weight Method Detection Limit of 0.03 ppm (mg/kg). Mercury samples shall be analyzed within 28 days of receipt. All other metals shall be analyzed within 180 days. DEP will not pay for results generated beyond these holding time specifications.

Polychlorinated biphenyls (PCBs) may be analyzed by SW-846 Method 8082 with an appropriate extraction method (3550C, 3540C or 3541). The laboratory must submit documentation to demonstrate the ability to achieve a wet weight Method Detection Limit of 0.036 ppm (mg/kg).

Please Complete:

Approximate Minimum Detection Limit lab is able to attain for each analyte (bidders must use the format below):

- a. Total Mercury: 0.03 mg/kg
- b. Total Selenium: 0.2 mg/kg
- c. Total PCB: 0.05 mg/Kg

Percent moisture shall be determined by drying an aliquot of the sample at 105 degrees Centigrade for 14 hours. The % moisture shall be calculated as follows:

$$\% \text{ Moisture} = \frac{(\text{g of sample} - \text{g of dry sample})}{\text{g of sample}} \times 100$$

Lipid analysis shall be performed using a gravimetric method. Dichloromethane shall be used as the extraction solvent in all lipid analyses. Details for lipid analysis are presented in *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1: Fish Sampling and Analysis*, Third Edition, Section 8.2.1. (See Attachment A)

Analysis of samples is not deemed complete until the data has been submitted to and accepted by DEP. Should the DEP not provide notice of acceptance within four weeks of

the date results were mailed, the vendor may consider the data to be acceptable by the DEP. The vendor shall be responsible for the proper disposal of all samples submitted to them by the DEP unless otherwise notified. The results of the analysis shall be submitted to the DEP no more than four (4) weeks after receipt of samples, unless DEP has granted written consent to extend this deadline.

Results shall be submitted as a written (printed) report. An electronic version of the data in spreadsheet format will also be required.

Step 4 – Quality Assurance/Quality Control

Three programs are to be utilized to assure reliable laboratory data: (1) the use and documentation of standard analytical methods, (2) analysis of duplicate and spiked samples at regular intervals each day to check analytical precision and accuracy, and (3) **analysis of an external Quality Control Sample (QCS) of comparable matrix having "known" concentrations of the analytes specified in this contract.** The National Institute of Standards and Technology has a Standard Reference Material – 1947, Lake Michigan Fish Tissue – is certified for Hg, Se, and selected PCB congeners. Regardless of which analytical methods are used in a laboratory, the methodology must be carefully documented. Standard methods that have been modified or entirely replaced because of recent advances in technologies may only be used when it has been given approval in the Federal Register. Documentation of procedures must be clear, honest, and adequately referenced; and the procedures shall be applied exactly as documented. The responsibility for results obtained from these procedures rests with the analyst and supervisor, both as representatives of the vendor.

To check the laboratory analytical accuracy, matrix spike and matrix spike duplicate analysis of samples shall be performed once per analytical batch consisting of 1 to 20 samples. The laboratory must calculate the Relative Percent Difference (RPD) between the matrix spike and the matrix spike duplicate. The acceptance limit for RPD shall be <20%.

To check the laboratory analytical precision, matrix spike and matrix spike duplicate analysis of samples shall be performed once per analytical batch consisting of 1 to 20 samples. The laboratory must calculate the percent recovery (%Rec) for each matrix spike and matrix spike duplicate. The acceptable range for recovery under this contract shall be 70-130 %Rec.

The percent recovery must be plotted out on accuracy quality control charts. "Out of Control" samples (samples which exceed $\pm 3\sigma$) are to be repeated and appropriate steps taken to locate and remedy the source of error. If an obvious error cannot be determined, the Method of Standard Additions must be used to verify element concentration in the sample. The DEP reserves the right to conduct unannounced examination of the laboratory's records to assure compliance.

Detailed guidance for maintaining quality assurance/quality control for fish tissue analysis is provided in *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1: Fish Sampling and Analysis*, Third Edition³, Section 8.3.

DEP will not pay for results lacking the aforementioned QA/QC requirements.

Step 5 – Legal Testimony

The selected vendor may be requested by the DEP to testify concerning the validity of the laboratory analysis. The vendor will only be required to testify to the following areas:

1. Time of notification by the DEP of sampling and by whom.
2. When and where samples were received by the vendor from DEP/DNR personnel.
3. Condition of sample.
4. How sample was preserved by the vendor.
5. Date and time(s) of analysis and by whom.
6. Chain of Custody procedures within the laboratory.
7. Methods used.
8. Results of analysis.

At no time will the vendor respond to questions concerning interpretation of results. The DEP shall reimburse the vendor for the costs of any such testimony.

Prime Vendor Responsibilities

A vendor who is awarded a contract, when performing work under the terms and conditions of this contract, is solely responsible for the satisfactory completion of the work. The prime vendor shall be responsible for ensuring that any subcontractors have all the necessary permits, and certifications (including WV State Laboratory Certification) to perform the work. DEP will consider the prime vendor to be the sole point of contact with regard to authorized work under the contract; however this provision does not prohibit the DEP from directly contacting subcontractors.

Subcontractors

The prime vendor shall not be allowed to subcontract any work or services under this contract to any other person, company, corporation, firm, organization or agency without prior written approval of the DEP.

³ Environmental Protection Agency Document EPA 823-B-00-007, November 2000. Available on-line at www.epa.gov/waterscience/fishadvice

ATTACHMENT A

*Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1: Fish Sampling and Analysis, Third Edition*¹.

Sections 7.2.2.6 through 7.2.2.9:
Scaling, Skinning and Filleting Fish and Preparation of Homogenate

Section 8.2.1:
Analytical Method for Lipid

Appendix J:
Procedures for Preparing Whole-Fish Homogenates

¹ Environmental Protection Agency Document EPA 823-B-00-007, November 2000. Available on-line at www.epa.gov/waterscience/fishadvice

Confidentiality

The vendor agrees that any and all data, analyses, materials, reports or other information, oral or written, prepared by the vendor with respect to this requisition shall, except for information which has been publicly available, be treated as confidential and shall not be utilized, released, published, or disclosed, by the vendor at any time for any purpose whatsoever other than to provide consultation or other service to the DEP.

Miscellaneous Provisions

The DEP personnel may, at their discretion, choose to deliver samples to the vendor's establishment rather than having them picked up by the vendor.

Any updates to the MDLs during the life of this contract shall be provided to the DEP, in writing, within one week of the update(s) completion.

The firm shall provide at no additional cost, any requested quality control / calibration information associated with a particular sample. Quality control / calibration information includes but is not limited to: values of standards used in calibration, date of last calibration, correlation coefficients of calibrations curves, instrument blank values, check standard values, spike/recovery values, duplicate values, dilution volumes, bench sheets, calculations and quality control charts.

Notice of any changes to the firm's certification status with regard to any of the parameters that the firm is certified to analyze, must be submitted to DEP, in writing, within ten (10) days of the time of status change.

