COMMONWEALTH of VIRGINIA

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Memorandum

SUBJECT: Water Guidance Memo No. 02-2002
Fish Kill Guidance Manual Second Edition

TO: Regional Directors (with attachment)

FROM: Larry Lawson, P.E., Director
Division of Water Program Coordination

DATE: March 4, 2002

COPIES: Environmental Field Managers, Regional Biologists, Regional Compliance and Enforcement Managers, Regional Water Permit Managers, and Alan Pollock (without attachment)

Attached is the second edition of the DEQ Fish Kill Investigation Guidance Manual that was developed in coordination with the Department of Game and Inland Fisheries and DEQ Regional and Central Office staffs. This manual supercedes Water Guidance Memo No. 98-2006, Fish Kill Investigation Guidance Manual, dated September 14, 1998. The manual should serve as a guide for fish kill investigations and employs procedures that are consistent with the U.S Fish and Wildlife Service and the American Fisheries Society published guidelines.

The document provides guidance regarding field equipment required for an investigation, field observations and sample collections for locating the source and extent of the kill, fish counting and fish cost assessment guidelines, documentation of expenses for cost recovery, etc.

If you have any questions on the contents of this manual, please feel free to contact Rick Browder with the Water Quality Standards & Biological Programs at 804/698-4134.

Disclaimer

This document provides procedural guidance to staff. This document is guidance only. It does not establish or affect legal rights or obligations. It does not establish a binding norm and is not finally determinative of issues addressed. Agency decisions in any particular case will be made by applying the State Water Control Law and the implementation regulations on the basis of the site specific facts.

LGL/scj

Attachment

VIRGINIA DEPARTMENT OF ENVIRONMENTAL QUALITY

FISH KILL INVESTIGATION GUIDANCE MANUAL

SECOND EDITION

WATER QUALITY STANDARDS AND BIOLOGICAL MONITORING PROGRAMS

RICHMOND, VIRGINIA

March 2002

EXECUTIVE SUMMARY

The Department of Environmental Quality (DEQ) Fish Kill Investigation Guidance Manual is designed to guide field personnel through an investigation of a fish kill event. It is intended for use in a first response scenario by trained personnel. It is not designed as a "cookbook" for untrained personnel or to be used as a substitute for formal training. Procedures outlined in this manual are the product of many fish kill investigations undertaken since the creation of the State Water Control Board in 1946, which was a predecessor to the DEQ. These procedures are consistent with agency guidelines published by the U.S. Fish and Wildlife Service and the American Fisheries Society.

This document covers the pertinent functions of a complete investigation and includes: defining the extent of the kill area; locating and stopping the source of the kill; notifying other appropriate parties; collecting the necessary information to substantiate the cause of the kill; determining the number and kind of fish killed; and presenting the data collected for potential enforcement action.

CHRONOLOGY OF THE GUIDANCE MANUAL AND ACKNOWLEDGEMENTS

This guidance manual was prepared by the Department of Environmental Quality (DEQ) Office of Water Quality Standards and Biological Programs (WQS & BP). On October 30, 1997 a draft version of the first version of the guidance was presented to personnel of the Department of Game and Inland Fisheries (DGIF) and to DEQ Central and Regional Office Staff who have fish kill investigation and cost recovery responsibilities at a meeting in Charlottesville, Virginia. On August 1998 the first version was finalized and presented to the DGIF and to the DEQ regional and central offices. On December 7, 2001 (WQS & BP) staff requested comments from the same and additional DGIF & DEQ staff members who participated in the 1997 Charlottesville meeting to update the Manual. Few changes to the manual were requested and in March 2002, the second version of the DEQ Fish Kill Guidance Manual was completed. The final preparation of the first and second versions of this guidance manual is due to the extensive effort by various DGIF and DEQ staff (listed below).

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PURPOSE/POLICY STATEMENT

The purposes of fish kill investigations are: to determine the cause of fish kill events in the waters of Virginia; to assess environmental damage caused by such kills; and to determine the parties responsible for the kill. Evidence gathered during investigations may become part of enforcement actions to seek reimbursement of investigation and fish replacement costs by the responsible party (State Water Control Law §62.1-44.15) and to address violations of Virginia's Water Quality Standards (9 VAC 25-260-20A). Fish kill investigations also serve as the impetus for further studies which may reveal environmental or human health concerns in cases where water quality standards are not being met and/or where the consumption of contaminated or diseased fish is possible.

Reports of fish kills are investigated on the assumption that fish kills represent a potentially serious loss of aquatic resources.

BACKGROUND

Fish kills may be obvious indicators of serious water quality degradation. In addition, the loss of the Commonwealth's natural resources merits investigation and, if possible, compensation for damages sustained. Fish kill investigations have been a necessary function of the State Water Control Board (SWCB) since its inception in 1946. In 1970, the State Water Control Law was modified to make the SWCB responsible for investigating fish kills. The modification also empowered the SWCB to recover the costs of investigations, the replacement costs of dead fish, and to impose penalties for violations (**see Appendix A** of applicable statutory and regulatory requirements). In 1993, the SWCB merged with the State Air Pollution Control Board, the Waste Management Board, and the Council on the Environment to form the Department of Environmental Quality (DEQ). The DEQ regulates water discharges, air emissions, and waste management in Virginia.

Responsibility for handling fish kill events lies in three divisions of the DEQ; Regional Offices (RO), the Central Office of Enforcement (OE), and the Water Quality Standards and Biological Programs unit (WQS&BP).

Investigations of fish kill events are conducted by the RO pollution response teams. Reports are forwarded to the RO enforcement staff and reside in RO files. The Central OE serve in a technical assistance capacity.

Throughout the course of an investigation, coordination among the RO's, OE, and WQS&BP is essential. The immediate staff time requirements of fish kill investigations may place hardships on all offices involved, possibly resulting in the postponement or cancellation of routinely scheduled work assignments. Although personnel are kept on call for such investigations, staff requirements may, at times, be expected to exceed that which is readily available.

This document is based on information contained in the U.S. Fish & Wildlife Service Field Manual for the Investigation of Fish Kills (1990) and the American Fisheries Society (AFS) Investigation and Valuation of Fish Kills (1992). Investigators should refer to those documents for detailed information regarding investigations of fish kills. Investigators are expected to set priorities and exercise varying degrees of technical judgement in carrying out fish kill investigations. This document is not to be viewed as a standard operating procedure.

RESPONSIBILITIES

Responsibility for the fish kill investigation program is shared among the RO, Central OE, and the Office of WQS&BP in the following manner.

Regional Offices (RO)

Investigate fish kills in their region. Log the initial report and assign a pollution incident report number (IR#) to the investigation. Coordinate notifications to other state and local agencies as appropriate.

Conduct fish kill investigations when appropriate and maintain a field log book.

Document all investigative costs. Obtain figures for the replacement costs of fish from the Department of Game and Inland Fisheries (DGIF) and figures for the costs of lab tests from the Division of Consolidated Laboratory Services (DCLS) if the kill is not due to natural causes.

Write the fish kill reports and maintain report files.

The report should be in a finished form so that it may be presented to the party responsible for the kill. Each report should contain at least the information required to complete the "Virginia Department of Environmental Quality Fish Kill Report/Notification", and its appropriate attachments (see Appendix B).

Regional OE evaluate fish kill reports to ensure that appropriate enforcement action and cost recovery may be undertaken when warranted and return written reports to the regional investigator when incomplete.

Request technical review by WQS&BP staff when needed.

Seek recovery of investigative and fish costs when appropriate. All proceedings concerning judicial action to be taken by the DEQ shall be coordinated through the Central OE. In addition, enforcement actions to be taken against major VPDES facilities and actions involving state-wide policy or precedent-setting issues, shall be reviewed with Central OE in advance of final disposition.

Central Office of Enforcement (OE)

Update enforcement procedural manual as necessary.

Provide interpretation and guidance on applicable statutory, regulatory and policy requirements, to the RO, as necessary.

Coordinate judicial action on fish kills with the Office of the Attorney General and the RO.

Review cases involving VPDES majors, state-wide policy and precedent-setting issues.

Assist in obtaining inspection warrants.

Water Quality Standards and Biological Programs (WQS&BP)

Update fish kill investigation procedural manuals and guidelines as necessary.

Provide technical assistance to the RO upon request.

Provide technical review of fish kill reports for RO upon request.

Provide guidance to regional investigators in obtaining the appropriate literature for reference data.

Assist RO with unusual or large fish kill investigations(i.e. fish counts, etc.).

PRELIMINARY TASKS

Receipt of the Fish Kill Report

Upon receipt of a fish kill report, as much information as possible should be obtained from the person reporting the fish kill.

Contact Information

The name, address, telephone number, and any other pertinent information about the caller should be recorded in case there is a need for later contact.

Fish Kill Location

The exact location and description of the kill site should be obtained, not only for the purpose of finding the kill area but also for trip preparation. Precise information on route numbers, intersections, railroad crossings, landmarks, etc. are essential. One should also ask whether the kill site is a lake requiring a large boat, or a small wadeable stream.

Magnitude of the Kill

The approximate size of the kill area in acres or stream miles, the species and sizes of fish, and the approximate number of fish involved should be obtained.

Condition of Fish and Other Observations

It is helpful to the investigator to know if the fish are still dying; such information will enable the investigator to determine how recently the kill occurred and the possibility of the pollutant still being present or released. Also, it is helpful to get the caller's opinion as to the time and cause of the fish kill (e.g., information on recent watershed activities upstream of the fish kill, such as pesticide spraying, fuel or chemical spills, agricultural or construction activity, dumping of fish by commercial fisherman, unusual odors, color of water, foam or oil sheen on the surface of the water. In addition, any obvious external marks or lesions on fish, etc.).

Notifications

The initial fish kill report is usually received by the RO or the Department of Emergency Services (DES) and may be shared via phone or facsimiles. Subsequent notifications within DEQ, and to other state (e.g. the Virginia Department of Health regarding threatened public and/or private water supplies, etc.), federal, local agencies, and private facilities are handled by the RO. It is recommended that the Virginia Department of Game and Inland Fisheries be notified when large or significant fish kills occur e.g., if a fish kill occurs in waters containing threatened or endangered species **(see Appendix C)**. Notifications to the above parties do not necessarily indicate that the RO Office desires assistance with field investigations. The RO will originate any request for assistance. In special cases where technical assistance in the investigation is required, the WQS&BP may be contacted for guidance.

FORMAL FISH KILL INVESTIGATION

A quick response to a fish kill is essential and the incident should be treated as an emergency response situation; however, some office preparation should take place prior to departing for the field in order to conduct an efficient investigation. The investigator should locate the fish kill area on maps. USGS topographical maps and Virginia county maps are ideal for field work and enable the investigator to perceive the terrain and water area involved in order to prepare appropriate equipment for the investigation. The investigator may perform a quick search of the pollution complaint files to determine if the fish kill is a continuing or recurring problem. Permit files and maps may also be searched to identify discharges that are present in the area.

Finally, before departing the office, all field equipment and sample containers should be checked to determine if they are sufficient to conduct the investigation.

The primary purpose of the fish kill investigation is to determine the cause of death (natural or otherwise), the mechanism of death (toxicosis, asphyxia, septicemia, etc.),conditions that lead to death, and the size and number of each species killed (Meyer and Barclay 1990). Field tests, sampling, and other observations should be geared toward answering these basic questions and to identify, if possible, a responsible party to stop or contain the pollution discharged and to prevent future fish kills. Every investigation must be handled on a case-by-case basis and no document can cover all aspects of an investigation. The following sections will aid in the use of specific techniques, either investigative or technical, and may be applied as necessary to individual fish kills.

Location/Confirmation

The first step in the investigation is to locate and confirm the kill, and determine if fish are still dying. Next, attempt to **locate the source** of the pollutant and take measures to **stop and contain** the pollutant with local Fire Department, DES, and/or responsible party assistance. **The DEQ does not expect employees to risk their personal safety in responding to chemical/hazardous spills or any pollution complaint**. In instances where hazardous chemicals, explosives, and flammable materials are involved, (particularly if one is not familiar with the material), **stand clear** and **contact the DES for help**.

Problems of Entry

Investigators on the scene have an obligation and a responsibility to report anything that appears harmful or damaging to the environment. As state employees, investigators should report any problems to the appropriate state agency. If an investigator deems that an inspection of a facility or posted property is appropriate or essential to the investigation, permission to enter the facility or posted property should be requested from a company official or property owner. Normally, immediate access will be granted, however, if an investigator is denied entry for any reason, the investigator should immediately contact RO management. Central OE may also be contacted for assistance in obtaining inspection warrants.

Field Tests

If fish are found dead or dying, the investigator should conduct initial field tests (i.e. pH, dissolved oxygen, temperature, chlorine, conductivity, salinity) in the immediate area where dead fish are found, using properly calibrated instruments. Calibration data should be logged to ensure that any field test measurements can be defended in court. All field test locations, date and time, method, and measurements should be documented in a waterproof field notebook and initialed by the investigator taking the test. Field tests may be used to trace the source of a spill or pollutant discharge and to delineate the area impacted by a spill.

Sampling

Once initial field tests have been made, water column, sediment, and/or fish samples should be collected for lab analyses. Reliable results can be obtained if a few basic principles are followed. For example, ensure that the sample taken is truly representative of the stream, use proper sampling techniques (see pages 8-1, 8-2), preserve and protect the samples until they are analyzed, and use proper sample chain of custody procedures (see Water Quality Assessment Operating Procedures Manual-legal sampling handling procedures and Guidance Memo No 00-2016 Chain of Custody Policy and Procedures in Appendix L). Be sure to collect samples of everything that is pertinent, for example all discharges in the area that may be involved. **REMEMBER: Any sample(s) collected in excess of those needed for validation of the cause or mechanism of the kill can always be discarded later**. Preserved (formalin or frozen) voucher specimens of fish submitted for testing should be kept to later confirm data if required.

General Observations

After appropriate field tests and samples have been taken, general observations and photographs and/or video of the fish kill should be made. As quickly as possible, establish the area of the kill. Estimate the number, size and species of fish involved. If fish are still dying, observe their behavior; whether they are listless, frantic, spiraling, suffering from a loss of equilibrium, etc. Examine the fish externally for gross abnormalities such as open lesions, hemorrhaging, etc. **(see Appendix D)**. Collect a few live fish and keep on ice **(see Pathology 8-6)**. A macroinvertebrate benthic survey should be conducted in nontidal streams or rivers to provide important clues as to the cause of the kill and to document the total impact of the pollution event (see Benthic Survey 8-3). Algal observations should also be made. Dense algal blooms may create a low dissolved oxygen condition in the water column during night time algal respiration and indirectly cause a fish kill. In addition, certain algae produce toxins that can directly cause a fish kill **(see Algae 8-9)**. General observations should be made as quickly as possible. Do not try to get specific details at this time. If additional help is needed for further investigation, request it at this time.

Reference or Control Station

Establishment of a reference station is necessary for comparison of the field test results, field samples, and observations of aquatic life. If the limits of the kill can be found, then a reference or control station should be selected either upstream from the kill or, if the kill is in a tidal estuary, far enough away from the kill area as not to be affected by tidal influence. If the kill has occurred in the headwaters of a stream, then another, comparable stream may be used as a reference. It would be beneficial to have a control or reference sample for each type of field test and/or sample collected.

Continued investigation (e.g. field testing, sampling, etc.) after initial observations and sampling may be necessary. The investigator should use the initial information as a starting point to begin a more detailed investigation/sample plan.

Press Release

Only after the initial investigation has been completed is there enough information to respond to inquiries by the press or other concerned parties. The size and character of some fish kills necessitates a quick response; however, the release of information will be conducted under the direct responsibility of the Regional Director under the same guidelines as other pollution events.

Counting Dead Fish

The DEQ has been given the responsibility for assessing the damage to aquatic life caused by pollution incidents. The State Water Control Law is very specific for fish kills: the SWCB shall have the duty "To investigate any large-scale killing of fish" (§62.1-44.15 (11) **see Appendix A**). Thus, the counting of fish killed is a responsibility of the DEQ staff. Just counting dead fish, unfortunately, is not enough. Since the costs of fish replacement are assigned by DGIF, the count must contain the necessary information for the DGIF to make assessments as well as for DEQ to determine the total natural resource damage. Species level identification should be obtained for all fish killed except for certain members of Cyprinidae (carps and minnows) (i.e. *Notropis* spp.) and Percidae (perch and darters) which are often similar in appearance. Counts of generalized cyprinids and percids should be conducted for replacement cost analysis. In waters containing endangered species, species level identification should be conducted for all species. Numerous publications exist that can help investigators identify fish to species level in the field. Two publications specific to fishes in Virginia are referenced at the end of this manual (Jenkins and Burkhead, 1993, and McIninch and Garman, 1998). In addition, the Department of Game and Inland Fisheries on-line Wildlife Information Database may be accessed at www.dgif.state.va.us/wildlife/index.cfm to quickly generate a list of fish and other aquatic and semi-aquatic animals including species listed as threatened, endangered, and special concern that are expected to occur within a three mile radius of the fish kill area.

The following procedure explains the methods used and the information required to ensure accurate and responsible fish kill counts.

Counts used in fish kill reports can come from several sources. Most counts will be done by the DEQ staff, but count information also has been obtained in the past from the DGIF, the Virginia Institute of Marine Science (VIMS), and the Virginia Marine Resources Commission (VMRC). Every count, no matter who makes it, must be conducted utilizing an acceptable technique (i.e. AFS guidelines). Accepting count information from other sources must be handled carefully as the DEQ will ultimately be responsible for the accuracy of the information reported.

Three primary methods may be employed to conduct fish kill counts: total count, standard procedural count, and estimates.

Total Count

The best method of counting fish is a total count of every fish, starting downstream and working upstream. The total count is the most indisputable method available, since it is an exact count of every fish the investigator saw.
The total count method is preferred and should be done whenever possible. However, in many cases, a kill will be of such magnitude that a total count will be impossible.

Standard Procedural Count

Lacking a total count, a standard procedural count involving the counting of fish within sampling areas and extrapolating the total number of fish killed, is the next best method. The DEQ utilizes the AFS "Investigation and Valuation of Fish Kills, Special Publication No. 24", 1992 and the "Sourcebook for Investigation and Valuation of Fish Kills", Supplement to Special Publication No. 24, 1993) as the official methodology for standard procedural counts **(see Appendix E)**. To conduct a standard procedural count, the furthest upstream and downstream locations that dead fish are found are first determined. Then the investigator delineates sample segments at regular intervals between the upstream and downstream locations on a map.

For most fish kills, a count of dead fish in one 100 yard segment per 1/2 mile of stream is acceptable. This type of count is commonly used by the staff and meets the minimum evidentiary requirements for legal action. However, the larger the percentage of affected stream counted, the more accurate the computed estimate will be. Therefore, the investigator may decide to count more than one 100 yard segment per 1/2 mile. Within each 1/2 mile section, the investigator must choose a representative area to count. Often access is limited but, if possible, randomly select a 100 yard reach of stream that is representative of the characteristics of the stream. The investigator must be careful not to bias the count, particularly if dams or other obstructions which accumulate fish are present. Keep in mind that the segment count is a sample and should be a good representative of the whole, not an exception. For example, if one encounters a dam or obstacle where many fish are trapped, the downstream count will be greatly influenced by the obstruction.

The distance between sample segments may have to be adjusted to accommodate the total length of the kill and the investigative resources available. Ascertaining the length of the kill is important before starting the count so the proper distance between segments can be determined. As a rule of thumb (it will vary depending on stream depth and accessibility), a two person counting team can count about three or four 100 yard segments in one day. Since it is desirable to make the count within a single day to avoid duplicate counts of drifting fish, the investigator must judge his or her segment length and distance carefully. For example, if only two people are available for counting dead fish and the fish kill is determined to be 10 river miles long, it will take the crew five to ten days to count 100 yard segments, spaced 1/2 mile apart as recommended by protocol. In this case, the investigator could call for assistance from other regional personnel; if additional assistance is not available, an alternative would be to make the segments about 2 or 2 1/2 miles apart. For large fish kills, as many as six to twelve persons may be involved in counts if the 1/2 mile segment guideline is followed.

Final fish counts for the stream segments should be recorded on the "Fish Kill Count Form" **(see Appendix F)**.

The number of fish killed for each species size class is calculated using either the mean number of fish counted per segment or an expansion factor for the total number of fish counted in the sample segments.

Example:
In a 2 mile long fish kill, 240 2-inch bluegills were counted in 4 segments of 100 yards, spaced 1/2 mile a part.

(a) Mean number of fish counted per segment:

mean number = 240 fish / 4 segments = 60 fish per segment

In 2 miles, there are 35.2 segments each 100 yards long, so

60 fish per segment x 35.2 segments = 2,112 2-inch bluegills killed;

2100 2-inch bluegills killed; estimate based on appropriate significant digits (AFS, 1992).

(b) Expansion factor:

$$\text{expansion factor} = \frac{1{,}760 \text{ yards per mile} \times 2.0 \text{ miles}}{4 \text{ segments} \times 100 \text{ Yards per segment}} = 8.80$$

240 fish x 8.80 = 2,112 Fish:

2100 2-inch bluegills killed; estimate based on appropriate significant digits (AFS, 1992).

Procedural counts have been modified for fish kills in narrow completely and incompletely accessible streams, narrow streams with drifting fish, wide streams, large meandering streams, lakes, and multiple day counts. Detailed procedures and counting examples are presented in **Appendix E**.

Estimate

If neither a total count nor a procedural count can be made, then an estimate should be made. Estimates have mostly been used for large menhaden kills, where a million fish may be a foot deep in a small channel. Estimates provide an idea of the magnitude of a fish kill.

There are several more important points to remember. The investigator should look closely at the fish species involved in the kill even if no replacement cost is to be assigned.

Primarily, one should remember that threatened or endangered species or rare endemic populations may be severely impacted. In addition, other aquatic or semi-aquatic animals may have been killed. Impacts to benthic invertebrates, amphibians, reptiles, birds, and mammals should be documented and the total environmental impact of the pollution incident should be reported.

It should be noted that counting procedures typically underestimate the number of fish killed for a variety of reasons. For example, fish may not be counted that are too deep or too small to be seen, or they may have been scavenged by predators. In addition, time is against investigators of fish kill events. For example, Hayne et. al. reported that estimates for the number of fish killed decrease by approximately 50% after 24 Hours of the fish kill (Nielson and Johnson, 1983). The underestimation may be reduced by more complex and more costly counting techniques, however, most state agencies reason that a rapid defensible estimate better serves the needs of the public than a more complete and costly study (AFS, 1993).

COSTS

Fish Cost Analysis

It is DEQ policy to have the DGIF determine the replacement costs of the fish that are killed. In order for the fish to be assigned a cost by DGIF, the fish must be correctly measured and identified, due to the fact that restocking values differ with the size and species of fish involved. Determining which information to obtain is facilitated by the publication "Investigation and Valuation of Fish Kills" (American Fisheries Society, 1992; **see Appendix E**). This publication represents the results of the AFS Pollution Committee work on the value of fish species and the cost of their replacement. These costs are now accepted by the Southeastern Division of AFS. Familiarization with this document will assist the investigator in determining what information to gather on fish counted in the field. In cases where only weight is acceptable, a representative collection of species sizes must be made. This collection is weighed in the field using accurate scales and the values expanded for the total number of fish killed. Fish that cannot be identified must be collected and identified later. Remember to record the number of the unknown fish at each segment and their size. In order for the DGIF to assign costs, information is recorded on a "Replacement Cost of Fish" form (**see Appendix G**). This form is officially transmitted to DGIF via the Regional Director. The form is dated and the fish kill number (IR #) is listed. To assist in the evaluation of replacement costs, the body of water and location of the kill is also noted. The most important part of the form is the itemized listing of fish killed. Each species is listed separately by size and number counted. Continuation sheets may be used if needed. The form is filled out by the investigator and sent directly to the Director of the Fisheries Division at DGIF via the Regional Director. DGIF assigns the costs, signs and returns the original copy to the regional investigator. The original form becomes part of the case file for each fish kill investigation.

Cost of Investigation

By law the DEQ may recover the costs incurred as a result of a fish kill investigation, as well as any cost incurred by the DGIF in investigating the kill **(Appendix A)**. Hence, investigators should keep records of investigative expenses as a basis for cost recovery. The Pollution Complaint Investigation Total Cost Summary Form **(see Appendix H)** is designed to document investigation costs. The form is used to record the actual costs incurred per individual during the investigation and the costs of any follow-up investigation, report writing, etc.. The form has several major parts. The first part deals with hourly labor costs. The grade is the grade the employee held at the time of the investigation. The grade standard hourly rate is multiplied times the number of hours spent on the investigation to arrive at a total labor cost. The next part of the form deals with vehicle mileage or other transportation costs and is self explanatory. The following part deals with food and lodging. These expenses are reported exactly as claimed for reimbursement on travel vouchers. Lastly, under materials and equipment, items may be included such as costs of ice, tolls, film, sample jars, laboratory costs (obtained from the catalog of laboratory services if conducted by DCLS), etc. The expenses are totaled and the form is signed and dated by the staff involved in the investigation. Disk one at the back of this manual includes an Excel program file (FISHKIL1.XLS) expense form that automatically calculates pay grade rates and totals all expenses. If DGIF personnel assist with an investigation they should also complete an expense form. The original form is submitted to the regional enforcement staff with the final report. The last part of the form is the sum of all costs for each investigator and materials and equipment expenses. If a responsible party is identified, this is the amount that will be requested for reimbursement.

CAUSES OF FISH KILLS

Fish kills may have many different causes which can be grouped under seven general categories:

1. Industrial Operations
2. Municipal Operations (domestic sewage systems)
3. Agriculture and related activities
4. Construction/other causes
5. Transportation operations/storage
6. Natural causes
7. Fish dumping from commercial operations

Industrial Operations

Fish kills can occur as a result of industrial operations. Once the outfall of a suspected industrial waste discharge has been located, an attempt should be made to identify the owner of the facility from which the outfall originates and encourage the owner or operator to halt the suspected toxic discharge. A sample of the discharge should be collected as soon as possible, preferably at the location where the waste leaves the facility property. If an in-plant inspection is warranted, contact the plant manager or person in charge and request a brief tour of the facility. If denied entry, contact Regional Office management or the Central Office of Enforcement. During a tour; the investigator can obtain general information concerning the products manufactured; raw materials used in the manufacturing process; quantities, sources, and characteristics of wastes generated; and waste treatment units if any. The plant manager may be able to supply a flow diagram of plant operations. The investigator should also request specific information concerning facility operations (i.e., accidental spills, etc.) immediately prior to the beginning of the fish kill.

Municipal Operations

Waste discharges from municipal or domestic sewage treatment plants may contain domestic sewage or industrial wastes combined with domestic sewage. These wastes may have been partially treated at the treatment plant or discharged untreated directly into a stream. Since the municipality or owner or operator of the sewage system is generally held responsible for any discharge from such a system, the owner/operator or their representative (i.e. city engineer, public works supervisor, a subdivision developer ,etc.) should be contacted when the samples of the suspect wastewater discharge are collected. The investigator should obtain information about plant operations. If the cause of the fish kill is determined to be the result of industrial waste discharging to a municipal treatment facility and then to a stream, data about the industry and its discharge should be obtained from municipal officials.

Agriculture and Related Activities

Fish kills can occur as a result of pollution from agricultural practices such as crop dusting, fertilizer application, and manure or other organic material discharges to a stream. Fish kills resulting from these agricultural operations are usually associated with runoff due to rainfall. The source or type of pollution may be difficult to identify and may involve a large nonpoint source area. Talking to local residents may help pinpoint the problem area. Runoff from fields, drainage ditches, and small streams leading to the kill area may provide good sampling sites to trace the cause. Noting changes in turbidity in the stream may help to locate possible sources of runoff. Analyses of sediment samples are usually more reliable than water samples where pesticides or herbicides are suspected as a cause of the kill.

Construction/Other Causes

Fish kills may result from mining activities as well as from such temporary or intermittent activities such as; mosquito spraying; construction activities involving chemicals, concrete, and oils; and weed spraying with herbicides or other toxic substances. As with agricultural activities, tracing the cause of these kills is difficult and may require extensive investigation.

Transportation Operations

Fish kills occurring as a result of transportation accidents/incidents are usually readily identified. The investigation should include specifics of the accident such as vehicle license number, vehicle owners, cargo type, DOT placard number, etc..

The **DEQ does not expect employees to risk their personal safety in responding to chemical/hazardous spills or any pollution complaint.** In instances where hazardous chemicals, explosives, and flammable materials are involved, (particularly if one is not familiar with the material), **stand clear** and **contact the DES for help**.

Natural Causes

There are several possible natural causes for fish kills:

1. Oxygen depletion due to ice and snow cover on surface waters.
2. Oxygen depletion at night because of plant respiration or at anytime during the day because of naturally occurring organic compounds in the water.
3. Abrupt temperature changes.
4. Epidemic and endemic diseases, parasites, and other naturally occurring biological causes.
5. Lake water inversion during vernal or autumnal turnover which results in toxic material or anoxic water being brought to the surface.
6. Poor lake management resulting in overcrowding or introduction of the wrong species.
7. Fish spawning stress.
8. *Pfiesteria piscicida* and *Pfiesteria* like microbes often called *Pfiesteria* complex are dinoflagellates that have been implicated in recent fish kills in coastal waters in North Carolina and in the Pocomoke River near the Virginia Maryland border on the Eastern Shore. The DEQ has developed response and safety plans to deal with *Pfiesteria* related fish kills. **See Appendix M** for a copy of the DEQ plans and for information regarding *Pfiesteria.* Up to date information regarding *Pfiesteria* fish kills may be found at the Virginia Institute of Marine Science Web site (www.vims.edu).

There are few truly "natural" kills. Almost all kills, including disease outbreaks, occur due to external stresses. It is just as important, therefore, to identify these environmental stresses as it is to identify the disease causing organism(s).

Fish Dumping from Commercial Operations

This problem occurs predominantly in the piedmont and tidewater regions but can occur anywhere there are commercial fishing operations. Most reported kills result from the emptying of nets or wash-down operations. In addition, commercial nets that break may release large numbers of dead or dying fish. Commercial fisherman in Virginia coastal waters are required to report such incidents to the VMRC.

ADDITIONAL METHODS TO ASSESS ENVIRONMENTAL IMPACTS

The following are additional methods of obtaining information that may assist in further enhancing or defining the cause(s) of fish kills and assessing environmental impacts. Their use is not mandatory, but one or more may be employed at the investigator's discretion.

Water Samples

Substances in solution or suspension largely determine the quality of water. Fish are affected both directly and indirectly by these substances. The addition of dissolved or suspended material to water or the altering of amounts of substances naturally found in the environment can be harmful to fish and thereby cause a fish kill. The analysis of water by collecting water samples is a standard procedure for fish kills. The actual collection is, therefore, of considerable importance. The fish kill investigator should use appropriate containers that have been properly cleaned. Otherwise, the chemical data received will be invalid.

Water samples should be collected in flowing water where the water is well mixed. Place the mouth of the container a few inches below the water surface. This is done to avoid collecting floating material (except where the material is the suspected pollutant). Air should be excluded from the container when samples are to be analyzed for dissolved oxygen, BOD, pH, acidity, alkalinity, chlorine, volatile organics, sulfur dioxide, and hydrogen sulfide. Table 3 in Section 2.0 of the Water Quality Assessment Operating Procedures Manual (the WQAOP manual) lists analytical parameters, recommended containers, preservation and holding times as mandated by EPA (40 CFR Part 136). The volume of sample required by the DCLS to analyze the requested parameters is also listed in Table 3. Specific preservation procedures for the most commonly sampled parameters are described in Section 2.0 of the WQAOP manual.

Also, be sure to identify and handle all samples properly as described in Section 5.0 of the WQAOP manual and DEQ Guidance Memo 00-2016 **(see Appendix L)**. Be certain that all sample tags and lab sheets are filled out completely. DCLS will reject and may discard any samples with improper documentation.

Knowing what parameters to sample for may be the most difficult part of collecting water samples. If the fish kill occurs below a sewage treatment plant, the investigator should collect samples for BOD, COD, nutrients, metals, organic priority pollutants and pesticides analysis if the area is industrialized. If a fish kill occurs below certain industrial discharges the nature of the industrial activity should determine the type of sample collection needed. For example, below a pulp or paper mill, investigators may collect samples for BOD, COD, pH, and phenols for analysis. In agricultural areas the investigator may collect samples for pesticides, metals, and nutrients from fertilizers for analysis.

Sediment Samples

In some cases, the collection of water samples is not enough. The investigator may have arrived after the toxic discharge has stopped, the pollutant may have passed downstream or it may have become diluted beyond recognition in the receiving water. This presents the investigator with a formidable problem. He or she must not only attempt to identify a pollutant no longer in the water but also must find a source which may no longer be discharging. It is at this point one may consider collecting sediment samples.

The sediment sample offers the investigator the opportunity to recover pollutant residues, some of which may have settled out or may have become attached to particles of material in the water column which have settled. Obviously, sediment itself has some different physical and chemical characteristics than the overlying water. Analysis of some parameters is not appropriate due to the differing chemistry or physical properties of sediment versus water.

Sediment samples do offer a good chance of identifying metals, pesticides, and some organic materials that may be the cause of the fish kill. The amount of a minimum sample (one pint) is nearly the same for all uses, but the location of the sediment collection may vary. In the case of fish kills, where very recent contamination is involved, one is interested in only a few top millimeters of sediment. A grab or scoop of sediment may dilute the recent surface contaminate beyond recognition. The type of sediment involved is also important and should be specified on the lab sheet. The absorption of some pollutants in silty mud may differ greatly from that of sand. As always, a good control is essential. Analysis of sediment samples is time- consuming and expensive. Such samples should be collected to identify a specific pollutant and the analysis cancelled if found unnecessary to verify the pollutant type. Detailed procedures for sampling sediments may be found in Section 2.0 of the WQAOP manual. Preserve and protect the samples until they are analyzed, and use proper sample chain of custody procedures (see Water Quality Assessment Operating Procedures Manual-legal sampling handling procedures and Guidance Memo No 00-2016 Chain of Custody Policy and Procedures in Appendix L).

Benthic Macroinvertebrate Surveys

The term "Benthic Macroinvertebrate" refers to invertebrate animals such as larval and adult insects, molluscs, aquatic worms, and crustaceans that live on the bottoms of lakes, streams, estuaries and ocean floors. Although susceptibility to toxic compounds varies among the invertebrate groups, a concentration of a toxic substance that will kill fish will also kill benthic organisms. After a compound toxic to benthic organisms enters the water via a spill or a discharge, the number of benthic organisms living on the bottom downstream from the spill or discharge will be reduced, compared to upstream control areas. The boundary between the affected and unaffected areas is usually distinct.

This difference in the concentrations of bottom dwelling organisms above and below a spill or discharge point makes benthic surveys useful in pinpointing the source of a toxic pollutant when its origin is unknown. After or even during a fish kill, by examining the benthic life as you proceed to the head of a kill area, you may discover the sharp boundary between the affected and unaffected zones. This boundary may point to a particular discharge in an area with many discharges close together. It may also be possible to trace the path of the toxicant into tributaries too small to contain permanent fish life, which might have otherwise been overlooked without a cursory benthic inspection. Even when the source of pollution is known, information on how the benthic life was affected still helps build your case against the polluter. The benthic studies utilize the EPA approved Rapid Bioassessment Protocols for Use in Wadable Streams and Rivers (EPA 841-B-99-002) to assess benthic communities. For the most meaningful results, the benthic macroinvertebrate survey should be completed within three weeks. Benthic organisms themselves are slow to recolonize. The differences between the affected and unaffected areas will remain distinct for several weeks, after which time, recolonization by benthic organisms drifting downstream with the current makes the differences continually less apparent. A benthic survey also helps to determine the severity of a fish kill and the total impact on the stream's biota. Since most benthic animals are a source of fish food, a severe reduction in their numbers over a lengthy stretch of stream would inhibit recolonization of the stream by fishes.

Bioassay Toxicity Testing

Bioassay toxicity tests are not recommended to investigate potential toxicants during fish kill investigations. Rather, if the specific chemical can be identified, the manufacturer or the Material Safety Data Sheet (MSDS) should be consulted to determine the toxicity of the chemical. Many chemicals are required to be tested on aquatic organisms before they are marketed and the information is available from one of these two sources. Another option is to search the EPA on-line AQUIRE Database at www.epa.gov/ecotox. This database provides information regarding the toxicity of chemicals to aquatic organisms.

If a toxicity test is to be conducted it must be performed by a contract laboratory (private or university) since the DEQ no longer has the capability to conduct bioassay toxicity testing.

Arranging a Toxicity Test for Fish Kill Investigations

1. Contact a contract laboratory to determine if a test can be performed and establish a contractual agreement in accordance with DEQ contract procedures, and arrange sample delivery. Due to time constraints, a base contract with a bioassay laboratory should be established before a fish kill event.

2. Collect at least 3 gallons of the material to be tested. Immediately store on ice.

3. Include information as to the composition of the sample and an MSDS if available. (This protects the staff from known hazards).

4. Ship the sample overnight or deliver the sample, on ice, to the laboratory selected for testing.

Chemical Analyses of Fish Tissue

Chemical analysis of fish tissue samples may prove to be of value in fish kill investigations. As with many investigative practices, the dead fish may yield information as to the cause of death. It is important to remember that fish tissue analyses are an indirect method of identifying the pollutant involved.

Affected fish must also be carefully chosen. They must be representative of the kill; they should be alive rather than dead. Fresh dead fish may be acceptable, but during the summer months significant deterioration of organs and muscle tissue may occur within hours. Individual fish make better samples than composites, since composites tend to average concentrations of pollutants and make range determinations difficult.

The use and analysis of fish samples is far too complex to cover fully here. The "DEQ Quality Assurance/Quality Control Project Plan for the Fish Tissue and Sediment Monitoring Program" gives a breakdown of fish handling procedures **(see Appendix I)**. Further handling and analysis is best determined on an individual basis.

Shellfish

Shellfish, when present, offer excellent opportunities for analysis of many metals, pesticides, and organic pollutants. Being filter feeders and unable to move about, oysters, clams, and even freshwater mussels tend to accumulate and concentrate pollutants in their tissues. Sample variation is decreased if animals of the same size are taken from a single location.

Handling of shellfish samples is relatively easy once the animal is obtained from the bottom. Since oysters and clams can live for days out of water, simply keep them cool and bring them in for shipment to the laboratory.

