SECTION 6

FIELD PROCEDURES

This section provides guidance on sampling design of screening and intensive studies and recommends field procedures for collecting, preserving, and shipping samples to a processing laboratory for target analyte analysis. Planning and documentation of all field procedures are emphasized to ensure that collection activities are cost-effective and that sample integrity is preserved during all field activities. This section also describes the implications that result when deviations occur in the recommended study design. Some of the deviations in study design most likely to occur include the use of unequal numbers of fish in composite samples, unequal numbers of replicate samples collected at different stations, and sizes of fish within a composite sample exceeding the recommendation for composite samples.

6.1 SAMPLING DESIGN

Prior to initiating a screening or intensive study, the program manager and field sampling staff should develop a detailed sampling plan. As described in Section 2, there are seven major parameters that must be specified prior to the initiation of any field collection activities:

- Site selection
- Target species (and size class)
- Target analytes
- Target analyte screening values
- Sampling times
- Sample type
- Replicate samples.

In addition, personnel roles and responsibilities in all phases of the fish and shellfish sampling effort should be defined clearly. All aspects of the final sampling design for a state's fish and shellfish contaminant monitoring program should be documented clearly by the program manager in a Work/QA Project Plan (see Appendix I). Routine sample collection procedures should be prepared as standard operating procedures (U.S. EPA, 1984b) to document the specific methods used by the state and to facilitate assessment of final data quality and comparability.

The seven major parameters of the sampling plan should be documented on a sample request form prepared by the program manager for each sampling site. The sample request form should provide the field collection team with readily available information on the study objective, site location, site name/number, target species and alternate species to be collected, target analytes to be evaluated, anticipated sampling dates, sample type to be collected, number and

size range of individuals to be collected for each composite sample, sampling method to be used, and number of replicates to be collected. An example of a sample request form is shown in Figure 6-1. The original sample request form should be filed with the program manager and a copy kept with the field logbook. The seven major parameters that must be specified in the sampling plan for screening and intensive studies are discussed in Sections 6.1.1 and 6.1.2, respectively.

6.1.1 Screening Studies (Tier 1)

The primary aim of screening studies is to identify frequently fished sites where commonly consumed fish and shellfish species are chemically contaminated and may pose a risk to human health. Ideally, screening studies should include all waterbodies where commercial, recreational, or subsistence fishing and shellfish harvesting are practiced.

6.1.1.1 Site Selection—

Sampling sites should be selected to identify extremes of the bioaccumulation spectrum, ranging from presumed undisturbed reference sites to sites where existing data (or the presence of potential pollutant sources) suggest significant chemical contamination. Where resources are limited, states initially should target those harvest sites suspected of having the highest levels of contamination and of posing the greatest potential health risk to local fish and shellfish consumers. Screening study sites should be located in frequently fished areas near

- Point source discharges such as
  - Industrial or municipal discharges
  - Combined sewer overflows (CSOs)
  - Urban storm drains

- Nonpoint source inputs such as
  - Landfills, Resource Conservation and Recovery Act (RCRA) sites, or Superfund Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) sites
  - Areas of intensive agricultural, silvicultural, or resource extraction activities or urban land development
  - Areas receiving inputs through multimedia mechanisms such as hydrogeologic connections or atmospheric deposition (e.g., areas affected by acid rain impacts, particularly lakes with pH <6.0 since elevated mercury concentrations in fish have been reported for such sites)

- Areas acting as potential pollutant sinks where contaminated sediments accumulate and bioaccumulation potential might be enhanced (i.e., areas where water velocity slows and organic-rich sediments are deposited)

- Areas where sediments are disturbed by dredging activities

Sample Request Form

Project Objective	☐ Screening Study	☐ Intensive Study
Sample Type	☐ Fish fillets only ☐ Shellfish (edible portions) *(Specify portions if other than whole _____)* ☐ Whole fish or portions other than fillet *(Specify tissues used if other than whole _____)*	☐ Fish fillets only ☐ Shellfish (edible portions) *(Specify portions if other than whole _____)* ☐ Whole fish or portions other than fillet *(Specify tissues used if other than whole _____)*
Target Contaminants	☐ All target contaminants ☐ Additional contaminants *(Specify _____)*	☐ Contaminants exceeding screening study SVs *(Specify _____ _____)*

INSTRUCTIONS TO SAMPLE COLLECTION TEAM

Project Number: _____ Site (Name/Number): _____

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

Target Species: Alternate Species: *(in order of preference)*

☐ Freshwater _____ _____

☐ Estuarine _____ _____

_____ _____

Proposed Sampling Dates: _____

Proposed Sampling Method: _____

☐ Electrofishing ☐ Mechanical grab or tongs

☐ Seining ☐ Biological dredge

☐ Trawling ☐ Hand collection

☐ Other *(Specify _____)*

Number of Sample Replicates: ☐ No field replicates (1 composite sample only)

☐ _____ field replicates

(Specify number for each target species)

Number of Individuals per Composite: _____ Fish per composite

_____ Shellfish per composite *(specify number to obtain 200 grams of tissue)*

Figure 6-1. Example of a sample request form.

- Unpolluted areas that can serve as reference sites for subsequent intensive studies or as "green areas" that states can designate for unrestricted consumption (see Appendix B). **Note:** Michigan sampled lakes that were in presumed unpolluted areas but discovered mercury contamination in fish from many of these areas and subsequently issued a fish consumption advisory for all of its inland lakes.

The procedures required to identify candidate screening sites near significant point source discharges are usually straightforward. It is often more difficult, however, to identify clearly defined candidate sites in areas affected by pollutants from nonpoint sources. For these sites, assessment information summarized in state Section 305(b) reports should be reviewed before locations are selected. State 305(b) reports are submitted to the EPA Assessment and Watershed Protection Division biennially and provide an inventory of the water quality in each state. The 305(b) reports often contain Section 319 nonpoint source assessment information that may be useful in identifying major sources of nonpoint source pollution to state waters. States may also use a method for targeting pesticide hotspots in estuarine watersheds that employs pesticide use estimates from NOAA's National Coastal Pollutant Discharge Inventory (Farrow et al., 1989).