Vendors must also submit the following with their bid: (see pages 2&3 of the Bid Schedule)

- Summary of experience with preparing and testing fish tissue samples. Bid submission must include names and contact information of previous clients requiring services of this type.
- Identification and West Virginia Laboratory Certification Number for any subcontractors that are used.

7. LABORATORY PROCEDURES I — SAMPLE HANDLING

scaleless fish, the pectoral fin spines should be clipped and saved (Versar, 1982). The scales, spines, or otoliths may be stored by sealing them in small envelopes (such as coin envelopes) or plastic bags labeled with, and cross-referenced by, the identification number assigned to the tissue specimen (Versar, 1982). Removal of scales, spines, or otoliths from each fish should be noted (by a check mark) on the sample processing record.

7.2.2.4 Sex Determination (Optional)—

Fish sex should be determined before filleting. To determine the sex of a fish, an incision should be made on the ventral surface of the body from a point immediately anterior to the anus toward the head to a point immediately posterior to the pelvic fins. If necessary, a second incision should be made on the left side of the fish from the initial point of the first incision toward the dorsal fin. The resulting flap should be folded back to observe the gonads. Ovaries appear whitish to greenish to golden brown and have a granular texture. Testes appear creamy white and have a smooth texture (Texas Water Commission, 1990). The sex of each fish should be recorded on the sample processing form.

7.2.2.5 Assessment of Morphological Abnormalities (Optional)—

Assessment of gross morphological abnormalities in finfish is optional. This assessment may be conducted in the field (see Section 6.3.1.5) or during initial inspection at the processing laboratory prior to filleting. States interested in documenting morphological abnormalities should consult Sinderman (1983) and review recommended protocols for fish pathology studies used in the Puget Sound Estuary Program (1990c) and those described by Goede and Barton (1990).

7.2.2.6 Scaling or Skinning—

To control contamination, separate sets of utensils and cutting boards should be used for skinning or scaling fish and for filleting fish. Fish with scales should be scaled and any adhering slime removed prior to filleting. Fish without scales (e.g., catfish) should be skinned prior to filleting. These fillet types are recommended because it is believed that they are most representative of the edible portions of fish prepared and consumed by sport anglers. However, it is the responsibility of each program manager, in consultation with state fisheries experts, to select the fillet or sample type most appropriate for each target species based on the dietary customs of local populations of concern.

A fish is scaled by laying it flat on a clean glass or PTFE cutting board or on one that has been covered with heavy duty aluminum foil and removing the scales and adhering slime by scraping from the tail to the head using the blade edge of a clean stainless steel, ceramic, or titanium knife. Cross-contamination is controlled by rinsing the cutting board and knife with contaminant-free distilled water between fish. If an aluminum-foil-covered cutting board is used, the foil should be

7. LABORATORY PROCEDURES I — SAMPLE HANDLING

changed between fish. The skin should be removed from fish without scales by loosening the skin just behind the gills and pulling it off between knife blade and thumb or with pliers as shown in Figure 7-3.

Once the scales and slime have been scraped off or the skin removed, the outside of the fish should be washed with contaminant-free distilled water and it should be placed on a second clean cutting board for filleting.

7.2.2.7 Filleting—

Filleting should be conducted only by or under the supervision of an experienced fisheries biologist. If gloves are worn, they should be talc- or dust-free, and of noncontaminating materials. Prior to filleting, hands should be washed with Ivory soap and rinsed thoroughly in tap water, followed by distilled water (U.S. EPA, 1991d). Specimens should come into contact with noncontaminating surfaces only. Fish should be filleted on glass or PTFE cutting boards that are cleaned properly between fish or on cutting boards covered with heavy duty aluminum foil that is changed between fish (Puget Sound Estuary Program, 1990d, 1990e). Care must be taken to avoid contaminating fillet tissues with material released from inadvertent puncture of internal organs. **Note:** If the fillet tissue is contaminated by materials released from the inadvertent puncture of the internal organs during resection, the state may eliminate the fillet tissue as a sample or, alternatively, the fillet tissue should be rinsed in contaminant-free, deionized distilled water and blotted dry. Regardless of the procedure selected, a notation should be made in the sample processing record.

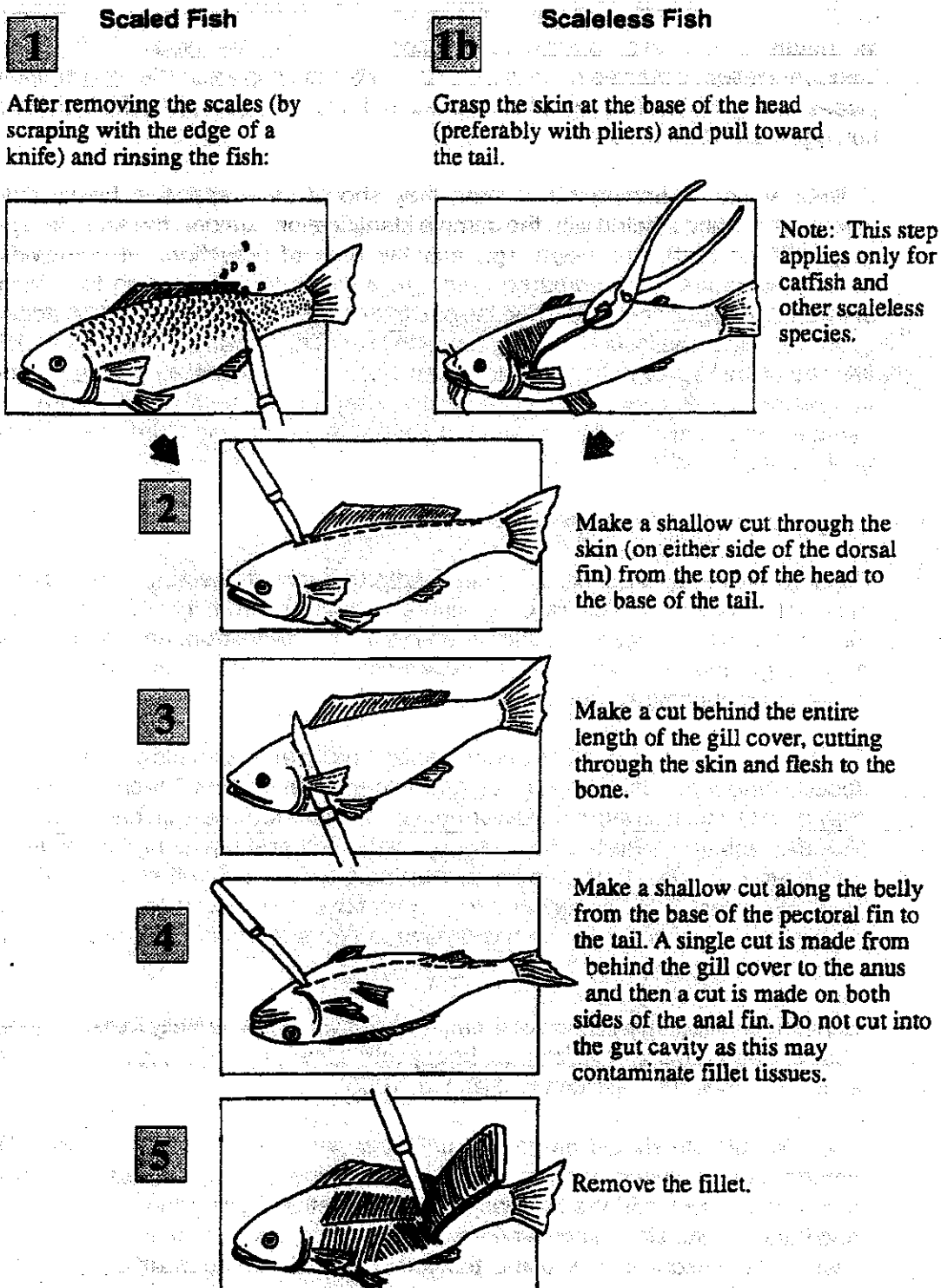
Ideally, fish should be filleted while ice crystals are still present in the muscle tissue. Therefore, if fish have been frozen, they should not be allowed to thaw completely prior to filleting. Fish should be thawed only to the point where it becomes possible to make an incision into the flesh (U.S. EPA, 1991d).