Control or reference samples are often a serious problem. Since collection is normally done in tidal areas, one must collect control samples a considerable distance from the affected area to avoid contamination. Unfortunately, shellfish may be of different sizes or not present at all if salinity is different in the control area.

For specific instructions on the preparation of shellfish samples **see Appendix I**.

Pathology

The causes behind fish disease may be divided into three categories:

1) Pathogenic organisms (bacterial, viral, fungal, or protozoan),

2) Toxins or poisons,

3) Physical stress or changes in water quality

Populations of fish may be affected by one or a combination of the above factors with a resulting fish kill. Investigation of fish kills and the diagnosis of diseases should be a step-by-step procedure. It should be the goal of the investigator to gather as much information as possible at a kill site so that the agent(s) behind the disease may be accurately determined.

Consult the "Fish Kill Pathological Examination Report" form for the water quality data required to perform the pathological examination **(see Appendix J)**.

Notations on conditions at a kill site and the affected species may often be as helpful to the diagnostician as samples sent to the lab. Investigators should pay close attention to the behavior of ailing fish and accurately record any abnormalities. Attention should be given to the species, size, appearance, and distribution of affected fish on-site. Thoughtful estimations of the numbers of sick or dying fish as well as the size of the water body are also helpful. Estimating the size of a kill can often be accomplished by counting the number of dead or dying fish per unit length of shoreline or surface area of water. Finally, measurement of water quality parameters (pH, DO, temperature, conductivity, etc.) may help in diagnosis.

Careful consideration should be given to the selection of diagnostic specimens to be sent in for analyses. Affected fish that are near death or freshly dead should be used whenever possible.

Poor or grossly decomposed samples not only provide little information, they may also act to confuse or mislead the examiner. Any specimens that were dead upon collection should be clearly noted for the diagnostician and they should be packaged separate from those collected alive, to prevent contamination. Samples for analysis should be collected as gently as possible, clearly labeled, and shipped or transferred for examination as soon as possible.

Fish for pathological examination are normally shipped to one of the following laboratories;

1) Leetown West Virginia Science Center, Contact Dr. Vicki Blazer (304) 725-8461 Ext. 434, Biological Resource Division, USGS - Eastern Region , 1700 Leetown Road, Kearneysville, West Virginia 25430.

2) The College of William & Mary, Virginia Institute of Marine Sciences, Contact Dr. Wolfgang Volgelbein, (804) 684-7261, e-mail: wolf@vims.edu, Chesapeake Bay Hall Room N107, N125, Gloucester Point, Va 23062

3) Virginia Tech, Contact Dr. Stephen Smith (540) 231-5131, e-mail: stsmith7@vt.edu, Biomed. Sciences & Pathology Phase II, Room 121, College of Veterinary Medicine, Blacksburg, Va 24061

The WQS&BP office should be contacted prior to shipping to coordinate the shipment of fish collected for pathological examination. Specimens that cannot be examined immediately must be preserved for future investigation. The majority of fish pathogens (especially ectoparasites) will quickly drop off or perish during the decay of host tissues. The investigator who selects diagnostic specimens must first make an assumption of the general cause of the disease in order to use the most suitable method of preservation. Common methods of preservation and probable uses are listed below.

CHILLING is generally used to preserve samples where the freezing or chemical fixation of host tissues would prevent the isolation or identification of disease agents. Whole fish or fish parts may be preserved in this manner if the time until examination is measured in hours. Bacteria, fungi, protozoans, as well as histological and blood samples are generally preserved by this method. Diseases in which the appearance of the fish tissues is important in diagnosis should also be preserved by this process. The best procedure for chilling is to place the specimen in a waterproof container and transfer on wet ice or under refrigeration.

FREEZING is used in those cases where living organisms are thought to be present in the specimen and the destruction of tissue cells is not important. Tissues thought to be harboring larger parasites may be preserved in this manner. Tissue samples to be analyzed for the presence of chemicals or toxins should also be preserved by freezing. Specimens should be frozen as soon as possible to avoid overgrowth of contaminating organisms. The samples should be placed in a waterproof container on dry ice or in a mechanical freezer.

All samples should remain frozen until they are ready for examination.

CHEMICAL preservatives are generally used when the viability of organisms is not necessary and contamination of tissues by fixatives does not hinder examination. Tissue samples and large animal parasites may be preserved in this manner. Specimens selected for chemical preservation should be small enough to allow penetration of the preservative within a short time. Fishes up to five cm can be placed directly into the preservative while larger specimens should be opened along the abdomen before being placed in the chemical. Common chemical preservatives include a 70% isopropyl alcohol or a 10% solution of formalin.

Fish samples are collected for pathological examination whenever the investigator feels that the kill is the result of pathogenic organisms or under conditions where unusual symptoms are noted. For the purposes of pathological analysis, fish kills may be divided into two groups, those kills which are the result of pathogenic organisms (which may be viral, bacterial, fungal, or protozoan) and those that are the result of the introduction of a toxic material or materials.

In the first group, the goal of the investigator is to determine the specific pathogen involved so that a more concise analysis of the cause of the kill is possible and, in some instances, so that appropriate remedial action may be taken. Important field observations would be species involved; size class, behavior, and appearance of affected fish; distribution of affected fish; and the normal water quality parameters of DO, pH, and temperature.

In the second group, fish samples are usually taken with an eye to toxicity testing. For example, a fish kill occurs and a given toxicant is suspected but the literature fails to adequately support this hypothesis. Pathological examinations are performed on the affected fish and abnormalities are noted. Later, a toxicity test is conducted using similar circumstances and the fish from this test also undergo pathological examination. If the abnormalities noted on the kill fish correspond to those noted on the test fish then the investigator has an expanded data base to support the original hypothesis. It is important that control fish also be examined so that any abnormalities noted can be directly attributed to the toxicant and not to factors such as other toxicants, spawning stress, seasonal variation, and the like.

It is important that the investigator understand that fish samples submitted for pathological examination (concerning toxicant caused fish kills) in no way replace fish tissue, sediment or water samples collected for chemical analyses.

Algae

Under certain circumstances and in particular periods of high algal productivity, algae or their toxic products may cause illness or death in man, fish, and other animals. There are two basic groups of algae responsible for fish kills. These are the bluegreen algae and the armored dinoflagellates. Although both groups occur in both fresh and saltwater, the bluegreen algae are known to cause toxicity problems in freshwater. In marine waters the predominant organisms causing toxicity are the dinoflagellates (see *Pfiesteria* page 7-3 and Appendix M) but other groups have also been implicated. Problems with dinoflagellates usually occur south of Virginia.

Instances of acute and often fatal poisoning of aquatic animals, farm animals, and birds are numerous, especially if the animals drink water containing the greatest concentration of plankton during an algal bloom. Most of the outbreaks of algal poisoning have occurred during periods of continuous hot weather, when the water had a high organic content, and when the floating algae were concentrated in the water body by wind or current. Algae samples should be taken if such conditions precede or occur during a fish kill.

Algae samples should be preserved (with copper sulfate and formalin or Lugol's solution) and, if possible, non-preserved samples should be collected in quart containers. The non-preserved samples should be stored and shipped at approximately 4°C. (see DEQ Sampling Techniques for Chlorophyll and Algae, Appendix K). It is acceptable to arrange for other state institutions to perform algal taxonomic identifications. Recently, Dr. Harold Marshall (phone 757 683-4204, e-mail hmarshal@odu.edu) at ODU has performed many algae identifications for the Tidewater and Piedmont regional offices. If an outside source is used, a contract and purchase order may be required.

Special Studies/Surveys

Follow-up surveys can be conducted if needed. Investigators may perform special studies/surveys to determine if the kill area is still receiving pollutants or whether the problem has been corrected. The survey can aid in determining the source of a pollutant and will also provide data on the effectiveness of a facility's waste treatment process. A macroinvertebrate benthic survey can be conducted by the regional biologist on the stream, lake, or estuary involved. The benthic survey can ascertain the effect of the discharge on the body of water which receives the wastes. The survey may also judge the severity of the effect of the discharge and the extent of the area affected. Some kills may involve only a few dead fish because the stream supported only a small number of fish, yet a benthic survey may reveal that the stream has been impacted for miles.

DATA INTERPRETATION AND REPORT PREPARATION

After all the field work and additional research has been completed, the chemical and biological laboratory samples have been analyzed, and the results returned to the investigator, the data are ready to be organized and interpreted into a final written report. The investigator should consider all phases of the investigation when determining the cause of the kill. The data should be arranged in a logical sequence to make interpretation as easy as possible.

The first step is to look at the field test results. These results should be compared to the DEQ Water Quality Standards (9 VAC 25-260-20.A) and any standards violation noted. Literature research is then necessary to determine if the violation is extreme enough to cause a fish kill. The WQS&BP can be contacted for assistance with the literature search. In addition, the internet may also be used to conduct quick information searches.

The investigator should then check the field observations. Does the fish behavior confirm the water quality violation demonstrated by the field tests as the cause of the kill or is another cause suspected? The investigator should check the pathologist's report for the possibility of a disease causing the kill. If this proves to be the case, the cause for the pathogen outbreak should be investigated further to determine the extent of water quality degradation.

The next step is to review the chemical and biological analysis results. Sample results in the affected area are compared to the control stations. Chemical data which appear abnormal are compared to data found in the literature. This is where the investigator may have to rely on toxicity tests for a source of comparison to his or her chemical data. Most literature values are specific to certain aquatic life and water conditions. Therefore, the need may arise for duplication of the specific conditions present at the time of the kill. Standards violations are fairly straightforward and require little additional work. The biological data can augment the chemical data and in some instances indicate the cause of the kill. Macroinvertebrate benthic analyses can be very useful for determining the extent of damage. The algal data are important because if the species present is identified as a producer of toxin, the algae could have directly caused the kill.

All of the above are not necessary to prove the cause of every kill. Important points in substantiating a kill are locating the source, the responsible party, and the causative agent.

As long as these three steps are covered, the report is complete. It should be remembered that any fish kill may require legal action to collect penalties, recover replacement costs of the dead fish and investigation costs, as well as to secure injunctive relief to prevent future kills. Keeping the legal possibilities in mind, the investigation should include as many of the steps above necessary to support a legal case.

Remember: The most serious error an investigator can make is to assume that conditions obvious to him in the field can be easily demonstrated in a final report. Data, sample analyses, photos, detailed notes in a waterproof field log book, other evidence, etc., must be able to convince someone with less knowledge of the subject than the investigator.

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APPENDIX A

STATE WATER CONTROL LAW §62.1-44.15

§ 62.1-44.15. Powers and duties.

It shall be the duty of the Board and it shall have the authority:

(1) [Repealed.]

(2) To study and investigate all problems concerned with the quality of state waters and to make reports and recommendations.

(2a) To study and investigate methods, procedures, devices, appliances, and technologies which could assist in water conservation or water consumption reduction.

(2b) To coordinate its efforts toward water conservation with other persons or groups, within or without the Commonwealth.

(2c) To make reports concerning, and formulate recommendations based upon, any such water conservation studies to ensure that present and future water needs of the citizens of the Commonwealth are met.

(3a) To establish such standards of quality and policies for any state waters consistent with the general policy set forth in this chapter, and to modify, amend or cancel any such standards or policies established and to take all appropriate steps to prevent quality alteration contrary to the public interest or to standards or policies thus established, except that a description of provisions of any proposed standard or policy adopted by regulation which are more restrictive than applicable federal requirements, together with the reason why the more restrictive provisions are needed, shall be provided to the standing committee of each house of the General Assembly to which matters relating to the content of the standard or policy are most properly referable. The Board shall, from time to time, but at least once every three years, hold public hearings pursuant to subsection B of § 2.2-4007 but, upon the request of an affected person or upon its own motion, hold hearings pursuant to § 2.2-4009, for the purpose of reviewing the standards of quality, and, as appropriate, adopting, modifying, or canceling such standards. Whenever the Board considers the adoption, modification, amendment or cancellation of any standard, it shall give due consideration to, among other factors, the economic and social costs and benefits which can reasonably be expected to obtain as a consequence of the standards as adopted, modified, amended or cancelled. The Board shall also give due consideration to the public health standards issued by the Virginia Department of Health with respect to issues of public health policy and protection. If the Board does not follow the public health standards of the Virginia Department of Health, the Board's reason for any deviation shall be made in writing and published for any and all concerned parties.

(3b) Except as provided in subdivision (3a), such standards and policies are to be adopted or modified, amended or cancelled in the manner provided by the Administrative Process Act (§ 2.2-4000 et seq.).

(4) To conduct or have conducted scientific experiments, investigations, studies, and research to discover methods for maintaining water quality consistent with the purposes of this chapter. To this end the Board may cooperate with any public or private agency in the conduct of such experiments, investigations and research and may receive in behalf of the Commonwealth any moneys which any such agency may contribute as its share of the cost under any such cooperative agreement. Such moneys shall be used only for the purposes for which they are contributed and any balance remaining after the conclusion of the experiments, investigations, studies, and research, shall be returned to the contributors.

(5) To issue, revoke or amend certificates under prescribed conditions for: (a) the discharge of sewage, industrial wastes and other wastes into or adjacent to state waters; (b) the alteration otherwise of the physical, chemical or biological properties of state waters; (c) excavation in a wetland; or (d) on and after October 1, 2001, the conduct of the following activities in a wetland: (i) new activities to cause draining that significantly alters or degrades existing wetland acreage or functions, (ii) filling or dumping, (iii) permanent flooding or impounding, or (iv) new activities that cause significant alteration or degradation of existing wetland acreage or functions.

(5a) All certificates issued by the Board under this chapter shall have fixed terms. The term of a Virginia Pollution Discharge Elimination System permit shall not exceed five years. The term of a Virginia Water Protection Permit shall be based upon the projected duration of the project, the length of any required monitoring, or other project operations

or permit conditions; however, the term shall not exceed fifteen years. The term of a Virginia Pollution Abatement permit shall not exceed ten years, except that the term of a Virginia Pollution Abatement permit for confined animal feeding operations shall be ten years. The Department of Environmental Quality shall inspect all facilities for which a Virginia Pollution Abatement permit has been issued at least once every five years, except that the Department shall inspect all facilities covered by the Virginia Pollution Abatement permit for confined animal feeding operations annually. Department personnel performing inspections of confined animal feeding operations shall be certified under the voluntary nutrient management training and certification program established in § 10.1-104.2. The term of a certificate issued by the Board shall not be extended by modification beyond the maximum duration and the certificate shall expire at the end of the term unless an application for a new permit has been timely filed as required by the regulations of the Board and the Board is unable, through no fault of the permittee, to issue a new permit before the expiration date of the previous permit.

(5b) Any certificate issued by the Board under this chapter may, after notice and opportunity for a hearing, be amended or revoked on any of the following grounds or for good cause as may be provided by the regulations of the Board:

1. The owner has violated any regulation or order of the Board, any condition of a certificate, any provision of this chapter, or any order of a court, where such violation results in a release of harmful substances into the environment or poses a substantial threat of release of harmful substances into the environment or presents a hazard to human health or the violation is representative of a pattern of serious or repeated violations which, in the opinion of the Board, demonstrates the owner's disregard for or inability to comply with applicable laws, regulations, or requirements;

2. The owner has failed to disclose fully all relevant material facts or has misrepresented a material fact in applying for a certificate, or in any other report or document required under this law or under the regulations of the Board;

3. The activity for which the certificate was issued endangers human health or the environment and can be regulated to acceptable levels by amendment or revocation of the certificate; or

4. There exists a material change in the basis on which the permit was issued that requires either a temporary or a permanent reduction or elimination of any discharge controlled by the certificate necessary to protect human health or the environment.

(6) To make investigations and inspections, to ensure compliance with any certificates, standards, policies, rules, regulations, rulings and special orders which it may adopt, issue or establish and to furnish advice, recommendations, or instructions for the purpose of obtaining such compliance. In recognition of §§ 32.1-164 and 62.1-44.18, the Board and the State Department of Health shall enter into a memorandum of understanding establishing a common format to consolidate and simplify inspections of sewage treatment plants and coordinate the scheduling of the inspections. The new format shall ensure that all sewage treatment plants are inspected at appropriate intervals in order to protect water quality and public health and at the same time avoid any unnecessary administrative burden on those being inspected.

(7) To adopt rules governing the procedure of the Board with respect to: (a) hearings; (b) the filing of reports; (c) the issuance of certificates and special orders; and (d) all other matters relating to procedure; and to amend or cancel any rule adopted. Public notice of every rule adopted under this section shall be by such means as the Board may prescribe.

(8a) To issue special orders to owners (i) who are permitting or causing the pollution, as defined by § 62.1-44.3, of state waters to cease and desist from such pollution, (ii) who have failed to construct facilities in accordance with final approved plans and specifications to construct such facilities in accordance with final approved plans and specifications, (iii) who have violated the terms and provisions of a certificate issued by the Board to comply with such terms and provisions, (iv) who have failed to comply with a directive from the Board to comply with such directive, (v) who have contravened duly adopted and promulgated water quality standards and policies to cease and desist from such contravention and to comply with such water quality standards and policies, (vi) who have violated the terms and provisions of a pretreatment permit issued by the Board or by the owner of a publicly owned treatment works to comply with such terms and provisions or (vii) who have contravened any applicable pretreatment standard

or requirement to comply with such standard or requirement; and also to issue such orders to require any owner to comply with the provisions of this chapter and any decision of the Board.

(8b) Such special orders are to be issued only after a hearing with at least thirty days' notice to the affected owners, of the time, place and purpose thereof, and they shall become effective not less than fifteen days after service as provided in § 62.1-44.12; provided that if the Board finds that any such owner is grossly affecting or presents an imminent and substantial danger to (i) the public health, safety or welfare, or the health of animals, fish or aquatic life; (ii) a public water supply; or (iii) recreational, commercial, industrial, agricultural or other reasonable uses, it may issue, without advance notice or hearing, an emergency special order directing the owner to cease such pollution or discharge immediately, and shall provide an opportunity for a hearing, after reasonable notice as to the time and place thereof to the owner, to affirm, modify, amend or cancel such emergency special order. If an owner who has been issued such a special order or an emergency special order is not complying with the terms thereof, the Board may proceed in accordance with § 62.1-44.23, and where the order is based on a finding of an imminent and substantial danger, the court shall issue an injunction compelling compliance with the emergency special order pending a hearing by the Board. If an emergency special order requires cessation of a discharge, the Board shall provide an opportunity for a hearing within forty-eight hours of the issuance of the injunction.

(8c) The provisions of this section notwithstanding, the Board may proceed directly under § 62.1-44.32 for any past violation or violations of any provision of this chapter or any regulation duly promulgated hereunder.

(8d) With the consent of any owner who has violated or failed, neglected or refused to obey any regulation or order of the Board, any condition of a permit or any provision of this chapter, the Board may provide, in an order issued by the Board against such person, for the payment of civil charges for past violations in specific sums not to exceed the limit specified in § 62.1-44.32 (a). Such civil charges shall be instead of any appropriate civil penalty which could be imposed under § 62.1-44.32 (a) and shall not be subject to the provisions of § 2.2-514. Such civil charges shall be paid into the state treasury and deposited by the State Treasurer into the Virginia Environmental Emergency Response Fund pursuant to Chapter 25 (§ 10.1-2500 et seq.) of Title 10.1, excluding civil charges assessed for violations of Article 9 (§ 62.1-44.34:8 et seq.) or 10 (§ 62.1-44.34:10 et seq.) of Chapter 3.1 of this title, or a regulation, administrative or judicial order, or term or condition of approval relating to or issued under those articles.

The amendments to this section adopted by the 1976 Session of the General Assembly shall not be construed as limiting or expanding any cause of action or any other remedy possessed by the Board prior to the effective date of said amendments.

(9) To make such rulings under §§ 62.1-44.16, 62.1-44.17 and 62.1-44.19 as may be required upon requests or applications to the Board, the owner or owners affected to be notified by certified mail as soon as practicable after the Board makes them and such rulings to become effective upon such notification.

(10) To adopt such regulations as it deems necessary to enforce the general water quality management program of the Board in all or part of the Commonwealth, except that a description of provisions of any proposed regulation which are more restrictive than applicable federal requirements, together with the reason why the more restrictive provisions are needed, shall be provided to the standing committee of each house of the General Assembly to which matters relating to the content of the regulation are most properly referable.

(11) To investigate any large-scale killing of fish.

(a) Whenever the Board shall determine that any owner, whether or not he shall have been issued a certificate for discharge of waste, has discharged sewage, industrial waste, or other waste into state waters in such quantity, concentration or manner that fish are killed as a result thereof, it may effect such settlement with the owner as will cover the costs incurred by the Board and by the Department of Game and Inland Fisheries in investigating such killing of fish, plus the replacement value of the fish destroyed, or as it deems proper, and if no such settlement is reached within a reasonable time, the Board shall authorize its executive secretary to bring a civil action in the name of the Board to recover from the owner such costs and value, plus any court or other legal costs incurred in connection with such action.

(b) If the owner is a political subdivision of the Commonwealth, the action may be brought in any circuit court within

the territory embraced by such political subdivision. If the owner is an establishment, as defined in this chapter, the action shall be brought in the circuit court of the city or the circuit court of the county in which such establishment is located. If the owner is an individual or group of individuals, the action shall be brought in the circuit court of the city or circuit court of the county in which such person or any of them reside.

(c) For the purposes of this subsection the State Water Control Board shall be deemed the owner of the fish killed and the proceedings shall be as though the State Water Control Board were the owner of the fish. The fact that the owner has or held a certificate issued under this chapter shall not be raised as a defense in bar to any such action.

(d) The proceeds of any recovery had under this subsection shall, when received by the Board, be applied, first, to reimburse the Board for any expenses incurred in investigating such killing of fish. The balance shall be paid to the Board of Game and Inland Fisheries to be used for the fisheries' management practices as in its judgment will best restore or replace the fisheries' values lost as a result of such discharge of waste, including, where appropriate, replacement of the fish killed with game fish or other appropriate species. Any such funds received are hereby appropriated for that purpose.

(e) Nothing in this subsection shall be construed in any way to limit or prevent any other action which is now authorized by law by the Board against any owner.

(f) Notwithstanding the foregoing, the provisions of this subsection shall not apply to any owner who adds or applies any chemicals or other substances that are recommended or approved by the State Department of Health to state waters in the course of processing or treating such waters for public water supply purposes, except where negligence is shown.

(12) To administer programs of financial assistance for planning, construction, operation, and maintenance of water quality control facilities for political subdivisions in this Commonwealth.

(13) To establish policies and programs for effective area-wide or basin-wide water quality control and management. The Board may develop comprehensive pollution abatement and water quality control plans on an area-wide or basin-wide basis. In conjunction with this, the Board, when considering proposals for waste treatment facilities, is to consider the feasibility of combined or joint treatment facilities and is to ensure that the approval of waste treatment facilities is in accordance with the water quality management and pollution control plan in the watershed or basin as a whole. In making such determinations, the Board is to seek the advice of local, regional, or state planning authorities.

(14) To establish requirements for the treatment of sewage, industrial wastes and other wastes that are consistent with the purposes of this chapter; however, no treatment shall be less than secondary or its equivalent, unless the owner can demonstrate that a lesser degree of treatment is consistent with the purposes of this chapter.

(15) To promote and establish requirements for the reclamation and reuse of wastewater that are protective of state waters and public health as an alternative to directly discharging pollutants into waters of the state. The requirements shall address various potential categories of reuse and may include general permits and provide for greater flexibility and less stringent requirements commensurate with the quality of the reclaimed water and its intended use. The requirements shall be developed in consultation with the Department of Health and other appropriate state agencies. This authority shall not be construed as conferring upon the Board any power or duty duplicative of those of the State Board of Health.

(16) To establish and implement policies and programs to protect and enhance the Commonwealth's wetland resources. Regulatory programs shall be designed to achieve no net loss of existing wetland acreage and functions. Voluntary and incentive-based programs shall be developed to achieve a net resource gain in acreage and functions of wetlands. The Board shall seek and obtain advice and guidance from the Virginia Institute of Marine Science in implementing these policies and programs.

Code 1950, § 62.1-27; 1968, c. 659; 1970, c. 638; 1972, c. 741; 1975, c. 335; 1976, c. 621; 1977, c. 32; 1978, c. 827; 1984, c. 11; 1985, cc. 249, 397; 1988, cc. 167, 328; 1989, c. 389; 1990, c. 717; 1991, cc. 239, 718; 1993, c. 456; 1994, c. 698; 1998, cc. 805, 863; 2000, cc. 972, 1032, 1054.)

APPENDIX B

DEQ FISH KILL REPORT/NOTIFICATION

VIRGINIA DEPARTMENT OF ENVIRONMENTAL QUALITY
FISH KILL REPORT/NOTIFICATION

Incident #_____ Stream_____Basin_____

City/County_____Region_____

Date Investigated_____Investigator_____

Reported by: Name_____

Address_____

Phone (Home)_____(Work)_____

Reported to: Name_____

Office_____Date_____

****

Date Kill Started_____Date Kill Ended_____Total # Killed_____

Fish Killed (common name)_____

_____

Location of Kill_____

Area Involved_____

Cause of Kill_____

Staff Hours_____ Total Cost of Investigation_____

Investigator's Signature_____Date_____

****

Attachments (Check all included forms. As a minimum Attachments A and B should be included for investigated kills.)

_____ A - Investigator's Expense
_____ B - Map
_____ C - Summary Memo
_____ D - Fish Count
_____ E - Fish Cost
_____ F - Fish Description
_____ G - Lab Analyses
_____ H - Lab Cost
_____ I - Benthic/Algal Results
_____ J - Bioassay Results
_____ K - Bioassay Costs
_____ L - Pathology Results
_____ M - Pathology Costs
_____ N - Total Costs

****

Remarks/Recommendations/Action Taken

Copies: Enforcement (orig.), Regional Office

APPENDIX C

DEPARTMENT OF GAME AND INLAND FISHERIES REGIONAL NOTIFICATION LIST

Dept. of Game & Inland Fisheries, Fisheries Division

P. O. Box 11104
Richmond, Virginia 23230-1104
804/367-1004
Fax: 804/367-2628

FAX TRANSMISSION COVER SHEET

Date *January 30, 2002*

To: *Rick Browder*

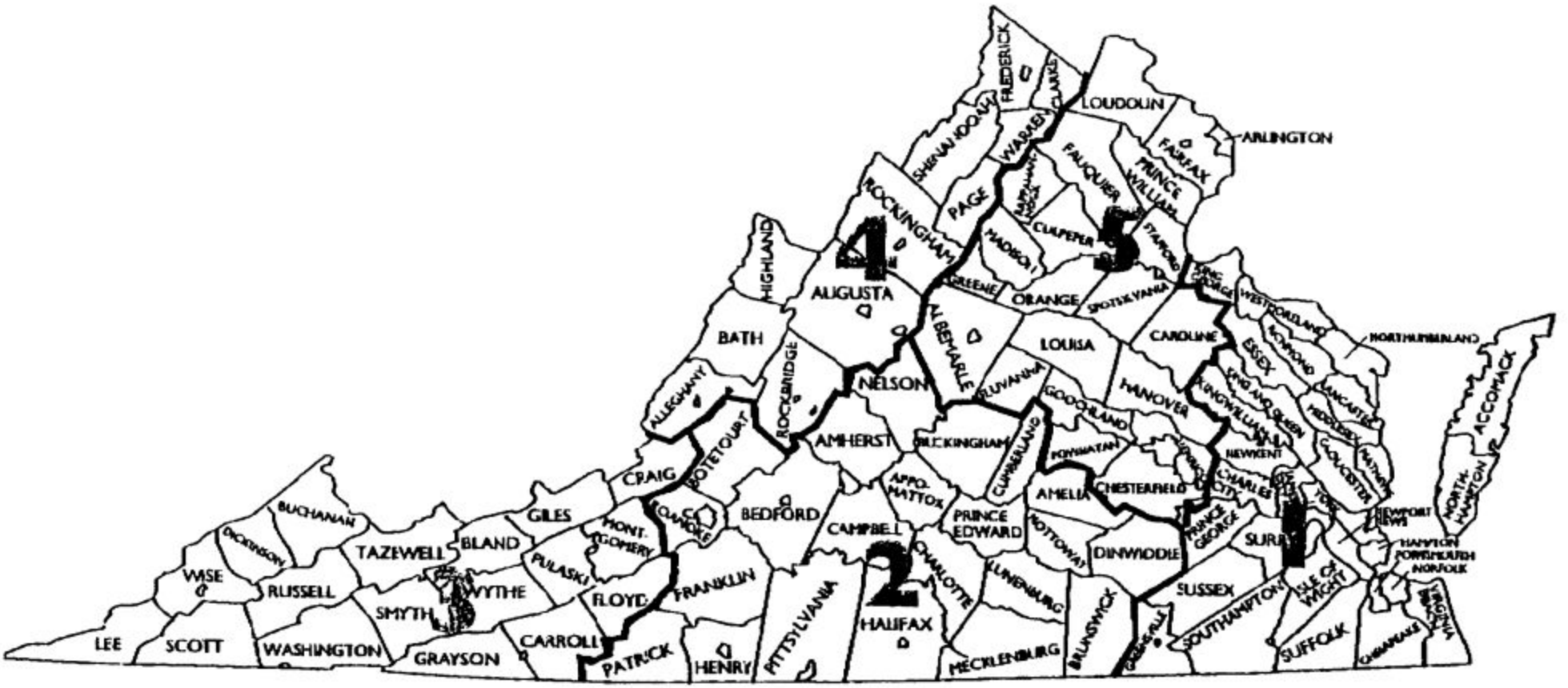
Fax: *698-4522*

Re: *VDGIF, Fisheries Division Biologists List*

Sender: *Gary F. Martel* GFM

YOU SHOULD RECEIVE 7 PAGE(S), INCLUDING THIS COVER SHEET. IF YOU DO NOT RECEIVE ALL THE PAGES, PLEASE CALL 804/367-0509.

Per our discussion, I am attaching a current list of Fisheries Division biologists. If we can be of further assistance, feel free to call.


LEE
SCOTT
WASHINGTON
WISE
RUSSELL
SMYTH
WYTHE
GRAYSON
CARROLL
BUCHANAN
DICKENSON
TAZEWELL
BLAND
GILES
PULASKI
FLOYD
PATRICK
HENRY
MONT-GOMERY
FRANKLIN
CRAIG
BOTETOURT
ROANOKE CO.
ALLEGHANY
BATH
HIGHLAND
ROCKBRIDGE
BEDFORD
PITTSYLVANIA
CAMPBELL
AMHERST
NELSON
AUGUSTA
ROCKINGHAM
SHENANDOAH
PAGE
WARREN
FREDERICK
CLARKE
LOUDOUN
FAUQUIER
CULPEPER
MADISON
GREENE
ORANGE
ALBEMARLE
BUCKINGHAM
APPO-MATTOX
CHARLOTTE
HALIFAX
PRINCE EDWARD
CUMBERLAND
LUNENBURG
MECKLENBURG
BRUNSWICK
AMELIA
NOTTOWAY
DINWIDDIE
GREENSVILLE
LOUISA
FLUVANNA
GOOCHLAND
POWHATAN
CHESTERFIELD
PRINCE GEORGE
SUSSEX
SOUTHAMPTON
SURRY
ISLE OF WIGHT
SUFFOLK
CHESAPEAKE
VIRGINIA BEACH
HANOVER
CAROLINE
KING GEORGE
STAFFORD
PRINCE WILLIAM
FAIRFAX
ARLINGTON
KING AND QUEEN
KING WILLIAM
NEW KENT
CHARLES CITY
JAMES CITY
YORK
WESTMORELAND
NORTHUMBERLAND
RICHMOND
ESSEX
LANCASTER
MIDDLESEX
GLOUCESTER
MATHEWS
ACCOMACK
NORTH-AMPTON
NEWPORT NEWS
HAMPTON
PORTSMOUTH
NORFOLK
1
2
3
4
5

FISHERIES DIVISION

Richmond Office

NAME	JOB TITLE	ADDRESS	OFFICE PHONE	HOME PHONE
Gary F. Martel	Director	4010 W. Broad St. Richmond, VA 23230	804-367-1004	
Samuel S. West	Engineering Technician	4010 W. Broad Street Richmond, VA 23230	804-367-1644	
Frances H. Anderson	Executive Secretary	4010 W. Broad Street Richmond, VA 23230	804-367-0509	
Fred D. Leckie, Jr.	Assistant Director Operations	4010 W. Broad Street Richmond, VA 23230	804-367-8944	
Christine L. Butor	Secretary	4010 W. Broad Street Richmond, VA 23230	804-367-8629	
Ron Southwick	Assistant Director Hatcheries	4010 W. Broad Street Richmond, VA 23230	804-367-1292	
Dean L. Fowler	Outreach Biologist	5806 Mooretown Road Williamsburg, VA 23188	757-253-4180	
Linda S. Stone	Secretary	4010 W. Broad Street Richmond, VA 23230	804-367-1293	
Anne Skalski-Windle	Aquatic Education Coordinator	4010 W. Broad Street Richmond, VA 23230	804-367-6778	
	Sport Fishing Education Specialist	4010 W. Broad Street Richmond, VA 23230	804-367-0141	

Region I

Mitchell D. Norman	Regional Fisheries Manager	500 Hinton Avenue Chesapeake, VA 23323	757-558-4731	

District 1

Work Area: Charles City, Essex, Gloucester, James City, King George, King William, King & Queen, Lancaster, Mathews, Middlesex, New Kent, Northumberland, Richmond, Westmoreland, York, and the cities of Hampton and Newport News.

Robert S. Greenlee	Fisheries Biologist	5806 Mooretown Road Williamsburg, VA 23188	757-253-4170	
Mukhtar A. Farooqi	Fisheries Biologist	5806 Mooretown Road Williamsburg, VA 23188	757-253-4172	
Douglas E. Smith	Fisheries Technician	5806 Mooretown Road Williamsburg, VA 23188	757-253-4172	

District 2

Work Area: Accomack, Greensville, Isle of Wight, Northampton, Prince George, Southampton, Surry, Sussex, and the cities of Chesapeake, Norfolk, Portsmouth, Suffolk, and Virginia Beach.

Christopher C. Long	Fisheries Biologist	500 Hinton Avenue Chesapeake, VA 23323	757/558-4773	

Region II

Arthur L. LaRoche, III	Regional Fisheries Manager	209 E. Cleveland Ave. Vinton, VA 24179	540-857-7704	
Angela Overstreet	Secretary	209 E. Cleveland Ave. Vinton, VA 24179	540-857-7704	

District 1

Work Area: Amelia, Appomattox, Buckingham, Brunswick, Charlotte, Cumberland, Dinwiddie, Halifax, Lunenburg, Mecklenburg, Nottoway, Prince Edward, and the city of Petersburg. (Hopewell)

Daniel P. Michaelson	Fisheries Biologist	H.C. 6, Box 46 Farmville, VA 23901	434-392 9645	
Victor J. DiCenzo	Fisheries Biologist	H.C. 6, Box 46 Farmville, VA 23901	434-392-9645	
Eric M. Brittle	Assistant Fisheries Biologist	H.C. 6, Box 46 Farmville, VA 23901	434-392-9645	

District 2

Work Area: Amherst, Bedford, Botetourt, Campbell, Franklin, Henry, Nelson, Patrick, Pittsylvania, Roanoke, and the cities of Lynchburg and Roanoke.

Daniel M. Wilson	Fisheries Biologist Senior	1132 Thomas Jefferson Road Forest, VA 24551-9223	434-525-7522	
Scott M. Smith	Fisheries Biologist	1132 Thomas Jefferson Road Forest, VA 24551-9223	434-525-7522	

Region III

William B. Kittrell, Jr.	Regional Fisheries Manager	1796 Highway Sixteen Marion, VA 24354	276-783-4860	

District 1

Work Area: Bland, Carroll, Craig, Floyd, Giles, Montgomery, Pulaski, and Wythe.

John C. Copeland	Fisheries Biologist Senior	2206 South Main Street Suite C Blacksburg, VA 24060	540-951-7923	
Joe Williams	Fisheries Biologist	2206 South Main Street Suite C Blacksburg, VA 24060	540-951-7923	

District 2

Work Area: Buchanan, Dickenson, Grayson, Lee, Scott, Russell, Smyth, Tazewell, Washington, and Wise.

Thomas M. Hampton	Fisheries Biologist Senior	1796 Highway Sixteen Marion, VA 24354	276-783-4860	
George C. Palmer	Fisheries Biologist	1796 Highway Sixteen Marion, VA 24354	276-783-4860	
Clifford R. Kirk	Fisheries Technician	1796 Highway Sixteen Marion, VA 24354	276-783-4860	

Region IV

Larry O. Mohn	Regional Fisheries Manager	P. O. Box 996 Verona, VA 24482	540-248-9360	

District 1

Work Area: Alleghany, Augusta, Bath, Highland, Rockbridge, and the cities of Lexington and Waynesboro

Paul E. Bugas, Jr.	Fisheries Biologist Senior	P. O. Box 996 Verona, VA 24482	540-248-9360	
Bradley A. Trumbo	Fisheries Technician	P. O. Box 996 Verona, VA 24482	540-248-9360	

District 2

Work Area: Clarke, Frederick, Page, Rockingham, Shenandoah, and Warren, and the Cities of Harrisonburg and Winchester

Stephen J. Reeser	Fisheries Biologist	P. O. Box 996 Verona, VA 24482	540-248-9360	

Region V

John W. Kauffman	Regional Fisheries Manager	Suite 100 900 Natural Resource Dr. Charlottesville VA 22903	434-296-4731	

District 1

Work Area: Albemarle, Chesterfield, Fluvanna, Goochland, Greene, Hanover, Henrico, Louisa, Madison, Powhatan, Rappahannock, and the cities of Charlottesville and Richmond.

Price P. Smith	Fisheries Biologist Senior	12104 Washington Highway Ashland, Va. 23005	804-752-5503	
Thomas P. Gunter, Jr.	Fisheries Biologist	12104 Washington Highway Ashland, Va. 23005	804-752-5503	
Alan Weaver	Fish Passage Coordinator	12104 Washington Highway Ashland, Va. 23005	804-752-5504	

District 2

Work Area: Arlington, Caroline, Culpeper, Fairfax, Fauquier, Loudon, Orange, Prince William, Spotsylvania, and Stafford.