It is important for states to identify and document at least a few unpolluted sites, particularly for use as reference sites in subsequent monitoring studies. Verification that targeted reference sites show acceptably low concentrations of contaminants in fish or shellfish tissues also provides at least partial validation of the methods used to select potentially contaminated sites. Clear differences between the two types of sites support the site-selection methodology and the assumptions about primary sources of pollution.

In addition to the intensity of subsistence, sport, or commercial fishing, factors that should be evaluated (Versar, 1982) when selecting fish and shellfish sampling sites include

- Proximity to water and sediment sampling sites
- Availability of data on fish or shellfish community structure
- Bottom condition
- Type of sampling equipment
- Accessibility of the site.

The most important benefit of locating fish or shellfish sampling sites near sites selected for water and sediment sampling is the possibility of correlating contaminant concentrations in different environmental compartments (water, sediment, and fish). Selecting sampling sites in proximity to one another is also more cost-effective in that it provides opportunities to combine sampling trips for different matrices.

Availability of data on the indigenous fish and shellfish communities should be considered in final site selection. Information on preferred feeding areas and

migration patterns is valuable in locating populations of the target species (Versar, 1982). Knowledge of habitat preference provided by fisheries biologists or commercial fishermen may significantly reduce the time required to locate a suitable population of the target species at a given site.

Bottom condition is another site-specific factor that is closely related to the ecology of a target fish or shellfish population (Versar, 1982). For example, if only soft-bottom areas are available at an estuarine site, neither oysters (*Crassostrea virginica*) nor mussels (*Mytilus edulis* and *M. californianus*) would likely be present because these species prefer hard substrates. Bottom condition also must be considered in the selection and deployment of sampling equipment. Navigation charts provide depth contours and the locations of large underwater obstacles in coastal areas and larger navigable rivers. Sampling staff might also consult commercial fishers familiar with the candidate site to identify areas where the target species congregates and the appropriate sampling equipment to use.

Another factor closely linked to equipment selection is the accessibility of the sampling site. For some small streams or land-locked lakes (particularly in mountainous areas), it is often impractical to use a boat (Versar, 1982). In such cases the sampling site should have good land access. If access to the site is by land, consideration should be given to the type of vegetation and local topography that could make transport of collection equipment difficult. If access to the sampling site is by water, consideration should be given to the location of boat ramps and marinas and the depth of water required to deploy the selected sampling gear efficiently and to operate the boat safely. Sampling equipment and use are discussed in detail in Section 6.2.1.

The selection of each sampling site must be based on the best professional judgment of the field sampling staff. Once the site has been selected, it should be plotted and numbered on the most accurate, up-to-date map available. Recent 7.5-minute (1:24,000 scale) maps from the U.S. Geologic Survey or blue line maps produced by the U.S. Army Corps of Engineers are of sufficient detail and accuracy for sample site mapping. The type of sampling to be conducted, water depth, and estimated time to the sampling site from an access point should be noted. The availability of landmarks for visual or range fixes should be determined for each site, and biological trawl paths (or other sampling gear transects) and navigational hazards should be indicated. Additional information on site-positioning methods, including Loran-C, VIEWNAV, TRANSIT (NAVSAT), GEOSTAR, and the NAVSTAR Global Positioning System (GPS), is provided in Battelle (1986), Tetra Tech (1986), and Puget Sound Estuary Program (1990a).

Each sampling site must be described accurately because state fish and shellfish contaminant monitoring data may be stored in a database available to users nationwide (see Section 9.2). For example, a sampling site may be defined as a 2-mile section of river (e.g., 1 mile upstream and 1 mile downstream of a reference point) or a 2-mile stretch of lake or estuarine/marine shoreline (U.S. EPA, 1990d). Each sampler should provide a detailed description of each site

using a 7.5-minute USGS map to determine the exact latitude and longitude coordinates for the reference point of the site. This information should be documented on the sample request form and field record sheets (see Section 6.2.3).

One additional consideration associated with sample site selection is whether the sampling area includes waters inhabited by threatened or endangered species. If such waterbodies are to be monitored, the state must obtain a permit from the U.S. Fish and Wildlife Service (USFWS) if their sampling effort could potentially impact a freshwater species (U.S. DOI, 1999) or from the National Marine Fisheries Service (NMFS) if their sampling effort could potentially impact any marine or anadromous species (U.S. DOC, 1999a, 1999b) covered under the Endangered Species Act (ESA) of 1973.

A species is listed under one of two categories, endangered or threatened, depending on its status and the degree of threat it faces. An endangered species is one that is in danger of extinction throughout all or a significant portion of its range. A threatened species is one that is likely to become endangered in the foreseeable future. The U.S. Fish and Wildlife Service maintains a list of all plant and animal species native to the United States that are candidates or proposed for possible addition to the Federal List. A complete listing of the current status of all threatened and endangered species as well as information about each USFWS region is available on-line on the USFWS website at
http://endangered.fws.gov/wildlife.html

Species information is also available by USFWS region having primary responsibility for that species. The seven major USFWS regions with their respective states are shown in Figure 6-2. States can obtain additional information by contacting the specific USFWS regional office and talking with the regional liaison for endangered species.

Freshwater Threatened and Endangered Species

State conservation agencies typically have cooperative agreements in place with the U.S. Fish and Wildlife Service. Under these agreements, any qualified employee of the state agency may take those endangered species covered by the cooperative agreement for conservation programs. Such taking of these species may be done provided it does not result in the following:

- Death or permanent disabling of the specimen
- Rremoval of the specimen from the state where the taking occurred
- Introduction of the specimen so taken, or of any progeny derived from the specimen, into an area beyond the historical range of the species
- Holding of the specimen in captivity for a period of more than 45 consecutive days.