Clean, high-quality stainless steel, ceramic, or titanium utensils should be used to remove one or both fillets from each fish, as necessary. The general procedure recommended for filleting fish is illustrated in Figure 7-3 (U.S. EPA, 1991d).

The belly flap should be included in each fillet. Any dark muscle tissue in the vicinity of the lateral line should not be separated from the light muscle tissue that constitutes the rest of the muscle tissue mass. Bones still present in the tissue after filleting should be removed carefully (U.S. EPA, 1991d).

If both fillets are removed from a fish, they can be combined or kept separate for duplicate QC analysis, analysis of different analytes, or archival of one fillet. Fillets should be weighed (either individually or combined, depending on the analytical requirements) and the weight(s) recorded to the nearest gram on the sample processing record.

7. LABORATORY PROCEDURES I — SAMPLE HANDLING



Source: U.S. EPA, 1991d.

Figure 7-3. Illustration of basic fish filleting procedure.

7. LABORATORY PROCEDURES I — SAMPLE HANDLING

If fillets are to be homogenized immediately, they should be placed in a properly cleaned glass or PTFE homogenization container. If samples are to be analyzed for metals only, plastic homogenization containers may be used. To facilitate homogenization, it may be necessary or desirable to chop each fillet into smaller pieces using a titanium or stainless steel knife prior to placement in the homogenization container.

If fillets are to be homogenized later, they should be wrapped in heavy duty aluminum foil and labeled with the sample identification number, the sample type (e.g., "F" for fillet), the weight (g), and the date of resection. If composite homogenates are to be prepared from only a single fillet from each fish, fillets should be wrapped separately and the designation "F1" and "F2" should be added to the sample identification number for each fillet. The individual fillets from each fish should be kept together. All fillets from a composite sample should be placed in a plastic bag labeled with the composite identification number, the individual sample identification numbers, and the date of resection and stored at ≤ -20 °C until homogenization.

7.2.2.8 Preparation of Individual Homogenates—

To ensure even distribution of contaminants throughout tissue samples and to facilitate extraction and digestion of samples, the fillets from individual fish must be ground and homogenized prior to analysis. The fillets from an individual fish may be ground and homogenized separately or combined, depending on the analytical requirements and the sample size.

Fish fillets should be ground and homogenized using an automatic grinder or high-speed blender or homogenizer. Large fillets may be cut into 2.5-cm cubes with high-quality stainless steel or titanium knives or with a food service band saw prior to homogenization. Parts of the blender or homogenizer used to grind the tissue (i.e., blades, probes) should be made of tantalum or titanium rather than stainless steel. Stainless steel blades and/or probes have been found to be a potential source of nickel and chromium contamination (due to abrasion at high speeds) and should be avoided.

Grinding and homogenization of tissue is easier when it is partially frozen (Stober, 1991). Chilling the grinder/blender briefly with a few chips of dry ice will also help keep the tissue from sticking to it (Smith, 1985).

The fillet sample should be ground until it appears to be homogeneous. The ground sample should then be divided into quarters, opposite quarters mixed together by hand, and the two halves mixed together. The grinding, quartering, and hand-mixing steps should be repeated at least two more times. If chunks of tissue are present at this point, the grinding and homogenization should be repeated. **Note:** Skin-on fillets are the fish fillet sample type recommended for use in state fish contaminant monitoring programs. However, skin-on fillets of some finfish species are especially difficult to homogenize completely. No chunks

7. LABORATORY PROCEDURES I — SAMPLE HANDLING

of tissue or skin should remain in the sample homogenate because these may not be extracted or digested efficiently and could bias the analytical results. If complete homogenization of skin-on fillets for a particular target species is a chronic problem or if local consumers are likely to prepare skinless fillets of the species, the state should consider analyzing skinless fillet samples. If the sample is to be analyzed for metals only, the ground tissue may be mixed by hand in a polyethylene bag (Stober, 1991). The preparation of each individual homogenate should be noted (marked with a check) on the sample processing record. At this time, individual homogenates may be either processed further to prepare composite homogenates or frozen separately and stored at ≤ -20 °C (see Table 7-1).

7.2.2.9 Preparation of Composite Homogenates—

Composite homogenates should be prepared from equal weights of individual homogenates. The same type of individual homogenate (i.e., either single fillet or combined fillet) should always be used in a given composite sample.

If individual homogenates have been frozen, they should be thawed partially and rehomogenized prior to weighing and compositing. Any associated liquid should be kept as a part of the sample. The weight of each individual homogenate used in the composite homogenate should be recorded, to the nearest gram, on the sample processing record.

Each composite homogenate should be blended as described for individual homogenates in Section 7.2.2.8. The composite homogenate may be processed immediately for analysis or frozen and stored at ≤ -20 °C (see Table 7-1).

The remainder of each individual homogenate should be archived at ≤ -20 °C with the designation "Archive" and the expiration date recorded on the sample label. The location of the archived samples should be indicated on the sample processing record under "Notes."

It is essential that the weights of individual homogenates yield a composite homogenate of adequate size to perform all necessary analyses. Weights of individual homogenates required for a composite homogenate, based on the number of fish per composite and the weight of composite homogenate recommended for analyses of all screening study target analytes (see Table 4-1), are given in Table 7-2. The total composite weight required for intensive studies may be less than that for screening studies if the number of target analytes is reduced significantly.

The recommended sample size of 200 g for screening studies is intended to provide sufficient sample material to (1) analyze for all recommended target analytes (see Table 4-1) at appropriate detection limits; (2) meet minimum QC requirements for the analyses of laboratory duplicate, matrix spike, and matrix spike duplicate samples (see Sections 8.3.3.4 and 8.3.3.5); and (3) allow for

7. LABORATORY PROCEDURES I — SAMPLE HANDLING

Table 7-2. Weights (g) of Individual Homogenates Required for Screening Study Composite Homogenate Sample^{a,b}

Number of fish per sample	Total composite weight		
	100 g (minimum)	200 g (recommended)	500 g (maximum)
3	33	67	167
4	25	50	125
5	20	40	100
6	17	33	84
7	14	29	72
8	13	25	63
9	11	22	56
10	10	20	50

^a Based on total number of fish per composite and the total composite weight required for analysis in screening studies. The total composite weight required in intensive studies may be less if the number of target analytes is reduced significantly.