Stephen J. Owens	Fisheries Biologist Senior	1320 Belman Road Fredericksburg VA 22401	540-899-4179	
John S. Odenkirk	Fisheries Biologist	1320 Belman Road Fredericksburg VA 22401	540-899-4179	
Scott W. Herrmann	fisheries Technician	1320 Belman Road Fredericksburg VA 22401	540-899-4179	

APPENDIX D

CLINICAL SIGNS

CLINICAL SIGNS

BEHAVIOR
- ☐ Gasping
- ☐ Flashing
- ☐ Lethargic
- ☐ Fin Twitching
- ☐ Convulsions
- ☐ In Shallow Water
- ☐ Around Inflow
- ☐ Around Drain
- ☐ Around Aeration
- ☐ Head up - tail down
- ☐ Head-tail whirling
- ☐ Long. axis whirling
- ☐ Pect. fins folded forward
- ☐ Anorexia
- ☐ Belly-up
- ☐ Loss of Balance
- ☐ Other

PHYSICAL – External Rate
- ☐ Normal
- ☐ Emaciated
- ☐ Depigmented
- ☐ Hyperpigmented
- ☐ Exophthalmia
- ☐ Endophthalmia
- ☐ Swollen Belly
- ☐ Scoliosis & Lordosis

FINS – Eroded
- ☐ Dorsal
- ☐ Pectoral
- ☐ Pelvic
- ☐ Anal
- ☐ Adipose
- ☐ Caudal

LESION – Shape
- ☐ Irregular
- ☐ Regular

LESION – Appearance
- ☐ Clean
- ☐ Dirty
- ☐ Yellow
- ☐ Red
- ☐ White

LESION – Location
- ☐ Fins
- ☐ Head
- ☐ Cranial Foramen
- ☐ Eyes
- ☐ Mouth
- ☐ Peduncle
- ☐ Ventral
- ☐ Dorsal
- ☐ Lateral

LESION – Size
- ☐ 1-5 mm
- ☐ 5-10 mm
- ☐ 1.25 cm
- ☐ 1-2.5 cm
- ☐ >2.5 cm

Histology Samples Taken ☐ _____

Tissues Taken _____

_____

HEMORRHAGES Rate
- ☐ Fins
- ☐ Head
- ☐ Mouth
- ☐ Eyes
- ☐ Peduncle
- ☐ Ventral
- ☐ Dorsal
- ☐ Lateral
- ☐ Vent
- ☐ Cranial Foramen

HEMORRHAGES – Size
- ☐ Petchiae
- ☐ Eccymoses
- ☐ Suffusion

ULCER – Location
- ☐ Fins
- ☐ Head
- ☐ Eyes
- ☐ Mouth
- ☐ Peduncle
- ☐ Ventral
- ☐ Dorsal
- ☐ Lateral

ULCER – Size
- ☐ 1-5 mm
- ☐ 5-10 mm
- ☐ 1.25 cm
- ☐ 1-2.25 cm
- ☐ >2.5 cm

ULCER – Shape
- ☐ Irregular
- ☐ Regular

ULCER – Appearance
- ☐ Clean
- ☐ Dirty
- ☐ Yellow
- ☐ Red

GILLS
- ☐ Normal
- ☐ Pale
- ☐ Brown
- ☐ Cherry Red
- ☐ Necrotic
- ☐ Hyperplasia
- ☐ Gas Bubbles
- ☐ Aneurisms
- ☐ Hyperemia
- ☐ Cellular Edema
- ☐ Golden Spherules
- ☐ Clubbed
- ☐ Swollen
- ☐ Puffy
- ☐ Other
- ☐ Hamburger Gill
- ☐ Postmortem Change

GAS BUBBLES Rate
- ☐ Gills
- ☐ Fins
- ☐ Skin

PHYSICAL – Internal
- ☐ Normal
- ☐ Postmortem Change

INTESTINE
- ☐ Normal
- ☐ Hemorrhagic
- ☐ Flaccid
- ☐ Gas
- ☐ Mucus
- ☐ Feces
- ☐ Fluid
- ☐ Intussusception

STOMACH
- ☐ Normal
- ☐ Hemorrhagic
- ☐ Gas
- ☐ Mucus
- ☐ Food
- ☐ Fluid

KIDNEY
- ☐ Normal
- ☐ Pale
- ☐ Hemorrhagic
- ☐ Swollen
- ☐ Brown
- ☐ Soft

LIVER
- ☐ Normal
- ☐ Pale
- ☐ Hemorrhagic
- ☐ Brown
- ☐ Black
- ☐ Mottled
- ☐ Congested

SPLEEN
- ☐ Congested
- ☐ Normal
- ☐ Mottled
- ☐ Brown
- ☐ Hemorrhagic

SWIM BLADDER
- ☐ Normal
- ☐ Hemorrhagic

BLOOD
- ☐ Normal
- ☐ Anemic
- ☐ Brown
- ☐ Black
- ☐ Cherry Red
- ☐ Methemoglobin
- ☐ Hct ____

COELOM
- ☐ Ascites
- ☐ Cloudy
- ☐ Bloody
- ☐ Clear
- ☐ Gas

DIAGNOSIS_____

PARASITES
A ☐ Ichthyoboda
B ☐ Ich
C ☐ Chilodon
D ☐ Trichodina
E ☐ Trichophrya
F ☐ Ambiphrya
G ☐ Epistylis
H ☐ Henneguya
I ☐ Monogenea (Gills)
J ☐ Gyrodactylus
K ☐ Yellow Grub
L ☐ White Grub
M ☐ Black Grub
N ☐ Lernaea
O ☐ Other_____
P ☐ Bodamonas
Q ☐ Apiosoma

BACTERIA
A ☐ A. hydrophila
B ☐ A. sobria
C ☐ Aeromonas sp.
D ☐ Plesiomonas shigelloides
E ☐ E. tarda
F ☐ E. ictaluri
G ☐ Flexibacter external
H ☐ Other myxobacteria
I ☐ Pseudomonas f.
J ☐ Pseudomonas sp.
K ☐ Klebsiella
L ☐ Enterobacter
M ☐ Proteus
N ☐ Unknown
O ☐ Other_____
P ☐ Flexibacter internal

FUNGI
A ☐ External
B ☐ Systemic
C ☐ Branchiomyces
D ☐ Other_____

VIRUSES
A ☐ CCV
B ☐ GSV
C ☐ Lymphocystis
D ☐ Other_____

WATER QUALITY
A ☐ Ammonia
B ☐ Nitrite
C ☐ Gas Bubble
D ☐ DO Depletion
E ☐ Thermal Shock
F ☐ pH
G ☐ Other_____
H ☐ Suspected DO Depletion

NUTRITIONAL
A ☐

TOXICITY
A ☐ Bluegreen algae
B ☐ Overtreatment_____
C ☐ Pesticides
D ☐ Other_____

MISCELLANEOUS
A ☐ Handling
B ☐ Genetic
C ☐ Tumors
D ☐ Crowding
E ☐ Moving
F ☐ Inadequate Sample_____
G ☐ Unknown
H ☐ Inspection
I ☐ Routine Check
J ☐ Other_____
K ☐ Hamburger Gill
L ☐ Anemia
M ☐ Winter Kill

Histology Results_____

_____

_____

Remarks_____

_____

APPENDIX E

AFS MONETARY VALUES OF FRESHWATER FISH AND FISH-KILL COUNTING GUIDELINES

Appendix E-1... American Fisheries Society. 1992. Investigation and valuation of fish kills. American Fisheries Society Publication 24. Bethesda, Maryland.

Appendix E-2... American Fisheries Society. 1993. Sourcebook for investigating and valuation of fish kills. American Fisheries Society Special Publication 24 (supplement). Bethesda, Maryland.

APPENDIX F

DEQ FISH KILL COUNT FORM

VA DEQ FISH KILL COUNT

Incident # ________ **Page____of _____**

Segment #	Species	Size Total Length (inches)	Number

APPENDIX G

REPLACEMENT COST OF FISH FORM

REPLACEMENT COST OF FISH

Incident #_____

Stream_____ **City/County**_____

Species (Common Name)	Size (Inches)	Number	Individual Costs	Total Cost

Grand Total_____

Signed_____
(Chief, Fish Div. DGIF)

Date_____

APPENDIX H

DEQ TOTAL COST SUMMARY

Department of Environmental Quality
Fish Kill Investigation Cost Calculation and Payment Instructions

1. Determine total DEQ fish kill investigation costs.

The agency fish kill investigation recovery worksheet file is found in DEQ intranet forms/admin/finance/finance forms. Each fish kill cost recovery case should be saved with the Pollution Incident Report Number (IR#), (formally called pollution complaint number, PC#) as the file name. Completed and signed originals of the form should be maintained with the appropriate cost support in the regional or central office fish kill investigation files.

a. The hourly rate is calculated from the employee's annual salary. Administrative and managerial oversight costs will be calculated using DEQ's established indirect cost rate of 28.5%. A standard benefit rate of 25% will be applied to direct salaries.
b. Standard mileage rates are established for pool cars (19 cents), agency owned (19 cents), and private vehicles (32.5 cents). Vehicle cost recovery is based on number of vehicle miles documented on the vehicle log sheet.
c. Actual costs for employee meals, lodging, and other travel expenses as documented on the employee's travel voucher will be recovered.
d. Actual laboratory sampling costs will be recovered based on the current catalog of sampling costs for the specific type of testing performed.
e. The cost of the use of agency boats will be calculated based on the standard hourly rate established for the boat motor. Costs will be calculated based on hours used to whole hours.
f. Other miscellaneous costs incurred such as ice, film processing, and apparel will be recovered based on the actual costs incurred. These costs are documented on the agency purchase requisition, petty cash voucher, or travel voucher. The costs of inventory or "stock" (ex. Gloves) items should be based on the documented current fair market price.

2. Determine replacement cost of the fish

3. The responsible party should send two separate checks for DEQ cost recovery and DGIF fish replacement. Each check should be made payable to "The Treasurer of Virginia" and MUST include the Pollution Incident Number (formally PC #) and refer to either DEQ cost or DGIF fish replacement costs. Both checks should be sent to the following DEQ lock box address:

Receipts Control
P.O. Box 10150
Richmond, VA 23240

Pollution Complaint Investigation
Time, Travel and Materials Cost Recovery Schedule
Expenses incurred by the Department of Environmental Quality
for Fish Kill Investigation
Spreadsheet completion instruction form

Hourly rates are established based on the employee's annual salary. Costs for administration and management oversight are included in the 28.5% indirect cost rate. The spreadsheet automatically calculates rates when annual salary and hours are added to spreadsheet.
Boat costs are standardized based on size of boat motor. See below for rates.

How to complete the form:

1) Enter the PC incident # in cell D6
2) Enter the Date in cell H6
3) Enter the site in cell D7
4) Enter the Region Code in D8
5) Enter the employee's last name & first initial in B11 for the 1st,B12 for the second employee, etc.
6) Matching the employee's number, enter their respective time codes in cells B21 through B25.
7) Enter the employee's annual salary in cells C21 through C25 respectively.
8) Enter time and units as 3.0; 3.25; etc. in cells D21 through D25.
8) Enter any travel dates in cells D31 /E31 through D35/E35.
9) Enter the number of miles traveled in either the Pool, Agency or Private column (cells F31/F35 , G31/G35 or H31/H35.)
10) Enter the total amount of Meals, Overnight Lodging and Other Travel costs in cells F, G, H 41/45 respectively.
11) For Additional Materials and or Equipment purchased/used complete the necessary cells under the appropriate headings in cells C50 through H 55. Laboratory costs are calculated based on the type of sample and sample cost. Refer to the catalog of lab services for individual sample costs. Other costs include ice, film, etc.
12) Print using the SmartIcon.
13) Save the File with the PC incident # as the file name in k:\agency\forms\costreco\ sub-directory.

Standard Boat Usage Rates by Motor Size:

100 hp and greater	$20.00 per hour
Under 100 hp	$15.00 per hour
Electro Fisher	$20.00 per hour

Pollution Incident Investigation
Time, Travel and Materials Cost Recovery Schedule
Expenses incurred by the Department of Environmental Quality
for Fish Kill Investigation

Incident Report (IR)		Date	
IR Site Location			
Region			

	Name	**Signature**		
Emp #1			Date	
Emp #2			Date	
Emp #3			Date	
Emp #4			Date	
Emp #5			Date	
Supervisor			Date	

Time Spent	**Time Code**	**Salary**	**Hours**	**Extended Cost**	**Benefits @ 25%**	**Indirect @28.5%**	**Total Time Costs**
Emp #1				0.00	0.00	0.00	0.00
Emp #2				0.00	0.00	0.00	0.00
Emp #3				0.00	0.00	0.00	0.00
Emp #4				0.00	0.00	0.00	0.00
Emp #5				0.00	0.00	0.00	0.00
Sub-Total Employee Time			0	0.00	0.00	0.00	0.00

Travel-Vehicle	**Date(s) From**	**To**	**No of Miles Pool @ 0.19**	**Agency @ 0.19**	**Private 0.325**	**Cost**
Emp #1						0.00
Emp #2						0.00
Emp #3						0.00
Emp #4						0.00
Emp #5						0.00
Sub-Total Employee Travel			0.00	0.00	0.00	0.00

Travel - Meal and Lodging

	Meals	**Lodging**	**Other**	**Costs**
Emp #1				0.00
Emp #2				0.00
Emp #3				0.00
Emp #4				0.00
Emp #5				0.00
Sub-Total Employee Meals and Lodging	0.00	0.00	0.00	0.00

Materials and Equipment Used **Activity**	**Date**	**Vendor**	**Hours/ Units**	**Rate/ Costs**	**Costs**
Boat Usage					0.00
Laboratory Analysis					0.00
Laboratory Analysis					0.00
Other- Specify					0.00

Other- Specify	0.00
Other- Specify	0.00
Sub-total Equipment and Supplies	0.00
Total Time, Travel and Materials Cost Recovery Schedule	0.00

APPENDIX I

DEQ FISH TISSUE AND SEDIMENT MONITORING PROGRAM (FORMERLY CORE PROGRAM) SAMPLING HANDLING PROCEDURE FROM THE USEPA. 2000. GUIDANCE FOR ASSESSING CHEMICAL CONTAMINANT DATA FOR USE IN FISH ADVISORIES, VOL. I. FISH SAMPLING AND ANALYSIS. WASHINGTON, DC.

Sample Handling

Fish samples should be carefully handled throughout the study to prevent contamination by the sampler's hands or field equipment. The following items are of particular importance:

sample preservation - dry or wet ice in field (sample frozen within 24 hrs.), freeze in lab
holding times - 6 month maximum for initial samples
sample storage - frozen
sample ID- sample number, fish collection data form, laboratory reporting form, bag labels
sample weight - 100 g **minimum** per composite

Fish handling procedure

The fish collected are to be handled with clean hands. Fish are wrapped in aluminum foil (dull side to the fish). If fish are to be analyzed as individual samples, they should be individually wrapped. For normal Core sampling, fish of the same sample may be wrapped in the same piece of foil. The wrapped fish are placed in plastic bags according to species or composite sample grouping and placed on ice. Usually, the three composite samples from each station are placed inside another plastic bag before being iced to decrease the chance of water infiltration. Once back from the field, the samples are immediately removed from ice and stored in the freezer until ready for shipment to DCLS contract laboratory.

Each DCLS lab prefers a 50 gram sample, however, the **minimum** sample weight requirements are listed below:

Metals 15 grams
Pesticides 35 grams
Trace Organics 35 grams

Laboratory reporting forms and sample identification tags for each sample should be completed upon conclusion of sample preparation for each station.

If samples are shipped to the DCLS laboratory, the DEQ laboratory coordinator should be contacted prior to submission of samples.

Laboratory Data Sheet Completion for STORET Data Entry

Correct completion of the laboratory data sheets is important to ensure that the fish tissue contaminant data is efficiently entered into the EPA STORET data base. CORE Procedure Appendix E lists the fish species commonly encountered while sampling in Virginia's waters along with the STORET numerical and alphabetic code. For other fish species STORET codes contact the STORET coordinator (see Section 2). STORET data printouts have a number of mislabeled columns. Appendix K is a copy of a STORET data retrieval. Please note that columns "Time of Day", "Medium" and "SMK or Depth" are incorrectly identified.

Appendix E

Fish Commonly Collected for Core Fish Tissue Program

PREDATOR SPECIES

COMMON NAME	STORET ALPHA CODE	STORET NUMERIC CODE
Bluegill	BGS	08
Bowfin	BF	68
Channel catfish	CHC	16
Flier	FR	412
Green sunfish	GSF	25
Largemouth bass	LMB	31
Longear sunfish	LSF	72
Oyster toadfish	TOAD	528
Pumpkinseed	SF	38
Rainbow trout	RBT	39
Redbreast sunfish	RBS	70
Rock bass	RKB	43
Smallmouth bass	SMB	47
Striped bass	STB	52
Sunfish	CSN	521
Weakfish (Grey trout)	WKF	180
White catfish	WHC	58
White crappie	WHS	59
White perch	WP	60
Yellow perch	YP	63

BOTTOM FEEDER SPECIES

COMMON NAME	STORET ALPHA CODE	STORET NUMERIC CODE
Carp	C	12
Croaker*	CRK	115
Fallfish	FFH	208
Gizzard shad	GSH	21
Menhaden	ATM	165
Northern hogsucker	NHO	94
Oyster	CRV	217
River Chub	RC	73
Shorthead redhorse	SRD	101
Silver redhorse	SRH	170
Snail bullhead	SBH	488
Spot*	SPT	181
Summer Flounder	SSF	183
White sucker	SWU	61
Yellow bullhead	YEB	62

*: also used as a predator for human health risk.

sh_spp

species of interest, dynamics of dispersion of pollutants of interest, or geographical location. Taking a simple random sample of lakes may not achieve sufficient coverage, whereas taking a stratified random sample approach may require more lakes be sampled than can be afforded. A conservative approach may be to look at the "worst case scenario". States may decide to sample the lakes that are believed to have the highest levels of pollutants, based on historical contaminant data, current water and sediment sampling results, or other variables. Another approach would be to select one or two of the factors described above ("representativeness"), stratify the lakes according to these factors, and select a random sample within each stratum. The set of factors for stratification may change every few years or so if it is deemed that some other factors are becoming more indicative of the levels of contamination.

6.2 SAMPLE COLLECTION

Sample collection activities should be initiated in the field only after an approved sampling plan has been developed. This section discusses recommended sampling equipment and its use, considerations for ensuring preservation of sample integrity, and field recordkeeping and chain-of-custody procedures associated with sample processing, preservation, and shipping.

6.2.1 Sampling Equipment and Use

In response to the variations in environmental conditions and target species of interest, fisheries biologists have had to devise sampling methods that are intrinsically selective for certain species and sizes of fish and shellfish (Versar, 1982). Although this selectivity can be a hindrance in an investigation of community structure, it is not a problem where tissue contaminant analysis is of concern because tissue contaminant data can best be compared only if factors such as differences in taxa and size are minimized.

Collection methods can be divided into two major categories, active and passive. Each collection method has advantages and disadvantages. Various types of sampling equipment, their use, and their advantages and disadvantages are summarized in Table 6-4 for fish and in Table 6-5 for shellfish. **Note:** Either active or passive collection methods may be used as long as the methods selected result in collection of a representative fish sample of the type consumed by local sport and subsistence fishers.

A basic checklist of field sampling equipment and supplies is shown in Table 6-6. Safety considerations associated with the use of a boat in sample collection activities are summarized in Table 6-7.

6.2.1.1 Active Collection—

Active collection methods employ a wide variety of sampling techniques and devices. Devices for fish sampling include electroshocking units, seines, trawls,

Table 6-4. Summary of Fish Sampling Equipment

Device	Use	Advantages	Disadvantages
ACTIVE METHODS			
Electrofishing	Shallow rivers, lakes, and streams.	Most efficiency nonselection method. Minimal damage to fish. Adaptable to a number of sampling conditions (e.g., boat, wading, shorelines). Particularly useful at sites where other active methods cannot be used (e.g., around snags and irregular bottom contours).	Nonselective–stuns or kills most fish. Cannot be used in brackish, salt, or extremely soft water. Requires extensive operator training. DANGEROUS when not used properly.
Seines	Shallow rivers, lakes, and streams. Shoreline areas of estuaries.	Relatively inexpensive and easily operated. Mesh size selection available for target species.	Cannot be used in deep water or over substrates with an irregular contour. Not completely efficient as fish can evade the net during seining operation.
Trawls	Various sizes can be used from boats in moderate to deep open bodies of water (10 to >70 m depths).	Effective in deep waters not accessible by other methods. Allows collection of a large number of samples.	Requires boat and trained operators.
Angling	Generally species selective involving use of hook and line.	Most selective method. Does not require use of large number of personnel or expensive equipment.	Inefficient and not dependable.
Purchasing specimens from commercial fishers	Only in areas where target species are commercially harvested.	Most cost-effective and efficient means of obtaining commercially valuable species from harvested waters.	Limited use; commercially harvested areas may not include sampling sites chosen for fish contaminant monitoring. The field collection staff should accompany the commercial fishers and should remove the required samples from the collection device. This will ensure the proper handling of the specimens and accurate recording of the collection time and sampling location.
PASSIVE METHODS			
Gill nets	Lakes, rivers, and estuaries. Where fish movement can be expected or anticipated.	Effective for collecting pelagic fish species. Relatively easy to operate. Requires less fishing effort than active methods. Selectivity can be controlled by varying mesh size.	Not effective for bottom-dwelling fish or populations that do not exhibit movement patterns. Nets prone to tangling or damage by large and sharp spined fish. Gill nets will kill captured specimens, which, when left for extended periods, may undergo physiological changes.
Trammel nets	Lakes, rivers, and estuaries. Where fish movement can be expected or anticipated. Frequently used where fish may be scared into the net.	Slightly more efficient than a straight gill net.	(Same as for gill nets.) Tangling problems may be more severe. Method of scaring fish into net requires more personnel or possibly boats in deep water areas.
Hoop, Fyke and Pound Nets	Shallow rivers, lakes, and estuaries when currents are present or when movements of fish are predictable. Frequently used in commercial operations.	Unattended operation. Very efficient in regard to long-term return and expended effort. Particularly useful in areas where active methods are impractical.	Inefficient for short term. Difficult to set up and maintain.
D-Traps	Used for long-term capture of slow-moving fish, particularly bottom species. Can be used in all environments.	Easy to operate and set. Unattended operation. Particularly useful for capturing bottom-dwelling organisms in deep waters or other types of inaccessible areas. Relatively inexpensive–often can be hand made.	Efficiency is highly variable. Not effective for pelagic fish or fish that are visually oriented. Less efficient for all species when water is clear rather than turbid. Not a good choice for a primary sampling technique, but available as backup for other methods.

Source: Versar, 1982.

Table 6-5. Summary of Shellfish Sampling Equipment

Device	Use	Advantages	Disadvantages
ACTIVE METHODS			
Seines	Shallow shoreline areas of estuaries.	Relatively inexpensive and easily operated. Mesh size selection available for target crustacean species (e.g., shrimp and crabs).	Cannot be used in deep water or over substrates with an irregular contour. Not completely efficient as crustaceans can evade the net during seining operation.
Trawls	Various sizes can be used from boats in moderate to deep open bodies of water (10 to >70 m depths).	Effective in deeper waters not accessible by other methods. Allows collection of a large number of samples.	Requires boat and trained operators.
Mechanical grabs Double-pole-operated grab buckets	Used from boat or pier. Most useful in shallow water areas less than 6 m deep including lakes, rivers, and estuaries.	Very efficiency means of sampling bivalves (e.g., clams and oysters) that are located on or buried in bottom sediments.	At depths greater than 6 m, the pole-operated devices become difficult to operate manually.
Tongs or double-handled grab sampler	Most useful in shallow water, lakes, rivers, and estuaries. Generally used from a boat.	Very efficient means of sampling oysters, clams, and scallops. Collection of surrounding or overlying sediments is not required and the jaws are generally open baskets. This reduces the weight of the device and allows the washing of collected specimens to remove sediments.	At depths greater than 6 m, the pole-operated devices become difficult to operate manually.
Line or cable-operated grab buckets			
Ekman grab	Used from boat or pier to sample soft to semisoft substrates.	Can be used in water of varying depths in lakes, rivers, and estuaries.	Possible incomplete closure of jaws can result in sample loss. Must be repeatedly retrieved and deployed. Grab is small and is not particularly effective in collecting large bivalves (calms and oysters).
Petersen grab	Deep lakes, rivers, and estuaries for sampling most substrates.	Large sample is obtained; grab can penetrate most substrates.	Grab is heavy, may require which for deployment. Possible incomplete closure of jaws can result is sample loss. Must be repeatedly retrieved and deployed.
Ponar grab	Deep lakes, rivers, and estuaries for sampling sand, silt, or clay substrates.	Most universal grab sampler. Adequate on most substrates. Large sample is obtained intact.	Possible incomplete closure of jaws can result in sample loss. Must be repeatedly retrieved and deployed.
Orange peel grab	Deep lakes, rivers, and estuaries for sampling most substrates.	Designed for sampling hard substrates.	Grab is heavy, may require winch for deployment. Possible incomplete closure of jaws can result in sample loss. Must be repeatedly retrieved and deployed. Grab is small and not particularly effective in collecting large bivalves (clams and oysters).
Biological or hydraulic dredges	Dragged along the bottom of deep waterbodies to collect large stationary invertebrates.	Qualitative sampling of large area of bottom substrate and benthic community. Length of tows can be relatively short if high density of shellfish exists in sampling area.	If the length of the tow is long, it is difficult to pinpoint the exact location of the sample collection area. Because of the scouring operation of the dredge, bivalve shells may be damaged. All bivalve specimens should be inspected and individuals with cracked or damaged shells should be discarded.

(continued)

Table 6-5. (continued)

Device	Use	Advantages	Disadvantages
ACTIVE METHODS (continued)			
Scoops, shovels	Used in shallow waters accessible by wading or SCUBA equipment for collection of hard clams *(Mercenaria mercenaria)* or soft-shell clam *(Mya arenaria).*	Does not require a boat; sampling can be done from shore.	Care must be taken not to damage the shells of bivalves while digging in substrate.
Scrapers	Used in shallow waters accessible by wading or SCUBA equipment for collection of oysters *(Crassostrea virginica)* or mussels *(Mytilus sp).*	Does not require a boat; sampling can be done from shore.	Care must be taken not to damage shells of bivalves while removing them from hard substrate.
Rakes	Used in shallow waters accessible by wading or can be used from a boat.	Does not require a boat; sampling can be done close to shore. Can be used in soft sediments to collect clams or scallops and can also be used to dislodge oysters or mussels that are attached to submerged objects such as rocks and pier pilings.	Care must be taken not to damage the shells of the bivalves while raking or dislodging them from the substrate.
Purchasing specimens from commercial fishers	Only in areas where target species are commercially harvested.	Most cost-effective and efficient means of obtaining bivalves for pollutant analysis from commercially harvested waters.	Limited use; commercially harvested areas may not include sampling sites chosen for shellfish contaminant monitoring. The field collection staff should accompany the commercial fishers and should remove the required samples from the collection device. This will ensure the proper handling of the specimens and accurate recording of the exact collection time and sampling location.
PASSIVE METHODS			
D-traps	Used for capture of slow-moving crustaceans (crabs and lobsters) that move about on or just above the substrate.	Can be used in a variety of environments. Particularly useful for capturing bottom-dwelling organisms in deep water or other inaccessible areas. Relatively inexpensive, can be hand made.	Catch efficiency is highly variable. Not a good choice for a primary sampling technique, but valuable as a backup for other methods.

Table 6-6. Checklist of Field Sampling Equipment and Supplies for Fish and Shellfish Contaminant Monitoring Programs

- ☐ Boat supplies
  - ☐ Fuel supply (primary and auxiliary supply)
  - ☐ Spare parts repair kit
  - ☐ Life preservers
  - ☐ First aid kit (including emergency phone numbers of local hospitals, family contacts for each member of the sampling team)
  - ☐ Spare oars
  - ☐ Nautical charts of sampling site locations
- ☐ Collection equipment (e.g., nets, traps, electroshocking device)
- ☐ Recordkeeping/documentation supplies
  - ☐ Field logbook
  - ☐ Sample request forms
  - ☐ Specimen identification labels
  - ☐ Chain-of-Custody (COC) Forms and COC tags or labels
  - ☐ Indelible pens
- ☐ Sample processing equipment and supplies
  - ☐ Holding trays
  - ☐ Fish measuring board (metric units)
  - ☐ Calipers (metric units)
  - ☐ Shucking knife
  - ☐ Balance to weigh representative specimens for estimating tissue weight (metric units)
  - ☐ Aluminum foil (extra heavy duty)
  - ☐ Freezer tape
  - ☐ String
  - ☐ Several sizes of plastic bags for holding individual or composite samples
  - ☐ Resealable watertight plastic bags for storage of Field Records, COC Forms, and Sample Request Forms
- ☐ Sample preservation and shipping supplies
  - ☐ Ice (wet ice, blue ice packets, or dry ice)
  - ☐ Ice chests
  - ☐ Filament-reinforced tape to seal ice chests for transport to the central processing laboratory

Table 6-7. Safety Considerations for Field Sampling Using a Boat

- Field collection personnel **should not** be assigned to duty alone in boats.
- Life preservers should be worn at all times by field collection personnel near the water or on board boats.
- If electrofishing is the sampling method used, there must be two shutoff switches--one at the generator and a second on the bow of the boat.
- All deep water sampling should be performed with the aid of an experienced, licensed boat captain.
- All sampling during nondaylight hours, during severe weather conditions, or during periods of high water should be avoided or minimized to ensure the safety of field collection personnel.
- All field collection personnel should be trained in CPR, water safety, boating safety, and first aid procedures for proper response in the event of an accident. Personnel should have local emergency numbers readily available for each sampling trip and know the location of the hospitals or other medical facilities nearest each sampling site.

and angling equipment (hook and line). Rotenone, a chemical piscicide, has been used extensively to stun fish prior to their collection with seines, trawls, or other sampling devices. Rotenone has not been found to interfere with the analysis of the recommended organic target analytes (see Table 4-1) when the recommended analysis procedures are used. See Section 8 for additional information on appropriate analysis methods for the recommended organic target analytes. Devices for shellfish sampling include seines, trawls, mechanical grabs (e.g., pole- or cable-operated grab buckets and tongs), biological and hydraulic dredges, scoops and shovels, rakes, and dip nets. Shellfish can also be collected manually by SCUBA divers. Although active collection requires greater fishing effort, it is usually more efficient than passive collection for covering a large number of sites and catching the relatively small number of individuals needed from each site for tissue analysis (Versar, 1982). Active collection methods are particularly useful in shallow waters (e.g., streams, lake shorelines, and shallow coastal areas of estuaries).

One aspect of sample collection that is of paramount importance is that the sampling team must ensure the collection of live, intact fish and shellfish for use in sample analysis for human risk assessment. It is highly desirable to collect live, intact fish and shellfish that have not been mutilated by the collection gear and that do not have any skin, shell, or carapace lacerations or fin deterioration that would allow body fluids to leak out of the specimen or contaminants to pass into the specimen after collection. For example, some fish collected by electro-shocking methods may have ruptured organs due to the electroshocking procedure. Fish that are found floating dead at a site should not be used for sample analysis for human risk assessments. For these reasons, EPA recommends that any specimens that show any skin, shell, or carapace lacerations or fin deterioration of any kind not used for chemical analysis.

Active collection methods have distinct disadvantages for deep water sampling. They require more field personnel and more expensive equipment than passive collection methods. This disadvantage may be offset by coordinating sampling efforts with commercial fishing efforts. Purchasing fish and shellfish from commercial fishers using active collection devices is acceptable; however, field sampling staff should accompany the commercial fishers during the collection operation to ensure that samples are collected and handled properly and to verify the sampling site location. The field sampling staff then remove the target species directly from the sampling device and ensure that sample collection, processing, and preservation are conducted as prescribed in sample collection protocols, with minimal chance of contamination. This is an excellent method of obtaining specimens of commercially important target species, particularly from the Great Lakes and coastal estuarine areas (Versar, 1982). More detailed descriptions of active sampling devices and their use are provided in Battelle (1975), Bennett, et al., (1970), Gunderson and Ellis (1986), Hayes (1983), Mearns and Allen (1978), Pitt (1981), Puget Sound Estuary Program (1990b), Versar (1982), and Weber (1973).

6.2.1.2 Passive Collection—

Passive collection methods employ a wide array of sampling devices for fish and shellfish, including gill nets, fyke nets, trammel nets, hoop nets, pound nets, and d-traps. Passive collection methods generally require less fishing effort than active methods but are usually less desirable for shallow water sample collection because of the ability of many species to evade these entanglement and entrapment devices. These methods normally yield a much greater catch than would be required for a contaminant monitoring program and are time consuming to deploy. In deep water, however, passive collection methods are generally more efficient than active methods. Crawford and Luoma (1993) caution that passive collection devices (e.g., gill nets) should be checked frequently to ensure that captured fish do not deteriorate prior to removal from the sampling device. Versar (1982, 1984) and Hubert (1983) describe passive sampling devices and their use in more detail. It is highly desirable to collect live, intact fish that have not been mutilated by the collection gear and that do not have any skin lacerations or fin deterioration. For these reasons, EPA recommends that fish captured in passive collection devices not remain in the water for more than 24 hours after the passive collection device is first deployed and that specimens that show any skin or fin deterioration or external lacerations of any kind not used for chemical analysis.

Purchasing fish and shellfish from commercial fishers using passive collection methods is acceptable; however, field sampling staff should accompany the fishers during both the deployment and collection operations to ensure that samples are collected and handled properly and to verify the sampling site location. The field sampling staff can then ensure that sample collection, processing, and preservation are conducted as prescribed in sample collection protocols, with minimal chance of contamination.

6.2.2 Preservation of Sample Integrity

The primary QA consideration in sample collection, processing, preservation, and shipping procedures is the preservation of sample integrity to ensure the accuracy of target analyte analyses. Sample integrity is preserved by prevention of loss of contaminants already present in the tissues and prevention of extraneous tissue contamination (Smith, 1985).

Loss of contaminants already present in fish or shellfish tissues can be prevented in the field by ensuring that the skin on fish specimens has not been lacerated by the sampling gear or that the carapace of crustaceans or shells of bivalves have not been cracked during sample collection resulting in loss of tissues and/or fluids that may contain contaminants. Once the samples have reached the laboratory, further care must be taken during thawing (if specimens are frozen) to ensure that all liquids from the thawed specimens are retained with the tissue sample as appropriate (see Sections 7.2.2, 7.2.3, and 7.2.4).

Sources of extraneous tissue contamination include contamination from sampling gear, grease from ship winches or cables, spilled engine fuel (gasoline or diesel), engine exhaust, dust, ice chests, and ice used for cooling. All potential sources of contamination in the field should be identified and appropriate steps taken to minimize or eliminate them. For example, during sampling, the boat should be positioned so that engine exhausts do not fall on the deck. Ice chests should be scrubbed clean with detergent and rinsed with distilled water after each use to prevent contamination. To avoid contamination from melting ice, samples should be placed in waterproof plastic bags (Stober, 1991). Sampling equipment that has obviously been contaminated by oils, grease, diesel fuel, or gasoline should not be used. All utensils or equipment that will be used directly in handling fish or shellfish (e.g., fish measuring board or calipers) should be cleaned in the laboratory prior to each sampling trip, rinsed in acetone and pesticide-grade hexane, and stored in aluminum foil until use (Versar, 1982). Between sampling sites, the field collection team should clean each measurement device by rinsing it with ambient water and rewrapping it in aluminum foil to prevent contamination.

Note: Ideally, all sample processing (e.g., resections) should be performed at a sample processing facility under cleanroom conditions to reduce the possibility of sample contamination (Schmitt and Finger, 1987; Stober, 1991). However, there may be some situations in which state staff find it necessary to fillet finfish or resect edible turtle or shellfish tissues in the field prior to packaging the samples for shipment to the processing laboratory. This practice should be avoided whenever possible. If states find that filleting fish or resecting other edible tissues must be performed in the field, a clean area should be set up away from sources of diesel exhaust and areas where gasoline, diesel fuel, or grease are used to help reduce the potential for surface and airborne contamination of the samples from PAHs and other contaminants. Use of a mobile laboratory or use of a portable resection table and enclosed hood would provide the best environment for sample processing in the field. General guidance for conducting sample

processing under cleanroom conditions is provided in Section 7.2.1. States should review this guidance to ensure that procedures as similar as possible to those recommended for cleanroom processing are followed. If sample processing is conducted in the field, a notation should be made in the field records and on the sample processing record (see Figure 7-2). Procedures for laboratory processing and resection are described in Section 7.2. Procedures for assessing sources of sample contamination through the analyses of field and processing blanks are described in Section 8.3.3.6.

6.2.3 Field Recordkeeping

Thorough documentation of all field sample collection and processing activities is necessary for proper interpretation of field survey results. For fish and shellfish contaminant studies, it is advisable to use preprinted waterproof data forms, indelible ink, and writing implements that can function when wet (Puget Sound Estuary Program, 1990b). When multicopy forms are required, no-carbon-required (NCR) paper is recommended because it allows information to be forwarded on the desired schedule and retained for the project file at the same time.

Four separate preprinted sample tracking forms should be used for each sampling site to document field activities from the time the sample is collected through processing and preservation until the sample is delivered to the processing laboratory. These are

- Field record form
- Sample identification label
- Chain-of-custody (COC) label or tag
- COC form.

6.2.3.1 Field Record Form—

The following information should be included on the field record for each sampling site in both **Tier 1** screening (Figures 6-3 and 6-4) and **Tier 2** intensive studies as appropriate (Figures 6-5 and 6-6):

- Project number
- Sampling date and time (give date in a Year 2000 compliant format [YYYYMMDD] and specify convention used for time, e.g., 24-h clock)
- Sampling site location (including site name and number, county/parish, latitude/longitude, waterbody name/segment number, waterbody type, and site description)
- Sampling depth (specify units of depth)
- Collection method
- Collectors' names and signatures
- Agency (including telephone number and address)

Field Record for Fish Contaminant Monitoring Program — Screening Study

Project Number: _____ Sampling Date and Time: _____

SITE LOCATION

Site Name/Number: _____

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

Waterbody Name/Segment Number: _____

Waterbody Type: ☐ RIVER ☐ LAKE ☐ ESTUARY

Site Description: _____

_____

Collection Method: _____

Collector Name: _____
(print and sign)

Agency: _____ Phone: (____) _____

Address: _____

_____

FISH COLLECTED

Bottom Feeder—Species Name: _____

Composite Sample #: _____ Number of Individuals: _____

Fish #	Length (mm)	Sex	Fish #	Length (mm)	Sex
001	_____	___	006	_____	___
002	_____	___	007	_____	___
003	_____	___	008	_____	___
004	_____	___	009	_____	___
005	_____	___	010	_____	___

$\frac{\text{Minimum size}}{\text{Maximum size}}$ x 100 = _____ >75% Composite mean length _____ mm

Notes (e.g., morphological anomalies): _____

_____

Predator—Species Name: _____

Composite Sample #: _____ Number of Individuals: _____

Fish #	Length (mm)	Sex	Fish #	Length (mm)	Sex
001	_____	___	006	_____	___
002	_____	___	007	_____	___
003	_____	___	008	_____	___
004	_____	___	009	_____	___
005	_____	___	010	_____	___

$\frac{\text{Minimum size}}{\text{Maximum size}}$ x 100 = _____ ≥ 75% Composite mean length _____ mm

Notes (e.g., morphological anomalies): _____

_____

Figure 6-3. Example of a field record for fish contaminant monitoring program—screening study.