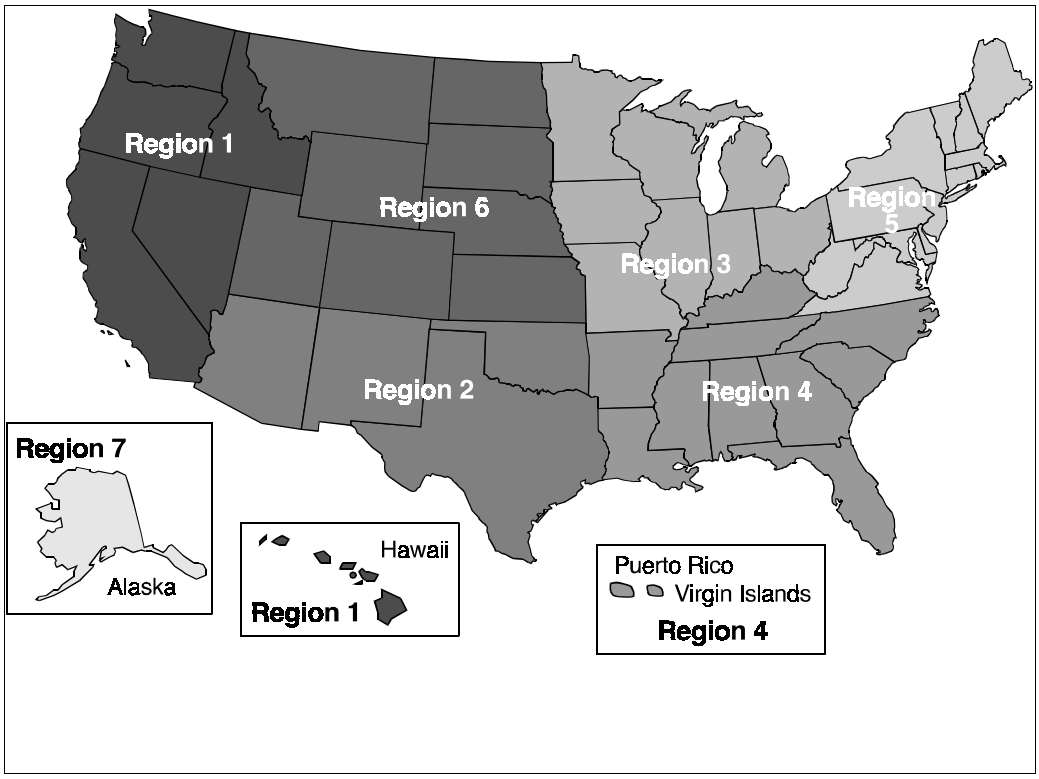


Figure 6-2. U.S. Fish and Wildlife Service Regions.

Additionally, any employee of a state conservation agency that is operating a conservation program with the USFWS (in accordance with section 6(c) of the Endangered Species Act) may take those threatened species of wildlife that are covered by an approved cooperative agreement to carry out conservation programs.

State agencies involved in designing and conducting fish sampling programs in freshwater systems may need to sample fish for human health risk assessments from areas inhabited by threatened or endangered species. In some of these waterbodies under study, threatened or endangered species may be collected incidental to the primary sampling objective. In these cases, the state agency involved in the primary sampling needs to check with the state conservation agency to determine whether a cooperative agreement between the state and the USFWS is in effect. Any questions about the permits for incidental taking of endangered or threatened species resulting from fish sampling programs should be reviewed with the appropriate USFWS regional endangered species liaison officer. If appropriate, the state must apply to the USFWS for an Incidental Take Permit (U.S. DOI, 1999). States are required to submit information on USFWS Form 3-200 with all of the following information provided as part of the permit application:

- A complete description of the sampling activity sought to be authorized
- The common and scientific names of the species sought to be covered by the permit, as well as the number, age, and sex of such species, if known.

The application must also include a conservation plan that specifies

- The impact that will likely result from such incidental taking
- What steps the applicant will take to monitor, minimize, and mitigate such impacts, the funding that will be available to implement such steps, and the procedures to be used to deal with unforseen circumstances
- What alternative actions to such incidental taking the applicant considered and the reasons why such alternatives are not proposed to be used
- Such other measures that the Director may require as being necessary or appropriate for purposes of the plan.

The completed application should be submitted to

U.S. Fish and Wildlife Service
Ecological Services/Endangered Species Permits
Attention: Regional Permit Coordinator
(see addresses below for each of the seven USFWS regional offices)

Region 1
Pacific Region
Eastside Federal Complex
911 NE 11th Avenue
Portland, OR 97232-4181

Region 2
Southwest Region
P.O. Box 1306
Albuquerque, NM 87103-1306

Region 3
Great Lakes and Big Rivers Region
1 Federal Drive
BHW Federal Building
Fort Snelling, MN 55111

Region 4
Southeast Region
1875 Century Boulevard, Suite 400
Atlanta, GA 30345-3319

Region 5
Northeast Region
300 Westgate Center Drive
Hadley, MA 01035-9589

Region 6
Mountain Prairie Region
134 Union Boulevard
Lakewood, CO 80228

Region 7
Alaska Region
300 Vintage Boulevard, Suite 201
Juneau, AK 99801-7125

States should expect to wait from 3 to 6 months to obtain such a permit and should plan and schedule their permit application submission accordingly.

Marine or Anadromous Threatened and Endangered Species

Each state that intends to sample fish as part of their tissue residue monitoring program and might collect endangered or threatened marine or anadromous species incidental to the purpose of their monitoring effort, must apply to the NMFS for an Incidental Take Permit (U.S. DOC, 1999a). Application forms and detailed instructions for completing these permit applications are available for downloading on the Internet at url: **http://www.nmfs.noaa.gov/prot_res/PR3/Permits/ESAPermit.html**. Users should click on <<Incident Take of Listed Species>> under Activity Category and select the PDF or HTML instructions.

States are required to submit information about the following:

- Type of permit
- Date of application
- Name, address, telephone, and fax number of the applicant
- A description of the endangered or threatened species, by common and scientific name, and a description of the status distribution, seasonal distribution, habitat needs, feeding habits, and other biological requirements of the affected species
- A detailed description of the proposed sampling activity, including
  - Anticipated dates and duration of sampling activity
  - Specific location of the activity (latitude and longitude coordinates)
  - An estimate of the total level of activity expected to be conducted

The application must also include a conservation plan based on the best scientific and commercial data available, which specifies

- Anticipated impact of the proposed activity on the listed species, including
  - Estimated number of animals of the listed species and, if applicable, the subspecies or population group and range
  - Type of anticipated taking, such as harassment, predation, competition for space and food, etc.
  - Effects of the take on the listed species, such as descaling, altered spawning activities, potential for mortality

- Anticipated impact of the proposed activity on the habitat of the species and the likelihood of restoration of the affected habitat

- Steps that will be taken to monitor, minimize, and mitigate such impacts, including

  — Specialized equipment, methods of conducting activities, or other means.
  — Detailed monitoring plans
  — Funding available to implement measures taken to monitor, minimize, and mitigate impacts.