^b Individual homogenates may be prepared from one or both filets from a fish. A composite homogenate should be prepared only from individual homogenates of the same type (i.e., either from individual homogenates each prepared from a single fillet or from individual homogenates each prepared from both filets).

reanalysis if the QC control limits are not met or if the sample is lost. However, sample size requirements may vary among laboratories and the analytical methods used. Each program manager must consult with the analytical laboratory supervisor to determine the actual weights of composite homogenates required to analyze for all selected target analytes at appropriate detection limits.

7.2.3 Processing Turtle Samples

Processing in the laboratory to prepare individual turtle homogenate samples for analysis (diagrammed in Figure 7-4) involves

- Inspecting individual turtles
- Weighing individual turtles
- Removing edible tissues
- Determining the sex of each turtle (optional)
- Determining the age of each turtle (optional)
- Weighing edible tissue or tissues
- Homogenizing tissues
- Preparing individual homogenate samples
- Preparing aliquots of the individual homogenates for analysis
- Distributing frozen aliquots to one or more analytical laboratories.

8. LABORATORY PROCEDURES II — SAMPLE ANALYSES

Note: Because the concentrations of contaminants, particularly nonpolar organics, are often correlated with the percentage of lipid in a tissue sample, contaminant data are often normalized to the lipid concentration before statistical analyses are performed. This procedure can, in some instances, improve the power of the statistical tests. States wishing to examine the relationship between contaminant concentrations and percentage of lipid should refer to Hebert and Keenleyside (1995) for a discussion of the possible statistical approaches.

8.2 ANALYTICAL METHODS

This section provides guidance on selecting methods for analysis of recommended target analytes. Analytical methods should include appropriate procedures for sample preparation (i.e., for digestion of samples to be analyzed for metals and for extraction and extract cleanup of samples to be analyzed for organics).

8.2.1 Lipid Method

It is recommended that a gravimetric method be used for lipid analysis. This method is easy to perform and is commonly used by numerous laboratories, employing various solvent systems such as chloroform/methanol (Bligh and Dyer, 1959), petroleum ether (California Department of Fish and Game, 1990; U.S. FDA, 1990), and dichloromethane (NOAA, 1993a; Schmidt et al., 1985). The results of lipid analyses may vary significantly (i.e., by factors of 2 or 3), however, depending on the solvent system used for lipid extraction (Randall et al., 1991; D. Swackhamer, University of Minnesota, personal communication, 1993; D. Murphy, Maryland Department of the Environment, Water Quality Toxics Division, personal communication, 1993). Therefore, to ensure consistency of reported results among fish contaminant monitoring programs, it is recommended that dichloromethane be used as the extraction solvent in all lipid analyses.

In addition to the effect of solvent systems on lipid analysis, other factors can also increase the inter- and intralaboratory variation of results if not adequately controlled (Randall et al., 1991). For example, high temperatures have been found to result in decomposition of lipid material and, therefore, should be avoided during extraction. Underestimation of total lipids can also result from denaturing of lipids by solvent contaminants, lipid decomposition from exposure to oxygen or light, and lipid degradation from changes in pH during cleanup. Overestimation of total lipids may occur if a solvent such as alcohol is used, which results in substantial coextraction of nonlipid material. It is essential that these potential sources of error be considered when conducting and evaluating results of lipid analyses.

APPENDIX J

RECOMMENDED PROCEDURES FOR PREPARING WHOLE FISH COMPOSITE HOMOGENATE SAMPLES

J.1 GENERAL GUIDELINES

Laboratory processing to prepare whole fish composite samples (diagrammed in Figure J-1) involves

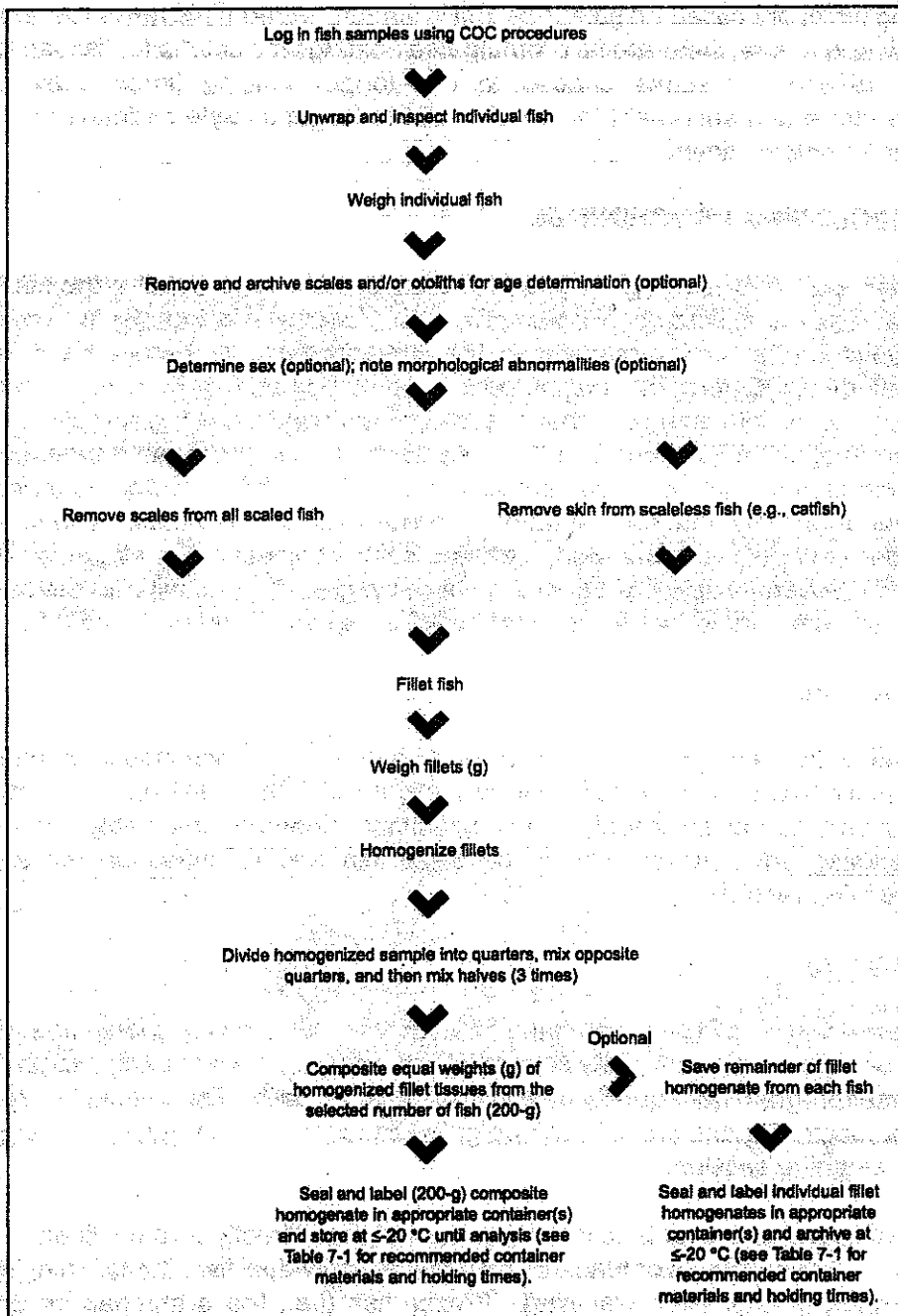
- Inspecting individual fish for foreign material on the surface and rinsing if necessary
- Weighing individual fish
- Examining each fish for morphological abnormalities (optional)
- Removing scales or otoliths for age determination (optional)
- Determining the sex of each fish (optional)
- Preparing individual whole fish homogenates
- Preparing a composite whole fish homogenate.