Field Record for Shellfish Contaminant Monitoring Program — Screening Study

Project Number: _____ Sampling Date and Time: _____

SITE LOCATION

Site Name/Number: _____

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

Waterbody Name/Segment Number: _____

Waterbody Type: ☐ RIVER ☐ LAKE ☐ ESTUARY

Site Description: _____

_____

Collection Method: _____

Collector Name: _____
(print and sign)

Agency: _____ Phone: (____) _____

Address: _____

_____

SHELLFISH COLLECTED

Bivalve Species Name: _____

Composite Sample #: _____ Number of Individuals: _____

Bivalve #	Size (mm)	Bivalve #	Size (mm)	Bivalve #	Size (mm)
001		018		035	
002		019		036	
003		020		037	
004		021		038	
005		022		039	
006		023		040	
007		024		041	
008		025		042	
009		026		043	
010		027		044	
011		028		045	
012		029		046	
013		030		047	
014		031		048	
015		032		049	
016		033		050	
017		034			

$\frac{\text{Minimum size}}{\text{Maximum size}}$ x 100 = _____ ≥ 75% Composite mean size _____ mm

Notes (e.g., morphological anomalies): _____

_____

Figure 6-4. Example of a field record for shellfish contaminant monitoring program—screening study.

Field Record for Fish Contaminant Monitoring Program — Intensive Study

Project Number: _____ Sampling Date and Time: _____

SITE LOCATION

Site Name/Number: _____

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

Waterbody Name/Segment Number: _____

Waterbody Type: ☐ RIVER ☐ LAKE ☐ ESTUARY

Site Description: _____

_____

Collection Method: _____

Collector Name: _____
(print and sign)

Agency: _____ Phone: (____) _____

Address: _____

_____

FISH COLLECTED

Species Name: _____ **Replicate Number**: _____

Composite Sample #: _____ Number of Individuals: _____

Fish #	Length (mm)	Sex (M, F, or I)	Fish #	Length (mm)	Sex (M, F, or I)
001	_____	___	006	_____	___
002	_____	___	007	_____	___
003	_____	___	008	_____	___
004	_____	___	009	_____	___
005	_____	___	010	_____	___

$\frac{\text{Minimum length}}{\text{Maximum length}}$ x 100 = _____ % Composite mean length _____ mm

Notes (e.g., morphological anomalies): _____

_____

Species Name: _____ **Replicate Number**: _____

Composite Sample #: _____ Number of Individuals: _____

Fish #	Length (mm)	Sex (M, F, or I)	Fish #	Length (mm)	Sex (M, F, or I)
001	_____	___	006	_____	___
002	_____	___	007	_____	___
003	_____	___	008	_____	___
004	_____	___	009	_____	___
005	_____	___	010	_____	___

$\frac{\text{Minimum length}}{\text{Maximum length}}$ x 100 = _____ ≥ 75% Composite mean length _____ mm

Notes (e.g., morphological anomalies): _____

_____

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Figure 6-5. Example of a field record for fish contaminant monitoring program—intensive study.

Field Record for Fish Contaminant Monitoring Program — Intensive Study (con.)

Project Number: _____ Sampling Date and Time: _____

SITE LOCATION:

Site Name/Number: _____

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

FISH COLLECTED

Species Name: _____ **Replicate Number**: _____

Composite Sample #: _____ Number of Individuals: _____

Fish #	Length (mm)	Sex (M, F, or I)	Fish #	Length (mm)	Sex (M, F, or I)
001			006		
002			007		
003			008		
004			009		
005			010		

Minimum length / Maximum length x 100 = _____ % Composite mean length _____ mm

Notes (e.g., morphological anomalies): _____

Species Name: _____ **Replicate Number**: _____

Composite Sample #: _____ Number of Individuals: _____

Fish #	Length (mm)	Sex (M, F, or I)	Fish #	Length (mm)	Sex (M, F, or I)
001			006		
002			007		
003			008		
004			009		
005			010		

Minimum length / Maximum length x 100 = _____ % Composite mean length _____ mm

Notes (e.g., morphological anomalies): _____

Species Name: _____ **Replicate Number**: _____

Composite Sample #: _____ Number of Individuals: _____

Fish #	Length (mm)	Sex (M, F, or I)	Fish #	Length (mm)	Sex (M, F, or I)
001			006		
002			007		
003			008		
004			009		
005			010		

Minimum length / Maximum length x 100 = _____ ≥ 75% Composite mean length _____ mm

Notes (e.g., morphological anomalies): _____

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Figure 6-5. (continued)

Field Record for Shellfish Contaminant Monitoring Program — Intensive Study

Project Number: _____ Sampling Date and Time: _____

SITE LOCATION

Site Name/Number: _____

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

Waterbody Name/Segment Number: _____

Waterbody Type: ☐ RIVER ☐ LAKE ☐ ESTUARY

Site Description: _____

Collection Method: _____

Collector Name: _____
(print and sign)

Agency: _____ Phone: (____) _____

Address: _____

SHELLFISH COLLECTED

Species Name: _____ **Replicate Number:** _____

Composite Sample #: _____ Number of Individuals: _____

Shellfish #	Size (mm)	Sex	Shellfish #	Size (mm)	Sex	Shellfish #	Size (mm)	Sex
001			018			035		
002			019			036		
003			020			037		
004			021			038		
005			022			039		
006			023			040		
007			024			041		
008			025			042		
009			026			043		
010			027			044		
011			028			045		
012			029			046		
013			030			047		
014			031			048		
015			032			049		
016			033			050		
017			034					

$\frac{\text{Minimum size}}{\text{Maximum size}} \times 100 =$ _____ ≥ 75% Composite mean size _____ mm

Notes (e.g., morphological anomalies): _____

Figure 6-6. Example of a field record for shellfish contaminant monitoring program—intensive study.

- Species collected (including species common and scientific name, composite sample number, individual specimen number, number of individuals per composite sample, number of replicate samples, total length/size [mm], sex [male, female, indeterminate])

Note: States should specify a unique numbering system to track samples for their own fish and shellfish contaminant monitoring programs.

- Percent difference in size between the smallest and largest specimens to be composited (smallest individual length [or size] divided by the largest individual length [or size] x 100; should be ≥75 percent) and mean composite length or size (mm)

- Notes (including visible morphological abnormalities, e.g., fin erosion, skin ulcers, cataracts, skeletal and exoskeletal anomalies, neoplasms, or parasites).

6.2.3.2 Sample Identification Label—

A sample identification label should be completed in indelible ink for each individual fish or shellfish specimen after it is processed to identify each sample uniquely (Figure 6-7). The following information should be included on the sample identification label:

- Species scientific name or code number
- Total length/size of specimen (mm)
- Specimen number
- Sample type: F (fish fillet analysis only)
  S (shellfish edible portion analysis only)
  W (whole fish analysis)
  O (other fish tissue analysis)

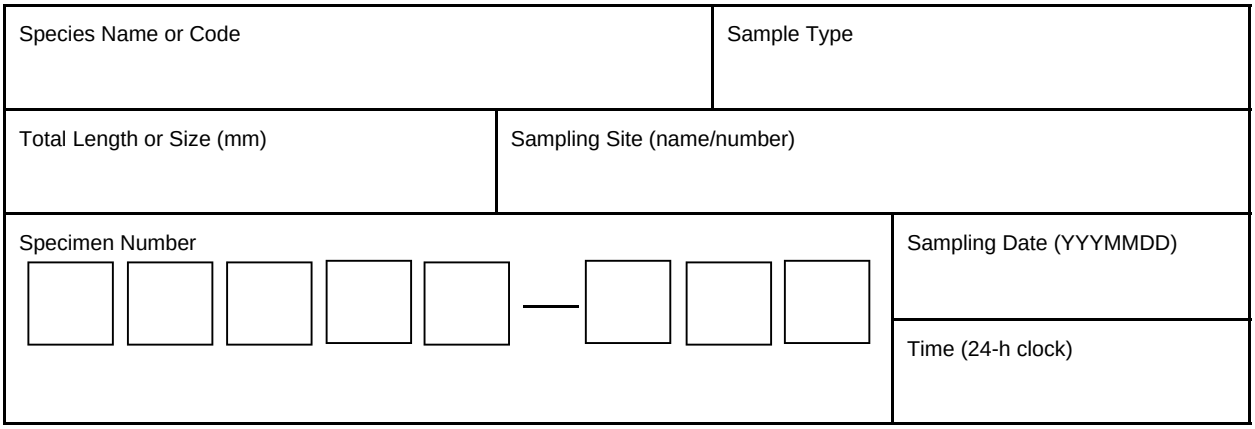


Figure 6-7. Example of a sample identification label.

- Sampling site—waterbody name and/or identification number
- Sampling date/time (give date in a Year 2000 compliant format [YYYYMMDD] and specify convention for time, e.g., 24-h clock).

A completed sample identification label should be taped to each aluminum-foil-wrapped specimen and the specimen should be placed in a waterproof plastic bag.

6.2.3.3 Chain-of-Custody Label or Tag—

A COC label or tag should be completed in indelible ink for each individual fish specimen. The information to be completed for each fish is shown in Figure 6-8.

<table>
<tr><td>Project Number</td><td colspan="5">Collection Agency (name, address, phone)</td></tr>
<tr><td colspan="2">Sampling Site (name and/or ID number)</td><td colspan="4">Sampler (name and signature)</td></tr>
<tr><td>Composition Number/Specimen Number(s)</td><td colspan="2">Chemical Analyses
☐ All target analytes
☐ Others (specify) _____</td><td colspan="3">Study Type</td></tr>
<tr><td rowspan="2">Sampling Date (YYYYMMDD) Time (24-h clock)</td><td colspan="2" rowspan="2">_____
_____</td><td rowspan="2">Screening</td><td>Intensive</td></tr>
<tr><td>Phase I ☐
Phase II ☐</td></tr>
<tr><td rowspan="2">Species Name or Code</td><td colspan="2">Processing</td><td colspan="2">Type of Ice</td></tr>
<tr><td>Whole Body</td><td>Resection</td><td>Wet</td><td>Dry</td></tr>
<tr><td colspan="5">Comments</td></tr>
</table>

Figure 6-8. Example of a chain-of-custody tag or label.

After all information has been completed, the COC label or tag should be taped or attached with string to the outside of the waterproof plastic bag containing the individual fish sample. Information on the COC label/tag should also be recorded on the COC form (Figure 6-9).

Because of the generally smaller size of shellfish, several individual aluminum-foil-wrapped shellfish specimens (within the same composite sample) may be placed in the same waterproof plastic bag. A COC label or tag should be completed in indelible ink for each shellfish composite sample. If more than 10 individual

Chain-of-Custody Record

Project Number	Collecting Agency (name, address, phone)	Sampling Date	Chemical Analyses: All Target Contaminants	Chemical Analyses: Specific Contaminants Only (Specify)	
Samplers (print and sign)		Container ____ of ____			

Composite Number	Specimen Nos.	Sampling Time	Study Type: Scr	Study Type: Int	Sampling Site (name/number)			Comments

Delivery Shipment Record	Deliver/Ship to: (name, address and phone)	Date/Time Shipped:
Delivery Method ☐ Hand carry ☐ Shipped		

Relinquished by: *(signature)*	Date / Time	Received by: *(signature)*	Relinquished by: *(signature)*	Date / Time	Received by: *(signature)*
Relinquished by: *(signature)*	Date / Time	Received for Central Processing Laboratory by: *(signature)*	Date / Time	Remarks:	

Laboratory Custody:

Released Name/Date	Received Name/Date	Purpose	Location

Figure 6-9. Example of a chain-of-custody record form.

shellfish are to be composited, several waterproof plastic bags may have to be used for the same composite. It is important not to place too many individual specimens in the same plastic bag to ensure proper preservation during shipping, particularly during summer months. Information on the COC label/tag should also be recorded on the COC form (Figure 6-9).

6.2.3.4 Chain-of-Custody Form—

A COC form should be completed in indelible ink for each shipping container (e.g., ice chest) used. Information recommended for documentation on the COC form (Figure 6-9) is necessary to track all samples from field collection to receipt at the processing laboratory. In addition, this form can be used for tracking samples through initial laboratory processing (e.g., resection) as described in Section 7.2.

Prior to sealing the ice chest, one copy of the COC form and a copy of the field record sheet should be sealed in a resealable waterproof plastic bag. This plastic bag should be taped to the inside cover of the ice chest so that it is maintained with the samples being tracked. Ice chests should be sealed with reinforced tape for shipment.

6.2.3.5 Field Logbook—

In addition to the four sample tracking forms discussed above, the field collection team should document in a field logbook any additional information on sample collection activities, hydrologic conditions (e.g., tidal stage), weather conditions, boat or equipment operations, or any other unusual activities observed (e.g., dredging) or problems encountered that would be useful to the program manager in evaluating the quality of the fish and shellfish contaminant monitoring data.

6.3 SAMPLE HANDLING

6.3.1 Sample Selection

6.3.1.1 Species Identification—

As soon as fish, shellfish, and turtles are removed from the collection device, they should be identified by species. Nontarget species or specimens of target species that do not meet size requirements (e.g., juveniles) should be returned to the water. Species identification should be conducted only by experienced personnel knowledgeable of the taxonomy of species in the waterbodies included in the contaminant monitoring program. Taxonomic keys, appropriate for the waters being sampled, should be consulted for species identification. Because the objective of both the screening and intensive monitoring studies is to determine the magnitude of contamination in specific fish, shellfish, and turtle species, it is necessary that all individuals used in a composite sample be of a single species. **Note:** Correct species identification is important and different species should never be combined in a single composite sample.

When sufficient numbers of the target species have been identified to make up a composite sample, the species name and all other appropriate information should be recorded on the field record forms (Figures 6-3 through 6-6).

Note: EPA recommends that, when turtles are used as the target species, target analyte concentrations be determined for each turtle rather than for a composite turtle sample.

6.3.1.2 Initial Inspection and Sorting—

Individual fish of the selected target species should be rinsed in ambient water to remove any foreign material from the external surface. Large fish should be stunned by a sharp blow to the base of the skull with a wooden club or metal rod. This club or rod should be used solely for the purpose of stunning fish, and care should be taken to keep it reasonably clean to prevent contamination of the samples (Versar, 1982). Small fish may be placed on ice immediately after capture to stun them, thereby facilitating processing and packaging procedures. Once stunned, individual specimens of the target species should be grouped by species and general size class and placed in clean holding trays to prevent contamination. All fish should be inspected carefully to ensure that their skin and fins have not been damaged by the sampling equipment, and damaged specimens should be discarded (Versar, 1982).

Freshwater turtles should be rinsed in ambient water and their external surface scrubbed if necessary to remove any foreign matter from their carapace and limbs. Each turtle should be inspected carefully to ensure that the carapace and extremities have not been damaged by the sampling equipment, and damaged specimens should be discarded (Versar, 1982). Care should be taken when handling large turtles, particularly snapping turtles; many can deliver severe bites. Particularly during procedures that place fingers or hands within striking range of the sharp jaws, covering the turtle's head, neck, and forelimbs with a cloth towel or sack and taping it in place is often sufficient to prevent injury to the field sampling crew (Frye, 1994).

After inspection, each turtle should be placed individually in a heavy burlap sack or canvas bag tied tightly with a strong cord and then placed in an ice-filled cooler. Placing turtles on ice will slow their metabolic rate, making them easier to handle. **Note:** It is recommended that each turtle be analyzed as an individual sample, especially if the target turtle species is not abundant in the waterbody being sampled or if the collected individuals differ greatly in size or age. Analysis of individual turtles can provide an estimate of the maximum contaminant concentrations to which recreational or substistence fishers are exposed. Target analyte concentrations in composite samples represent averages for a specific target species population. The use of these values in risk assessment is appropriate if the objective is to estimate the average concentration to which consumers of the target species are exposed over a long period of time. The use of long exposure periods (e.g., 70 years) is typical for the assessment of

carcinogenic effects, which may be manifest over an entire lifetime (see Volume II of this guidance series). Noncarcinogenic effects, on the other hand, may cause acute health effects over a relatively short period of time (e.g., hours or days) after consumption. The maximum target analyte contaminant concentration may be more appropriate than the average target analyte concentration for use with noncarginogenic target analytes (U.S. EPA, 1989d). This is especially important for those target analytes for which acute exposures to very high concentrations may be toxic to consumers.

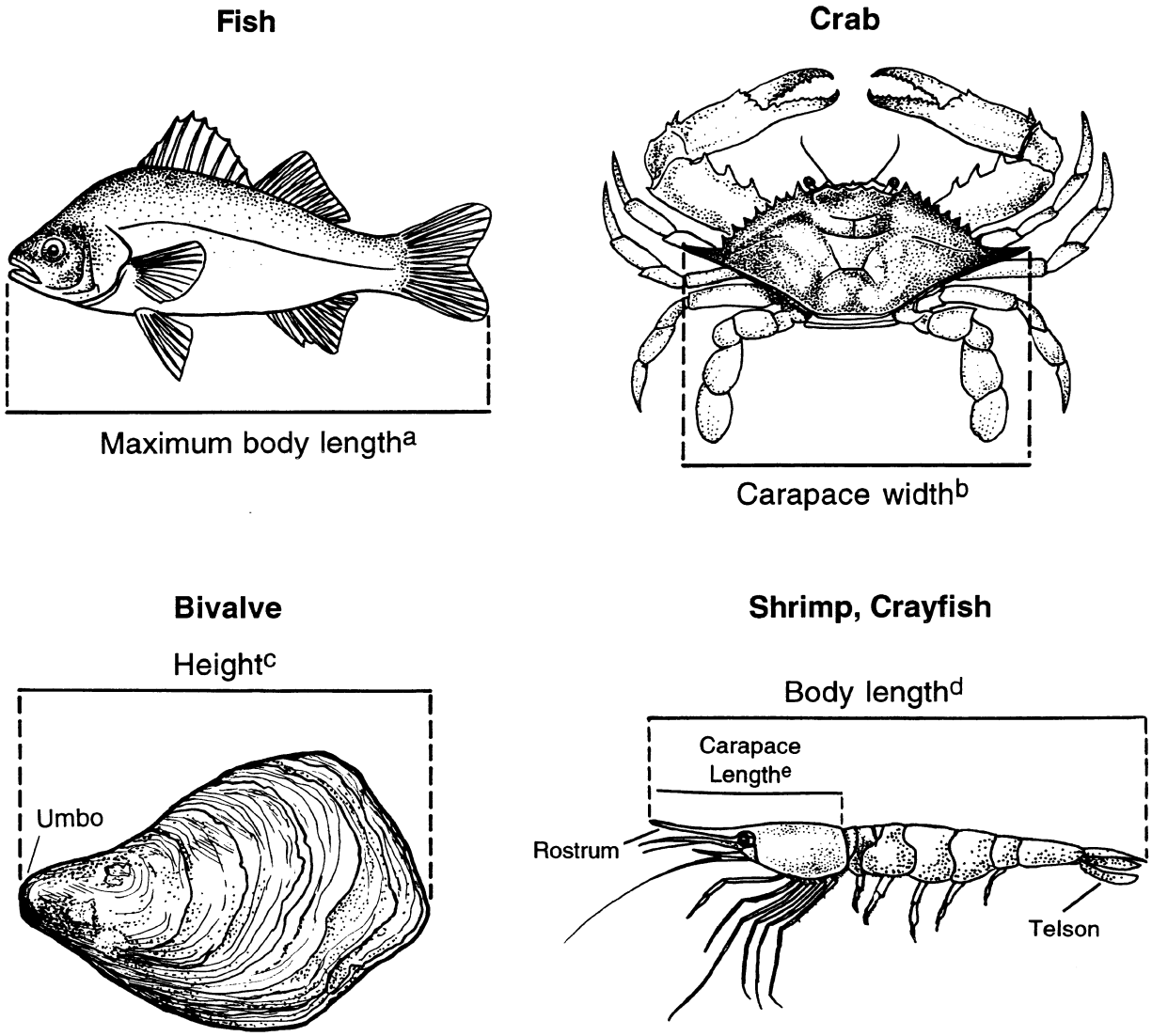
Stone et al. (1980) reported extremely high concentrations of PCBs in various tissues of snapping turtles from a highly contaminated site on the Hudson River. Contaminant analysis of various turtle tissues showed mean PCB levels of 2,991 ppm in fatty tissue, 66 ppm in liver tissue, and 29 ppm in eggs as compared to 4 ppm in skeletal muscle. Clearly, inclusion of the fatty tissue, liver, and eggs with the muscle tissues as part of the edible tissues will increase observed residue concentrations over those detected in muscle tissue only. States interested in using turtles as target species should review Appendix C for additional information on the use of individual samples in contaminant monitoring programs.

Bivalves (oysters, clams, scallops, and mussels) adhering to one another should be separated and scrubbed with a nylon or natural fiber brush to remove any adhering detritus or fouling organisms from the exterior shell surfaces (NOAA, 1987). All bivalves should be inspected carefully to ensure that the shells have not been cracked or damaged by the sampling equipment and damaged specimens should be discarded (Versar, 1982). Crustaceans, including shrimp, crabs, crayfish, and lobsters, should be inspected to ensure that their exoskeletons have not been cracked or damaged during the sampling process, and damaged specimens should be discarded (Versar, 1982). After shellfish have been rinsed, individual specimens should be grouped by target species and placed in clean holding trays to prevent contamination.

A few shellfish specimens may be resected (edible portions removed) to determine wet weight of the edible portions. This will provide an estimate of the number of individuals required to ensure that the recommended sample weight (200 g) is attained. **Note:** Individuals used to determine the wet weight of the edible portion should not be used for target analyte analyses.

6.3.1.3 Length or Size Measurements—

Each fish within the selected target species should be measured to determine total body length (mm). To be consistent with the convention used by most fisheries biologists in the United States, maximum body length should be measured as shown in Figure 6-10. The maximum body length is defined as the length from the anterior-most part of the fish to the tip of the longest caudal fin ray (when the lobes of the caudal fin are compressed dorsoventrally) (Anderson and Gutreuter, 1983).



[a] Maximum body length is the length from the anterior-most part of the fish to the tip of the longest caudal fin ray (when the lobes of the caudal fin are compressed dorsoventrally (Anderson and Gutreuter, 1983).

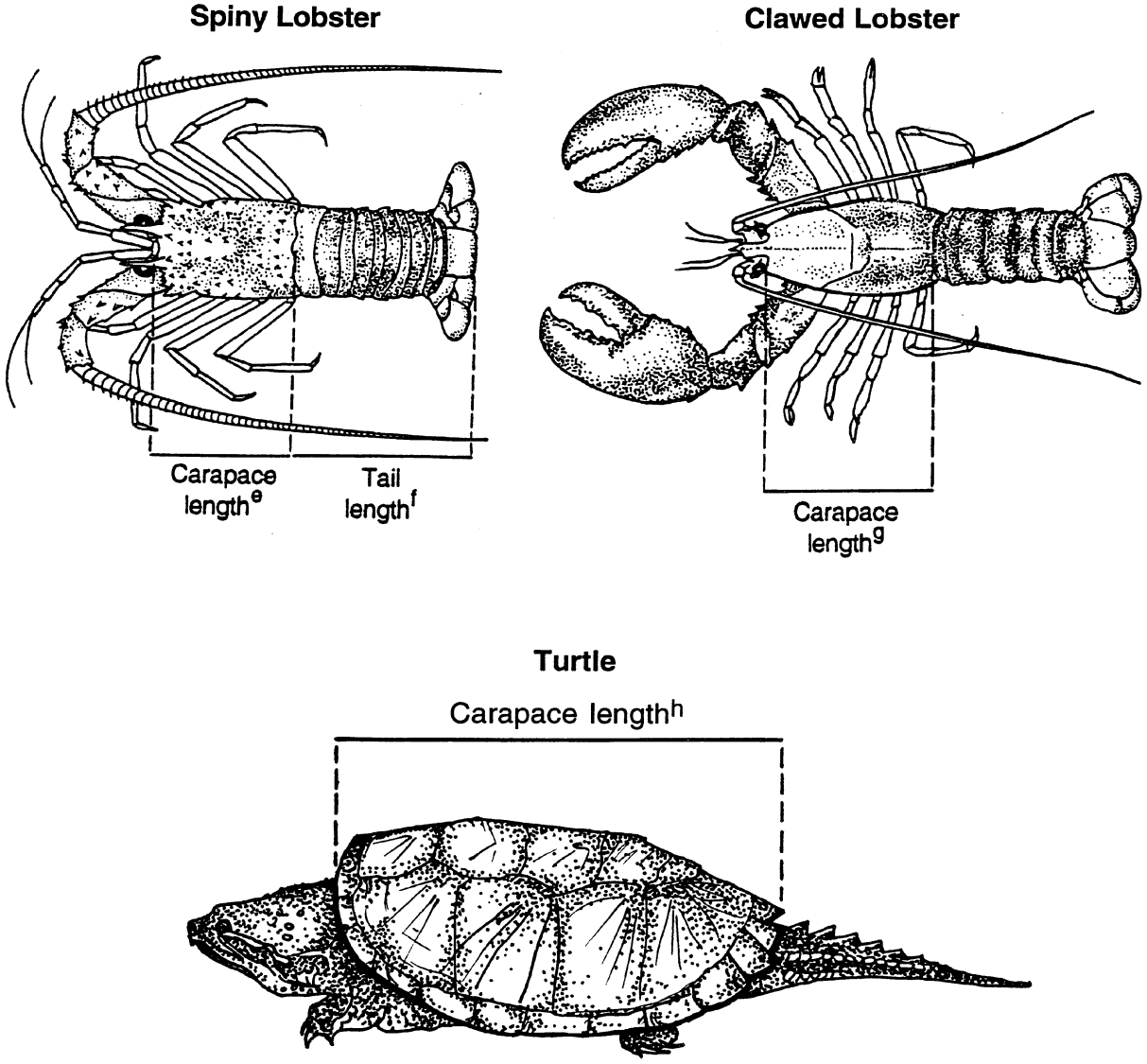
[b] Carapace width is the lateral distance across the carapace (from tip of spine to tip of spine (U.S. EPA, 1990c).

[c] Height is the distance from the umbo to the anterior (ventral) shell margin (Galtsoff, 1964).

[d] Body length is the distance from the tip of the rostrum to the tip of the telson (Texas Water Commission, 1990).

[e] Carapace length is distance from top of rostrum to the posterior margin of the carapace.

Figure 6-10. Recommended measurements of body length and size for fish, shellfish, and turtles.



[e] Carapace length is the distance from the anterior-most edge of the groove between the horns directly above the eyes, to the rear edge of the top part of the carapace as measured along the middorsal line of the back (Laws of Florida Chapter 46-24.003).

[f] Tail length is the distance measured lengthwise along the top middorsal line of the entire tail to rear-most extremity (this measurement shall be conducted with the tail in a flat straight position with the tip of the tail closed) (Laws of Florida Chapter 46-24.003).

[g] Carapace length is the distance from the rear of the eye socket to the posterior margin of the carapace (New York Environmental Conservation Law 13-0329.5.a and Massachusetts General Laws Chapter 130).

[h] Carapace length is the straight-line distance from the anterior margin to the posterior margin of the shell (Conant and Collins, 1991).

Figure 6-10. (continued)

Each turtle within the selected target species should be measured to determine total carapace length (mm). To be consistent with the convention used by most herpetologists in the United States, carapace length should be measured as shown in Figure 6-10. The maximum carapace length is defined as the straight line distance from the anterior edge of the carapace to the posterior edge of the carapace (Conant and Collins, 1991).

For shellfish, each individual specimen should be measured to determine the appropriate body size (mm). As shown in Figure 6-9, the recommended body measurements differ depending on the type of shellfish being collected. Height is a standard measurement of size for oysters, mussels, clams, scallops, and other bivalve molluscs (Abbott, 1974; Galtsoff, 1964). The height is the distance from the umbo to the anterior (ventral) shell margin. For crabs, the lateral width of the carapace is a standard size measurement (U.S. EPA, 1990c); for shrimp and crayfish, the standard measurement of body size is the length from the rostrum to the tip of the telson (Texas Water Commission, 1990); and for lobsters, two standard measurements of body size are commonly used. For clawed and spiny lobsters, the standard size is the length of the carapace. For spiny lobsters, the length of the tail is also used as a standard size measurement.

6.3.1.4 Sex Determination (Optional)—

An experienced fisheries biologist can often make a preliminary sex determination for fish by visual inspection. The body of the fish should not be dissected in the field to determine sex; sex can be determined through internal examination of the gonads during laboratory processing (Section 7.2.2.4).

An experienced herpetologist can often make a preliminary sex determination of a turtle by visual inspection in the field. The plastron (ventral portion of the carapace) is usually flatter in the female and the tail is less well developed than in the male. The plastron also tends to be more concave in the male (Holmes, 1984). For the common snapping turtle (*Chelydra serpentina*), the cloaca of the female is usually located inside or at the perimeter of the carapace, while the cloaca of the male extends slightly beyond the perimeter of the carapace. The carapace of the turtle should never be resected in the field to determine sex; sex can be determined through internal examination of the gonads during laboratory processing (Section 7.2.3.4.). For shellfish, a preliminary sex determination can be made by visual inspection only for crustaceans. Sex cannot be determined in bivalve molluscs without shucking the bivalves and microscopically examining gonadal material. Bivalves should not be shucked in the field to determine sex; sex determination through examination of the gonads can be performed during laboratory processing if desired (Section 7.2.4.2).

6.3.1.5 Morphological Abnormalities (Optional)—

If resources allow, states may wish to consider documenting external gross morphological conditions in fish from contaminated waters. Severely polluted

aquatic habitats have been shown to produce a higher frequency of gross pathological disorders than similar, less polluted habitats (Krahn et al., 1986; Malins et al., 1984, 1985; Mix, 1986; Sinderman, 1983; and Sinderman et al., 1980).

Sinderman et al. (1980) reviewed the literature on the relationship of fish pathology to pollution in marine and estuarine environments and identified four gross morphological conditions acceptable for use in monitoring programs:

- Fin erosion
- Skin ulcers
- Skeletal anomalies
- Neoplasms (i.e., tumors).

Fin erosion is the most frequently observed gross morphological abnormality in polluted areas and is found in a variety of fishes (Sinderman, 1983). In demersal fishes, the dorsal and anal fins are most frequently affected; in pelagic fishes, the caudal fin is primarily affected.

Skin ulcers have been found in a variety of fishes from polluted waters and are the second most frequently reported gross abnormality. Prevalence of ulcers generally varies with season and is often associated with organic enrichment (Sinderman, 1983).

Skeletal anomalies include abnormalities of the head, fins, gills, and spinal column (Sinderman, 1983). Skeletal anomalies of the spinal column include fusions, flexures, and vertebral compressions.

Neoplasms or tumors have been found at a higher frequency in a variety of polluted areas throughout the world. The most frequently reported visible tumors are liver tumors, skin tumors (i.e., epidermal papillomas and/or carcinomas), and neurilemmomas (Sinderman, 1983).

The occurrence of fish parasites and other gross morphological abnormalities that are found at a specific site should be noted on the field record form. States interested in documenting morphological abnormalities in fish should review the protocols for fish pathology studies recommended in the Puget Sound Estuary Program (1990c) and those described by Goede and Barton (1990).

6.3.2 Sample Packaging

6.3.2.1 Fish—

After initial processing to determine species, size, sex, and morphological abnormalities, each fish should be individually wrapped in extra heavy duty aluminum foil. Spines on fish should be sheared to minimize punctures in the aluminum foil packaging (Stober, 1991). The sample identification label shown in Figure 6-7 should be taped to the outside of each aluminum foil package, each individual fish should be placed into a waterproof plastic bag and sealed, and the

COC tag or label should be attached to the outside of the plastic bag with string or tape. All of the packaged individual specimens in a composite sample should be kept together (if possible) in one large waterproof plastic bag in the same shipping container (ice chest) for transport. Once packaged, samples should be cooled on ice immediately.

6.3.2.2 Turtles—

After inital processing to determine the species, size (carapace length), and sex, each turtle should be placed on ice in a separate burlap or canvas bag and stored on ice for transport to the processing laboratory. A completed sample identification label (Figure 6-7) should be attached with string around the neck or one of the turtle's extremities and the COC tag or label should be attached to the outside of the bag with string or tape. **Note:** Bagging each turtle should not be undertaken until the specimen has been sufficiently cooled to induce a mild state of torpor, thus facilitating processing. The samplers should work rapidly to return each turtle to the ice chest as soon as possible after packaging as the turtle may suddenly awaken as it warms thus becoming a danger to samplers (Frye, 1994). As mentioned in Section 6.3.1, states should analyze turtles individually rather than compositing samples. This is especially important when very few specimens are collected at a sampling site or when specimens of widely varying size or age are collected.

Note: When a large number of individual specimens in the same composite sample are shipped together in the same waterproof plastic bag, the samples must have adequate space in the bag to ensure that contact with ice can occur, thus ensuring proper preservation during shipping. This is especially important when samples are collected during hot weather and/or when the time between field collection and delivery to the processing laboratory approaches the maximum shipping time (Table 6-8).

6.3.2.3 Shellfish—

After initial processing to determine species, size, sex, and morphological abnormalities, each shellfish specimen should be wrapped individually in extra heavy duty aluminum foil. A completed sample identification label (Figure 6-7) should be taped to the outside of each aluminum foil package. **Note**: Some crustacean species (e.g., blue crabs and spiny lobsters) have sharp spines on their carapace that might puncture the aluminum foil wrapping. Carapace spines should never be sheared off because this would destroy the integrity of the carapace. For such species, one of the following procedures should be used to reduce punctures to the outer foil wrapping:

- Double-wrap the entire specimen in extra heavy duty aluminum foil.
- Place clean cork stoppers over the protruding spines prior to wrapping the specimen in aluminum foil.

Table 6-8. Recommendations for Preservation of Fish, Shellfish, and Turtle Samples from Time of Collection to Delivery at the Processing Laboratory

Sample type	Number per composite	Container	Preservation	Maximum shipping time
Fish[a]				
Whole fish (to be filleted)	3-10	Extra heavy duty aluminum foil wrap of each fish.[b] Each fish is placed in a waterproof plastic bag.	Cool on wet ice or blue ice packets (preferred method) or Freeze on dry ice only if shipping time will exceed 24 hours	24 hours 48 hours
Whole fish	3-10	Same as above.	Cool on wet ice or blue ice packets or Freeze on dry ice	24 hours 48 hours
Shellfish[a]				
Whole shellfish (to be resected for edible tissue)	3-50[c]	Extra heavy duty aluminum foil wrap of each specimen.[b] Shellfish in the same composite sample may be placed in the same waterproof plastic bag.	Cool on wet ice or blue ice packets (preferred method) or Freeze on dry ice if shipping time will exceed 24 hours	24 hours 48 hours
Whole shellfish	3-50[c]	Same as above.	Cool on wet ice or blue ice packets or Freeze on dry ice	24 hours 48 hours
Whole turtles (to be resected for edible tissue)	1[d]	Heavy burlap or canvas bags.	Cool on wet ice or blue ice packets (preferred method) or Freeze on dry ice if shipping time to exceed 24 hours	24 hours 48 hours

[a] Use only individuals that have attained at least legal harvestable or consumable size.
[b] Aluminum foil should not be used for long-term storage of any sample (i.e., whole organisms, fillets, or homogenates) that will be analyzed for metals.
[c] Species and size dependent. For very small shellfish species, more than 50 individuals may be required to achieve the 200-g composite sample mass recommended for screening studies.
[d] Turtles should be analyzed as individual rather than as composite samples.

- Wrap the spines with multiple layers of foil before wrapping the entire specimen in aluminum foil.

All of the individual aluminum-foil-wrapped shellfish specimens (in the same composite sample) should be placed in the same waterproof plastic bag for transport. In this case, a COC tag or label should be completed for the composite sample and appropriate information recorded on the field record sheet and COC form. The COC label or tag should then be attached to the outside of the plastic

bag with string or tape. For composite samples containing more than 10 shellfish specimens or especially large individuals, additional waterproof plastic bags may be required to ensure proper preservation. Once packaged, composite samples should be cooled on ice immediately. **Note**: When a large number of individual specimens in the same composite sample are shipped together in the same waterproof plastic bag, the samples must have adequate space in the bag to ensure that contact with ice can occur; thus ensuring proper preservation during shipping. This is especially important when samples are collected during hot weather and/or when the time between field collection and delivery to the processing laboratory approaches the maximum shipping time (Table 6-8).

6.3.3 Sample Preservation

The type of ice to be used for shipping should be determined by the length of time the samples will be in transit to the processing laboratory and the sample type to be analyzed (Table 6-8).

6.3.3.1 Fish, Turtles, or Shellfish To Be Resected—

Note: Ideally fish, turtles, and shellfish specimens should not be frozen prior to resection if analyses will include edible tissue only because freezing may cause some internal organs to rupture and contaminate fillets or other edible tissues (Stober, 1991; U.S. EPA, 1986b). Wet ice or blue ice (sealed prefrozen ice packets) is recommended as the preservative of choice when the fish fillet, turtle meat, or shellfish edible portions are the primary tissues to be analyzed. Samples shipped on wet or blue ice should be delivered to the processing laboratory within 24 hours (Smith, 1985; U.S. EPA, 1990d). If the shipping time to the processing laboratory will exceed 24 hours, dry ice should be used.

Note: One exception to the use of dry ice for long-term storage is if fish or shellfish are collected as part of extended offshore field surveys. States involved in these types of field surveys may employ shipboard freezers to preserve samples for extended periods rather than using dry ice. Ideally, all fish should be resected in cleanrooms aboard ship prior to freezing.