- Alternative actions to such taking that were considered and the reasons why those alternatives are not being used.

- A list of all sources of data used in preparation of the plan, including reference reports, environmental assessments and impact statements, and personal communications with recognized experts on the species or activity who may have access to data not published in the current literature.

The application may be submitted electronically if possible (either by e-mail or by mailing a diskette), but one signed original of the complete application must be sent to

Chief, Endangered Species Division
National Marine Fisheries Service, F/PR3
1315 East-West Highway
Silver Spring, Maryland 20910
Telephone (301) 713-1401, Fax (301) 713-0376

States should expect to wait from 3 to 6 months to obtain such a permit and should plan and schedule their permit application submission accordingly.

Threatened or Endangered Sea Turtles

States planning on sampling fish in marine waters inhabited by threatened or endangered species of sea turtles must apply to the NMFS for a Sea Turtle Incidental Take Permit (U.S. DOC, 1999b).

Application forms and detailed instructions for completing these permit applications are available for downloading on the Internet at **http://www.nmfs.noaa.gov/prot_res/PR3/Permits/ESAPermit.html**.

States are required to submit a cover letter including information on the following:

- Type of permit

- Date of application

- Name, address, telephone, and fax number of the applicant

- A description of each endangered or threatened sea turtle species impacted by the activity, by common and scientific name, and a description of the status, geographic distribution, seasonal distribution, habitat needs, feeding habits, and other biological requirements of the affected species

- A detailed description of the proposed sampling activity (fishery season), including

  - Anticipated dates and duration of sampling activity
  - Specific location of the activity (latitude and longitude coordinates) and fishery effort in that area
  - Other relevant information (e.g., gear description.)

The application must also submit a Conservation Plan based on the best scientific and commercial data available. The Conservation Plan must emphasize techniques, gear types, and general practices to mitigate takes. The Conservation Plan may involve development of new gear types or modification of fishing practices and include the following information

- Anticipated impact of the activity on the listed species of sea turtle, including
  - Estimated number of animals of the listed species impacted, their geographic range, and, if applicable, the subspecies or population group,
  - Type of anticipated taking, such as capture, harassment, predation, competition for space and food, nature of injury
  - Effects of the impact on the listed species, such as descaling, altered reproductive activities, potential for mortality, effects of repeated submergence

- Anticipated impact of the proposed activity on the habitat of the species and the likelihood of restoration of the affected habitat

- Steps that will be taken to monitor, minimize, and mitigate such impacts, including
  - Detailed monitoring plans (e.g., observer programs)
  - Detailed enforcement plans (e.g., monitoring Turtle Excluder Device compliance)
  - Specialized equipment, methods of conducting activities, or other mitigation techniques.
  - Detailed funding plan to implement measures taken to monitor, minimize, and mitigate impacts.

- Alternatives to the activity considered and the reasons why those alternatives are not being used.

- A list of all sources of data used in preparation of the plan, including reference reports, environmental assessments and impact statements, and personal communications with recognized experts on the species or activity who may have access to data not published in the current literature.

- Other measures the Assistant Administrator of NMFS may require as necessary or appropriate for the purposes of the plan.

The following criteria are considered for permit issuance:

- Status of the stock and/or species to be incidentally taken

- Likely direct and indirect impacts of the activity on sea turtles

- Availability and effectiveness of monitoring and enforcement programs

- Public comments received during the 30-day public notice and comment period

- Adequate funding for the Conservation Plan

- The fact that taking will not appreciably reduce the likelihood of survival and recovery of the species in the wild.

An issued permit would

- Require regular reporting and rights of inspection

- Identify species and number of animals allowed to be taken incidentally

- Specify the authorized method of incidental taking

- Require procedures for captured sea turtles (i.e., resuscitation techniques, disposal)

- Potentially impose administrative fees

- Establish duration of the permit

- Specify any other terms or conditions that the Assistant Administrator of NMFS identifies as necessary or appropriate

- The application may be submitted electronically if possible (either by e-mail or by mailing a diskette), but one signed original of the complete application must be sent to

Chief, Endangered Species Division
National Marine Fisheries Service, F/PR
1315 East-West Highway
Silver Spring, Maryland 20910
Telephone (301) 713-1401, Fax (301) 713-0376

States should expect to wait from 3 to 6 months to obtain such a permit and should plan and schedule their permit application submission accordingly.

6.1.1.2 Target Species and Size Class Selection—

After reviewing information on each sampling site, the field collection staff should identify the target species that are likely to be found at the site. Target species recommended for screening studies in freshwater systems are shown in Tables 3-1, 3-2, and 3-4. Tables 3-10 through 3-16 list recommended species for estuarine/marine areas. In freshwater ecosystems, one bottom-feeding and one predator fish species should be collected. In estuarine/marine ecosystems, either one bivalve species and one finfish species or two finfish species should be collected. Second- and third-choice target species should be selected in the event that the recommended target species are not collected at the site. The same criteria used to select the recommended target species (Section 3.2) should be used to select alternate target species. In all cases, the primary selection criterion should be that the target species is commonly consumed locally and is of harvestable size.

EPA recognizes that resource limitations may influence the sampling strategy selected by a state. If monitoring resources are severely limited, precluding performance of any Tier 2 intensive studies (Phase I and Phase II), EPA recommends three sampling options to states for collecting additional samples during the screening studies. These options are:

1. Collecting one composite sample for each of three size (age) classes of each target species
2. Collecting replicate composite samples for each target species
3. Collecting replicate composite samples for each of three size (age) classes of each target species.

Option 1 (single composite analysis for each of three size classes) provides additional information on size-specific levels of contamination that may allow states to issue an advisory for only the most contaminated size classes while allowing other size classes of the target species to remain open to fishing. The state could analyze the composite sample from the largest size class first. If any SVs are exceeded, analysis of the smaller size class composite samples could be conducted. This option, however, does not provide any additional information for estimating the variability of the contamination level in any specific size class. To obtain information for estimating the variability of the contamination level in the target species, states could separately analyze each individual fish specimen in

any composite that exceeded the SVs. **Note:** This option of analyzing individual fish within a composite sample is more resource-intensive with respect to analytical costs but is currently used by some Great Lakes states.