Whole fish should be shipped on wet or blue ice from the field to the sample processing laboratory if next-day delivery is assured. Fish samples arriving in this manner (chilled but not frozen) should be weighed, scales and/or otoliths removed, and the sex of each fish determined within 48 hours of sample collection. The grinding/homogenization procedure may be carried out more easily and efficiently if the sample has been frozen previously (Stober, 1991). Therefore, the samples should then be frozen (≤ -20 °C) in the laboratory prior to being homogenized.

If the fish samples arrive frozen (i.e., on dry ice) at the sample processing laboratory, precautions should be taken during weighing, removal of scales and/or otoliths, and sex determination to ensure that any liquid formed in thawing remains with the sample. **Note:** The liquid will contain target analyte contaminants and lipid material that should be included in the sample for analysis.

The thawed or partially thawed whole fish should then be homogenized individually, and equal weights of each homogenate should be combined to form the composite sample. Individual homogenates and/or composite homogenates may be frozen; however, frozen individual homogenates must be rehomogenized before compositing, and frozen composite homogenates must be rehomogenized before aliquotting for analysis. The maximum holding time from sample collection to analysis for mercury is 28 days at ≤ -20 °C; for all other analytes, the holding time is 1 year at ≤ -20 °C (Stober, 1991). Recommended container materials,

APPENDIX J



COC = Chain of custody.

Figure J-1. Laboratory sample preparation and handling for whole fish composite homogenate samples.

preservation temperatures, and holding times are given in Table J-1. **Note:** Holding times in Table J-1 are maximum times recommended for holding samples from the time they are received at the laboratory until they are analyzed. These holding times are based on guidance that is sometimes administrative rather than technical in nature; there are no promulgated holding time criteria for tissues (U.S. EPA, 1995b). If states choose to use longer holding times, they must demonstrate and document the stability of the target analyte residues over the extended holding times.

J.2 SAMPLE PROCESSING PROCEDURES

Fish sample processing procedures are discussed in more detail in the sections below. Each time custody of a sample or set of samples is transferred from one person to another during processing, the Personal Custody Record of the chain-of-custody (COC) form that originated in the field (Figure 6-8) must be completed and signed by both parties so that possession and location of the samples can be traced at all times (see Section 7.1). As each sample processing procedure is performed, it should be documented directly in a bound laboratory notebook or on standard forms that can be taped or pasted into the notebook. The use of a standard form is recommended to ensure consistency and completeness of the record. Several existing programs have developed forms similar to the sample processing record for whole fish composite samples shown in Figure J-2.

J.2.1 Sample Inspection

Individual fish received for filleting should be unwrapped and inspected carefully to ensure that they have not been compromised in any way (i.e., not properly preserved during shipment). Any specimen deemed unsuitable for further processing and analysis should be discarded and identified on the sample processing record.

J.2.2 Sample Weighing

A wet weight should be determined for each fish. All samples should be weighed on balances that are properly calibrated and of adequate accuracy and precision to meet program data quality objectives. Balance calibration should be checked at the beginning and end of each weighing session and after every 20 weighings in a weighing session.

Fish shipped on wet or blue ice should be weighed directly on a foil-lined balance tray. To prevent cross contamination between individual fish, the foil lining should be replaced after each weighing. Frozen fish (i.e., those shipped on dry ice) should be weighed in clean, tared, noncontaminating containers if they will thaw before the weighing can be completed. Liquid from the thawed sample must be

APPENDIX J

Table J-1. Recommendations for Container Materials, Preservation, and Holding Times for Fish, Shellfish, and Turtle Tissues from Receipt at Sample Processing Laboratory to Analysis

Analyte	Matrix	Sample container	Storage	
			Preservation	Holding time ^a
Mercury	Tissue (whole specimens, homogenates)	Plastic, borosilicate glass, quartz, and PTFE	Freeze at ≤ -20 °C	28 days ^b
Other metals	Tissue (whole specimens, homogenates)	Plastic, borosilicate glass, quartz, and PTFE	Freeze at ≤ -20 °C	6 months ^c
Organics	Tissue (whole specimens, homogenates)	Borosilicate glass, quartz, PTFE, and aluminum foil	Freeze at ≤ -20 °C	1 year ^d
Metals and organics	Tissue (whole specimens, homogenates)	Borosilicate glass, quartz, and PTFE	Freeze at ≤ -20 °C	28 days (mercury); 6 months (for other metals); and 1 year (for organics)
Lipids	Tissue (whole specimens, homogenates)	Plastic, borosilicate glass, quartz, PTFE	Freeze at ≤ -20 °C	1 year

PTFE = Polytetrafluoroethylene for Teflon.

^a Maximum holding times recommended by U.S. EPA (1995b).

^b This maximum holding time is also recommended by the Puget Sound Estuary Program (1990). The California Department of Fish and Game (1990) and the USGS National Water Quality Assessment Program (Crawford and Luoma, 1993) recommend a maximum holding time of 6 months for all metals, including mercury.

^c This maximum holding time is also recommended by the California Department of Fish and Game (1990), the 301(h) monitoring program (U.S. EPA, 1986), and the USGS National Water Quality Assessment Program (Crawford and Luoma, 1993). The Puget Sound Estuary Program (1990) recommends a maximum holding time of 2 years.

^d This maximum holding time is also recommended by the Puget Sound Estuary Program (1990). The California Department of Fish and Game (1990) and the USGS National Water Quality Assessment Program (Crawford and Luoma, 1993) recommend a more conservative maximum holding time of 6 months. EPA (1995a) recommends a maximum holding time of 1 year at ≤ -10 °C for dioxins and dibenzofurans.