6.3.3.2 Fish, Turtles, or Shellfish for Whole-Body Analysis—

At some sites, states may deem it necessary to collect fish, turtles, or shellfish for whole-body analysis if a local subpopulation of concern typically consumes whole fish, turtles, or shellfish. If whole fish, turtles, or shellfish samples are to be analyzed, either wet ice, blue ice, or dry ice may be used; however, if the shipping time to the processing laboratory will exceed 24 hours, dry ice should be used.

Dry ice requires special packaging precautions before shipping by aircraft to comply with U.S. Department of Transportation (DOT) regulations. The *Code of Federal Regulations* (49 CFR 173.217) classifies dry ice as Hazard Class 9 UN1845 (Hazardous Material). These regulations specify the amount of dry ice

that may be shipped by air transport and the type of packaging required. For each shipment by air exceeding 5 pounds of dry ice per package, advance arrangements must be made with the carrier. Not more than 441 pounds of dry ice may be transported in any one cargo compartment on any aircraft unless the shipper has made special written arrangements with the aircraft operator.

The regulations further specify that the packaging must be designed and constructed to permit the release of carbon dioxide gas to prevent a buildup of pressure that could rupture the package. If samples are transported in a cooler, several vent holes should be drilled to allow carbon dioxide gas to escape. The vents should be near the top of the vertical sides of the cooler, rather than in the cover, to prevent debris from falling into the cooler. Wire screen or cheesecloth should be installed in the vents to keep foreign materials from contaminating the cooler. When the samples are packaged, care should be taken to keep these vents open to prevent the buildup of pressure.

Dry ice is exempted from shipping certification requirements if the amount is less than 441 pounds and the package meets design requirements. The package must be marked "Carbon Dioxide, Solid" or "Dry Ice" with a statement indicating that the material being refrigerated is to be used for diagnostic or treatment purposes (e.g., frozen tissue samples).

6.3.4 Sample Shipping

The fish, turtle, and shellfish samples should be hand-delivered or shipped to the processing laboratory as soon as possible after collection. The time the samples were collected and time of their arrival at the processing laboratory should be recorded on the COC form (Figure 6-9).

If the sample is to be shipped rather than hand-delivered to the processing laboratory, field collection staff must ensure the samples are packed properly with adequate ice layered between samples so that sample degradation does not occur. In addition, a member of the field collection staff should telephone ahead to the processing laboratory to alert them to the anticipated delivery time of the samples and the name and address of the carrier to be used. Field collection staff should avoid shipping samples for weekend delivery to the processing laboratory unless prior plans for such a delivery have been agreed upon with the processing laboratory staff.

SECTION 7

LABORATORY PROCEDURES I — SAMPLE HANDLING

This section provides guidance on laboratory procedures for sample receipt, chain-of-custody, processing, distribution, analysis, and archiving. Planning, documentation, and quality assurance and quality control of all laboratory activities are emphasized to ensure that (1) sample integrity is preserved during all phases of sample handling and analysis, (2) chemical analyses are performed cost-effectively and meet program data quality objectives, and (3) data produced by different states and regions are comparable.

Laboratory procedures should be documented in a Work/QA Project Plan (U.S. EPA, 1980b) as described in Appendix I. Routine sample processing and analysis procedures should be prepared as standard operating procedures (SOPs) (U.S. EPA, 1984b).

7.1 SAMPLE RECEIPT AND CHAIN-OF-CUSTODY

Fish, shellfish, and turtle samples may be shipped or hand-carried from the field according to one or more of the following pathways:

- From the field to a state laboratory for sample processing and analysis
- From the field to a state laboratory for sample processing and shipment of composite sample aliquots to a contract laboratory for analysis
- From the field to a contract laboratory for sample processing and analysis.

Sample processing and distribution for analysis ideally should be performed by one processing laboratory. Transportation of samples from the field should be coordinated by the sampling team supervisor and the laboratory supervisor responsible for sample processing and distribution (see Section 6.3.4). An accurate written custody record must be maintained so that possession and treatment of each sample can be traced from the time of collection through analysis and final disposition.

Fish, shellfish, and turtle samples should be brought or shipped to the sample processing laboratory in sealed containers accompanied by a copy of the sample request form (Figure 6-1), a chain-of-custody form (Figure 6-9), and the field records (Figures 6-3 through 6-6). Each time custody of a sample or set of samples is transferred, the Personnel Custody Record of the COC form must be completed and signed by both parties. Corrections to the COC form should be made in indelible ink by drawing a single line through the original entry, entering

the correct information and the reason for the change, and initialing and dating the correction. The original entry should never be obscured.

When custody is transferred from the field to the sample processing laboratory, the following procedure should be used:

- Note the shipping time. If samples have been shipped on wet or blue ice, check that the shipping time has not exceeded 24 hours.

- Check that each shipping container has arrived undamaged and that the seal is intact.

- Open each shipping container and remove the copy of the sample request form, the COC form, and the field records.

- Note the general condition of the shipping container (samples iced properly with no leaks, etc.) and the accompanying documentation (dry, legible, etc.).

- Locate individuals in each composite sample listed on the COC form and note the condition of their packaging. Individual specimens should be properly wrapped and labeled. Note any problems (container punctured, illegible labels, etc.) on the COC form.

- If individuals in a composite are packaged together, check the contents of each composite sample container against the field record for that sample to ensure that the individual specimens are properly wrapped and labeled. Note any discrepancies or missing information on the COC form.

- Initial the COC form and record the date and time of sample receipt.

- Enter the following information for each composite sample into a permanent laboratory record book and, if applicable, a computer database:

  — Sample identification number (specify conventions for the composite sample number and the specimen number) **Note:** EPA recommends processing and analysis of turtles as individual samples.

  — Receipt date (use Year 2000 comliant format [YYYYMMDD])

  — Sampling date (use Year 2000 comliant format [YYYYMMDD])

  — Sampling site (name and/or identification number)

  — Fish, turtle, and shellfish species (scientific name or code number)

  — Total length of each fish, carapace length of each turtle, or size of each shellfish (mm)

- If samples have been shipped on wet or blue ice, distribute them immediately to the technician responsible for resection (see Section 7.2). See Section 7.2.3 for the procedure for processing turtle samples as individual samples. If samples have been shipped on dry ice, they may be distributed immediately to the technician for processing or stored in a freezer at ≤-20 °C for later processing. Once processed, fillets or edible portions of fish, turtles, or shellfish or tissue homogenates, should be stored according to the procedures described in Section 7.2 and in Table 7-1. **Note**: Holding times in Table 7-1 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, 1995i). 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.

7.2 SAMPLE PROCESSING

This section includes recommended procedures for preparing composite homogenate samples of fish fillets and edible portions of shellfish and individual samples of edible portions of freshwater turtles as required in screening and intensive studies. Recommended procedures for preparing whole fish composite homogenates are included in Appendix J for use by states in assessing the potential risk to local subpopulations known to consume whole fish or shellfish.

7.2.1 General Considerations

All laboratory personnel performing sample processing procedures (see Sections 7.2.2, 7.2.3, and 7.2.4) should be trained or supervised by an experienced fisheries biologist. Care must be taken during sample processing to avoid contaminating samples. Schmitt and Finger (1987) have demonstrated that contamination of fish flesh samples is likely unless the most exacting clean dissection procedures are used. Potential sources of contamination include dust, instruments, utensils, work surfaces, and containers that may contact the samples. All sample processing (i.e., filleting, removal of other edible tissue, homogenizing, compositing) should be done in an appropriate laboratory facility under cleanroom conditions (Stober, 1991). Cleanrooms or work areas should be free of metals and organic contaminants. Ideally, these areas should be under positive pressure with filtered air (HEPA filter class 100) (California Department of Fish and Game, 1990). Periodic wipe tests should be conducted in clean areas to verify the absence of significant levels of metal and organic contaminants. All instruments, work surfaces, and containers used to process samples must be of materials that can be cleaned easily and that are not themselves potential sources of contamination. More detailed guidance on establishing trace metal cleanrooms is provided in U.S. EPA (1995a).

Table 7-1. Recommendations for Container Materials, Preservation, and Holding Times for Fish, Shellfish, and Turtle Tissues from Receipt at Sample Processing Laboratory to Analysis

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

PTFE = Polytetrafluoroethylene (Teflon).

[a] Maximum holding times recommended by EPA (1995i).

[b] This maximum holding time is also recommended by the Puget Sound Estuary Program (1990e). 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, 1986b), and the USGS National Water Quality Assessment Program (Crawford and Luoma, 1993). The Puget Sound Estuary Program (1990e) recommends a maximum holding time of 2 years.

[d] This maximum holding time is also recommended by the Puget Sound Estuary Program (1990e). 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. U.S. EPA (1995b) recommends a maximum holding time of 1 year at ≤-10 °C for dioxins/furans.

To avoid cross-contamination, all equipment used in sample processing (i.e., resecting, homogenizing, and compositing) should be cleaned thoroughly before each composite sample is prepared. Verification of the efficacy of cleaning procedures should be documented through the analysis of processing blanks or rinsates (see Section 8.3.3.6).

Because sources of organic and metal contaminants differ, it is recommended that duplicate samples be collected, if time and funding permit, when analyses of both organics and metals are required (e.g., for screening studies). One sample can then be processed and analyzed for organics and the other can be processed independently and analyzed for metals (Batelle, 1989; California Department of Fish and Game, 1990; Puget Sound Estuary Program, 1990c, 1990d). If fish are of adequate size, separate composites of individual fillets may be prepared and

analyzed independently for metals and organics. If only one composite sample is prepared for the analyses of metals and organics, the processing equipment must be chosen and cleaned carefully to avoid contamination by both organics and metals.

Suggested sample processing equipment and cleaning procedures by analysis type are discussed in Sections 7.2.1.1 through 7.2.1.3. Other procedures may be used if it can be demonstrated, through the analysis of appropriate blanks, that no contamination is introduced (see Section 8.3.3.6).

7.2.1.1 Samples for Organics Analysis—

Equipment used in processing samples for organics analysis should be of stainless steel, anodized aluminum, borosilicate glass, polytetrafluoroethylene (PTFE), ceramic, or quartz. Polypropylene and polyethylene (plastic) surfaces, implements, gloves, and containers are a potential source of contamination by organics and should not be used. If a laboratory chooses to use these materials, there should be clear documentation that they are not a source of contamination. Filleting should be done 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 after each filleting. Tissue should be removed with clean, high-quality, corrosion-resistant stainless steel or quartz instruments or with knives with titanium blades and PTFE handles (Lowenstein and Young, 1986). Fillets or tissue homogenates may be stored in borosilicate glass, quartz, or PTFE containers with PTFE-lined lids or in heavy duty aluminum foil (see Table 7-1).

Prior to preparing each composite sample, utensils and containers should be washed with detergent solution, rinsed with tap water, soaked in pesticide-grade isopropanol or acetone, and rinsed with organic-free, distilled, deionized water. Work surfaces should be cleaned with pesticide-grade isopropanol or acetone, washed with distilled water, and allowed to dry completely. Knives, fish scalers, measurement boards, etc., should be cleaned with pesticide-grade isopropanol or acetone followed by a rinse with contaminant-free distilled water between each fish sample (Stober, 1991).

7.2.1.2 Samples for Metals Analysis—

Equipment used in processing samples for metals analyses should be of quartz, PTFE, ceramic, polypropylene, or polyethylene. The predominant metal contaminants from stainless steel are chromium and nickel. If these metals are not of concern, the use of high-quality, corrosion-resistant stainless steel for sample processing equipment is acceptable. Quartz utensils are ideal but expensive. For bench liners and bottles, borosilicate glass is preferred over plastic (Stober, 1991). Knives with titanium blades and PTFE handles are recommended for performing tissue resections (Lowenstein and Young, 1986). Borosilicate glass bench liners are recommended. Filleting may be done 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 after each fish. Fillets or tissue homogenates may be stored in plastic, borosilicate glass, quartz, or PTFE containers (see Table 7-1).

Prior to preparing each composite sample, utensils and containers should be cleaned thoroughly with a detergent solution, rinsed with tap water, soaked in acid, and then rinsed with metal-free water. Quartz, PTFE, glass, or plastic containers should be soaked in 50 percent $HN0_3$, for 12 to 24 hours at room temperature. **Note:** Chromic acid should not be used for cleaning any materials. Acids used should be at least reagent grade. Stainless steel parts may be cleaned as stated for glass or plastic, omitting the acid soaking step (Stober, 1991).

7.2.1.3 Samples for Both Organics and Metals Analyses—

As noted above, several established monitoring programs, including the Puget Sound Estuary Program (1990c, 1990d), the NOAA Mussel Watch Program (Battelle, 1989), and the California Mussel Watch Program (California Department of Fish and Game, 1990), recommend different procedures for processing samples for organics and metals analyses. However, this may not be feasible if fish are too small to allow for preparing separate composites from individual fillets or if resources are limited. If a single composite sample is prepared for the analyses of both organics and metals, precautions must be taken to use materials and cleaning procedures that are noncontaminating for both organics and metals.

Quartz, ceramic, borosilicate glass, and PTFE are recommended materials for sample processing equipment. If chromium and nickel are not of concern, high-quality, corrosion-resistant stainless steel utensils may be used. Knives with titanium blades and PTFE handles are recommended for performing tissue resections (Lowenstein and Young, 1986). Borosilicate glass bench liners are recommended. Filleting should be done 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 after each filleting. Fillets or tissue homogenates should be stored in clean borosilicate glass, quartz, or PTFE containers with PTFE-lined lids.

Prior to preparing each composite sample, utensils and containers should be cleaned thoroughly with a detergent solution, rinsed with tap water, soaked in 50 percent HNO_3, for 12 to 24 hours at room temperature, and then rinsed with organics- and metal-free water. **Note:** Chromic acid should not be used for cleaning any materials. Acids used should be at least reagent grade. Stainless steel parts may be cleaned using this recommended procedure with the acid soaking step method omitted (Stober, 1991).

Aliquots of composite homogenates taken for metals analysis (see Section 7.3.1) may be stored in plastic containers that have been cleaned according to the

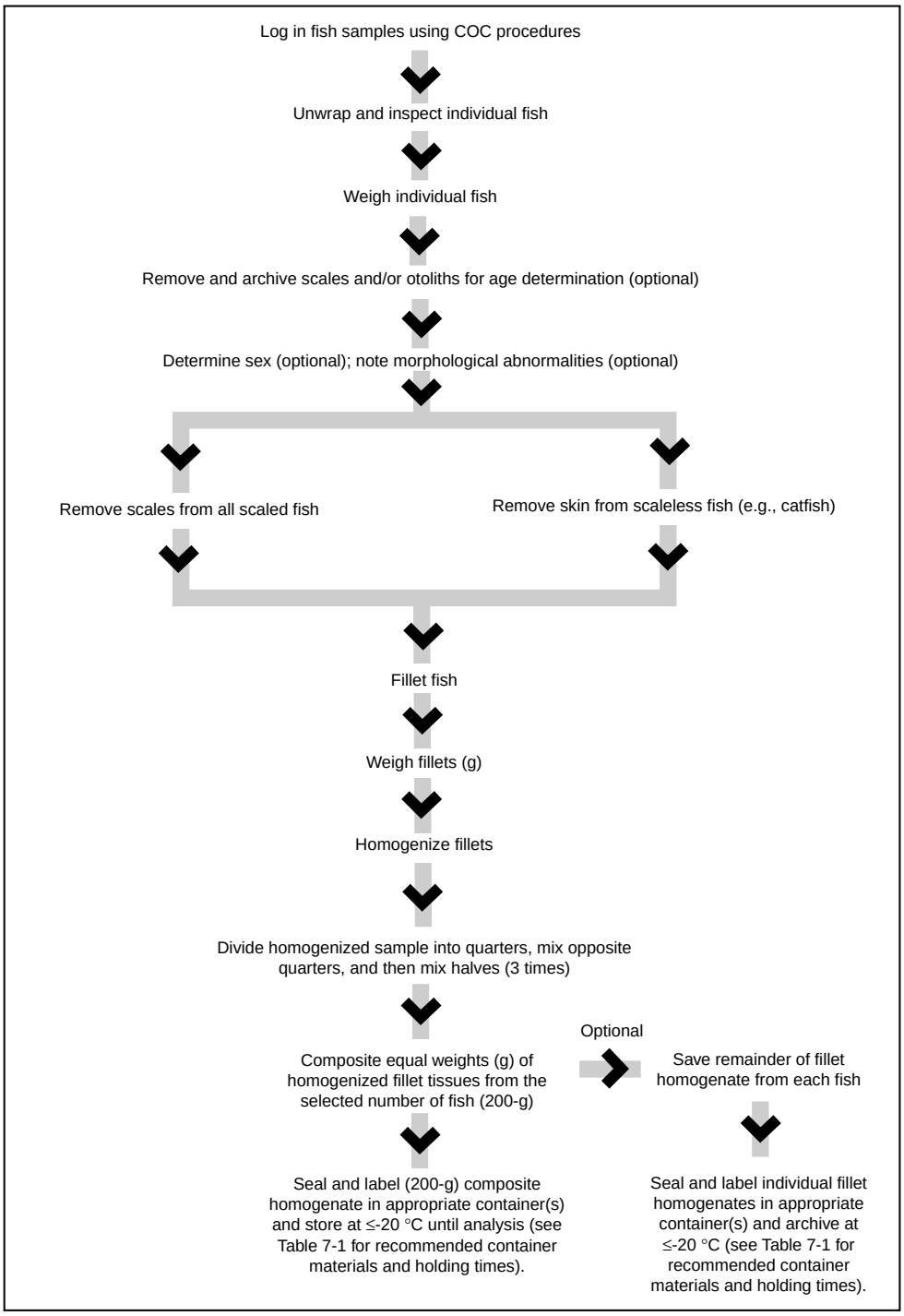
procedure outlined above, with the exception that aqua regia must not be used for the acid soaking step.

7.2.2 Processing Fish Samples

Processing in the laboratory to prepare fish fillet composite homogenate samples for analysis (diagrammed in Figure 7-1) involves

- Inspecting individual fish
- Weighing individual fish
- Removing scales and/or otoliths for age determination (optional)
- Determining the sex of each fish (optional)
- Examining each fish for morphological abnormalities (optional)
- Scaling all fish with scales (leaving belly flap on); removing skin of scaleless fish (e.g., catfish)
- Filleting (resection)
- Weighing fillets
- Homogenizing fillets
- Preparing a composite homogenate
- Preparing aliquots of the composite homogenate for analysis
- Distributing frozen aliquots to one or more analytical laboratories.

Whole fish should be shipped or brought to the sample processing laboratory from the field on wet or blue ice within 24 hours of sample collection. Fillets should be resected within 48 hours of sample collection. Ideally, fish should not be frozen prior to resection because freezing may cause internal organs to rupture and contaminate edible tissue (Stober, 1991; U.S. EPA, 1986b). However, if resection cannot be performed within 48 hours, the whole fish should be frozen at the sampling site and shipped to the sample processing laboratory on dry ice. Fish samples that arrive frozen (i.e., on dry ice) at the sample processing laboratory should be placed in a ≤ -20 °C freezer for storage until filleting can be performed. The fish should then be partially thawed prior to resection. **Note:** If the fillet tissue is contaminated by materials released from the rupture of the internal organs during freezing, the state may eliminate the fillet tissue as a sample or, alternatively, the fillet tissues should be rinsed in contaminant-free, distilled deionized



COC = Chain of custody.

Figure 7-1. Preparation of fish fillet composite homogenate samples.

water and blotted dry. Regardless of the procedure selected, a notation should be made in the sample processing record.

Sample processing procedures are discussed in the following sections. Data from each procedure should be recorded directly in a bound laboratory notebook or on forms that can be secured in the laboratory notebook. A sample processing record for fish fillet composites is shown in Figure 7-2.

7.2.2.1 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.

7.2.2.2 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. **Note:** Liquid from the thawed whole fish sample will come not only from the fillet tissue but from the gut and body cavity, which are not part of the final fillet sample. Consequently, inclusion of this liquid with the sample may result in an overestimate of target analyte and lipid concentrations in the fillet homogenate. Nevertheless, it is recommended, as a conservative approach, that all liquid from the thawed whole fish sample be kept in the container as part of the sample.

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

7.2.2.3 Age Determination (Optional)—

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

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

Project Number: _____ Sampling Date and Time: _____

STUDY PHASE: Screening Study ☐; Intensive Study: Phase I ☐ Phase II ☐

SITE LOCATION

Site Name/Number: _____

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

Waterbody Name/Segment Number: _____ Waterbody Type: _____

Sample Type (bottom feeder, predator, etc.) _____ **Species Name:** _____

Composite Sample #: _____ Replicate Number: _____ Number of Individuals: _____

					First Fillet (F1) or Combined Fillets (C)			Second Fillet (F2)		
Fish #	**Weight (g)**	**Scales/Otoliths Removed (✓)**	**Sex (M,F)**	**Resection Performed (✓)**	**Weight (g)**	**Homogenate Prepared (✓)**	**Wt. of Homog. for Composite (g)**	**Weight (g)**	**Homogenate Prepared (✓)**	**Wt. of Homog. for Composite (g)**
001										
002										
003										
004										
005										
006										
007										
008										
009										
010										
Analyst										
Date										

Total Composite Weight (g) **(F1 or C)** _____ **(F2)** _____

Notes: _____

Figure 7-2. Sample processing record for fish contaminant monitoring program—fish fillet composites.

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

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—

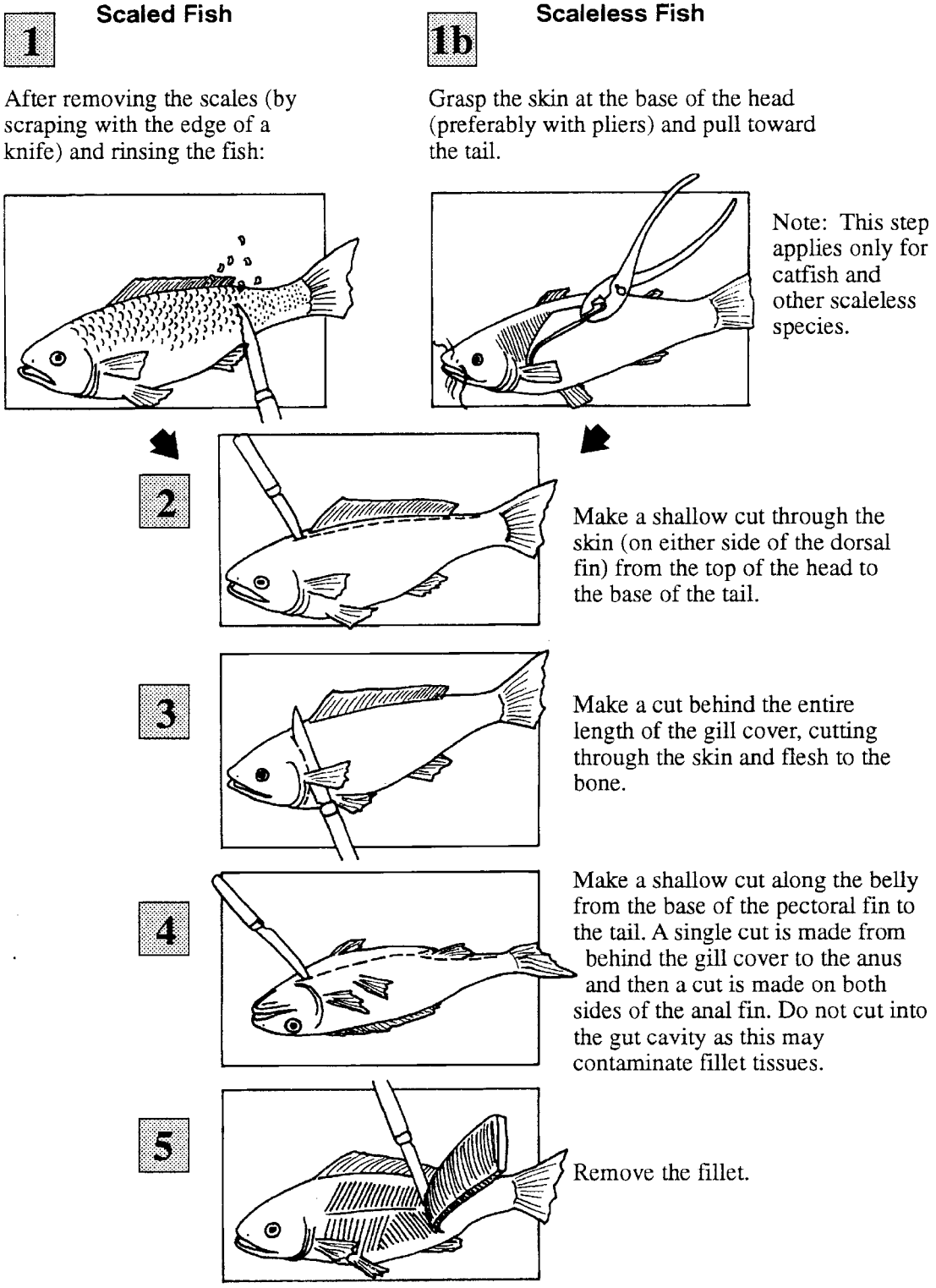
Filleting should be conducted only by or under the supervision of an experienced fisheries biologist. If gloves are worn, they should be talc- or dust-free, and of noncontaminating materials. Prior to filleting, hands should be washed with Ivory soap and rinsed thoroughly in tap water, followed by distilled 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.



Source: U.S. EPA, 1991d.

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

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 $\leq$-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

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

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 fillets 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 fillets).

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.

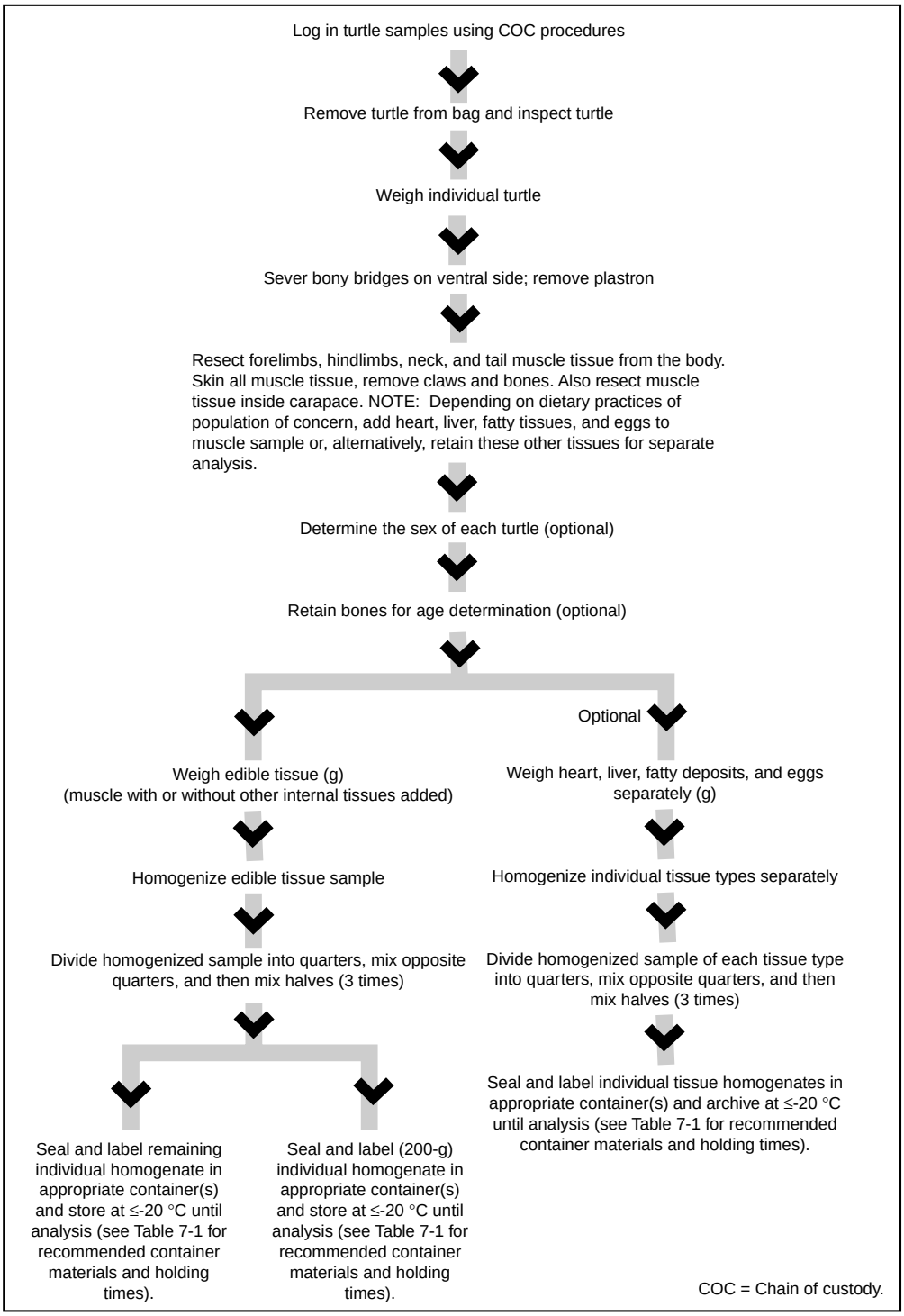


Figure 7-4. Preparation of individual turtle homogenate samples.

Whole turtles should be shipped or brought to the sample processing laboratory from the field on wet or blue ice within 24 hours of sample collection. The recommended euthanizing method for turtles is freezing (Frye, 1994) and a minimum of 48 hours or more may be required for large specimens. Turtles that arrive on wet or blue ice or frozen (i.e., on dry ice) at the sample processing laboratory should be placed in a $\leq$-20 °C freezer for storage until resection can be performed. If rupture of internal organs is noted for an individual turtle, the specimen may be eliminated as a sample or, alternatively, the edible tissues should be rinsed in distilled deionized water and blotted dry.

Sample processing procedures are discussed in the following sections. Data from each procedure should be recorded directly in a bound laboratory notebook or on forms that can be secured in the laboratory notebook. A sample processing record for individual turtle samples is shown in Figure 7-5.

7.2.3.1 Sample Inspection—

Turtles received for resection should be removed from the canvas or burlap collection bags 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.

7.2.3.2 Sample Weighing—

A wet weight should be determined for each turtle. 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.

Turtles euthanized by freezing should be weighed in clean, tared, noncontaminating containers if they will thaw before the weighing can be completed. **Note:** Liquid from the thawed whole turtle sample will come not only from the muscle tissue but from the gut and body cavity, which may not be part of the desired edible tissue sample. Consequently, inclusion of this liquid with the sample may result in an overestimate of target analyte and lipid concentrations in the edible tissue homogenate. Nevertheless, it is recommended, as a conservative approach, that all liquid from the thawed whole turtle be kept in the container as part of the sample.

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

Sample Processing Record for Turtle Contaminant Monitoring Program — Individual Samples

Project Number: _____ Sampling Date and Time: _____

STUDY PHASE: Screening Study ☐; Intensive Study: Phase I ☐ Phase II ☐

SITE LOCATION

Site Name/Number: _____

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

Waterbody Name/Segment Number: _____ Waterbody Type: _____

Sample Type (bottom feeder, predator, etc.) _____ **Species Name:** _____

Composite Sample #: _____ Replicate Number: _____ Number of Individuals: _____

Turtle #	Weight (g)	Carapace Length (mm)	Sex (M,F)	Resection Performed (✓)	Tissue Type Used	Tissue Weight (g)	Homogenate Prepared (✓)
001							
002							
003							
004							
005							
006							
007							
008							
009							
010							
Analyst							
Date							

Total Composite Weight (g) _____

Notes: *Define tissues used in edible sample; indicate whether fatty tissues, liver, heart, eggs, or other tissues are being analyzed individually or with muscle tissue as part of the edible sample.*

Figure 7-5. Sample processing record for a contaminant monitoring program—individual turtle samples.

7.2.3.3 Removal of Edible Tissues—

Edible portions of a turtle should consist only of those tissues that the population of concern might reasonably be expected to eat. Edible tissues should be clearly defined in site-specific sample processing protocols. A brief description of the edible portions used should also be provided on the sample processing record. General procedures for removing edible tissues from a turtle are illustrated in Appendix K.

Resection 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 resection, hands should be washed with soap and rinsed thoroughly in tap water, followed by distilled water (U.S. EPA, 1991d). Specimens should come into contact with noncontaminating surfaces only. Turtles should be resected on glass or PTFE cutting boards that are cleaned properly between each turtle or on cutting boards covered with heavy duty aluminum foil that is changed between each turtle (Puget Sound Estuary Program, 1990d, 1990e). A turtle is resected by laying it flat on its back and removing the plastron by severing the two bony ridges between the forelimbs and hindlimbs. Care must be taken to avoid contaminating edible tissues with material released from the inadvertent puncture of internal organs.

Ideally, turtles should be resected while ice crystals are still present in the muscle tissue. Thawing of frozen turtles should be kept to a minimum during tissue removal to avoid loss of liquids. A turtle 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 the muscle tissue and, depending on dietary or culinary practices of the population of concern, some of the other edible tissues from each turtle. The general procedure recommended for resecting turtles is illustrated in Figure 7-6.

Skin on the forelimbs, hindlimbs, neck, and tail should be removed. Claws should be removed from the forelimbs and hindlimbs. Bones still present in the muscle tissue after resection should be removed carefully (U.S. EPA, 1991d) and may be used in age determination (see Section 7.2.3.5).

To control contamination, separate sets of utensils and cutting boards should be used for skinning muscle tissue and resecting other internal tissues from the turtle (e.g., heart, liver, fatty deposits, and eggs). These other tissue types are recommended for inclusion with the muscle tissue as part of the edible tissue sample because it is believed that they are most representative of the edible portions of turtles that are prepared and consumed by sport anglers and subsistence fishers. Alternatively, states may choose to analyze some of these other lipophilic tissues separately. It is the responsibility of each program manager, in consultation with state fisheries experts, to select the tissue sample

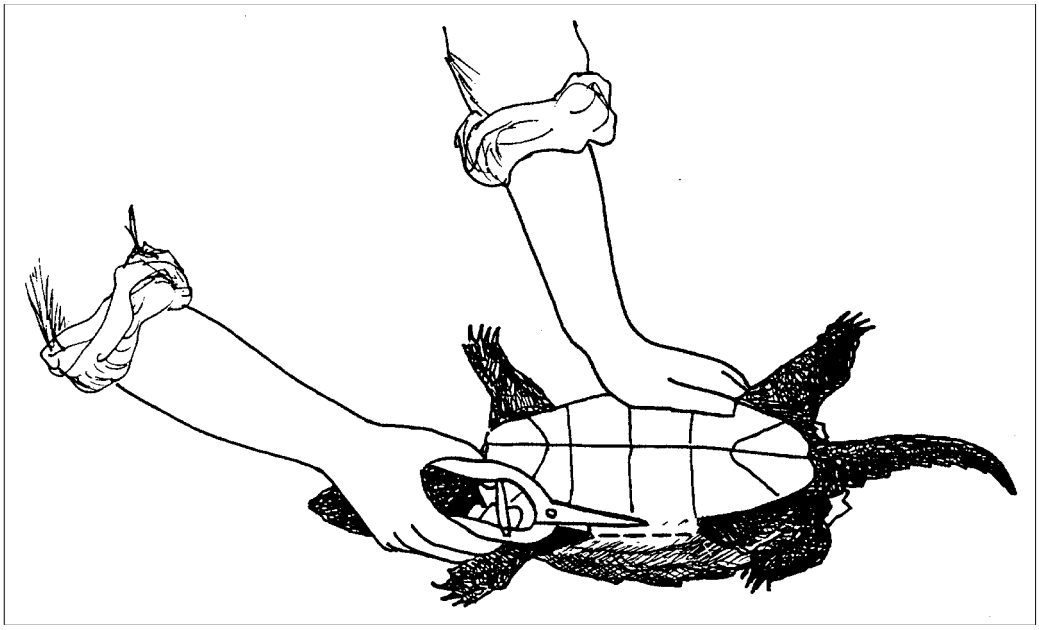


Figure 7-6. Illustration of basic turtle resection procedure.

type most appropriate for each target species based on the dietary customs of local populations of concern.

The edible turtle tissues should be weighed and the weight recorded to the nearest gram on the sample processing record. If the state elects to analyze the heart, liver, fatty deposits, or eggs separately from the muscle tissue, these other tissues should be weighed separately and the weights recorded to the nearest gram in the sample processing record.

If the tissues 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 of the large pieces of muscle tissue into smaller pieces using a titanium or stainless steel knife prior to placement in the homogenization container.

If the tissues 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., "M" for muscle, "E" for eggs, or "FD" for fatty deposits), the weight (g), and the date of resection. The individual muscle tissue samples from each turtle should be packaged together and given an individual sample identification number. The date of resection should be recorded and the sample should be stored at $\leq$-20°C until homogenization. **Note**: State staff may determine that the most appropriate sample type is muscle tissue only, with internal organ tissues analyzed separately (liver, heart, fatty deposits, or eggs). Alternatively, state staff may determine that the most appropriate sample type is muscle tissue with several other internal organs included as the turtle tissue sample. This latter

sample type typically will provide a more conservative estimate of contaminant residues, particularly with respect to lipophilic target analytes (e.g., PCBs, dioxins, and organochlorine pesticides).

7.2.3.4 Sex Determination (Optional)—

Turtle sex should be determined during resection if it has not already been determined in the field. Once the plastron is removed, the ovaries or testes can be observed posterior and dorsal to the liver. Each ovary is a large egg-filled sac containing yellow spherical eggs in various stages of development (Ashley, 1962) (see Appendix K). Each testes is a spherical organ, yellowish in color, attached to the ventral side of each kidney. The sex of each turtle should be verified and recorded on the sample processing form.

7.2.3.5 Age Determination (Optional)—

Age provides a good indication of the duration of exposure to pollutants (Versar, 1982). Several methods have been developed for estimating the age of turtles (Castanet, 1994; Frazer et al., 1993; Gibbons, 1976). Two methods are appropriate for use in contaminant monitoring programs where small numbers of animals of a particular species are to be collected and where the animals must be sacrificed for tissue residue analysis. These methods include (1) the use of external annuli (scute growth marks) on the plastron and (2) the use of growth rings on the bones.