Option 2 (replicate analyses of one size class) provides additional statistical power that would allow states to estimate the variability of contamination levels within the one size class sampled; however, it does not provide information on size-specific contamination levels.

Option 3 (replicate analyses of three size classes) provides both additional information on size-specific contamination levels and additional statistical power to estimate the variability of the contaminant concentrations in each of three size classes of the target species. If resources are limited, the state could analyze the replicate samples for the largest size class first; if the SVs are exceeded, analysis of the smaller size class composite samples could then be conducted.

Note: The correlation between increasing size (age) and contaminant tissue concentration observed for some freshwater finfish species (Voiland et al., 1991) may be much less evident in estuarine/marine finfish species (G. Pollock, California Environmental Protection Agency, personal communication, 1993). The movement of estuarine and marine species from one niche to another as they mature may change their exposure at a contaminated site. Thus, size-based sampling in estuarine/marine systems should be conducted only when it is likely to serve a potential risk management outcome.

6.1.1.3 Target Analyte Selection—

All 25 recommended target analytes listed in Table 4-1 should be considered for inclusion in screening studies unless reliable historic tissue, sediment, or pollutant source data indicate that an analyte is not present at a level of concern for human health. Additional regional or site-specific target analytes should be included in screening studies when there is indication or concern that such contaminants are a potential health risk to local fish or shellfish consumers. Historic data on water, sediment, and tissue contamination and priority pollutant scans from known point source discharges or nonpoint source monitoring should be reviewed to determine whether analysis of additional analytes is warranted.

6.1.1.4 Target Analyte Screening Values—

To enhance national consistency in screening study data, states should use the target analyte screening values listed in Tables 5-3 and 5-4 to evaluate tissue contaminant data. Specific methods used to calculate SVs for noncarcinogenic and carcinogenic target analytes, including examples of SVs calculated for selected subpopulations, are given in Sections 5.1 and 5.2. If target analytes different from those default SVs shown in Tables 5-3 and 5-4 are included in a screening study, these calculation procedures should be used to estimate SVs based on typical exposure assumptions for the fish-consuming public for the

additional compounds. **Note:** If the state chooses to use a different risk level or consumption rate to address site-specific considerations, the corresponding SVs should be calculated prior to initiation of chemical analyses to ensure that the detection limits of the analytical procedures are sufficiently low to allow reliable quantitation at or below the chosen SV. If analytical methodology is not sensitive enough to reliably quantitate target analytes at or below selected SVs (see Sections 5.2 and 8.2.2 and Table 8-4), program managers must determine appropriate fish consumption guidance based on lowest detectable concentrations or provide justification for adjusting SVs to values at or above achievable method detection limits. It should be emphasized that when SVs are below method detection limits, the failure to detect a target analyte cannot be assumed to indicate that there is no cause for concern for human health effects.

6.1.1.5 Sampling Times—

If program resources are sufficient, biennial screening of waterbodies is recommended where commercial, recreational, or subsistence harvesting is commonly practiced (as identified by the state). Data from these screenings can then be used in the biennial state 305(b) reports to document the extent of support of Clean Water Act goals. If biennial screening is not possible, then waterbodies should be screened at least once every 5 years.

Selection of the most appropriate sampling period is very important, particularly when screening studies may be conducted only once every 2 to 5 years. **Note:** For screening studies, sampling should be conducted during the period when the target species is most frequently harvested (U.S. EPA, 1989d; Versar, 1982).

In fresh waters, as a general rule, the most desirable sampling period is from late summer to early fall (i.e., August to October) (Phillips, 1980; Versar, 1982). The lipid content of many species (which represents an important reservoir for organic pollutants) is generally highest at this time. Also, water levels are typically lower during this time, thus simplifying collection procedures. This late summer to early fall sampling period should not be used, however, if (1) it does not coincide with the legal harvest season of the target species or (2) the target species spawns during this period. **Note:** If the target species can be legally harvested during its spawning period, however, then sampling to determine contaminant concentrations should be conducted during this time.

A third exception to the late summer to early fall sampling recommendation concerns monitoring for the organophosphate pesticides. Sampling for these compounds should be conducted during late spring or early summer within 1 to 2 months following pesticide application because these compounds are degraded and metabolized relatively rapidly compared to organochlorine pesticides. **Note:** The target species should be sampled during the spring only if the species can be legally harvested at this time.

In estuarine and coastal waters, the most appropriate sampling time is during the period when most fish are caught and consumed (usually summer for recreational and subsistence fishers). For estuarine/marine shellfish (bivalve molluscs and crustaceans), two situations may exist. The legal harvesting season may be strictly controlled for fisheries resource management purposes or harvesting may be open year round. In the first situation, shellfish contaminant monitoring should be conducted during the legal harvest period. In the second situation, monitoring should be conducted to correspond to the period when the majority of harvesting is conducted during the legal season. state staff may have to consider different sampling times for target shellfish species if differences in the commercial and recreational harvesting period exist.

Ideally, the sampling period selected should avoid the spawning period of the target species, including the period 1 month before and 1 month after spawning, because many aquatic species are subject to stress during spawning. Tissue samples collected during this period may not always be representative of the normal population. For example, feeding habits, body fat (lipid) content, and respiration rates may change during spawning and may influence pollutant uptake and clearance. Collecting may also adversely affect some species, such as trout or bass, by damaging the spawning grounds. Most fishing regulations protect spawning periods to enhance propagation of important fishery species. Species-specific information on spawning periods and other life history factors is available in numerous sources (e.g., Carlander, 1969; Emmett et al., 1991; Pflieger, 1975; Phillips, 1980). In addition, digitized life history information is available in many states through the Multistate Fish and Wildlife Information Systems (1990) on the web at **http://fwie.fw.vt.edu**.

Exceptions to the recommended sampling periods for freshwater and estuarine/ marine habitats will be determined by important climatic, regional, or site-specific factors that favor alternative sampling periods. For many states, budgetary constraints may require that most sampling be conducted during June, July, and August when temporary help or student interns are available for hire. The actual sampling period and the rationale for its selection should be documented fully and the final data report should include an assessment of sampling period effects on the results.