Sample Processing Record for Fish Contaminant Monitoring Program — Whole Fish Composites

Project No. _____ Sampling Date and Time: _____

STUDY PHASE: Screening : Intensive: Phase I Phase II

SITE LOCATION

Site Name/Number: _____

County/Parish: _____ Lat./Long.: _____

State Waterbody Segment Number: _____ Waterbody Type: _____

Bottom Feeder — Species Name: _____

Composite Sample #: _____ Number of Individuals: _____

Fish #	Weight (g)	Scales/Otoliths Removed (✓)	Sex (M, F)	Homogenate Prepared (✓)	Weight of homogenate taken for composite (g)
001	_____	_____	_____	_____	_____
002	_____	_____	_____	_____	_____
003	_____	_____	_____	_____	_____
004	_____	_____	_____	_____	_____
005	_____	_____	_____	_____	_____
006	_____	_____	_____	_____	_____
007	_____	_____	_____	_____	_____
008	_____	_____	_____	_____	_____
009	_____	_____	_____	_____	_____
010	_____	_____	_____	_____	_____
Analyst Initials/Date	_____ / _____	_____ / _____	_____ / _____	_____ / _____	_____ / _____
Total Composite Homogenate Weight					_____

Predator — Species Name: _____

Composite Sample #: _____ Number of Individuals: _____

Fish #	Weight (g)	Scales/Otoliths Removed (✓)	Sex (M, F)	Homogenate Prepared (✓)	Weight of homogenate taken for composite (g)
001	_____	_____	_____	_____	_____
002	_____	_____	_____	_____	_____
003	_____	_____	_____	_____	_____
004	_____	_____	_____	_____	_____
005	_____	_____	_____	_____	_____
006	_____	_____	_____	_____	_____
007	_____	_____	_____	_____	_____
008	_____	_____	_____	_____	_____
009	_____	_____	_____	_____	_____
010	_____	_____	_____	_____	_____
Analyst Initials/Date	_____ / _____	_____ / _____	_____ / _____	_____ / _____	_____ / _____
Total Composite Homogenate Weight					_____

Notes: _____

Figure J-2. Example of a sample processing record for fish contaminant monitoring program—whole fish composites.

kept in the container as part of the sample because it will contain lipid material that has separated from the tissue (Stober, 1991).

All weights should be recorded to the nearest gram on the sample processing record and/or in the laboratory notebook.

J.2.3 Age Determination

Age provides a good indication of the duration of exposure to pollutants (Versar, 1982). A few scales or otoliths (Jearld, 1983) should be removed from each fish and delivered to a fisheries biologist for age determination. For most warm water inland gamefish, 5 to 10 scales should be removed from below the lateral line and behind the pectoral fin. On soft-rayed fish such as trout and salmon, the scales should be taken just above the lateral line (WDNR, 1988). For catfish and other scaleless fish, the pectoral fin spines should be clipped and saved (Versar, 1982). The scales, spines, or otoliths may be stored by sealing them in small envelopes (such as coin envelopes) or plastic bags labeled with, and cross-referenced by, the identification number assigned to the tissue specimen (Versar, 1982). Removal of scales, spines, or otoliths from each fish should be noted (by a check mark) on the sample processing record.

J.2.4 Sex Determination (Optional)

To determine the sex of a fish, an incision should be made on the ventral surface of the body from a point immediately anterior to the anus toward the head to a point immediately posterior to the pelvic fins. If necessary, a second incision should be made on the left side of the fish from the initial point of the first incision toward the dorsal fin. The resulting flap should be folded back to observe the gonads. Ovaries appear whitish to greenish to golden brown and have a granular texture. Testes appear creamy white and have a smooth texture (Texas Water Commission, 1990). The sex of each fish should be recorded on the sample processing record.

J.2.5 Assessment of Morphological Abnormalities (Optional)

Assessment of gross morphological abnormalities in finfish is optional. This assessment may be conducted in the field (see Section 6.3.1.5) or during initial inspection at the central processing laboratory prior to filleting. States interested in documenting morphological abnormalities should consult Sinderman (1983) and review recommended protocols for fish pathology studies used in the Puget Sound Estuary Program (1990).

J.2.6 Preparation of Individual Homogenates

To ensure even distribution of contaminants throughout tissue samples, whole fish must be ground and homogenized prior to analyses.

Smaller whole fish may be ground in a hand crank meat grinder (fish < 300 g) or a food processor (fish 300-1,000 g). Larger (>1,000 g) fish may be cut into 2.5-cm cubes with a food service band saw and then ground in either a small or large homogenizer. To avoid contamination by metals, grinders and homogenizers used to grind and blend tissue should have tantalum or titanium blades and/or probes. Stainless steel blades and probes have been found to be a potential source of nickel and chromium contamination (due to abrasion at high speeds) and should be avoided.

Grinding and homogenization of biological tissue, especially skin from whole fish samples, is easier when the tissue is partially frozen (Stober, 1991). Chilling the grinder/homogenizer briefly with a few chips of dry ice will reduce the tendency of the tissue to stick to the grinder.

The ground sample should be divided into quarters, opposite quarters mixed together by hand, and the two halves mixed back together. The grinding, quartering, and hand mixing should be repeated two more times. If chunks of tissue are present at this point, the grinding/homogenizing should be repeated. No chunks of tissue should remain because these may not be extracted or digested efficiently. If the sample is to be analyzed for metals only, the ground tissue may be mixed by hand in a polyethylene bag (Stober, 1991). Homogenization of each individual fish should be noted on the sample processing record. At this time, individual whole fish homogenates may be either composited or frozen and stored at ≤ -20 °C in cleaned containers that are noncontaminating for the analyses to be performed (see Table J-1).

J.2.7 Preparation of Composite Homogenates

Composite homogenates should be prepared from equal weights of individual homogenates. If individual whole fish homogenates have been frozen, they should be thawed partially and rehomogenized prior to compositing. Any associated liquid should be maintained as a part of the sample. The weight of each individual homogenate that is used in the composite homogenate should be recorded, to the nearest gram, on the sample processing record.

Each composite homogenate should be blended by dividing it into quarters, mixing opposite quarters together by hand, and mixing the two halves together. The quartering and mixing should be repeated at least two more times. If the sample is to be analyzed only for metals, the composite homogenate may be mixed by hand in a polyethylene bag (Stober, 1991). At this time, the composite homogenate may be processed for analysis or frozen and stored at ≤ -20 °C (see Table J-1).

The remainder of each individual homogenate should be archived at ≤ -20 °C with the designation "Archive" and the expiration date recorded on the sample label. The location of the archived samples should be indicated on the sample processing record under "Notes."

It is essential that the weights of individual homogenates yield a composite homogenate of adequate size to perform all necessary analyses. Weights of individual homogenates required for a composite homogenate, based on the number of fish per composite and the weight of composite homogenate recommended for analyses of all screening study target analytes (see Table 4-1), are given in Table J-2. The total composite weight required for intensive studies may be less than in screening studies if the number of target analytes is reduced significantly.

The recommended sample size of 200 g for screening studies is intended to provide sufficient sample material to (1) analyze for all recommended target analytes (see Table 4-1) at appropriate detection limits, (2) meet minimum QA and QC requirements for the analyses of replicate, matrix spike, and duplicate matrix spike samples (see Section 8.3.3.4), and (3) allow for reanalysis if the QA and QC control limits are not met or if the sample is lost. However, sample size requirements may vary among laboratories and the analytical methods used. Therefore, it is the responsibility of each program manager to consult with the analytical laboratory supervisor to determine the actual weights of composite homogenates required to analyze for all selected target analytes at appropriate detection limits.