The surface of epidermal keratinous scutes on the plastron of turtle shells develops successive persistent grooves or growth lines during periods of slow or arrested growth (Zangerl, 1969). Because these growth rings are fairly obvious, they have been used extensively for estimating age in various turtle species (Cagle, 1946, 1948, 1950; Gibbons, 1968; Legler, 1960; Sexton, 1959). This technique is particularly useful for younger turtles where the major growth rings are more definitive and clear cut than in older individuals (Gibbons, 1976). However, a useful extension of the external annuli method is presented by Sexton (1959) showing that age estimates can be made for adults on which all annuli are not visible. This method involves visually examining the plastron of the turtle during the resection or tagging the plastron with the sample identification number of the turtle and retaining it for later analysis.

The use of bone rings is the second method that may be used to estimate age in turtles (Enlow and Brown, 1969; Peabody, 1961). Unlike the previous visual method, this method requires that the bones of the turtle be removed during resection and retained for later analysis. The growth rings appear at the surface or inside primary compacta of bone tissues. There are two primary methods for observing growth marks: either directly at the surface of the bone as in flat bones using transmitted or reflected light or inside the long bones using thin sections (Castanet, 1994; Dobie, 1971; Galbraith and Brooks, 1987; Hammer, 1969; Gibbons, 1976; Mattox, 1935; Peabody, 1961). The methods of preparation of

whole bones and histological sections of fresh material for growth mark determinations are now routinely performed. Details of these methods can be found in Castanet (1974 and 1987), Castanet et al. (1993), and Zug et al. (1986). State staff interested in using either of these methods for age determination of turtles should read the review articles by Castanet (1994) and Gibbons (1976) for discussions of the advantages and disadvantages of each method, and the associated literature cited in these articles on turtle species of particular interest within their jurisdictions.

7.2.3.6 Preparation of Individual Homogenates—

To ensure even distribution of contaminants throughout tissue samples and to facilitate extraction and digestion of samples, the edible tissues from individual turtles must be ground and homogenized prior to analysis. The various tissues from an individual turtle may be ground and homogenized separately, or combined, depending on the sampling program's definition of edible tissues.

Turtle tissues should be ground and homogenized using an automatic grinder or high-speed blender or homogenizer. Large pieces of muscle or organ tissue (e.g., liver or fatty deposits) 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 tissue 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. No chunks of tissue should remain because these may not be extracted or digested efficiently and could bias the analytical results. This is particularly true when lipophilic tissues (e.g., fatty deposits, liver, or eggs) are not completely homogenized throughout the sample. Portions of the tissue sample that retain unhomogenized portions of tissues may exhibit higher or lower residues of target analytes than properly homogenized 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 frozen separately and stored at $\leq$-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."

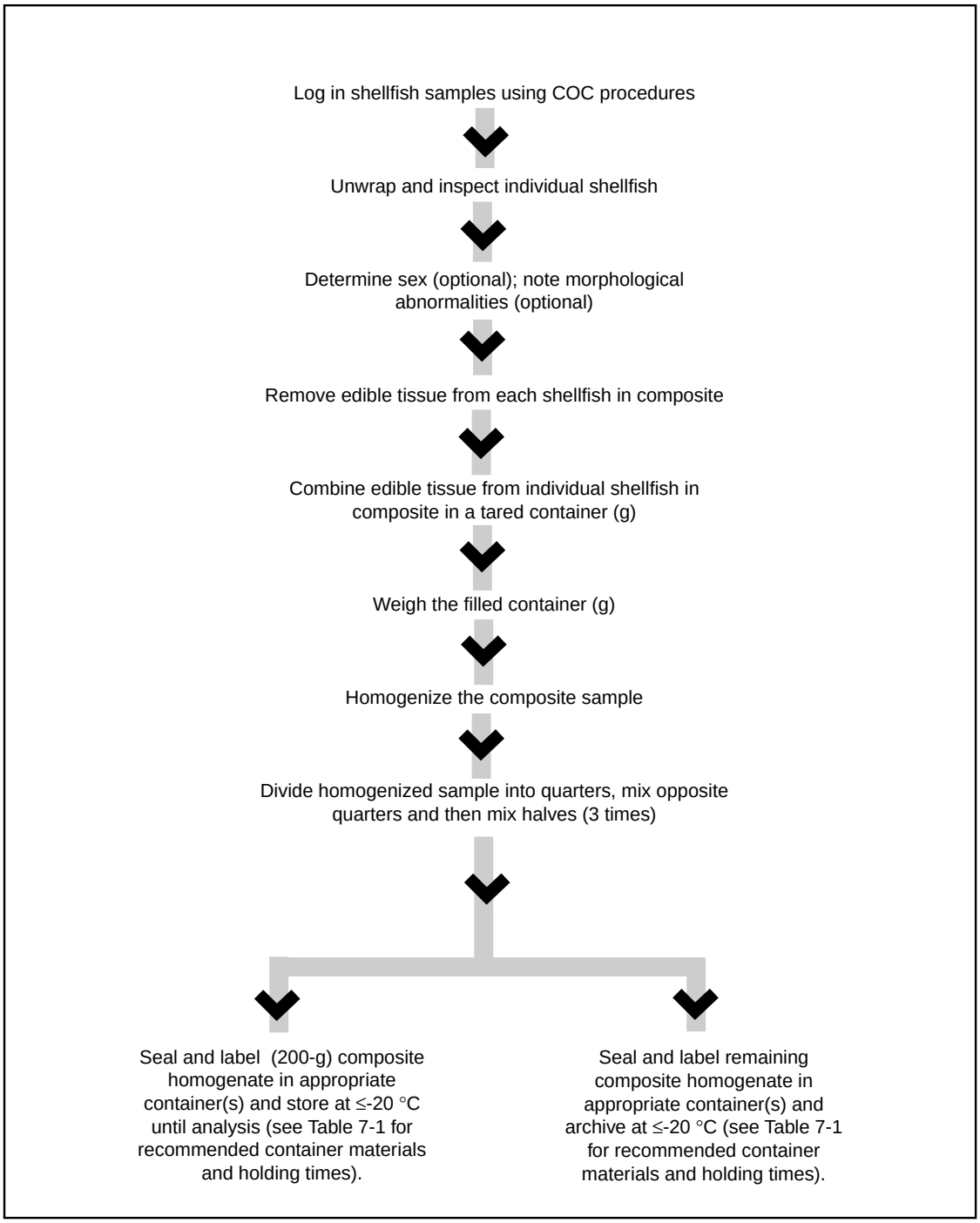
It is essential that the weight of individual homogenate samples is of adequate size to perform all necessary analyses. The recommended sample size of 200 g for screening studies is intended to provide sufficient sample material to (1) analyze for all recommended target analytes (see Table 4-1) at appropriate 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 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 homogenates required to analyze for all selected target analytes at appropriate detection limits. The total sample weight required for intensive studies may be less than that for screening studies if the number of target analytes is reduced significantly.

7.2.4 Processing Shellfish Samples

Laboratory processing of shellfish to prepare edible tissue composite homogenates for analysis (diagrammed in Figure 7-7) involves

- Inspecting individual shellfish
- Determining the sex of each shellfish (optional)
- Examining each shellfish for morphological abnormalities (optional)
- Removing the edible parts from each shellfish in the composite sample (3 to 50 individuals, depending upon the species)
- Combining the edible parts in an appropriate noncontaminating container
- Weighing the composite sample
- Homogenizing the composite sample
- Preparing aliquots of the composite homogenate for analysis
- Distributing frozen aliquots to one or more analytical laboratories.

Sample aliquotting and shipping are discussed in Section 7.3; all other processing steps are discussed in this section. Shellfish samples should be processed following the general guidelines in Section 7.2.1 to avoid contamination. In



COC = Chain of custody.

Figure 7-7. Preparation of shellfish edible tissue composite homogenate samples.

particular, it is recommended that separate composite homogenates be prepared for the analysis of metals and organics if resources allow. A sample processing record for shellfish edible tissue composite samples is shown in Figure 7-8.

Shellfish samples should be shipped or brought to the sample processing laboratory either on wet or blue ice (if next-day delivery is assured) or on dry ice (see Section 6.3.3). Shellfish samples arriving on wet ice or blue ice should have edible tissue removed and should be frozen to $\leq$-20°C within 48 hours after collection. Shellfish samples that arrive frozen (i.e., on dry ice) at the processing laboratory should be placed in a $\leq$-20°C freezer for storage until edible tissue is removed.

7.2.4.1 Sample Inspection—

Individual shellfish 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.

7.2.4.2 Sex Determination (Optional)—

The determination of sex in shellfish species is impractical if large numbers of individuals of the target species are required for each composite sample.

For bivalves, determination of sex is a time-consuming procedure that must be performed after shucking but prior to removal of the edible tissues. Once the bivalve is shucked, a small amount of gonadal material can be removed using a Pasteur pipette. The gonadal tissue must then be examined under a microscope to identify egg or sperm cells.

For crustaceans, sex also should be determined before removal of the edible tissues. For many species, sex determination can be accomplished by visual inspection. Sexual dimorphism is particularly striking in many species of decapods. In the blue crab, *Callinectes sapidus*, the female has a broad abdomen suited for retaining the maturing egg mass or sponge, while the abdomen of the male is greatly reduced in width. For shrimp, lobsters, and crayfish, sexual variations in the structure of one or more pair of pleopods are common. States interested in determining the sex of shellfish should consult taxonomic keys for specific information on each target species.

7.2.4.3 Assessment of Morphological Abnormalities (Optional)—

Assessment of gross morphological abnormalities in shellfish 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 removal of the edible tissues. States interested in documenting morphological abnormalities should consult Sinderman and Rosenfield (1967), Rosen (1970), and Murchelano (1982) for

Sample Processing Record for Shellfish Contaminant Monitoring Program — Edible Tissue Composites

Project Number: _____ Sampling Date and Time: _____

STUDY PHASE: Screening Study ☐; Intensive Study: Phase I ☐ Phase II ☐

SITE LOCATION

Site Name/Number: _____

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

Waterbody Name/Segment Number: _____ Waterbody Type: _____

SHELLFISH COLLECTED

Species Name: _____

Description of Edible Tissue _____

Composite Sample #: _____ Number of Individuals: _____

Shellfish #	Included in Composite (✓)	Shellfish #	Included in Composite (✓)	Shellfish #	Included in Composite (✓)
001		018		035	
002		019		036	
003		020		037	
004		021		038	
005		022		039	
006		023		040	
007		024		041	
008		025		042	
009		026		043	
010		027		044	
011		028		045	
012		029		046	
013		030		047	
014		031		048	
015		032		049	
016		033		050	
017		034			

Preparation of Composite:

Weight of container + shellfish _____ g

Weight of container (tare weight) _____ g

Total weight of composite _____ g ÷ _____ (# of specimens) = _____ (Average weight of specimen)

Notes: _____

Analyst _____ Date _____

Figure 7-8. Sample processing record for shellfish contaminant monitoring program—edible tissue composites.

detailed information on various pathological conditions in shellfish and review recommended protocols for pathology studies used in the Puget Sound Estuary Program (1990c).

7.2.4.4 Removal of Edible Tissue—

Edible portions of shellfish should consist only of those tissues that the population of concern might reasonably be expected to eat. Edible tissues should be clearly defined in site-specific sample processing protocols. A brief description of the edible portions used should also be provided on the sample processing record. General procedures for removing edible tissues from a variety of shellfish are illustrated in Appendix L.

Thawing of frozen shellfish samples should be kept to a minimum during tissue removal to avoid loss of liquids. Shellfish should be rinsed well with organics- and metal-free water prior to tissue removal to remove any loose external debris.

Bivalve molluscs (oysters, clams, mussels, and scallops) typically are prepared by severing the adductor muscle, prying open the shell, and removing the soft tissue. The soft tissue includes viscera, meat, and body fluids (Smith, 1985). Byssal threads from mussels should be removed with a knife before shucking and should not be included in the composite sample.

Edible tissue for **crabs** typically includes all leg and claw meat, back shell meat, and body cavity meat. Internal organs generally are removed. Inclusion of the hepatopancreas should be determined by the eating habits of the local population or subpopulations of concern. If the crab is soft-shelled, the entire crab should be used in the sample. Hard- and soft-shelled crabs must not be combined in the same composite (Smith, 1985).

Typically, **shrimp** and **crayfish** are prepared by removing the cephalothorax and then removing the tail meat from the shell. Only the tail meat with the section of intestine passing through the tail muscle is retained for analysis (Smith, 1985). Edible tissue for **lobsters** typically includes the tail and claw meat. If the tomalley (hepatopancreas) and gonads or ovaries are consumed by local populations of concern, these parts should also be removed and analyzed separately (Duston et al., 1990).

7.2.4.5 Sample Weighing—

Edible tissue from all shellfish in a composite sample (3 to 50 individuals) should be placed in an appropriate preweighed and labeled noncontaminating container. The weight of the empty container (tare weight) should be recorded to the nearest gram on the sample processing record. All fluids accumulated during removal of edible tissue should be retained as part of the sample. As the edible portion of each shellfish is placed in the container, it should be noted on the sample processing record. When the edible tissue has been removed from all shellfish

in the composite, the container should be reweighed and the weight recorded to the nearest gram on the sample processing record. The total composite weight should be approximately 200 g for screening studies. If the number of target analytes is significantly reduced in intensive studies, a smaller composite homogenate sample may suffice (see Section 7.2.2.9). At this point, the composite sample may be processed for analysis or frozen and stored at ≤-20°C (see Table 7-1).

7.2.4.6 Preparation of Composite Homogenates—

Composite samples of the edible portions of shellfish should be homogenized in a grinder, blender, or homogenizer that has been cooled briefly with dry ice (Smith, 1985). For metals analysis, tissue may be homogenized in 4-oz polyethylene jars (California Department of Fish and Game, 1990) using a Polytron equipped with a titanium generator. If the tissue is to be analyzed for organics only, or if chromium and nickel contamination are not of concern, a commercial food processor with stainless steel blades and glass container may be used. The composite should be homogenized to a paste-like consistency. Larger samples may be cut into 2.5-cm cubes with high-quality stainless steel or titanium knives before grinding. If samples were frozen after dissection, they can be cut without thawing with either a knife-and-mallet or a clean bandsaw. The ground samples should be divided into quarters, opposite quarters mixed together by hand, and the two halves mixed together. The quartering and mixing should be repeated at least two more times until a homogeneous sample is obtained. No chunks should remain in the sample because these may not be extracted or digested efficiently. At this point, the composite homogenates may be processed for analysis or frozen and stored at ≤-20 °C (see Table 7-1).

7.3 SAMPLE DISTRIBUTION

The sample processing laboratory should prepare aliquots of the composite homogenates for analysis, distribute the aliquots to the appropriate laboratory (or laboratories), and archive the remainder of each composite homogenate.

7.3.1 Preparing Sample Aliquots

Note: Because lipid material tends to migrate during freezing, frozen composite homogenates must be thawed and rehomogenized before aliquots are prepared (U.S. EPA, 1991d). Samples may be thawed overnight in an insulated cooler or refrigerator and then homogenized. Recommended aliquot weights and appropriate containers for different types of analyses are shown in Table 7-3. The actual sample size required will depend on the analytical method used and the laboratory performing the analysis. Therefore, the exact sample size required for each type of analysis should be determined in consultation with the analytical laboratory supervisor.

Table 7-3. Recommended Sample Aliquot Weights and Containers for Various Analyses

Analysis	Aliquot weight (g)	Shipping/storage container
Metals	1-5	Polystyrene, borosilicate glass, or PTFE jar with PTFE-lined lid
Organics	20-50	Glass or PTFE jar with PTFE-lined lid
Dioxins/furans	20-50	Glass or PTFE jar with PTFE-lined lid

PTFE = Polytetrafluoroethylene (Teflon).

The exact quantity of tissue required for each digestion or extraction and analysis should be weighed and placed in an appropriate container that has been labeled with the aliquot identification number, sample weight (to the nearest 0.1 g), and the date aliquots were prepared (Stober, 1991). The analytical laboratory can then recover the entire sample, including any liquid from thawing, by rinsing the container directly into the digestion or extraction vessel with the appropriate solvent. It is also the responsibility of the processing laboratory to provide a sufficient number of aliquots for laboratory duplicates, matrix spikes, and matrix spike duplicates so that the QC requirements of the program can be met (see Sections 8.3.3.4 and 8.3.3.5), and to provide extra aliquots to allow for reanalysis if the sample is lost or if QC control limits are not met.

It is essential that accurate records be maintained when aliquots are prepared for analysis. Use of a carefully designed form is recommended to ensure that all the necessary information is recorded. An example of a sample aliquot record is shown in Figure 7-9. The composite sample identification number should be assigned to the composite sample at the time of collection (see Section 6.2.3.1) and carried through sample processing (plus "F1," "F2," or "C" if the composite homogenate is comprised of individual or combined fillets). The aliquot identification number should indicate the analyte class (e.g., MT for metals, OR for organics, DX for dioxins) and the sample type (e.g., R for routine sample; RS or a routine sample that is split for analysis by a second laboratory; MS1 and MS2 for sample pairs, one of which will be prepared as a matrix spike). For example, the aliquot identification number may be WWWWW-XX-YY-ZZZ, where WWWWW is a 5-digit sample composite identification number, XX indicates individual (F1 or F2) or combined (C) fillets, YY is the analyte code, and ZZZ is the sample type.

Blind laboratory duplicates should be introduced by preparing two separate aliquots of the same composite homogenate and labeling one aliquot with a "dummy" composite sample identification. However, the analyst who prepares the laboratory duplicates must be careful to assign a "dummy" identification number that has not been used for an actual sample and to indicate clearly on the

Fish and Shellfish Monitoring Program
Sample Aliquot Record

Aliquot prepared by _____ Date _____ Time _____
(name)

Comments _____

Samples from:

Project No. _____ Site # _____ ☐ Screening study Intensive study ☐ Phase I ☐ Phase II

Composite Sample ID	Analyte Code ___ ___		Analyte Code ___ ___		Analyte Code ___ ___	
	Aliquot ID	Aliquot Weight (g)	Aliquot ID	Aliquot Weight (g)	Aliquot ID	Aliquot Weight (g)
Archive Location:	**Analyze for:** _____ **Ship to:**		**Analyze for:** _____ **Ship to:**		**Analyze for:** _____ **Ship to:**	

Page ____ of ____

Figure 7-9. Example of a fish and shellfish monitoring program sample aliquot record.

processing records that the samples are blind laboratory duplicates. The analytical laboratory should not receive this information.

When the appropriate number of aliquots of a composite sample have been prepared for all analyses to be performed on that sample, the remainder of the composite sample should be labeled with "ARCHIVE" and the expiration date and placed in a secure location at ≤-20 °C in the sample processing laboratory. The location of the archived samples should be indicated on the sample aliquot record. Unless analyses are to be performed immediately by the sample processing laboratory, aliquots for sample analysis should be frozen at ≤-20 °C before they are transferred or shipped to the appropriate analytical laboratory.

7.3.2 Sample Transfer

The frozen aliquots should be transferred on dry ice to the analytical laboratory (or laboratories) accompanied by a sample transfer record such as the one shown in Figure 7-10. Further details on federal regulations for shipping biological specimens in dry ice are given in Section 6.3.3.2. The sample transfer record may include a section that serves as the analytical laboratory COC record. The COC record must be signed each time the samples change hands for preparation and analysis.

Date _____ _____ _____ Time ___:___ (24-h clock)
YYYY MM DD

Released by: _____
(name)

At: _____
(location)

Shipment Method _____
Shipment Destination _____

Date _____ _____ _____ Time ___:___ (24-h clock)
YYYY MM DD

Released by: _____
(name)

At: _____
(location)

Comments _____

Study Type: ☐ Screening — Analyze for: ☐ Trace metals ☐ Organics ☐ Lipid

Intensive Phase 1 ☐ Phase II ☐ — Analyze for *(specify)* _____

Sample IDs:

Laboratory Chain of Custody

Relinquished by	Received by	Purpose	Location

Figure 7-10. Example of a fish and shellfish monitoring program sample transfer record.

APPENDIX J

DEQ FISH KILL PATHOLOGICAL EXAMINATION REPORT

VIRGINIA DEPARTMENT OF ENVIRONMENTAL QUALITY
FISH KILL PATHOLOGICAL EXAMINATION REPORT

POLLUTION COMPLAINT NO._____ COUNTY_____
REGION_____
SITE DESCRIPTION_____
_____
SUBMITTED BY_____
OFFICE/DIV_____
PHONE_____
DATE SHIPPED_____ SHIPMENT METHOD_____
DATE RECEIVED_____
TYPE OF SAMPLES SUBMITTED: Fish___ Water___ Other___

Collection Data

COLLECTED BY_____
OFFICE/DIV_____
PHONE_____
FISH SIZE (Inches) Fry___ 1-6___ 7-12___ 13-18___ >18___
FISH
SPECIES_____
SAMPLE CAPTURED: Alive___ Dead___ Sick___ Feeding___ Unknown___
Hook___ Cast Net___ Seine___ Hand___ Dip Net___
Shot___ Unknown___

NUMBER OF FISH IN SAMPLE_____
SAMPLE CONDITION: Alive___ Dead___ Iced___ Frozen___ Preserved
_____

Pond/Stream Data

DISSOLVED OXYGEN: A.M._____ P.M._____ PH_____ NO_2_____ CO_2_____
HARDNESS_____ ALKALINITY_____ TEMPERATURE_____ SALINITY_____
AMMONIA_____ CONDUCTIVITY_____
OTHER_____
POND ACREAGE_____ STREAM FLOW_____ COLOR_____
OTHER_____
_____
WEATHER: Clear___ Cloudy___ Calm___ Windy___ Rain___ Temp_____

Mortality Data

TOTAL NUMBER DEAD_____ DURATION OF KILL_____

REMARKS_____

APPENDIX K

DEQ SAMPLING TECHNIQUES FOR CHLOROPHYLL AND ALGAE

Sampling Techniques for Chlorophyll

1) Collect chlorophyll samples 1 ft below the surface. Phytoplankton populations are concentrated near the surface and depth composites samples will generally yield too little algal biomass for interpretable results.

2) Chlorophyll samples must be processed by the contract laboratory within 24 hours of collection. Chlorophyll samples are routinely submitted by all regional offices via CEDS/WQM to DCLS as part of the ambient monitoring program. There are two sample collection methods based on whether the samples are field filtered or lab filtered.

   The Group code CHLOR is a lab filtered sample and requires a single sample collected in a 2 liter HDP Amber Bottle preserved at 4 °C with Magnesium carbonate.

   The Group code FCHLR is a field filtered sample which requires a known volume of sample (typically 300 ml) to be filtered through a single 55 mm GF/F Glass fiber filter. The filter is frozen and stored in the dark. **Field filtered are preferred if the filter apparatus is available at the time of sample collection.**

   Multiple collection methods are available to serve different program requirements. These are outlined in CEDS. Charlie Morgan in the DEQ office of Water Quality Programs should be contacted for lab liaison assistance and updates regarding sample procedures.

Sampling Techniques for Algae

Collection

Algae samples for identification are usually collected near the surface in a 1 quart cubitainer or similar container of known volume.

Storage and Preservation

If refrigerated or kept on ice soon after collecting most algae can be kept alive for short periods (a day or two). For long term storage samples should be preserved in a liquid (listed below). Even with ideal preservation, examination of fresh material is sometimes essential for an accurate identification. For example, motile algae should be examined while their flagella and other structures are intact.

Liquid Preservation

A. Formalin

Preserve approximately 1 quart of sample with 40 ml of 2% Formalin and 1 ml of copper sulfate solution (see below). **Formalin is a diluted solution of formaldehyde which is thought to be carcinogenic. Contact with eyes, skin, and air passages should be avoided.** Algae samples can be stored in dilute formalin for a number of years.

B. Lugol's Solution

Add 3-4 ml of Lugol's solution (see below) to a one-quart cubitainer.

Lugol's solution is commonly used for short-term storage (i.e. few months to a year).

Collection Information

The collectors name, date, sample location, and preservation method should be included on the sample tag. Include other information if needed to help identify the algae (e.g. sample number, color and texture, size of algae, floating or submerged, whether the water is saline, brackish, or fresh, and etc.).

Sample Identification

Algae samples may be identified by DEQ RO Staff or outside academic institutions. Recently, Dr. Harold Marshall (phone 757 683-4204, e-mail hmarshal@odu.edu) at Old Dominion University has performed many algae identifications for the Tidewater and Piedmont regional offices. If samples are submitted to an outside institution, a contract and purchase order may be required.

Reagents

The following reagents may be used for chlorophyll or algae preservation.

Magnesium Carbonate

1 gram $MgCO_3$ added to 100 ml distilled water.

Copper Sulfate Solution (Saturated)

21 Grams $CuSO_4$ crystals added to 100 ml distilled water. Stir to dissolve.

2% Formalin Solution:

5 ml 40% aqueous formaldehyde solution (formalin). Formalin is added to 95 ml distilled water. **Formalin is a diluted solution of formaldehyde which is thought to be carcinogenic. Contact with eyes, skin, and air passages should be avoided.**

Lugol Solution

Dissolve 20 g potassium iodide and 10 g iodine crystals in 200 ml distilled water containing 20 ml glacial acetic acid. Preserve sample with 3-4 ml per quart cubitainer.

APPENDIX L

DEQ Chain of Custody Policy and Procedures Guidance Memo No. 00-2016.

MEMORANDUM

DEPARTMENT OF ENVIRONMENTAL QUALITY

SUBJECT: Guidance Memo No. 00-2016
Chain of Custody Policy and Procedures

TO: Regional Directors

FROM: John M. Daniel, Jr.
Air Program Coordination Director

Larry G. Lawson, P.E.
Water Program Coordination Director

Karen Jackson Sismour
Waste Program Coordination Director

DATE: October 23, 2000

COPIES: Dennis H. Treacy, David Johnson, David Paylor, Ralph J. Mayer, Compliance, Enforcement and Monitoring Senior Management Team, Remediation Managers Senior Management Team, Permit and Planning Managers Senior Management Team, Matthew Dullaghan

POLICY STATEMENT

It shall be the policy of the Department of Environmental Quality ("DEQ") that all samples will be collected using the following chain of custody procedures to ensure the integrity of samples so they can be used as admissible evidence to enforce the Commonwealth's environmental laws and regulations.

Samples taken in all cases involving a facility, permit, certificate, order or potential violation of a regulation or law shall follow chain of custody procedures. Samples taken for ambient environmental monitoring do not require chain of custody procedures. Variances to these procedures may be granted for those samples taken for special studies, on a case by case basis, only by the joint written agreement of the

appropriate Compliance and Enforcement Managers ("CEM") and Media Division Director.

The Division of Consolidated Laboratory Services ("DCLS") shall be used for the analysis of all samples except another laboratory may be used upon prior written approval of the appropriate CEM and Media Division Director. The chain of custody procedures used by the alternate laboratory shall be reviewed by the appropriate CEM and Media Division Director to ascertain that it meets DEQ's chain of custody policy and procedure requirements. Only following such review shall authorization be given to use an alternate laboratory.

Samples taken by DEQ's VOC Sampling and Analysis Program shall be sent to the City of Philadelphia Department of Public Health Air Management Services Laboratory, Philadelphia, Pennsylvania or DCLS if they are capable of performing the required testing.

I. PURPOSE AND SCOPE

The following procedures are used by all DEQ employees to ensure accountability for and documentation of sample integrity from the time all samples are collected until receipt by the receiving laboratory. These procedures are intended to document each stage of the sample's life cycle (*i.e.*, collection, transport, and delivery). Only the DEQ Director or his designee can authorize exceptions to this policy.

II. DEFINITIONS

2.1. **Custody-Physical Possession or Control.** A sample is "under custody" if it is in the possession or under the control of the Sample Custodian so as to prevent tampering or alteration of its characteristics. A sample is under custody if:

2.1.1. It is in your possession or in your view after assuming possession,

2.1.2. It was in your possession and then you locked or sealed the sample in a manner to prevent tampering, or

2.1.3. It is in a secured area. A secured area should have restricted access, locked storage facilities and be locked at all times when not attended (*e.g.,* inside a locked cooler, locked vehicle, or locked storage area).

2.2 **Sample.** A portion of an environmental or source matrix that is collected and used to characterize that matrix.

2.3 **Sample Custodian.** The person possessing the sample.

2.4 **Chain of Custody.** A process whereby a sample is maintained under physical possession or control. Chain of custody procedures are one piece of a large quality assurance program to assure data and conclusions are defensible in a legal or regulatory situation.

2.5 **Sample Submission Forms.** DEQ or the laboratory provides the forms used to record sample collection information, test(s) requested and result reporting instructions.

2.6 **Sample Set.** Collection of samples collected during one sampling event.

III. SAMPLE COLLECTION

3.1 **Sampling.** Samples are routinely collected by DEQ employees using standard collection procedures defined by media specific Standard Operating Procedures ("SOPs").

3.2 **Custody Assignment.** The sampler shall ensure proper collection, preservation and labeling of the sample. The sampler will also initiate the chain of custody documentation process, prepare sample submission information, and prepare and store samples for transport to the laboratory. Since as few people as possible should handle samples, the sampler is responsible for the initial custody of the sample.

3.3 **Consultation.** Information regarding the collection, preservation, transport, testing and sample custody may be obtained around-the-clock, seven days a week, from DCLS even when not using DCLS.

3.4 **Sample Kits.** Collection kits with containers, preservatives and sampling instructions may be provided by DCLS. DCLS is available for consultation purposes if there are questions about sample collection and preservation regardless of the laboratory used.

3.5 **Sample Identification.** To ensure samples are traceable, samples shall be clearly labeled immediately upon collection. Labeling information may vary by media SOPs, but labels must be written legibly, using a ballpoint (indelible) pen, unique for the sample/case and firmly fixed to the sample. The sample label shall contain the unique sample number or identification, sample type, name of sampler, and date and time of collection.

3.6 **Sample Preservation.** Sample preservation instructions are provided in sample kit collection instructions and in agency SOPs. Sample preservation actions shall be documented in field logs, on chain of custody forms, on lab sheets, and on sample labels.

IV. SAMPLING DOCUMENTATION

4.1 **Field Logbooks**. In any sampling effort, there are field information and measurements that need to be recorded. This information shall be retained in a sampler's field log. Examples of information entered include: purpose of sampling, producer, type of sample, address, sample composition, description of sampling point, sampling method, date and time of collection, sample identification number, field data, and preservative. This record may be considered evidence and part of the larger aspect of data defensibility. Logbooks shall be kept in a safe place.

4.2 **Custody Forms.** Agency chain of custody forms shall be used when submitting a sample for analysis. Attachment 1 is the General DEQ Chain of Custody Form and Attachment 2 is the DEQ VOC Chain of Custody Form. Chain of custody forms shall be completed by the sampler at the time of sample collection and shall be submitted with each sample set. The

completed form shall be signed by the sampler and dated (chain of custody block) and placed in a waterproof carrier (*e.g.*, zip-lock bag) if it is a water sample. The form shall be packaged with the sample for transport to the laboratory. The original form shall be returned to the sampler along with the results of the tests that are performed. The original chain of custody form and laboratory results is then filed in the appropriate case file.

4.3 **Sample Submittal and Test Request Forms.** With each sample submitted to the laboratory for analysis, the sampler shall include the following information:

- 4.3.1 The analytical request
- 4.3.2 Sample identification
- 4.3.3 Field data
- 4.3.4 The chain of custody form
- 4.3.5 Copies of applicable documents (*e.g.*, MSDS, sample formulations (if applicable))
- 4.3.6 Any other information required to meet laboratory testing and reporting requirements
- 4.3.7 This information may be submitted by:
  - 4.3.7.1 An agency lab sheet that is completed following established agency SOPs and packaged and shipped with the chain of custody form with the sample
  - 4.3.7.2 An electronic file generated by the agency's Comprehensive Environmental Database System ("CEDS")

V. SAMPLE PACKAGING, TRANSPORT AND TRANSFER OF CUSTODY

5.1 **Sample Packaging.** The correct preparation and preservation of samples for transport are critical to ensure sample integrity.

5.1.1 The sampler should contact the laboratory if unsure of any aspect of sample collection, preservation, packaging and transport.

5.1.2 Samples must be labeled, tightly sealed in the appropriate container and double bagged in plastic where applicable.

5.1.3 Evidence tape shall be used to seal sample containers. The tape shall be placed across the container in a manner that tampering attempts would be obvious. In all cases, the initials/mark of the person sealing the evidence must be placed on, across or under the seal.

5.1.4 The chain of custody form and other documentation are to be sealed tightly in a plastic zip-lock bag.

5.1.5 Samples and documentation are then placed in an appropriate transport container (*e.g.,* cardboard mailer, stryofoam box, paint can, plastic box or cooler) and padded (*e.g.,* bubble wrap, styrofoam peanuts) as appropriate.

5.1.6 If not immediately delivered to the laboratory by the sampler, containers shall be locked (personal padlock) and sealed (custody seal). When samples are ready for shipment to DCLS lockable transport containers, locks and custody seals provided by DCLS shall be used. DCLS will retain all keys for locks. Once locked and sealed, containers provided by DCLS may be opened only by DCLS staff. Samples that are ready for shipment to laboratories other than DCLS shall be shipped in tamperproof transport containers.

5.1.7 The sampler is responsible for preservation prior to and during transport. If ice is required, it will need to be contained in a way to prevent leakage. Special shipping measures can be briefly described on the chain of custody form and should be described in detail in the field logbook.

5.2 **Sample Transport.** Samples are to be delivered to DCLS or other prior approved laboratory by one of the following means. Regardless of the mode of transport, arrangements are to be coordinated with the laboratory and delivered as soon as possible.

5.2.1 Immediate Delivery by Sample Custodian. Samples are to be delivered to the Sample Records Management section of DCLS or other prior approved laboratory as soon as possible after collection. The sample shall remain in the Sample Custodian's possession or sight at all times.

5.2.2 Routine and Special Courier. DCLS couriers shall be used to deliver legal and regulatory samples to DCLS. Samples will be packaged as described in § 5.1. DCLS provides special lock boxes and coolers for this purpose. Lock boxes and coolers may be delivered to courier pick-up sites for delivery. The DCLS courier routinely picks samples up in the late afternoon and delivers them to the laboratory the next day. A special pick-up can be arranged for any time of night or day. When received by the laboratory custodian, the package will be inspected to ascertain whether tampering occurred and these actions recorded on the chain of custody form.

5.2.3 Common Carriers. Common carrier delivery services (*e.g.*, United Parcel Service, Federal Express) are often the only practical means of delivering samples to laboratories when DCLS cannot provide the required testing and an alternate laboratory must be used. Common carrier delivery services must commit to safeguard all cargo, assuring that it will not be tampered with and will be delivered promptly. Carriers generally specify the type of shipping container to be used when accepting the carrier's services.

5.2.4 Holding Area. Until the courier or the common carrier picks up the container, the container either must be locked or kept in a locked area.

Attachment 1

Shipping Seal Number _____

VIRGINIA DEPARTMENT OF ENVIRONMENTAL QUALITY
629 EAST MAIN STREET, RICHMOND, VIRGINIA 23240

CHAIN OF CUSTODY RECORD

SAMPLERS: *(Signatures)* _____ Date Submitted: _____ Agency Case No.: _____

Agency (Print or Type): _____

Address: _____

Phone No.: _____ Fax No.: _____

E-Mail: _____

If this is an Emergency (Priority1) sample, please print 24-hour contact name and number. _____

STATION ID	DATE	TIM'E	STATION DESCRIPTION	COMP.	GRAB	TESTS TO BE RUN IN LAB	Observations, Field Tests, Remarks Made in Field

Relinquished by: *(Signature)*	Date/ Time	Received by: *(Signature)*	Relinquished by: *(Signature)*	Date/ Time	Received by: *(Signature)*
Relinquished by: *(Signature)*	Date/ Time	Received by: *(Signature)*	Relinquished by: *(Signature)*	Date/ Time	Received by: *(Signature)*
Relinquished by: *(Signature)*	Date/ Time	Received in Laboratory by: *(Signature)*	Date/ Time	Shipping seal received n place? ____ Seal No. _____ Lab Remarks:	

Original to Accompany Shipment; copy to Sampler; Copy to Transporter

Attachment 2

COATING SAMPLE CHAIN OF CUSTODY RECORD

INSPECTOR, AGENCY AND ADDRESS: BRENDA EGGLESTON 629 EAST MAIN STREET P.O. BOX 10009 RICHMOND, VA 23240	*SOURCE NAME AND PERMIT #:*	
SAMPLE ID NUMBER:	*AMS LAB #*	
COATING NAME/TYPE:		
PRODUCT CODE:		
LOT OR BATCH #:		
PROCEDURE: [ ] EPA METHOD 24 [ ] EPA METHOD 24A	*ANALYSIS REQUIRED:* [ ] VOC CONTENT & DENSITY [ ] WATER [ ] EXEMPT SOLVENTS	*PROPERTIES:* [ ] BASE INK [ ] MULTI-COMPONENT COATING [ ] SAMPLE PRESERVATION ACTIONS TAKEN DURING SHIPMENT
REMARKS:		
PERSON RESPONSIBLE FOR SAMPLE (INSPECTOR'S SIGNATURE):	*TIME:*	*DATE:*
PLANT WITNESS (SIGNATURE):		

RELINQUISHED BY:	*RECEIVED BY:*	*TIME:*	*DATE:*	*REASON FOR TRANSFER:*
RELINQUISHED BY:	*RECEIVED BY:*	*TIME:*	*DATE:*	*REASON FOR TRANSFER:*
RELINQUISHED BY:	*RECEIVED BY:*	*TIME:*	*DATE:*	*REASON FOR TRANSFER:*
RELINQUISHED BY:	*RECEIVED BY:*	*TIME:*	*DATE:*	*REASON FOR TRANSFER:*
RELINQUISHED BY:	*RECEIVED BY:*	*TIME:*	*DATE:*	*REASON FOR TRANSFER:*
RELINQUISHED BY:	*RECEIVED BY:*	*TIME:*	*DATE:*	*REASON FOR TRANSFER:*
RELINQUISHED BY:	*RECEIVED BY:*	*TIME:*	*DATE:*	*REASON FOR TRANSFER:*

For Facility Inspections, FI program code,

Run Ids will be: XVA9999999	where X = Regional identifier (e.g. S = SWRO), 9999999 = Permit number minus the outfall number. e.g. SVA0001015 Total of 10 characters
Station Ids will be: VA9999999-999	where 9999999 = Permit number to match run ID and -999 = outfall number e.g. VA0001015-001 Total of 13 characters

For Facility Inspections, FI program code where no permit currently exists,

Run Ids will be LHS120XKKKK	where X = Regional identifier, K = alpha/numeric regionally derived ID e.g. LHS120SJMHP Total of 11 characters
Station Ids will be LHS120KKKKZ	where K = alpha/numeric code regionally derived ID to match run ID Z = alpha/numeric code regionally derived for outfall designation e.g. LHS120JMHP1 Total of 11 characters

For Incident Responses, IR program code,

Run Ids will be IRYYYYX9999	YYYY = four digit year X = Regional identifier 9999 = numeric incident code e.g. IR2000S0089 Total of 11 characters
Station Ids will be IRYYYY9999Z	YYYY = four digit year 9999 = numeric incident code to match run ID Z = alpha/numeric code regionally derived for station designation e.g. IR20000089B Total of 11 characters

For UST, LUST, AST Samplings, UI and UR program codes,

Run Ids will be PCYYYYX9999	YYYY = four digit year X = regional identifier 9999 = numeric incident code e.g. PC2000S3422 Total of 11 characters
Station Ids will be PCYYYY9999Z	YYYY = four digit year 9999 = numeric incident code to match run ID Z = alpha/numeric code regionally derived for station designation e.g. PC20003422B Total of 11 characters

ATTACHMENT 1

SAMPLE COLLECTION CONTACT LIST

DIRECTORS:

Dr. James L. Pearson, DGS Deputy Director for Consolidated Laboratories
Office: 804-786-7905; Pager: 1-800-946-4646 then dial 141-6949.