6.1.1.6 Sample Type—

Composite samples of fish fillets or of the edible portions of shellfish are recommended for analysis of target analytes in screening studies (U.S. EPA 1987b; 1989d). For health risk assessments, the recommended composite sample type for chemical analysis should be based on both the study objectives and the sample type consumed by the target population of concern. For example, using skinless fillets for assessing mercury exposures for members of the general population and most recreational fishers is most conservative. Because mercury is differentially concentrated in muscle tissue, leaving the skin on the fish fillet actually results in a lower mercury concentration per gram of skin-

on fillet than per gram of skin-off fillet (Gutenmann and Lisk, 1991). In addition, few consumers in the general population eat the skin of the fish, which justifies its removal for analysis, particularly when monitoring concerns are directed solely at mercury contamination. Analysis of skinless fillets may also be more appropriate for some target species such as catfish and other scaleless finfish species. In contrast, using whole fish with skin-on as the sample type for assessing PCBs, dioxins/furans, or organochlorine pesticide exposures in populations of Native Americans, Asian Americans, Caribbean-Americans, or other ethnic groups that consume whole fish in a stew or soup is warranted because these contaminants accumulate in fatty tissues of the fish. Cooking the whole fish to make a stew or soup releases the PCBs, dioxins/furans, or organochlorine contaminants into the broth; thus, the whole fish should be analyzed to mirror the way the consumer prepares the fish. Similarly, using skin-on fillets with belly-flap included for most other scaled fish to evaluate PCB, dioxin/furan, or organochlorine pesticide exposures in the general fishing population or among recreational fishers is appropriate since this is a standard filleting method (see Sections 7.2.2.6 and 7.2.2.7). This method also allows for the inclusion of the fatty belly flap tissue and skin in which organochlorines, PCBs, and dioxins/furans concentrate and takes into account the fact that some consumers may not neatly trim the more highly contaminated fatty tissue from the edible muscle fillet tissue.

For shellfish samples, the recommended composite sample type for chemical analysis also should be based on both the study objectives and the sample type consumed by the target population at risk. The specific tissues considered to be edible will vary among target shellfish species (see Section 7.2.4.4) based on local consumer preference. For example, several states (Maine, Massachusetts, New Hampshire, New Jersey and New York) have issued advisories for a variety of contaminants (PCBs, dioxins/furans, or cadmium) in specific glands or tissues of crustaceans such as lobsters and crabs. Some consumers of lobsters, *Homarus americanus,* enjoy eating the tomalley (digestive gland of the lobster), which has been shown to contain higher concentrations of chemical contaminants than the claw, leg, or tail meat typically consumed by members of the general population. For this reason, the tomalley should be analyzed separately if the target population consumes this organ so that a determination can be made as to whether contaminant concentrations in the tomalley only, or in the claw, leg, and tail meat are above levels of human health concern. Similarly, for the blue crab, *Callinectes sapidus,* as well as other crab species, the hepatopancreas (digestive gland) is consumed by some individuals and has also been found to contain higher concentrations of contaminants than claw, leg, or body muscle tissue. If the target population of concern consumes the hepatopancreas, then to best evaluate the risk of consumption from this tissue, it should be analyzed separately from the claw, leg, and body muscle tissue. A precise description of the sample type (including the number and size of the individual crustaceans in the composite) should be documented in the program record for each target species.

A similar situation exists with respect to selection of the appropriate sample type for bivalve molluscs. For example, while most individuals in the general population consume whole oysters (e.g.,*Crassostrea virginica or C. gigas)*, clams (e.g., *Mercenaria mercenaria*) or mussels (e.g., *Mytilus edulis or M. californianus*), only the adductor muscle tissue is typically consumed of the scallops (*Aropecten irradians or A. gibbus*). For bivalves in general, the adductor muscle is typically less contaminated than gill, mantle, and digestive organ tissues primarily due to the filter-feeding nature of these animals. Therefore, the adductor muscle of scallops should be analyzed separately for the general population. If the whole body of the scallop is to be consumed as part of a stew or soup by the target population of concern, the state should also conduct analysis of the whole body of the scallop as part of a risk assessment. A precise description of the sample type (including the number and size of the individual bivalves in the composite) should be documented in the program record for each target species.

For freshwater turtles also, the study objectives and sample type consumed by the target population at risk must be of primary consideration. However, EPA recommends use of individual turtle samples rather than composite samples for evaluating turtle tissue contamination. As with shellfish, the tissues of freshwater turtles considered to be edible vary based on the dietary and culinary practices of local populations (see Section 7.2.3.3). For example, New York and Minnesota have advisories for snapping turtles that recommend that consumers who wish to eat turtle meat should trim away all fat and discard the liver and eggs of the turtle (if they are still in the female's body cavity) prior to cooking. These three tissues (fat, liver, and eggs) have been shown to accumulate extremely high concentrations of a variety of contaminants in comparison to muscle tissue (Bishop et al., 1996; Bonin et al. 1995; Bryan et al., 1987; Hebert et al., 1993; Olafsson et al., 1983; 1987; Ryan et al., 1986; and Stone et al., 1980). States should consider monitoring pollutant concentrations in all three tissues in addition to muscle tissue. If residue analysis reveals the presence of high concentrations of contaminants in liver, eggs, and fatty tissue, but not in the muscle tissue, then the state can make the general recommendation to consumers to discard the three most lipophilic tissues to reduce the risk of exposure. This action is most useful when such lipophilic contaminants such as dioxins/furans, PCBs, and organochlorine pesticides are the contaminants involved.

Note: Composite samples are homogeneous mixtures of samples from two or more individual organisms of the same species collected at a particular site and analyzed as a single sample. Because the costs of performing individual chemical analyses are usually higher than the costs of sample collection and preparation, composite samples are most cost-effective for estimating average tissue concentrations of target analytes in target species populations. Besides being cost-effective, composite samples also ensure adequate sample mass to allow analyses for all recommended target analytes. A disadvantage of using composite samples, however, is that extreme contaminant concentration values for individual organisms are lost.