J.3 REFERENCES

- California Department of Fish and Game. 1990. *Laboratory Quality Assurance Program Plan*. Environmental Services Division, Sacramento, CA.
- Crawford, J.K., and S.N. Luoma. 1993. *Guidelines for Studies of Contaminants in Biological Tissues for the National Water-Quality Assessment Program*. USGS Open-File Report 92-494. U.S. Geological Survey, Lemoyne, PA.
- Jearld, A. 1983. Age determination. pp. 301-324. In: *Fisheries Techniques*. L.A. Nielsen and D. Johnson (eds.). American Fisheries Society, Bethesda, MD.
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- Sinderman, C. J. 1983. An examination of some relationships between pollution and disease. *Rapp. P. V. Reun. Cons. Int. Explor. Mer.* 182:37-43.
- Stober, Q. J. 1991. *Guidelines for Fish Sampling and Tissue Preparation for Bioaccumulative Contaminants*. Environmental Services Division, Region 4, U.S. Environmental Protection Agency, Athens, GA.

Texas Water Commission. 1990. *Texas Tissue Sampling Guidelines*. Texas Water Commission, Austin, TX.

U.S. EPA (U.S. Environmental Protection Agency). 1986. *Bioaccumulation Monitoring Guidance: 4. Analytical Methods for U.S. EPA Priority Pollutants and 301(h) Pesticides in Tissues from Marine and Estuarine Organisms*. EPA-503/6-90-002. Office of Marine and Estuarine Protection, Washington, DC.

U.S. EPA (U.S. Environmental Protection Agency). 1995a. *Method 1613b. Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS*. Final Draft. Office of Water, Office of Science and Technology, Washington, DC.

U.S. EPA (Environmental Protection Agency). 1995b. *QA/QC Guidance for Sampling and Analysis of Sediments, Water, and Tissues for Dredged Material Evaluations—Chemical Evaluations*. EPA 823-B-95-001. Office of Water, Washington, DC, and Department of the Army, U.S. Army Corps of Engineers, Washington, DC.

Versar, Inc. 1982. *Sampling Protocols for Collecting Surface Water, Bed Sediment, Bivalves and Fish for Priority Pollutant Analysis—Final Draft Report*. EPA Contract 68-01-6195. Prepared for U.S. EPA Office of Water Regulations and Standards. Versar, Inc., Springfield, VA.

WDNR (Wisconsin Department of Natural Resources). 1988. *Fish Contaminant Monitoring Program—Field and Laboratory Guidelines (1005.1)*. Madison, WI.

State of West Virginia VENDOR PREFERENCE CERTIFICATE

Certification and application* is hereby made for Preference in accordance with **West Virginia Code, §5A-3-37**. (Does not apply to construction contracts). **West Virginia Code, §5A-3-37**, provides an opportunity for qualifying vendors to request (at the time of bid) preference for their residency status. Such preference is an evaluation method only and will be applied only to the cost bid in accordance with the **West Virginia Code**. This certificate for application is to be used to request such preference. The Purchasing Division will make the determination of the Resident Vendor Preference, if applicable.

1. **Application is made for 2.5% resident vendor preference for the reason checked:**
 Bidder is an individual resident vendor and has resided continuously in West Virginia for four (4) years immediately preceding the date of this certification; or,
 Bidder is a partnership, association or corporation resident vendor and has maintained its headquarters or principal place of business continuously in West Virginia for four (4) years immediately preceding the date of this certification; or 80% of the ownership interest of Bidder is held by another individual, partnership, association or corporation resident vendor who has maintained its headquarters or principal place of business continuously in West Virginia for four (4) years immediately preceding the date of this certification; or,
 Bidder is a nonresident vendor which has an affiliate or subsidiary which employs a minimum of one hundred state residents and which has maintained its headquarters or principal place of business within West Virginia continuously for the four (4) years immediately preceding the date of this certification; or,
2. **Application is made for 2.5% resident vendor preference for the reason checked:**
 Bidder is a resident vendor who certifies that, during the life of the contract, on average at least 75% of the employees working on the project being bid are residents of West Virginia who have resided in the state continuously for the two years immediately preceding submission of this bid; or,
3. **Application is made for 2.5% resident vendor preference for the reason checked:**
 Bidder is a nonresident vendor employing a minimum of one hundred state residents or is a nonresident vendor with an affiliate or subsidiary which maintains its headquarters or principal place of business within West Virginia employing a minimum of one hundred state residents who certifies that, during the life of the contract, on average at least 75% of the employees or Bidder's affiliate's or subsidiary's employees are residents of West Virginia who have resided in the state continuously for the two years immediately preceding submission of this bid; or,
4. **Application is made for 5% resident vendor preference for the reason checked:**
 Bidder meets either the requirement of both subdivisions (1) and (2) or subdivision (1) and (3) as stated above; or,
5. **Application is made for 3.5% resident vendor preference who is a veteran for the reason checked:**
 Bidder is an individual resident vendor who is a veteran of the United States armed forces, the reserves or the National Guard and has resided in West Virginia continuously for the four years immediately preceding the date on which the bid is submitted; or,
6. **Application is made for 3.5% resident vendor preference who is a veteran for the reason checked:**
 Bidder is a resident vendor who is a veteran of the United States armed forces, the reserves or the National Guard, if, for purposes of producing or distributing the commodities or completing the project which is the subject of the vendor's bid and continuously over the entire term of the project, on average at least seventy-five percent of the vendor's employees are residents of West Virginia who have resided in the state continuously for the two immediately preceding years.

Bidder understands if the Secretary of Revenue determines that a Bidder receiving preference has failed to continue to meet the requirements for such preference, the Secretary may order the Director of Purchasing to: (a) reject the bid; or (b) assess a penalty against such Bidder in an amount not to exceed 5% of the bid amount and that such penalty will be paid to the contracting agency or deducted from any unpaid balance on the contract or purchase order.

By submission of this certificate, Bidder agrees to disclose any reasonably requested information to the Purchasing Division and authorizes the Department of Revenue to disclose to the Director of Purchasing appropriate information verifying that Bidder has paid the required business taxes, provided that such information does not contain the amounts of taxes paid nor any other information deemed by the Tax Commissioner to be confidential.

Under penalty of law for false swearing (West Virginia Code, §61-5-3), Bidder hereby certifies that this certificate is true and accurate in all respects; and that if a contract is issued to Bidder and if anything contained within this certificate changes during the term of the contract, Bidder will notify the Purchasing Division in writing immediately.

Bidder: Bio-Chem Testing, Inc Signed: [Signature]
 Date: 09-16-09 Title: President

*Check any combination of preference consideration(s) indicated above, which you are entitled to receive.