Dr. Thomas York, Analytical Services Bureau Director,DCLS,
Office: 804-786-7905; Pager: 804-418-9922.

CUSTOMER SERVICE & PLANNING, KITS MANAGEMENT SYSTEM:

Thomas Lindfors, Customer Service-Planning Group, DCLS,
Office: 804-786-0307; Pager: 804-418-9924

Grace LaPlace, Program Support Technician, DCLS,
Office: 804-786-3756

CUSTOMER SERVICE LABORATORY SUPPORT, SAMPLE RECEIVING MANAGEMENT:

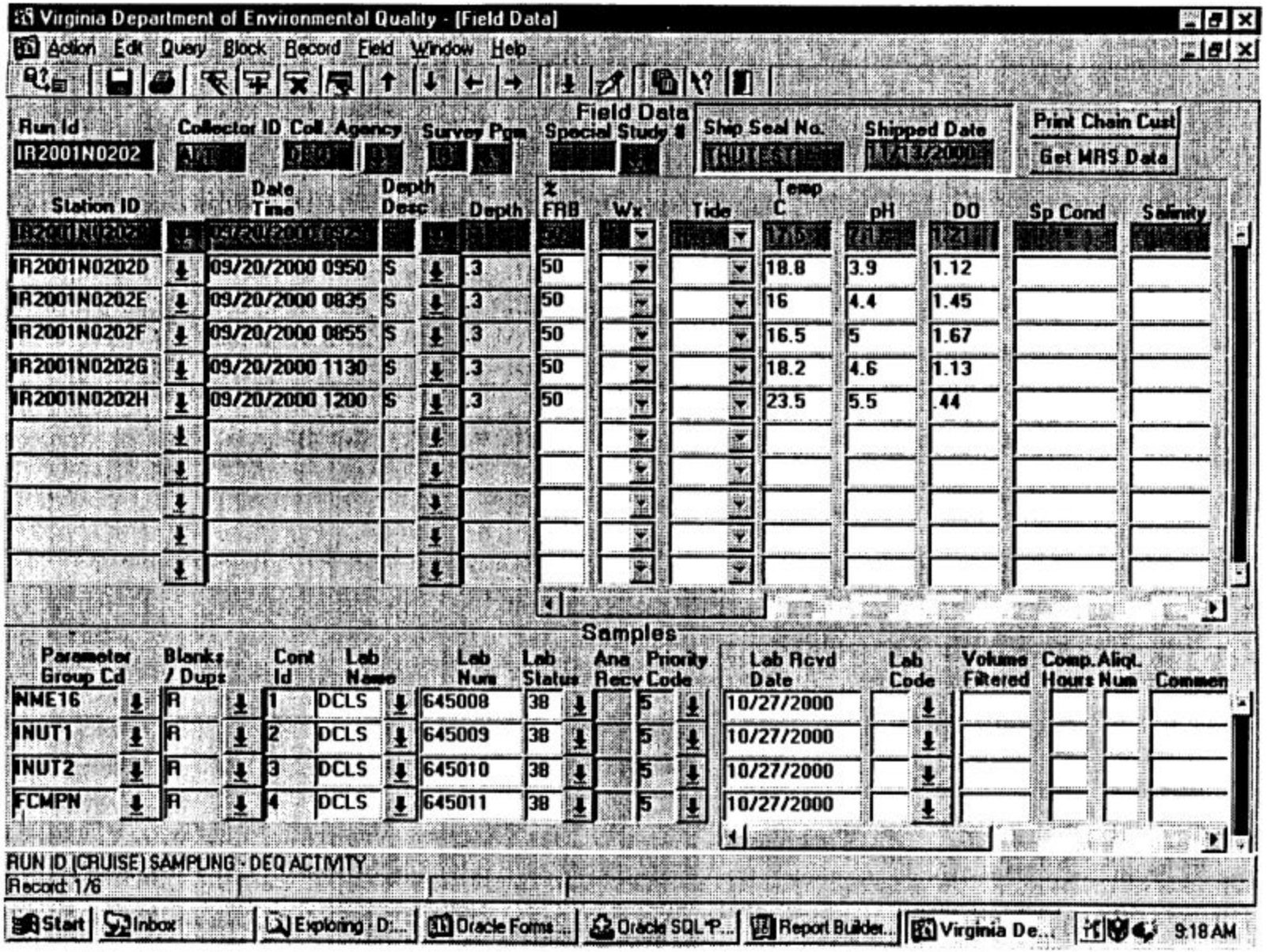
Glorious Bennett, Customer Service-Lab Support Group, DCLS,
Office: 804-786-2413; Pager: 804-418-9932

COURIER SERVICE:

Thomas Lindfors, Customer Service-Planning Group, DCLS,
Office: 804-786-0307; Pager: 804-418-9924

EMERGENCY SERVICE OFFICER:

Pager: 804-418-9923


Virginia Department of Environmental Quality - [Field Data]
Action Edit Query Block Record Field Window Help
Field Data
Run Id
IR2001N0202
Collector ID
Coll. Agency
Survey Pgm
Special Study #
Ship Seal No.
Shipped Date
Print Chain Cust
Get MRS Data
Station ID
Date Time
Depth Desc
Depth
% FRB
Wx
Tide
Temp C
pH
DO
Sp Cond
Salinity
IR2001N0202D 09/20/2000 0950 S .3 50 18.8 3.9 1.12
IR2001N0202E 09/20/2000 0835 S .3 50 16 4.4 1.45
IR2001N0202F 09/20/2000 0855 S .3 50 16.5 5 1.67
IR2001N0202G 09/20/2000 1130 S .3 50 18.2 4.6 1.13
IR2001N0202H 09/20/2000 1200 S .3 50 23.5 5.5 .44
Samples
Parameter Group Cd
Blanks / Dups
Cont Id
Lab Name
Lab Num
Lab Status
Ana Recv
Priority Code
Lab Rcvd Date
Lab Code
Volume Filtered
Comp. Aliqt. Hours
Num
Commen
NME16 R 1 DCLS 645008 38 5 10/27/2000
NUT1 R 2 DCLS 645009 38 5 10/27/2000
NUT2 R 3 DCLS 645010 38 5 10/27/2000
FCMPN R 4 DCLS 645011 38 5 10/27/2000
RUN ID (CRUISE) SAMPLING - DEQ ACTIVITY
Record: 1/6
Start
Inbox
Exploring D:...
Oracle Forms ...
Oracle SQL*P...
Report Builder...
Virginia De...
9:18 AM

Shipping Seal No.
THUTEST

629 EAST MAIN STREET, RICHMOND, VIRGINIA 23240

CHAIN OF CUSTODY RECORD

SAMPLERS: *(Signatures)* ALT

Agency: Central Office

Address: 629 E. MAIN STREET

RICHMOND VA 23219

Phone No: Fax No: 8046984561

E-mail:

Shipped Date: 11/13/2000 RUN ID IR2001N0202

If this is an Emergency (Priority1) sample, please print 24-hour contact name and number

FIRST LAST M 8041234567

TESTS TO BE RUN IN LAB		Group Code	FCMP	INUT1	INUT2	NME1																	Observations, Field Tests, Remarks Made i
STATION ID	**Coll Date Time**		Typ	Typ	Typ	Typ																	
IR2001N0202B	09/20/2000 09:25		R	R	R	R																	
IR2001N0202D	09/20/2000 09:50		R	R	R	R																	
IR2001N0202E	09/20/2000 08:35		R	R	R	R																	
IR2001N0202F	09/20/2000 08:55		R	R	R	R																	
IR2001N0202G	09/20/2000 11:30		R	R	R	R																	
IR2001N0202H	09/20/2000 12:00		R	R	R	R																	

Relinquished by: (Signature)	Date/ Time	Received by: (*Signature*)	Relinquished by: (*Signature*)	Date/ Time	Received by: (*Signature*)
Relinquished by: (Signature)	Date/ Time	Received by: (*Signature*)	Relinquished by: (*Signature*)	Date/ Time	Received by: (*Signature*)
Relinquished by: (Signature)	Date/ Time	Received in Laboratory by: (*Signature*)	Date/ Time	Shipping seal received intact ? No. of _____	Lab Remarks:

Implementing the Chain of Custody Procedures

The Chain of Custody Record (COCR) shall provide documentation of everyone who has custody of the samples. The sample collector starts the COCR when the samples are collected. The COCR travels with the samples that are listed on the COCR. The COCR must contain the written signature of everyone that has custody of the samples and must document the relinquishment and receipt of the samples between sample custodians.

Transferring COC of samples from person to person

When custody of the samples changes, i.e. when samples are transferred from one person to another, the Custody Record must show the samples being relinquished by one person and received by another in the presence of each other.. The custody of the samples and the responsibility of maintaining sample integrity are transferred during this process. The transfer process is documented on the bottom of the Chain of Custody Record form.

Transferring COC of samples from person to DCLS via courier

This transfer is the same as above except that the transfer is not face to face. Both the collector and receiver document the integrity of the shipping container and the samples therein. The actual sample transfer is via courier in a tamper-proof shipping container.

Preparing the COCR Form

The COCR form may be prepared using one of two methods, CEDS or manually. The preferred method is to use the CEDS system and the COCR form printed from information entered into the CEDS system. When using lab sheets, a multiple-part preprinted carbonless form is used. The COCR does not take the place of a lab sheet or CEDS data shipment. Consideration must be taken of the number of samples being shipped, as **a separate form must be completed for each cooler shipped**. Care must be taken to ensure that the information on the form concerning the samples exactly matches that found on the samples. Samples must be rejected if they do not match the COCR form.

Sample Priority

Using the COCR does not in itself indicate the samples are priority. The priority number should be recorded on the COCR in the space where the 24-hour contact information is located. When using CEDS, this information is also captured in the field data screen and shipped to DCLS. When using lab sheets, each lab sheet must have the priority code entered. Advance notice of priority samples, or samples being delivered on a weekend, should be given the lab by contacting persons on the contact list. Only high priority samples or samples with a short holding time should shipped to the lab for weekend delivery.

SHIPPING SEAL NUMBER

This is number of the wire seal provided by DCLS that will be used to seal the individual coolers. All coolers not delivered in person to DCLS must have a shipping seal in place with the shipping seal number recorded on the COCR. The seal number is unique and entering it on the COCR makes that record unique. When the cooler is received by DCLS they will compare the number of the unbroken seal on the cooler with the seal number that is recorded on the COCR.

FORM NUMBER

The multi-part forms will have a unique form number in the top right hand portion of the page. This number will be used to identify the form from all others when no shipping seal number is used.

SAMPLERS

The person who collects the samples or is present when the samples are collected and labeled and takes initial custody of the samples signs the COCR in this area.

CASE NO. OR PC NO. OR VPDES NO OR OTHER

This area is used to record the reference number for the sampling event. The number is program specific. Program protocols should be followed when entering this number. When using CEDS this reference number is referred to as the RUN ID.

LAT. & LONG (optional)

The latitude and longitude may be entered at the top of the page if known. This is used to identify the location of the sampling event. If more than one site is sampled during the sampling event, a lat/long must be entered for each station and lat/long space at the top of the page should be left empty.

REGION OR UNIT and ADDRESS

Enter the name of the region or central office unit responsible for collecting the samples and the address of the region or unit. This address may be used when returning certificates of analysis.

PHONE & FAX NUMBERS AND E-MAIL

Enter this contact information for the collector. This information will be used to contact the collector if questions arise concerning the samples or analysis.

24-HOUR CONTACT INFORMATION

Enter the sample priority in this area. If the samples have a high priority (1), enter any and all information that can be used 24-hours a day to confer about sample analysis and results. Examples of information are; home, work, pager and cell phone numbers as well as e-mail addresses.

STATION ID

This is the brief description of the station at which the samples listed (on the same line) were collected. Limit the description to information necessary for you to uniquely identify the station from all others collected. This information must match the information on the sample tags and in the field log.

DATE/TIME

This is the date and time at which the samples were collected. The date is in the form MMDDYYYY and time is recorded in military time.

BASIC STATION DESCRIPTION OR CONTAINER TYPE (optional)

This space is used for additional station description information if necessary. For situations where pre-cleaned containers are used, the container description information may be entered here.

COMP/.GRAB (optional)

This space may be used to identify samples other than routine grab samples. In the case of composite samples, the number of samples or time frame of the composite should be entered. If a horizontal or vertically integrated sample is collected, the information may be entered in the station description or under "FIELD OBSERVATIONS".

TESTS TO BE RUN IN THE LAB

Enter the group code from the DCLS catalog of services which contains the analysis desired. If you have questions concerning the appropriate group code, contact DCLS using the contact list.

OBSERVATIONS & FIELD TESTS (optional)

Enter field observations or field tests. These should be entered in the field log and on the lab sheets as well.

RELINQUISHED BY:, DATE/TIME, RECEIVED BY:

This area is used to record transfers of the sample custody. The person with custody of the samples (initially the collector) must relinquish the sample custody in the presence of the person receiving custody of the samples (except when shipping samples to DCLS). This is accomplished by the custodian signing the COCR form in "relinquished by" section and entering the date and time. The new custodian must sign the COCR form in the "received by" section in the company of the original custodian. This process is followed in any subsequent changes in sample custody. Space is provided for four such transactions plus the final acceptance of custody by DCLS and the date and time the samples are received by DCLS.

SHIPPING SEAL RECEIVED INTACT, NO. OF, LAB REMARKS

This area is used by DCLS to record the number of the seal broken to gain access to the contents of the cooler. They will note if the seal is intact. They will also note any other remarks such as the condition of the samples, ice present, etc.

Using CEDS

CEDS can be used for shipping sample information to DCLS and for printing the COCR from the field data entered in the field data screen.
Since the COCR is printed from CEDS after returning from the field, this method may not be used when sample custody is transferred from person to person in the field.
When using CEDS for shipping sample information to the lab, stations must be established in the station screen and all the pertinent information must be entered in the field data screen using established protocols.
Additionally, you will need to **record the Shipping Seal Number** you will be using on the cooler and the **Shipped Date**. Review the information on-screen with the information on the sample tags and in the field log before printing the COCR form. Information on the COCR form may be changed in CEDS and reprinted if errors are made up until the time that the data is shipped to DCLS.
Click on the <PRINT CHAIN CUST> button on the field data screen to print the COCR form. Three copies will be printed.
Sign one copy as "Relinquished by:" and complete the date/time at which the samples are relinquished or locked in the cooler. Place it inside the cooler in a zip-loc type waterproof container.
One copy is put in a zip-loc type waterproof cover and is taped to the outside front of the cooler.
The collector retains one copy with the field log.

Multiple-part Preprinted Form

Complete the multi-part form using the definitions above. All sections not indicated as optional must be completed.. Make certain that the information on the form exactly matches the information on the sample tags, the field log, and the labsheets. Minor mistakes made while filling out the form may be corrected by crossing them out and initialing the crossed-out portion. Major mistakes will require a new form to be completed. The form with the mistake must be destroyed.

Sign the bottom portion of the COCR as "Relinquished by:" and complete the date/time at which the samples are relinquished or locked in the cooler.
The original signed copy is to be sent inside the cooler in a zip-loc type waterproof container.
One copy is put in a zip-loc type waterproof cover and is taped to the outside front of the cooler.
The collector retains one copy.

Preparing Samples for Shipment

Before shipping samples make absolutely sure that the information on the sample tags exactly matches the information on the COCR, the field log, and either lab sheets or the CEDS field data entry screen.
Individual samples must be sealed with a custody seal tape or evidence tape in such a way as to prevent the caps from working loose and the sample tags from coming off. This is generally accomplished by wrapping the wire sample tag securely around the neck of the sample container and leaving the ends of the wires exposed. The tape is then used to tightly span between moving and stationary parts of the sample cap securing the ends of the wire under the tape.
Put samples in a large waterproof bag and put into shipping cooler. Ice samples by putting the ice between the cooler and the waterproof bag. The maximum weight of the cooler is 60#.
Record the wire shipping container seal number on the COCR. Put the original signed copy of the COCR form inside the cooler inside of a waterproof zip-loc type bag. If used, include the lab sheets inside the zip-loc bag as well. Close and lock the cooler by closing the hasp (the bail over the eyelet) and securing it using the padlock supplied by DCLS. Put the wire seal individually through both the bail and the eyelet of the hasp. Do not put the wire seal through the eyelet in the same manner as the lock. Before closing the wire seal, make absolutely sure the serial number of the wire seal matches the number entered on the COCR form.

The sealed, locked cooler containing the signed COCR form may be placed in the regionally designated location to be picked up by the DCLS courier. The sealed, locked cooler is considered to now be under the custody of DCLS.

Problems

The sample tags don't match either the CEDS information or the lab sheets, or the COCR form.

The collector can correct these errors only if their field log contains information that will rectify the error. A correction of this type must be meticulously documented in the field log. Only the collector can make changes of this type. If the samples have been shipped, the collector will have to go to DCLS to make the corrections.

Cooler is locked before all the samples are placed inside.

The collector must develop a separate COCR for the additional samples and ship them in a separate COC cooler. The samples listed on the first cooler's COCR but missing will be marked as not received by DCLS. The change in coolers' contents must be documented in the field log.

Cooler is sealed but not locked before all the samples are placed inside.

The seal may be broken to inspect the cooler contents and to add or remove samples prior to padlocking the shipment. If the seal is broken, a new COCR must be developed.

- In CEDS, return to the field data screen (prior to data shipment @ 0900 and 2200, check for the lab send date in the CEDS field data screen), change the seal number, print a new COCR and replace all three of the original COCRs.
- When using lab sheets, you may change the seal number on each of the three copies of the form and initial the change on each sheet. This change should be documented in the field log as well.

You don't have any wire seals.

You or someone else who assumes custody of the samples must accompany the samples to DCLS. Samples delivered by the sample custodian don't require locks or custody seals. They do require container seals.

The wrong wire seal number is recorded on the COCR.

If the cooler is locked, you must accompany the samples to DCLS.
If the cooler is not locked you may change the wire seal. *(see above, breaking the wire seal)*

The Personal Field Log

The personal field log is a legal document used to record information concerning all aspects of an investigation. The log must have bound and numbered pages. The log should be kept in a secure place. Only the owner of book should make notations in the personal field log.
At a minimum, the field log should be used to record information which links that section of the field log with the information found on the COCR. The following information should be used a page headers:

- Investigation identification information such as PC, or permit number
- Date of investigation

The field log should also contain information that supports but does not duplicate information found on the COCR. This includes, but is not limited to:

- Names, addresses, phone numbers of complainant, permit holder, operators, etc.
- Detailed descriptions of the sampling sites
- Variations if any from the WQA SOP manual
- Types of samples collected (grab, straight timed composite including time frame, volume weighted composite, cross-section composite, vertically integrated composite
- Pre and post meter calibration information
- QAQC samples collected
- Detailed observations of the site including physical lay of the land such as upstream, up-gradient, east/west, etc.
- Detailed information included as comments in CEDS or on the lab sheets such as "expect high BOD"
- Documentation of changes to the COCR.

★ SHIPPING SEAL #:

APPENDIX M

Pfiesteria and the DEQ *Pfiesteria* response and safety plans.

Chesapeake Bay Fish Lesions and *Pfiesteria*

Virginia Sea Grant Program • Virginia Institute of Marine Science • September 1997

Recent fish kills and closures in the Pocomoke River have increased public concern over *Pfiesteria piscicida*, a microorganism that has been linked to large fish kills in North Carolina. In response to concerns about an unusual number of fish with lesions reported in Chesapeake Bay, especially the Pocomoke River. A Virginia *Pfiesteria* Task Force was formed in May 1997. The Task Force includes members of the Virginia Marine Resources Commission (VMRC), The Virginia Department of Health (VDH), The Virginia Department of Environmental Quality (DEQ), and research scientists from Virginia Institute of Marine Science (VIMS), and Old Dominion University (ODU). The Task Force has developed an information network that enables members to respond quickly and efficiently to events.

What causes lesions (open sores) on fish?

There are many possible causes for fish lesions including physical injury in nets or traps, bites by other fish or birds, toxic chemicals, and infectious disease agents such as viruses, bacteria and fungi. On the basis of laboratory experiments, we now have to add toxins released by *Pfiesteria* to the list of possible causes. Currently, it is very difficult to determine the original cause of a lesion unless an obvious parasite is present because basic knowledge of the physiologic and environmental factors related to lesions in fish is insufficient.

Open sores that expose the underlying musculature are the most difficult kind of lesion to assess. The skin and mucus of a fish are effective barriers against infection by bacteria, which are always present in Chesapeake Bay waters. However, that barrier can be broken by a variety of causes including injury, general stress to toxic chemicals (including *Pfiesteria* toxins). When the skin/mucus barrier is broken, the area is usually rapidly colonized by bacteria which further erode the tissue and produce an open lesion or sore that may penetrate deep into the musculature. In such cases, the cause of the original break in the skin/mucus barrier that lead to the lesion is difficult to determine with our present state of knowledge.

Have there been unusually high numbers of fish lesions during 1997?

No, not in most areas of Chesapeake Bay. Some fish lesions occur every summer in Chesapeake Bay and based on information from VMRC, DEQ and VIMS and also from agencies in Maryland, the incidence of lesions on Chesapeake Bay fish during 1997 is not unusually high and there is no indication that fish populations are facing serious problems.

The Pocomoke River, located on the Eastern Shore near the Virginia-Maryland border, may be an exception. Commercial fishermen have reported what they consider to be unusually high numbers of fish lesions in the Pocomoke River and there were low- to moderate-level fish kills in the river during August. These lesions and kills have been linked to the toxic dinoflagellate *Pfiesteria piscicida*, but the link is as yet circumstantial.

Have fish kills occurred in Chesapeake Bay in the past?

Small- to moderate-scale fish kills, usually of small menhaden, occur occasionally in tidal creeks during the summer months. These kills are usually caused by low oxygen content of the water, but other possible causes, now including *Pfiesteria*, are routinely investigated.

Who should I call if I see a fish kill or see fish with lesions?

A few fish here and there with lesions, or even a few washed up dead on the beach, is not an uncommon occurrence and there is little cause for concern. However, if large numbers of fish with lesions or dead fish are observed, the appropriate agencies should be notified. The

Virginia Department of Environmental Quality (DEQ) has responsibility for investigating fish kills.

What is *Pfiesteria* and is it related to red tides?

Contrary to some recent press reports, *Pfiesteria piscicida* is not a virus or bacterium and it is not an infectious agent; fish or other organisms cannot become infected with *Pfiesteria*. *Pfiesteria piscicida* is a dinoflagellate, a microscopic, free-floating, single-celled organism with two flagella for locomotion. Most dinoflagellates are plants (called algae or phytoplankton) that gain energy from photosynthesis. However, many species of dinoflagellates, including *Pfiesteria*, do not photosynthesize, but behave like animals and consume algae or bits of organic matter. Normally, *Pfiesteria* feeds on algae cells. However, under certain circumstances that are not understood, and only in the presence of live fish, *Pfiesteria* can release a toxin that can cause sloughing of the surface layer of fish skin and, in high concentrations, can kill fish. *Pfiesteria* has been implicated in fish kills in North Carolina and in the Pocomoke River near the Virginia/ Maryland border on the Eastern Shore.

There are two reasons that the public may connect *Pfiesteria* and "red tides". *Pfiesteria* is a dinoflagellate and red tides are typically, but not always, caused by dinoflagellates. *Pfiesteria* is known to be toxic to fish and red tides are often, but not always, toxic to marine life. Despite these similarities, there are important distinctions to be made between *Pfiesteria* and red tides, especially for the Chesapeake Bay region. *Pfiesteria* is reported to kill fish when it occurs at low concentrations in the water, typically a few hundred cells per milliliter (.00026 gallons) of water. This is not a sufficient concentration of cells to discolor the water and *Pfiesteria* has never been reported to cause discolored water.

Red tides (also called red water or mahogany water) are typically caused by the dense accumulation, typically thousands of cells per milliliter of water, of dinoflagellates near the surface. Red tides are common occurrences in the Chesapeake Bay and its tributaries. They can occur at any time of year but usually are most common during July and August. Unlike other coastal regions of the United States where red tides result in fish death and bans on eating shellfish, red tides in the Chesapeake Bay to date have not been toxic to marine life. This lack of toxicity is because the species of dinoflagellates causing red tides in Chesapeake Bay are not toxic species. Red tides are typically categorized as a type of Harmful Algal Bloom (HAB), whether they are harmful to aquatic life or not. There is increasing interest in HABs worldwide because of the perception that they are becoming much more numerous, are often toxic to marine life, and are likely caused by man's influence on coastal areas. Because of their lack of toxicity to date, there has been less urgency to study red tides in the Chesapeake Bay and it is not clear what causes them and whether they are becoming more numerous.

Does *Pfiesteria* occur in Virginia?

Pfiesteria is known to occur from the Gulf of Mexico along the east coast as far north as Delaware Bay. It has been reported in Virginia waters near the mouth of the Pocomoke River and it is known to occur in Maryland. *Pfiesteria* is probably an ubiquitous organism that occurs all along the east coast in low numbers.

Is there a relationships between *Pfiesteria* and environmental degradation?

Popular press reports of *Pfiesteria* and its possible effect on fish often suggest that nutrient enrichment of estuaries and coastal waters from a variety of land-derived sources is a principal cause of *Pfiesteria* proliferation and activity. Some scientific literature suggests a similar relationship. Manure from hog and chicken production facilities is often identified as a source of nutrients. The association between *Pfiesteria* and nutrient enrichment is also fostered by the tendency to associate *Pfiesteria* with algal blooms, which are well documented to result, in part, from nutrient enrichment of natural waters. However, as discussed above, *Pfiesteria* is not an algae and does not make its own food by photosynthesis and does not require dissolved nitrogen and phosphorous (two typical nutrients) in the water for its nutrition. *Pfiesteria* eats other microscopic plants and animals. Because it is an animal and not a plant it is less likely to respond directly to nutrient enrichment. To the extent that its preferred food is microscopic algae, one

might expect *Pfiesteria* to be more abundant where its preferred food is more abundant. Thus, it might be indirectly linked to nutrient enrichment through its food supply.

In general, the Chesapeake Bay and its tributaries are not as enriched with nutrients as the Pamlico Sound and its tributaries in North Carolina, yet *Pfiesteria* has been reported from various locations in the Chesapeake Bay and has been linked to fish kills and human health problems in the Pocomoke River. However, based on common indices of nutrient enrichment used for the Chesapeake Bay, the Pocomoke River is not considered to be highly enriched. Other, more enriched areas of the Bay have not experienced fish kills or fish with lesions. The carefully controlled scientific experiments which identify nutrient enrichment as a stimulus to *Pfiesteria* are few and others are currently being conducted. Until more results are available it is not possible to say with confidence why *Pfiesteria* occurs where it does and why it becomes toxic when and where it does.

Can Chesapeake Bay expect large-scale fish kills similar to North Carolina?

When fish with lesions were first observed in the Pocomoke River there was doubt about the possible role of *Pfiesteria* as a cause because of the lack of large numbers of dead fish on the surface. In North Carolina, where *Pfiesteria* has been reported to be the cause of fish kills, there are reports of large numbers of dead fish, often hundreds of thousands to millions, during fish kills. Recent fish kills in the Pocomoke River, attributable to *Pfiesteria*, report thousands to perhaps tens of thousands of dead fish, much lower numbers than observed in North Carolina.

One possible explanation for the fewer numbers of dead fish in the Chesapeake Bay region as a result of Pfiesteria may be differences in hydrography between these two regions. The Pamlico Sound and Neuse River estuary in North Carolina are very shallow, poorly flushed estuaries with weak tidal currents. By contrast, the Chesapeake Bay and its tributaries are typically deeper, better flushed and have stronger tidal currents. If Pfiesteria in the sediments are detecting fish in the overlying water column by means of chemical cues from the fish, as some scientists believe, and if fish death is caused by a chemical toxin produced by Pfiesteria, then it is possible that the greater the dispersion of these chemical cues and toxins by water currents and circulation, the fewer fish will be detected and killed. Also, in deeper water fish may be less concentrated.

There is much yet to be learned about Pfiesteria and its role in fish lesions and death, but this is one possible reason that Pfiesteria reported in the Chesapeake Bay region may not be as much as a problem as reported for North Carolina.

Is it safe to eat Virginia seafood?

YES, Chesapeake Bay seafood is safe. Consumers should use common sense and avoid dead fish or fish with sores, but otherwise there is no reason to avoid eating Virginia seafood. There have been no reports of adverse effects on human health from eating shellfish (crabs, oysters, etc.) harvested in the vicinity of fish kills, but little information is available on this subject.

Does Pfiesteria affect humans?

A variety of symptoms have been reported by commercial watermen and other citizens in North Carolina, Maryland and Virginia and by researchers who cultured *Pfiesteria* in the laboratory. Symptoms, including sores, fatigue and short-term memory loss, have only been associated with laboratory exposure, or with large-scale fish kills in North Carolina and with fish kills in the Pocomoke River in Maryland and Virginia. Portions of the Pocomoke River were closed periodically during August because of possible human health concerns. Establishing a definite link between generalized symptoms and *Pfiesteria* is difficult, but health officials are studying the situation carefully. Your local health department has up to date information.

For more information, visit the VIMS web site:
http//www.vims.edu

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Marine Resource Advisory No. 65

July 1997
Update, September 1997

DEPARTMENT OF ENVIRONMENTAL QUALITY

Operational Response Plan

For *Pfiesteria* Related Fish Kills

Pfiesteria Incident Response Team

PIRT

May 18, 1998

TABLE OF CONTENTS

INTRODUCTION

The purpose of this document is to serve as the operational plan for Department of Environmental Quality (DEQ) staff response to suspected *Pfiesteria* related fish kill events.

DEQ responsibility for fish kill investigations is clearly established by Virginia Code §62.1-44.15(11), and staff guidance on conducting these investigations is provided in the draft revised October, 1997 edition of the Fish Kill Investigation Guidance Manual. Therefore, this plan does not duplicate information in the agency fish kill investigation document but rather provides guidance specific to suspected *Pfiesteria* related events.

The plan establishes a DEQ seven day a week, 24 hour a day *Pfiesteria* response program from May 1 through October 31 of each year. DEQ's Tidewater (TRO), Piedmont (PRO), and Northern Regional (NRO) Offices will each have a *Pfiesteria* response person on call who will handle all calls and reports of fish with lesions, fish acting erratically and fish kills. They will respond with the assistance of enforcement officers from the Virginia Marine Resources Commission (VMRC), who will provide transportation to the fish kill site with their boats for the DEQ *Pfiesteria* responder. The DEQ *Pfiesteria* responder will collect the samples needed to determine if a *Pfiesteria* event is occurring; the Virginia Department of Health (VDH) will use this information to determine if a public advisory warning (yellow light) or water segment closure (red light) decision is necessary (see Appendix B for a discussion of these criteria). DEQ will also have On Scene Coordinators (OSC) on call that will contact all the other agencies and institutions involved and set up a more complete DEQ crew for follow-up work, if a *Pfiesteria* related fish kill event is confirmed. In addition, a Central Office (CO) DEQ response team and OSC staff are available as backup support to the agency regional offices.

OVERVIEW OF THE COMMONWEALTH'S PFIESTERIA PROGRAMS

In addition to the DEQ *Pfiesteria* response plan described in this document, Virginia has developed and is implementing a multi-agency/institution effort for response, monitoring and research on *Pfiesteria piscicida* and *Pfiesteria* Complex Organisms (PCOs). A *Pfiesteria* task force was designated in 1997 and all the agencies and institutions represented on the task force have been involved in the development of Virginia's plan for responding to *Pfiesteria* events. The task force is comprised of 13 members from five agencies and institutions: Virginia Department of Health (VDH), Virginia Institute of Marine Science (VIMS), Department of Environmental Quality (DEQ), Old Dominion University (ODU) and the Virginia Marine Resources Commission (VMRC). In addition, there are a number of auxiliary agencies and academic institutions which share a direct or indirect role in task force efforts: Department of Game and Inland Fisheries (VDGIF), Department of Conservation and Recreation (DCR), the Chesapeake Bay Local Assistance Department (CBLAD), the Marine Products Board (VMPB), Department of Agriculture and Consumer Services (VDACS), Virginia Commonwealth University (VCU) and Virginia Polytechnic Institute and State University (VPISU).

The Commonwealth's *Pfiesteria* monitoring, response, and research activities involve extensive participation by ODU, VIMS, VDH, VCU, DEQ and VMRC. VIMS, ODU and DEQ have been active participants in Virginia's Chesapeake Bay Program for years. ODU brings extensive experience in research and monitoring of phytoplankton and zooplankton. VIMS brings its extensive experience in research and monitoring of phytoplankton and fish health. VDH is undertaking an extensive study funded by the Centers for Disease Control (CDC) of human health effects and is handling the application of health advisories and closures. VIMS will be undertaking a major study of the Great Wicomico River and the fish health monitoring as part of their normal fish surveys in 1998. ODU will be undertaking the screening of water samples by light microscopy for PCOs and will culture sediment samples to facilitate *Pfiesteria piscicida* identification. Both VIMS and ODU are developing scanning electron microscopy capabilities for identification of *Pfiesteria* and similar organisms. Virginia will still rely on North Carolina State University (NCSU) for identification of the toxic forms of *Pfiesteria piscicida* for at least the near term. VIMS is in the process of gearing up for a P-3 laboratory that will enable them to identify the toxic forms.

DEQ, VIMS, and ODU will be supporting VDH's health impacts study through CDC. VIMS and DEQ will perform twice monthly

monitoring of 14 stations identified to support the cohort health study. DEQ will complement the health study with an extensive network of stations for PCO and water quality sampling. A total of 24 stations in small estuaries on the eastern and western shores of the Bay and in the major rivers of the Bay will be monitored for standard water quality parameters, PCOs, chlorophyll "a" and a full range of nutrients. These 24 stations are inclusive of the 14 VDH cohort health study stations.

The data from this water quality and PCO monitoring along with data from *Pfiesteria* event response will complement data from DEQ's 153 ambient water quality monitoring stations on the Bay and data from DEQ's Chesapeake Bay Program monitoring stations. It is believed these data will provide useful information on the spatial distribution of *Pfiesteria* and the environmental factors that result in toxic blooms.

PFIESTERIA RESPONSE

PFIESTERIA INCIDENT RESPONSE TEAMS - PIRT

DEQ has four PIRTs, one from each of the three regional offices (PRO, TRO, NRO and, one from the Central Office). Each team has a initial responder trained in all aspects of *Pfiesteria* response including personal safety. Each region will have an initial responder on call at all times and a list of secondary responders that includes their pager and/or home telephone numbers so they can be activated if needed.

AFTER HOURS

After hours fish kills will be reported by Department of Emergency Services (DES) through the normal regional after hours pollution response protocols. DEQ pollution response staff should obtain as much information as available; but most importantly, the name and telephone number of the person reporting the fish kill to DES. This information will then be referred to the initial response staff member of the appropriate DEQ regional *Pfiesteria* Incident Response Team (PIRT).

ALL FISH KILL REPORTS

All fish kill reports are referred to an initial *Pfiesteria* responder during the "*Pfiesteria* Season" from May through October. Each region shall ensure that at least one initial responder is accessible by pager and cellular phone at all times during regular business hours and after hours. Initial responders will have full training in safety and all required safety equipment (Appendix A); they will be trained in fish species identification.

If a name and telephone number is available, the first step is for the initial responder to call the person who reported the fish kill to DES to get first hand information.

If the event is in brackish or salt water (east of I-95) and there are fish with lesions, fish acting erratically, or the fish are menhaden, then the fish kill should be treated as a possible *Pfiesteria* incident. Any menhaden fish kill should be investigated even if there appears to be an obvious cause.

Any fish kills in waters not likely to have a *Pfiesteria* incident should be handled by existing fish kill response protocols. However, to avoid having the person taking the call make a judgement on whether it is likely to be *Pfiesteria* waters and because the same staff handle both types of fish kills, all fish kills should be referred to the initial responder on the PIRT.

PFIESTERIA RESPONSE - Initial Investigation

Once the *Pfiesteria* responder has determined that the incident should be investigated as a possible *Pfiesteria* incident, the responder will contact VMRC. See Appendix F for the *Pfiesteria* response process and actions.

When requesting assistance from VMRC, call 1-800-541-4646, which is VMRC's twenty-four (24) hour toll free Operations number. If this call is made Monday through Friday between 7:00am and 5:00pm, the dispatcher will contact the appropriate Area Supervisor, who would be responsible for the geographic location in which the incident is occurring.

When calling VMRC, provide them your name, title, department, as well as a number at which you can be reached until contacted. If you have not been contacted within thirty minutes, call the Operations number again and advise the dispatcher that you have not been contacted. It is possible that the page was not transmitted or received due to technical difficulty. The dispatcher will either relay to you pertinent data or will page again.

If the call to VMRC is made on a Friday after 5:00pm and before 7:00am Monday,the dispatcher will contact the on-call Supervisor who will arrange for the appropriate response. The same time-frame parameters for a response will be in effect.

The responder will arrange to meet VMRC at a mutually acceptable boat launch as close as possible to the fish kill. During regular office hours the responder can take a DEQ regional boat and crew, if the crew is readily available; however, VMRC should still be contacted.