In screening studies, EPA recommends that states analyze one composite sample for each of two target species at each screening site. Organisms used in a composite sample

- Must all be of the same species
- Should satisfy any legal requirements of harvestable size or weight, or at least be of consumable size if no legal harvest requirements are in effect
- Should be of similar size so that the smallest individual in a composite is no less than 75 percent of the total length (size) of the largest individual
- Should be collected at the same time (i.e., collected as close to the same time as possible but no more than 1 week apart) [**Note:** This assumes that a sampling crew was unable to collect all fish needed to prepare the composite sample on the same day. If organisms used in the same composite are collected on different days (no more than 1 week apart), they should be processed within 24 hours as described in Section 7.2 except that individual fish may have to be filleted and frozen until all the fish to be included in the composite are delivered to the laboratory. At that time, the composite homogenate sample may be prepared.]
- Should be collected in sufficient numbers to provide a 200-g composite homogenate sample of edible tissue for analysis of recommended target analytes.

Individual organisms used in composite samples must be of the same species because of the significant species-specific bioaccumulation potential. Accurate taxonomic identification is essential in preventing the mixing of closely related species with the target species. **Note:** Individuals from different species should not be used in a single composite sample (U.S. EPA, 1989d, 1990d).

For cost-effectiveness, EPA recommends that states collect only one size class for each target species and focus on the larger individuals commonly harvested by the local population. Ideally, each composite sample for a specific species should contain the same number of individual fish and the individuals within each target species composite should be of similar size within a target size range so that the composite samples for a particular species are comparable over a wide geographic area. This is particularly important when states want to compare data on an individual species that might be used to establish a statewide advisory.

For persistent chlorinated organic compounds (e.g., DDT, dioxin, PCBs, and toxaphene) and methylmercury, the larger (older) individuals within a population are generally the most contaminated (Phillips, 1980; Voiland et al., 1991). As noted earlier, this correlation between increasing size and increasing contaminant concentration is most striking in freshwater finfish species but is less evident in estuarine and marine species. Size is used as a surrogate for age, which

provides some estimate of the total time the individual organism has been at risk of exposure. Therefore, the primary target size range ideally should include the larger individuals harvested at each sampling site. In this way, the states will maximize their chances of detecting high levels of chemical contamination in the single composite sample collected for each target species. If this ideal condition cannot be met, the field sampling team should retain individuals of similar length that fall within a secondary target size range.

Individual organisms used in composite samples should be of similar size (WDNR, 1988). **Note:** Ideally, for fish or shellfish, the total length (or size) of the smallest individual in any composite sample should be no less than 75 percent of the total length (or size) of the largest individual in the composite sample (U.S. EPA, 1990d). For example, if the largest fish is 200 mm, then the smallest individual included in the composite sample should be at least 150 mm. In the California Mussel Watch Program, a predetermined size range (55 to 65 mm) for the target bivalves (*Mytilus californianus and M. edulis*) is used as a sample selection criterion at all sampling sites to reduce size-related variability (Phillips, 1988). Similarly, the Texas Water Commission (1990) specifies the target size range for each of the recommended target fish species collected in the state's fish contaminant monitoring program.

Individual organisms used in a composite sample ideally should be collected at the same time so that temporal changes in contaminant concentrations associated with the reproduction cycle of the target species are minimized.

Each composite sample should contain 200 g of tissue so that sufficient material will be available for the analysis of all recommended target analytes. A larger composite sample mass may be required when the number of target analytes is increased to address regional or site-specific concerns. However, the tissue mass may be reduced in the **Tier 2** intensive studies (Phase I and II) when a limited number of specific analytes of concern have been identified (see Section 7.2.2.9). Given the variability in size among target species, only approximate ranges can be suggested for the number of individual organisms to collect to achieve adequate mass in screening studies (U.S. EPA, 1989d; Versar, 1982). For fish, 3 to 10 individuals should be collected for a composite sample for each target species; for shellfish, 3 to 50 individuals should be collected for a composite sample. In some cases, however, more than 50 small shellfish (e.g., mussels, shrimp, crayfish) may be needed to obtain the recommended 200-g sample mass. **Note:** The same number of individuals should be used in each composite sample for a given target species at each sampling site.

Deviations from the recommended study design have implications that may make the statistical analyses more complicated. The statistical methods for analyzing composite samples are made tractable and easier-to-use by simplifying the study design. Using equal numbers of fish in replicate composite samples is one way to do this. For example, with equal numbers of fish, the arithmetic average of the replicate composite measurements is an unbiased estimator of the population

mean. When unequal numbers are used, the arithmetic average is no longer unbiased. Instead, a weighted average of the composite measurements is calculated, where the weight for each composite reflects the number of fish it is made up of. Oftentimes fish are lost or damaged prior to compositing. When several fish are damaged or lost, the allocation of the remaining fish to composites may be reconfigured to allow equal numbers of fish in composites. If this is not possible, care should be taken to adjust the statistical procedures to account for the unequal allocations.

The use of sizes of fish exceeding the size range recommended for compositing may introduce more variability. If it is the size range within each composite that is broadened (e.g., 100-200 mm instead of 150-200 mm), the variability within the composite may increase. If additional composites are made with fish exceeding the recommended size ranges (e.g., adding composites of fish of size 300-450 mm when the target size is no more than 250 mm), this may increase the variability between composites of different size ranges. Overall inferences made from composites of different size ranges will have increased variability associated with them (e.g., wider confidence intervals).

Differences in the numbers of replicates at different sampling locations may complicate any comparisons to be made between locations or overall conclusions to be obtained by combining the results from different sampling locations. As with unequal numbers of fish in composites, unequal numbers of replicate samples complicate the statistical calculations. The appropriate weighted estimates should be used when combining information from different sampling locations. Consider, for instance, a state that monitors five lakes each year. If the state uses the same target fish species, the same number of fish per composite and the same size ranges, the overall mean level of contamination will be a straightforward average over the five locations if the same number of replicates are used at each location. However, if unequal numbers of replicates are used, the information contributed by each location is not the same and must be weighted accordingly.

As alluded to above, one limitation of using composite samples is that information on extreme levels of chemical contamination in individual organisms is lost. Therefore, EPA recommends that the residual individual homogenates be saved to allow for analyses of individual specimens if resources permit (Versar, 1982). Analysis of individual homogenates allows states to estimate the underlying population variance which, as described in Section 6.1.2.6, facilitates sample size determination for the intensive studies. Furthermore, individual homogenates may also be used to provide materials for split and spike samples for routine QC procedures either for composites or individual organisms (see Section 8.3). The circumstances in which the analysis of individual fish samples might be preferred over the analysis of composite samples is described in more detail in Appendix C.

Recommended sample preparation procedures are discussed in Section 7.2.