RFQ No. DEP 14746

STATE OF WEST VIRGINIA
Purchasing Division

PURCHASING AFFIDAVIT

VENDOR OWING A DEBT TO THE STATE:

West Virginia Code §5A-3-10a provides that: No contract or renewal of any contract may be awarded by the state or any of its political subdivisions to any vendor or prospective vendor when the vendor or prospective vendor or a related party to the vendor or prospective vendor is a debtor and the debt owed is an amount greater than one thousand dollars in the aggregate.

PUBLIC IMPROVEMENT CONTRACTS & DRUG-FREE WORKPLACE ACT:

West Virginia Code §21-1D-5 provides that: Any solicitation for a public improvement construction contract shall require each vendor that submits a bid for the work to submit at the same time an affidavit that the vendor has a written plan for a drug-free workplace policy in compliance with Article 1D, Chapter 21 of the West Virginia Code. A public improvement construction contract may not be awarded to a vendor who does not have a written plan for a drug-free workplace policy in compliance with Article 1D, Chapter 21 of the West Virginia Code and who has not submitted that plan to the appropriate contracting authority in timely fashion. For a vendor who is a subcontractor, compliance with Section 5, Article 1D, Chapter 21 of the West Virginia Code may take place before their work on the public improvement is begun.

ANTITRUST:

In submitting a bid to any agency for the state of West Virginia, the bidder offers and agrees that if the bid is accepted the bidder will convey, sell, assign or transfer to the state of West Virginia all rights, title and interest in and to all causes of action it may now or hereafter acquire under the antitrust laws of the United States and the state of West Virginia for price fixing and/or unreasonable restraints of trade relating to the particular commodities or services purchased or acquired by the state of West Virginia. Such assignment shall be made and become effective at the time the purchasing agency tenders the initial payment to the bidder.

I certify that this bid is made without prior understanding, agreement, or connection with any corporation, firm, limited liability company, partnership or person or entity submitting a bid for the same materials, supplies, equipment or services and is in all respects fair and without collusion or fraud. I further certify that I am authorized to sign the certification on behalf of the bidder or this bid.

LICENSING:

Vendors must be licensed and in good standing in accordance with any and all state and local laws and requirements by any state or local agency of West Virginia, including, but not limited to, the West Virginia Secretary of State's Office, the West Virginia Tax Department, West Virginia Insurance Commission, or any other state agencies or political subdivision. Furthermore, the vendor must provide all necessary releases to obtain information to enable the Director or spending unit to verify that the vendor is licensed and in good standing with the above entities.

CONFIDENTIALITY:

The vendor agrees that he or she will not disclose to anyone, directly or indirectly, any such personally identifiable information or other confidential information gained from the agency, unless the individual who is the subject of the information consents to the disclosure in writing or the disclosure is made pursuant to the agency's policies, procedures and rules. Vendors should visit www.state.wv.us/admin/purchase/privacy for the Notice of Agency Confidentiality Policies.

Under penalty of law for false swearing (West Virginia Code §61-5-3), it is hereby certified that the vendor acknowledges the information in this said affidavit and is in compliance with the requirements as stated.

Vendor's Name: Bio-Chem Testing, Inc.

Authorized Signature: [Signature] Date: 09-16-09

Homogenization & Analysis of Animal Tissue

DEP14746

Vendor's Bid Sheet

Vendors Name: Bio-Chem Testing, Inc.

The DEP reserves the right to request additional information and supporting documentation regarding unit prices when the unit price appears to be unreasonable.

ITEM NO.	QUANTITY	DESCRIPTION	UNIT PRICE	AMOUNT
1.0		Bids must include the following information: (Bidders must use the format provided below)		
1.a	30 ea	Cost per fish to prepare fillets from whole fish samples	\$ 11.00	\$ 330
1.b	200 ea	Cost per sample (up to 10 fillets) to prepare homogenate of fillet specimens	\$ 11.50	\$ 2,300
1.c	100 ea	Cost per fish to prepare homogenate for whole-fish samples (Small fish may be grouped and considered one sample)	\$ 11.50	\$ 1,150
1.d	40 ea	Cost per sample to prepare homogenate for aquatic insect samples	\$ 14.50	\$ 580
1.e	50 ea	Cost per sample to prepare homogenate for bird egg samples (samples may include eggshell and mature embryos with well-developed boney tissue)	\$ 15.50	\$ 775
1.f	300 ea	Cost per sample to perform % moisture analysis	\$ 11.50	\$ 3,450
1.g	200 ea	Cost per sample to perform % lipids analysis	\$ 65.00	\$ 13,000
1.h	200 ea	Cost per sample to perform Hg analysis	\$ 28.00	\$ 5,600
1.i	200 ea	Cost per sample to perform PCB's analysis	\$ 88.00	\$ 17,600
1.j	100 ea	Cost per sampe to perform Se analysis	\$ 25.00	\$ 2,500
2.0	1,000 miles	Cost per mile for sample pick-up at DEP Charleston Headquarters	00	\$ 00
3.0	10 hours	Cost per hour for professional staff representation of data in legal/administrative meetings	\$ 100	\$ 1,000
TOTAL COST				\$ 48,285

***Note: Quantities are estimates for bidding purposes only.

DEP14746 Bid Sheet continued...

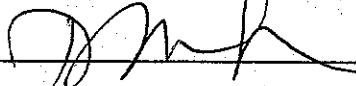
Please Complete:

Approximate Minimum Detection Limit lab is able to attain for each analyte (bidders must use the format below):

- a. Total Mercury: 0.03 mg/kg
 b. Total Selenium: 0.2 mg/kg
 c. Total PCB: 0.05 mg/Kg

WV Lab Certification #: 220

* To be subcontracted to WVDEP certified lab.

Name of Laboratory Contact: Mukesh Shab
 Contact's Signature: 
 Contact's Phone Number: 304-757-8954
 Contact's Address: 5 Weatheridge Drive
Hurricane, WV 25526

DEP14746 Bid Sheet continued...

SUMMARY OF EXPERIENCE

1. Client: US Army Corps of Engineers
 Contact Name: Elana Woody
 Phone #: 304-576-3301

Address/Contact Information:

PO Box 9
Apple Grove, WV 25502-0009

Experience: Prepare fillets from whole fish, homogenate of fillet, prepare homogenate for insects, bird eggs. Analysis of fish for % moisture, lipid contents, metals contents, PCB contents, Selenium, and Mercury contents.

2. Client: Potesta & Associates
 Contact Name: Lisa Burges
 Phone #: 304-342-1400

Address/Contact Information:

7052 MacCorkle Ave SE
Charleston, WV 25304

Experience: Prepare fillets from whole fish, homogenate of fillets and analyze for % moisture, Selenium & PCB contents.

3. Client: West Virginia Dept. of Environment metal Protection
 Contact Name: Jenice Smithson
 Phone #: 304-926-0499 *1051

Address/Contact Information:

601 57th Street SE
Charleston, WV 25304

Experience: Prepare fillets from whole fish, homogenate of fillet, insects, bird eggs. Analyzing at all above for or in part Selenium, % moisture, lipid & PCB contents.

***Additional References and Experience Information may be attached to this form.