The initial *Pfiesteria* responder will always take a complete package of *Pfiesteria* response equipment and supplies. This package of equipment and supplies will already be loaded in a vehicle and ready to go. The vehicle can be at work for after hours response, if the responder is within a short commute of work; if not, the vehicle will be taken home on weekends and holidays.

Pfiesteria Response - Confirmation of a Pfiesteria Event

If a site investigation confirms a possible *Pfiesteria* event, the responder will contact the regional OSC for the appropriate regional PIRT, who will contact VIMS and ODU to arrange for sample delivery and to notify them of the event (Appendix C). The regional OSC will also contact the VDH *Pfiesteria* contact and DEQ management. Contacts with news media shall follow the DEQ guidance to ensure that complete and accurate information is provided to the media.

Next, the responder will determine the extent of the fish kill and take appropriate samples for verification of the presence of *Pfiesteria*.

The regional OSC will activate a follow-up response. The OSC will use the contact list to call members of the PIRT by using their home and/or pager numbers to get three staff members to accompany the initial responder on a follow-up response.

The OSC will call individual PIRT members from his/her own region first, then Central Office and then, if needed, one of the other regions.

A contact list of DEQ PIRT members and OSCs will be maintained and kept current by the Scientific Research Unit (SRU) within the Central Office. It is the responsibility of each regional office to immediately notify SRU of any changes in the regional contact list in order to keep the list current.

Every effort should be made to perform a follow-up response the next morning. Two DEQ staff will serve as the boat crew along with the initial responder and the third will bring an additional vehicle for the delivery of samples. The OSC also may respond to coordinate activities from a local site.

The follow-up response will be for the collection of *Pfiesteria* complex organisms (PCOs) samples, water quality samples, sediment, fish samples and to do a fish lesion percentage count.

RED LIGHT AND YELLOW LIGHT DECLARATION RESPONSE

The authority and responsibility to make the declaration of a Red or Yellow Light incident currently resides with the VDH (Appendix B). DEQ's role is to investigate fish kills and to gather the information (water samples, fish samples and fish with lesion counts) that VDH needs to make a determination.

If a Red light or Yellow light incident is declared, routine monitoring will be required until the "Undo Criteria" are met and the situation returns to normal. During a Yellow light incident, an OSC may not be necessary, but the regional office may need assistance to maintain the routine monitoring.

If an incident is declared a Red light incident, then a DEQ command center should be established and a rotation of PIRT and/or members will be put in place. If there is an active fish kill and the initial responder eliminates other causes and observes fish with lesions, the incident should be treated as a Red Light incident until a formal determination can be made by VDH.

ON SCENE COORDINATOR

An OSC is required when a "Red Light Waters" declaration is issued, i.e. large scale or ongoing *Pfiesteria* fish kill. Each of the four response teams have designated OSCs.

The responsibilities of the OSC include the following:

- contacting VIMS and ODU to arrange for receipt of the samples from the initial response
- setting up the follow-up response
- ensure that all authorities that need to be informed of the incident have been informed including the VDH *Pfiesteria* response contact and DEQ management
- calling in whatever resources are necessary including requesting Virginia Emergency Services's (VES) assistance
- coordinate with VES the notifcation of local governments
- establish a rotation schedule for the response teams
- ensure the availability of all equipment needed for the incident on an ongoing basis
- ensure the availability of all supplies needed on an ongoing basis
- ensure transportation of samples to the appropriate facilities and delivery of supplies

- liaison with the agency public information personnel to deal with media (VDH currently is lead agency for providing *Pfiesteria* information related to human health effects, CO and RD's also have assigned duties).

- ensure PCO counts, fish lesion analysis and other data are forwarded as quickly as possible to the VDH as appropriate.

EQUIPMENT/FUNDS

- **Financial Resources will be critical to the response teams and OSC.** Each regional office shall maintain an adequate reserve of petty cash to purchase supplies and support at the local scene, especially for sustained incidents.

- **Sampling Equipment.** Each region shall ensure that an adequate supply of sampling equipment is on hand in quantities sufficient to handle 30 days of continuous sampling.

- **Safety Equipment.** All safety equipment including decontamination equipment shall be available at each regional office. (The exception is water needed for field decontamination which will be obtained on an as needed basis.)

- **Command Center.** If needed, a command center will be provided by DES. The center will include cell phone, fax, computer with modem, and printer.

- **Decontamination capabilities.** Each regional office shall have some type of spraying capability available. Silt fencing and straw bales will be purchased from a local supplier as needed.

- **Portable sanitary facilities.** Local supplies will be identified through local officials.

- **Large supply of potable water.** A bottled water supplier could provide a source of potable water unless water is also needed for decontamination. If a source of decontamination water is needed, the OSC will first contact local officials and then if necessary contact the National Guard, VDOT, etc.

- **Boat fuel.** Boat fuel will normally be obtained from the nearest VDOT facility. However, fuel will have to be purchased from a commercial service station or marina after hours and on weekends and in situations where the time required to travel to a VDOT facility would adversely impact the investigation.

- **Tools.** Tools for minor repairs of boats, trailers, sampling

equipment will be brought on scene. These are currently available at the regional offices.

- **Meals and drinks.** These can be obtained from a local supplier.

- **Ice and Coolers.** DEQ regional offices should have on hand sufficient coolers for the investigation. Local suppliers can be used for ice.

FIELD SAMPLING DURING RESPONSE TO PFIESTERIA RELATED FISH KILLS

The following sampling protocols are designed for initial and follow-up response to fish kills that may be related to PCOs, which are also referred to as Harmful Algal Blooms (HABs). Due to the possible human health implications from *Pfiesteria*, staff will follow the Safety Plan For Pfiesteria Response, as specified in Appendix A.

INITIAL RESPONSE FOR WATER QUALITY MONITORING

It is important to arrive and sample while the kill is still in progress. Because of the critical nature of the initial response, a protocol has been developed for conducting investigations when available staff may be limited. A single DEQ responder, using vessel transportation provided by VMRC, Shellfish Sanitation (VDH), or VDGIF, will arrive on site and collect the basic samples as needed.

Some common relationships associated with *Pfiesteria* like fish kills are:

1. Oxygen levels are usually not depressed.
2. Water may be discolored around kill.
3. Live fish may exhibit erratic movements.
4. Presence of fish lesions, especially in juvenile menhaden.

First Response Equipment Kits. The following sampling equipment should be maintained in field kits to minimize response time.

1. Quart cubitainers (20)
2. Gallon cubitainers (5)
3. TOC bottles (5)
4. 2 L Chlorophyll bottles fixed with $MgCO_3$ (5)
5. Lugol's solution (25 ml)**
6. Drop bottle with H_2SO_4
7. Lab Tags (30)
8. Lab Sheets (10)
9. Chain of custody sheets (5)
10. Plastic bags of various sizes for fish samples

11. Discrete sampling bottle (Labline, Alpha, Kemmerer, etc.)
12. Personal protective equipment
13. Appropriate meters for field parameters (pH, DO,salinity)
14. Secchi disk
15. GPS
16. Field notebook/pens
17. Cellular phone and contact list
18. Fish Kill Manual
19. Binoculars
20. Nautical charts
21. 3 - 500 ml Open Mouth Glass Jars
22. Stainless Steel Pan
23. Stainless Steel Scoop

**Lugol's Solution:

20 g Potassium iodide (KI)
10 g Iodine crystals
200 ml distilled water containing 20 ml glacial acetic acid

Field Observations. All field measurements and observations will be recorded in a bound and numbered notebook.

1. Note any discoloration of the water.
2. Note any erratic movements or behavior by the fish.
3. Note size, species, and number of fish.
4. Observe fish for any unusual marks such as lesions, net marks, hook marks, abrasions, or parasites. Note the location on the fish and describe these marks.
5. Record tide, weather conditions, and any other pertinent information.
6. Record sampling coordinates using a GPS.

Field Parameters. Measure the following field parameters:

1. Dissolved oxygen
2. Temperature
3. Conductivity
4. Salinity
5. pH
6. Secchi depth

Fish Enumeration. Follow the procedures described in the American Fisheries Society's Fish Kill Counting Guidelines (Appendix F of the 1997 DEQ Fish Kill Investigation Guidance Manual) for determining fish counts.

Fish Samples. Use the following guidelines in collecting fish for lesion analysis.

1. Collect only distressed or dying fish.

2. Place fish in plastic bags and keep on ice (**Do not freeze or preserve with formalin**). Include an ID

tag with date, time, location, collector, and phone number.

3. Deliver fish as soon as possible, preferably the same day, to Dr. Wolfgang Vogelbein or David Zwerner at VIMS - Aquatic Animal Disease Diagnostic Center, 1st floor, Chesapeake Bay Hall. Call first to make arrangements (Appendix C). If Dr. Vogelbein or his associates cannot be reached, keep the fish on ice until the next business day.

4. Include all field information and a map or description of the location with GPS coordinates.

5. If fish are collected by private citizens, verify that they meet the collection criteria and refer them to the Aquatic Animal Disease Diagnostic Center. If the fish were not collected properly or not moribund when collected, they should not be used.

Pfiesteria Samples. For *Pfiesteria* identification use 1 qt. cubitainers to collect from 1 ft. below the surface 3 replicate non-preserved samples and 3 replicate samples preserved with Lugol's solution (3-4 ml per qt.). Samples are to be in labeled containers kept out of the sunlight in a cooler or at room temperature. *Pfiesteria* samples are to be delivered to Dr. Harold Marshall or his alternate at ODU - Phytoplankton Lab. Call first to make arrangements (See Appendix C). Include the field information on the chain of custody form (Appendix D) and a map or description of the location with the Global Positioning Satellite (GPS) coordinates. Copies of this information are to be maintained by DEQ.

Water Quality Parameters. Water samples should be taken in conjunction with the *Pfiesteria* samples. Collect samples for nutrients (LNUT), BOD/suspended solids (NME1), total organic carbon (TOC), chlorophyll, and any additional parameters that may aid in identifying the cause of the fish kill. For depths greater than 4 meters, surface and bottom samples are to be collected for LNUT, NME1, and TOC. Also, field parameters are to be recorded at 2 m intervals. Label all samples with station location, date, time and collector. On the lab tags include a warning to DCLS that the samples may contain *Pfiesteria*.

Sediment Samples. Take 2 grab samples (taken with a 6" X 6" petite ponar) at the shallow areas close to the shoreline. Transfer the top 2 cm of each grab into a stainless steel pan and mix to combine the samples. Using clean 500 ml glass jars, collect 3 replicate sediment aliquots from the stainless steel pan. Label all samples with station location, date, time, and collector. Deliver the sediment samples with the *Pfiesteria* water samples to Dr. Marshall at ODU.

FOLLOW-UP WATER QUALITY MONITORING

Follow-up monitoring protocols are designed to provide additional data (i.e., to VDH for establishing advisories and closures).

Follow-Up Monitoring Equipment. The following equipment is needed.

1. Quart cubitainers
2. Gallon cubitainers
3. TOC bottles
4. 500 ml open mouth short glass jars
5. 2 L Chlorophyll bottles fixed with $MgCO_3$
6. 25 ml Lugol's solution
7. Drop bottle with H_2SO_4
8. Lab Tags
9. Lab Sheets
10. NCSU chain of custody sheets
11. Plastic bags of various sizes for fish samples
11. Discrete sampling bottle (Labline, Alpha, Kemmerer, etc.)
12. Personal protective equipment
13. Appropriate meters for field parameters (pH, DO, Salinity)
14. Secchi disk
15. GPS
16. Field notebook/pens
17. Cellular phone and contact list
18. Fish Kill Manual
19. Binoculars
20. Ponar dredge
21. Stainless steel pan
22. Stainless steel scoop
23. Field filtration equipment
24. 2 16' otter trawls
25. Large barrel for dumping catch
26. Sorting pans for fish ID
27. Fish measuring board
28. Heavy duty gloves
29. Cast net
30. Nautical charts

Pfiesteria Sampling. Follow the same protocols specified for the initial response sampling.

Water Quality Parameters. Water samples should be taken in conjunction with the *Pfiesteria* samples. Collect the samples using the Chesapeake Bay Program nutrient series (NTNP, PP, PNC), unfiltered nutrients (LNUT), BOD/suspended solids (NME1), total organic carbon (TOC), and chlorophyll. For depths greater than 4 meters, surface and bottom samples are to be collected for all parameters except chlorophyll. Also, field parameters are to be recorded at 2 m intervals. Label all samples with station location, date, time, and name of collector. On the lab tags include a warning to DCLS that the samples may contain *Pfiesteria*.

Sediment Samples. Follow the same protocols specified for the initial response sampling.

Fish Trawl Samples. Fish trawl survey results will be needed to provide VDH with the percent lesion data necessary for establishing advisories and closures. Trawls will be taken using a 16 ft. otter trawl with a 1/4" mesh bag on the cod end. It is recommended that two otter trawls be on board in case one is damaged during trawling. The starting and ending location of each trawl should be determined using a GPS and recorded. Trawls should be made on relatively level substrates (check nautical charts for presence of submerged pilings, obstructions, etc.) at 4-10 ft depths for approximately 2.5 to 5 minutes. The duration of the trawl is to be recorded. Enumerate and record species, length, and presence/absence of lesions for each fish. If numerous fish of one species are collected (i.e. several hundred anchovies), record only the minimum and maximum lengths. In areas with no access by boat, cast nets can be used from shore, piers or bridges. Follow the same protocols for storage and delivery of fish for lesion analysis that was specified in the initial response fish sampling guidance.

FIELD DATA RECORDS

All field measurements and observations are to be recorded in a bound and numbered notebook. All results are to be forwarded to the DEQ Central Office Water Quality Assessments (WQA) unit as soon as received.

DATA MANAGEMENT

WQA unit has the responsibility for maintaining a central data base for physical, chemical, and biological data related to *Pfiesteria* events. (Please refer to the data analysis flow chart in Appendix E).

One special study code will be established for these data sets for use by all regions and central office units, to make them easier to track and retrieve from STORET. Physical, chemical and biological data measured in the field and laboratory will be sent via labsheets to WQA where it will be manually entered into an Access database. When the STORETX database application is available, all data will be stored there until such time as a national *Pfiesteria* database is available that meets the needs of the Commonwealth.

Geolocation standards are routinely established for all data collected. The regional and central office planning staffs are now equipped with ARCVIEW software and supporting hardware. The ability to display data on maps could be a critical way to relay information to other agencies and the public.

FIELD SAMPLING TRAINING

All regional and central office DEQ staff are required - prior to their participation in the agency *Pfiesteria* response program - to either receive training or demonstrate competency in five subject areas:

1. Enumeration of dead fish during fish kill events, including knowledge of agency fish kill investigation guidance manual (1997) and American Fishery Society fish kill counting guidelines (Appendix F of 1997 DEQ Fish Kill Investigation Guidance Manual),

2. Collection, preservation and shipment of water and sediment samples for use in laboratory identification of *Pfiesteria*,

3. Use of otter trawl, beach seine, and gill nets to collect fish for fish lesion surveys,

4. Collection and identification of saltwater and estuarine fish species, especially juvenile species, and

5. Field examination of fish for external abnormalities and lesions.

It is the responsibility of the central office Scientific Research Unit (SRU), to ensure PIRT member competency in these five subject areas and to provide remedial training as needed.

Charter members of the regional and central office PIRTs attended a two day field sampling seminar at the Gloucester Point VIMS campus. The first day and half of the winter seminar included lecture and lab instructions in the five subject areas described above. The classroom training was followed by a field exercise in deployment of fish collection gear.

Equivalent training will be scheduled by SRU on an "as needed basis" for replacement PIRT members. Prior to April 1 of each year, SRU staff will evaluate the need for any additional training or refresher courses and arrange for such staff training as resources allow.

CO team members, not routinely involved in field work, must be trained in operation of dissolved oxygen, pH, and conductivity/salinity meters, basic safe boating skills, and procedures for collecting and submitting samples to DCLS. Before May 1 of each year, they are required to participate in at least two regional monitoring boat runs in order to review all aspects of field monitoring.

Members of the CO PIRT will also be required by the DEQ's Safety Officer to train periodically with their respirators and other safety equipment under conditions similar to what would be encountered in an incident response. If a team member's normal work responsibilities do not include field work, this acclimation training may also be accomplished by periodically assisting in regional Chesapeake Bay tributary monitoring trips or other *Pfiesteria* monitoring activities.

APPENDIX A

DEQ SAFETY PLAN FOR PFIESTERIA RESPONSE

DEQ SAFETY PLAN
FOR *PFIESTERIA* RESPONSE

INTRODUCTION

DEQ is charged with investigating major fish kills in Virginia waters. It also has agreements with several state agencies to participate in coordinated responses to events which may be caused by Pfiesteria or Pfiesteria like organisms (PLOs).

These response duties will require the taking of water samples and specimens of fish during or as soon as possible after a fish kill or during an event where fish are in distress from causes which cannot be readily determined. Additional sampling of water, sediment and fish will be required for a period of at least 30 days if the presence of Pfiesteria is confirmed.

The Pfiesteria organism and presumably other similar organisms produce toxins which cause immobilization and death in fish and are also associated with lesions on the fish.

The greatest problem with people has occurred in a laboratory where the organism was cultured for scientific study. It is believed that the contact with the toxins from Pfiesteria came from inhaling aerosol from the culture tanks over a period of at least several weeks. The symptoms included memory loss and problems related to performing such tasks as arithmetic computation. There have been reports of lesions on the skin of exposed persons.

The department must take prudent measures when intentionally requiring its employees to enter areas suspected of being an active site of release of the toxin(s). When using precautions such as foul weather gear and respirators in warm weather conditions, employees will experience stress from heat. The situation must be managed such that the risk to employees from working in hot conditions does not present an unacceptable risk.

This plan is intended only for those responding to incidents and the follow-up to incidents. It is intended to be a living document to be modified as conditions change and more becomes known about the nature of Pfiesteria and the problems associated with it.

SAFETY STRATEGY

I Control exposures to risks from vehicle, normal boating and aquatic work using conventional best practices, such as seat belts, following regulations and laws, avoiding operations in inclement weather.

II Reduce potential exposures to Pfiesteria Toxins by:

A. Restricting area to only necessary personnel.
B. Confining the potential exposure to as short a time as possible.
C. Rotating crews so that the same persons are not exposed day after day.
D. Preventing contact with potential toxins in water using PPE, i.e. foul weather gear
E. Reduce potential respiratory exposure to toxins in aerosols by using proven respiratory protection scheme and devices.
F. Decontaminations procedures used prior to removal of PPE.

III Manage stress caused by using PPE and respirators in hot weather to prevent impact on employee health and safety. The measures include:

A. Medical screening to prevent exposure of susceptible individuals.
B. Hydration prior to use of PPE.
C. Monitoring of individuals while wearing PPE for indications of problems.
D. Use of work/rest scheme to prevent individuals from exceeding safe exposures.
E. Use of multiple crews to reduce the amount of time each individual has to spend in PPE.
F. First aid of all operational staff to allow effective response to heat and other problems encountered by individuals as a result of field activities.
G. Reduction of solar load by using light colored (reflective) PPE. Shade to be provided where possible.
H. Use of aprons and shoulder length gloves instead of full coverage foul weather gear or full coverage gear to reduce heat load when work conditions (calm water and lack of agents causing aerosols) allow.

IV Report problems and modify programs as necessary. Review of entire safety program at least before after the end of the "Pfiesteria Season" in October and before the end of each calender year.

PRE-ACTION PHASE:

A Medical examinations will be conducted for all employees who are directly involved with response to a Suspected Pfiesteria Incident. These medical examinations are intended to accomplish several objectives.

1. Determine the overall state of health of the individual in order to prevent individuals who have medical problems which might be aggravated by conditions such as working in hot protective gear and equipment during the response activity from being exposed to conditions where they might be susceptible.

2. Qualification to wear respiratory protection.

3 Establish a baseline for comparison in the event that there are suspected problems due to exposure to Pfiesteria or other harmful agents during a response.

B. Each employee in the sampling parties (and boat crews) will be "fit tested" for a respirator.

C. First Aid and CPR training to be conducted prior to participating in the active phase of response. This is intended as a general measure to limit the impact of any accident or physical problems which might occur during response activities. In particular, individuals wearing protective clothing and respirators on warm days may have problems tolerating heat.

D. Training in the use of personal protective equipment such as respirators, foul weather gear, aprons, boots, respirators and the use of ensembles while performing specific tasks.

This training will consist of familiarization and use of the equipment in a classroom or wearing the equipment while performing specific tasks where Pfiesteria is not suspected to be present within the boat storage portion of a building and on the water. Instruction will include specific procedures for donning and doffing protective clothing, respirators and gloves. Decontamination of boats, equipment, respirators and other PPE will be demonstrated.

E. Practice use of the PPE including respirators will be conducted on a weekly basis for all boat crews. A record will be kept of the practice which includes the name, date, type, of equipment and duration of use.

F. Personal flotation gear will be tested with protective equipment, including a full face respirator in a po

G. Establishment of SOPs for tasks or groups of tasks by supervisors. These will be established by the supervisor of the field crew or boat crew after a documented risk analysis using worksheet provided by the Safety Officer. They will include a description of the task, operational instructions and will spec

List of SOPs to be available:

1. Sampling During the event (where dying or distressed fish are present)

2. Calm Conditions (apron or bib foul weather pants with respirator)
3. Filtering Samples
4. Sampling After the Event
5. Cleaning Equipment and tools

A copy of the written, approved SOP will be provided to each individual involved before an operation is conducted. This includes VMRC, VIMS and Health Department personnel who are working with DEQ employees on the same task.

H. It is recommended that sampling and boat crews practice use of PPE during activities which are not response operations and that a log is kept of such practices. This practice should occur at least once a week and the time of the practice should be extended as long as possible to obtain experience and allow the body to adapt to the use of the equipment.

I. Equipment/gear malfunction will be evaluated by the person in charge. All malfunctioning PPE required in the SOP (including respirators) will be replaced or repaired before continuing to conduct the mission. Questionable equipment will be taken out of service and repaired, replaced or tagged for evaluation at the base of operations.

DEPLOYMENT PHASE

Deployment will be such that the entry team or vessel will be at the target area in the early morning, late afternoon or other times to prevent exposure to the hottest portion of the day. When a fish kill is reported at night, deployment should be as early as possible the next day. When an active problem is reported during daylight hours and it is necessary to respond as soon as possible to get the best samples possible, entry will be as brief as possible.

Equipment will be selected or drawn from storage for a specific response. It may be stowed together in a vehicle or boat. There will be a checklist for safety equipment, based on the SOPs to be used, which will be completed before responding to the scene or to the point where a boat will be launched.

Sufficient drinking water and liquid replenishment will be aboard each boat and with each work party. This may be stored in coolers which are protected from contamination. Boat and sampling crews are required to decontaminate and remove PPE (except boots and foul weather gear) and to wash hands and face with potable water before eating, drinking or smoking, after entry is made into target areas.

A quantity of potable water will be available for use in decontamination by use of a pump up sprayer. Each boat will be equipped with a pump capable of delivering a stream of water from the body of water in which the boat is floating to be used in decontamination. This pump should have sufficient capacity to provide a stream of water through a device such as a garden hose. The pump should be checked shortly after launching and prior to reaching the target area.

Appropriate sun block and clothing will be available to protect employees for over exposure to sunlight. Sunglasses which meet the requirements for eye protection (ANSI standards for safety glasses and other types of eye protection as appropriate) will be used protect the eyes from sunlight or uv during sampling.

Additional clothing for the crew, food and personal items must be stowed so that they will not become contaminated with waters from the target area. It is recommended that food and beverage should be consumed prior to departing for target area.

Safety equipment and respirators which are missing or malfunction will be replaced or repaired before deployment occurs unless there is means to deliver replacement equipment before launching or usage will occur.

OPERATIONAL PHASE

There will be one person on each boat or in each operational party who is in charge. That person will determine when a specific SOP will be used.

If the OSC is deployed and establishes a command post, the safety designee for that region will be deployed to the command post and James Saunders, DEQ Safety Officer, will be notified (pager 804-246-4137).

Operations during the response will be conducted as specified in the SOPs which apply.

If it is necessary to deviate from SOPs, it is the supervisor's responsibility to document the deviation and the rational for the deviation. (Notice of the deviation must be provided to the Agency Safety Officer and also will be included in the written report of the response.)

When an entry into PPE is made, the time shall be recorded in the safety log or field notes. An entry in the same record will be made at the conclusion of the use of PPE.

Prior to donning of PPE except for the bib portion of foul weather gear and boots, each worker will consume as much water or an electrolyte replenishing fluid such as "GATOR AID" as he or she can tolerate. This should occur several minutes before the active use of full PPE commences.

After entering the target area, breaks for consumption of food or liquid replenishment will only be taken after leaving the target area, washing down of the PPE and boat area with water not contaminated with water from the target area, and washing of hands and face with potable water.

The target area should be approached from up wind if possible. A marker or a distance from the target area which is protected from mist and spray will be established for each site according to the condition encountered. PPE will be donned as required by the SOP before entering the target area.

If it is determined from observation or measurement that the cause of the event is other than Pfiesteria, use of PPE for protection from Pfiesteria toxins can be discontinued.

Work will commence as soon as possible after entering the target area and the work party or vessel will withdraw as soon as possible to an area which is not contaminated by any spray from the target area. After samples are secured, PPE and all surfaces of sampling tools, boat and exposed equipment will be washed with clean ambient water (another fluid or potable water can be used). The exterior of PPE (except the cartridges of respirators) will be washed with potable water and the equipment will be doffed in the manner indicated during training. Respirator cartridges may be used for an entire day unless they become blocked form water.

Containers of samples will be sealed and placed in secure coolers or other containers prior to removal of and PPE designed to prevent splash or skin contact.

If it may be necessary to use the same equipment to re-enter the area or another area the same day, the equipment shall be air dried prior to reuse so that the interior is not wet. At the close of the day equipment shall be placed in a plastic bag, secured by means of tape or a knot, to be taken ashore and cleaned and dried. If conditions do not allow for drying, disposable protective clothing may be used.

POST OPERATIONAL PHASE:

After returning to the base of operations, PPE shall be cleaned and disinfected as specified by the manufacturer or by training. Respirator cartridges will be discarded at the end of each day's operation.

The boat and equipment on the boat will be cleaned and washed down with fresh water and detergent.

All employees who have entered the target area must shower, including shampoo.

A written report of the operation shall include the names of members of the sampling parties , time and duration of use of respiratory protection and full PPE. The report shall also note any unusual event or incident and must note any deviations from SOPs and the rational for these deviations.

Safety equipment which did not function properly will be repaired or replaced.

APPENDIX B

PFIESTERIA-RELATED PUBLIC HEALTH ACTION LEVELS FOR VIRGINIA TIDAL RIVERS AND ESTUARIES

Pfiesteria-Related Public Health Action Levels for Virginia Tidal Rivers and Estuaries -- Virginia Department of Health

"RED LIGHT" WATERS

Action: Closure of a water area to recreational and commercial uses. Pass-through permitted. Geographic limits will take into account availability of easily recognized (by public) existing markers, and provide an appropriate buffer around the kill area based on visual observation of fish and testing of water for (Pfiesteria Complex Organisms) PCOs. Criteria may change as more knowledge is gained. Closure will prohibit water contact in area, including fishing, swimming, water skiing, other recreational activities and commercial fishing and shellfishing. Public service announcements will be made and signs posted where appropriate.

Meaning: It is reasonable to assume that water exposure may pose a risk to human health from toxins produced by PCOs.

Do Criteria: Presence of a fish kill which low DO or other common factors are ruled out as a cause and fish have deep ulcers or, if no ulcers, water samples examined in a VDH recognized laboratory reveal a significant number of PCOs by light microscopy.

Undo Criteria: No ongoing fish kill for 7 days and fish surveys show deep ulcers in less that 20% of a minimum sample of 50 of any one fish species.*

"YELLOW LIGHT" WATERS

Action: Health advisory for water area is issued via media, posters, signs, or handouts for marine police and water related businesses. Advisory will alert public to the current status and trigger more intensive monitoring. Advisory will reinforce avoidance of fish that are dead, dying, or have deep ulcers. Unannounced inspection of commercial edible fishing will verify that fish with deep ulcers are not entering human food chain. Advisory will recommend avoidance of intense exposure to water (primary contact recreation, e.g. swimming, water skiing). Criteria may change as more knowledge is gained. Recreational and commercial harvesting of fish and shellfish will not be affected. Geographic limits will take into account availability of easily recognized (by public) existing markers, and provide an appropriate buffer around the area of concern based on visual observation of fish and testing of water for PCOs.

Do Criteria: Fish surveys show deep ulcers in more that 20% of a minimum sample of 50 fish of any one species* and water samples examined in a VDH recognized laboratory reveal a **signficant** number of PCOs by light microscopy. There is no evidence of a fish kill as described above.

Undo Criteria: Fish surveys show deep ulcers in less than 20% of a minimum sample of 50 fish of any one species* and water samples examined in a VDH recognized laboratory reveal an **insignificant** number of PCOs. There is no evidence of a fish kill as described above.

OTHER WATERS

General Advisory: Look for posted signs and follow the advise on them. Do not swim in water in which there are dead or dying fish or that looks stagnant, muddy or smells unpleasant. Try to avoid swallowing river, stream, bay or lake water, especially if you are immunocompromised. Prevent broken skin from directly contacting natural bodies of water. Bathe skin with soap and water after contact with natural bodies of water. Do not eat fish or shellfish that have sores or do not appear healthy or have been taken from areas with large numbers of dead and dying fish (fish kills). Report symptoms of illness that you believe are related to contact with water to your physician, your local health department or the toll-free Pfiesteria line (888-238-6154).

*If during follow-up monitoring less than 50 fish of a species are collected, a determination of whether to act will be made after consultation with the Task Force.

APPENDIX C

PFIESTERIA RESPONSE CONTACT LIST

Pfiesteria Response Contact List

VESSEL AVAILABILITY FOR INITIAL RESPONSE

Virginia Marine Resource Commission.

Twenty-four hour toll-free operations number, 1-800-541-4646.

Virginia Department of Game and Inland Fisheries.

Gary Martel, (804-367-1004) or regional fisheries biologists.

VHD Division of Shellfish Sanitation:

Norfolk Office (757-683-8461)
Accomack County Office (757-787-5864)
White Stone Office (804-435-1095)

PFIESTERIA COMPLEX ORGANISM ANALYSIS AND VERIFICATION

Old Dominion University:

Dr. Harold Marshall, (757-683-4204, hmarshal@odu.edu),
Phytoplankton Lab, Mills Godwin Building, Norfolk, 23529

Virginia Institute of Marine Science, College of William and Mary:

Dr. Larry Haas, (804-684-7258, lhaas@vims.edu)

FISH PATHOLOGY

Virginia Institute of Marine Science, College of William and Mary

Dr. Wolfgang Vogelbein, VIMS Aquatic Animal Disease Diagnostic Center (daytime 804-642-7261; after hours 804-642-9522, wolf@vims.edu)

David Zwerner (804-642-7249), Waterman's Hall, Gloucester Point.

VIRGINIA DEPARTMENT OF HEALTH

Dr. Suzanne Jenkins, State Epidemiologist (804-786-6029, sjenkins@vdh.state.va.us).

APPENDIX D

CHAIN OF CUSTODY FORM

DEPARTMENT OF ENVIRONMENTAL QUALITY - CHAIN OF CUSTODY REPORT

Organization:

Date:

River:

Collected by (signature): _____ _____ _____ _____

River and Atmospheric Condition (daily temp./wind speed & direction/visual appearance/distressed fish/abnormal appearance)-

_____

_____

_____

Sample Location	Sample Time	Sample Depth	Phytoplankton Preserved	Phytoplankton Unpreserved	Other Samples collected	Physical Data Depth Profile	DO mg/L	Salinity ppt	Temp ,C	Secchi Depth	Fish Kill Occurring #'s & Type

Relinquished by: Date: Time: Note:

Received in the field by: Date: Time: Note:

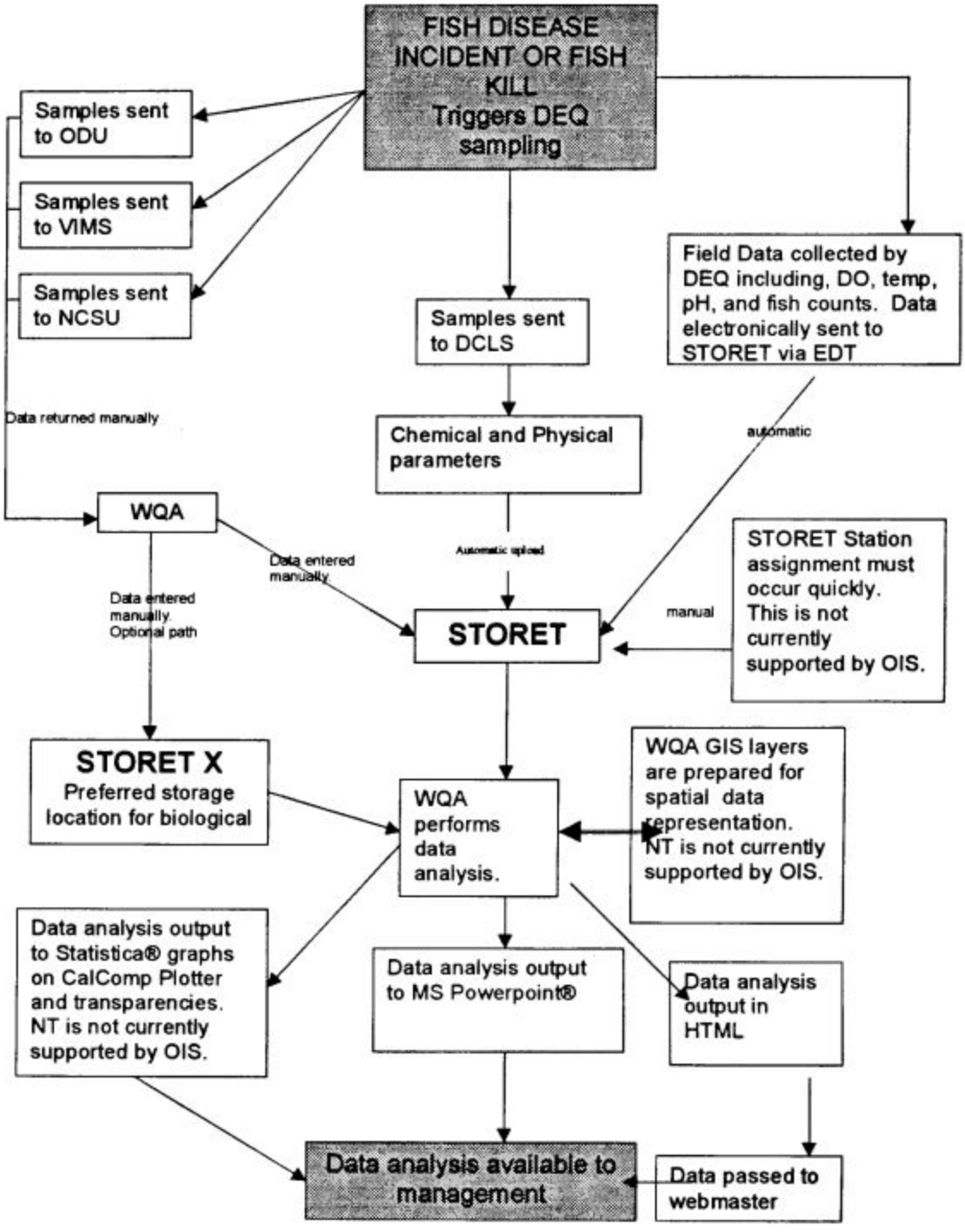
Relinquished by: Date: Time: Note:

Received into the lab by: Date: Time: Note:

APPENDIX E

DATA FLOW CHART FOR *PFIESTERIA* ANALYSIS

NOTE: This flow chart may not print - contact DEQ for a copy.


FISH DISEASE INCIDENT OR FISH KILL Triggers DEQ sampling
Samples sent to ODU
Samples sent to VIMS
Samples sent to NCSU
Samples sent to DCLS
Field Data collected by DEQ including, DO, temp, pH, and fish counts. Data electronically sent to STORET via EDT
Data returned manually
Chemical and Physical parameters
automatic
WQA
Data entered manually
Automatic upload
STORET Station assignment must occur quickly. This is not currently supported by OIS.
Data entered manually Optional path
manual
STORET
STORET X
Preferred storage location for biological
WQA GIS layers are prepared for spatial data representation. NT is not currently supported by OIS.
WQA performs data analysis.
Data analysis output to Statistica® graphs on CalComp Plotter and transparencies. NT is not currently supported by OIS.
Data analysis output to MS Powerpoint®
Data analysis output in HTML
Data analysis available to management
Data passed to webmaster

APPENDIX F

PFIESTERIA RESPONSE PROCESS

COMMONWEALTH OF VIRGINIA
PFIESTERIA RESPONSE PROCESS

INITIAL REPORTS

- ❑ Work Hours - Calls handled at DEQ Regional Offices
  - ✳ Tidewater Regional Office (757-518-2000)
  - ✳ Piedmont Regional Office (804-527-5020)
  - ✳ Northern Regional Office (703-583-3800)
- ❑ After Hours - Calls received at DES (1-800-468-8892)
  - ✳ DES Dispatcher Pages on-call DEQ Responder
- ❑ DEQ Responder decides if field investigation is needed

INITIAL FIELD INVESTIGATION (if needed)

- ❑ DEQ Responder notifies VMRC and arranges field meeting
- ❑ VMRC and DEQ conduct initial field investigation
  - ✳ D.O., pH, and temperature measurements
  - ✳ Visual observations
  - ✳ VMRC and DEQ collect water quality and PCO samples
- ❑ DEQ decides if field response is needed

FIELD RESPONSE (if needed)

- ❑ DEQ On-Scene Coordinator is notified
  - ✳ Activates DEQ Response Team(s) as needed
  - ✳ Notifies Task Force, Secretariats, Local Authorities etc.
  - ✳ Coordinates activities on-site
- ❑ DEQ Response Team(s) collect data
  - ✳ Fish Lesion Counts
  - ✳ Fish Samples sent to VIMS for analysis
  - ✳ PCO Samples sent to ODU for analysis
  - ✳ Water Quality Samples sent to DCLS for analysis
- ❑ All Data sent to VDH as quickly as possible
- ❑ VDH evaluates data and decides action level
  - ✳ Yellow Light - Public Advisory issued
  - ✳ Red Light - water body closed or restricted
- ❑ VMRC enforces water body restrictions
- ❑ VDH decides status changes & handle press relations