6.1.1.7 Replicate Samples—

The collection of sufficient numbers of individual organisms from a target species at a site to allow for the independent preparation of more than one composite sample (i.e., sample replicates) is strongly encouraged but is **option** in screening studies. If resources and storage are available, single replicate (i.e., duplicate) composite samples should be collected at a minimum of 10 percent of the screening sites (U.S. EPA, 1990d). The collection and storage of replicate samples, even if not analyzed at the time due to inadequate resources, allow for followup QC checks. These sites should be identified during the planning phase and sample replication specifications noted on the sample request form. If replicate field samples are to be collected, states should follow the guidance provided in Section 6.1.2.7. **Note:** Additional replicates must be collected at each site for each target species if statistical comparisons with the target analyte SVs are required in the state monitoring programs. The statistical advantages of replicate sampling are discussed in detail in Section 6.1.2.7.

6.1.2 Intensive Studies (Tier 2)

The primary aim of intensive studies is to characterize the magnitude and geographic extent of contamination in harvestable fish and shellfish species at those screening sites where concentrations of target analytes in tissues were found to be above selected SVs. Intensive studies should be designed to verify results of the screening study, to identify specific fish and shellfish species and size classes for which advisories should be issued, and to determine the geographic extent of the fish contamination. In addition, intensive studies should be designed to provide data for states to tailor their advisories based on the consumption habits or sensitivities of specific local fish-consuming subpopulations.

State staff should plan the specific aspects of field collection activities for each intensive study site after a thorough review of the aims of intensive studies (Section 2.2) and the fish contaminant data obtained in the screening study. All the factors that influence sample collection activities should be considered and specific aspects of each should be documented clearly by the program manager on the sample request form for each site.

6.1.2.1 Site Selection—

Intensive studies should be conducted at all screening sites where the selected SV for one or more target analytes was exceeded. The field collection staff should review a 7.5-minute (1:24,000 scale) USGS hydrologic map of the study site and all relevant water, sediment, and tissue contaminant data. The site selection factors evaluated in the screening study (Section 6.1.1.1) must be reevaluated before initiating intensive study sampling.

States should conduct **Tier 2** intensive studies in two phases if program resources allow. **Phase I intensive studies** should be more extensive investigations of the magnitude of tissue contamination at suspect screening sites. **Phase II intensive studies** should define the geographic extent of the contamination around these suspect screening sites in a variety of size (age) classes for each target species. The field collection staff must evaluate the accessibility of these additional sites and develop a sampling strategy that is scientifically sound and practicable.

Selection of Phase II sites may be quite straightforward where the source of pollutant introduction is highly localized or if site-specific hydrologic features create a significant pollutant sink where chemically contaminated sediments accumulate and the bioaccumulation potential might be enhanced (U.S. EPA, 1986d). For example, upstream and downstream water quality and sediment monitoring to bracket point source discharges, outfalls, and regulated disposal sites showing contaminants from surface runoff or leachate can often be used to characterize the geographic extent of the contaminated area. Within coves or small embayments where streams enter large lakes or estuaries, the geographic extent of contamination may also be characterized via multilocational sampling to bracket the areas of concern. Such sampling designs are clearly most effective where the target species are sedentary or of limited mobility (Gilbert, 1987). In addition, the existence of barriers to migration, such as dams, should be taken into consideration.

Site selection considerations should also include the number of samples necessary to characterize different waterbody types (lakes, rivers, estuaries, and coastal marine waters) based on both the hydrodynamics of the waterbody type including waterbody size as well as the inherent migratory nature of the species under consideration. Typically, as the size of a waterbody increases (from small lakes to larger lakes to Great Lakes or from streams, to rivers, to estuaries, to coastal marine waters), the number of samples that need to be collected to maintain a selected statistical power (i.e., 70 percent) as well as the number of sampling stations needed to define the area that should be under advisory both increase. For example, fish inhabiting relatively small lakes are likely to be exposed to a relatively homogeneous aquatic environment of contaminant concentrations. In a riverine, estuarine, or coastal situation, however, the hydrodynamics of the ecosystem can greatly affect the magnitude and nature of contamination in the water that fish encounter as they move up and downstream of areas with distinct nonpoint and point source inputs of contamination. Thus, the amount of time that any fish spends exposed to the contamination may be highly variable as compared to the relatively homogeneous exposures that might occur in smaller, less hydrologically dynamic lake ecosystems.

Overlayed on the hydrodynamic differences of each type of ecosystem and the spatial distribution of both nonpoint and point sources of pollution that can be encountered in larger ecosystems are the inherent behavioral differences in fish and shellfish species with respect to the size of their home range as well as to whether, at some time or times in their life cycle, they migrate widely to other

more or less contaminated areas. Consider the bluegill sunfish, a common inhabitant of small lakes and creeks. The home range for this species is typically less than 0.25 acres (~1,000 m^2) in lakes and does not exceed 28 m in streams (Carlander, 1969; Hardy, 1978). Smallmouth bass, a riverine species, have a home range of 500 to 4,500 m^2, but typically migrate up to 45 km (28 miles) (Reid and Rabeni, 1989; Todd and Rabeni, 1989). In contrast, many Great Lake fish species, as well as riverine, estuarine, and marine species migrate considerable distances during spawning periods. Several Great Lakes species also move upstream considerable distances into tributary rivers to spawn. Lake trout in the Great Lakes have been found to migrate up to 300 km (186 miles) with larger fish migrating 300 miles (483 km) (Daly et al., 1962; Mills, 1971; Willers, 1991). For many marine species, estuaries are the spawning areas for the adults and nursery areas for the developing juveniles, who eventually travel offshore as adults and return again to the estuaries to spawn. For these species, migratory or seasonal movements both from inshore to offshore areas and north and south migrations along the coasts can take place. Obviously, the number of samples needed to define an area under advisory for bluegill sunfish inhabiting a relatively homogeneous environment with respect to contaminant concentrations is quite different from that required for the more mobile species like the smallmouth bass and lake trout.

For shellfish, similar considerations are necessary. Bivalve molluscs like the oyster or mussel cement themselves to hard substrate as young spat and are unable to move away from pollution effects once they have settled out of the water column. Although clams and scallop species are slightly more mobile, they also typically stay in the general area in which they first settled out of the water column. For crustaceans like the blue crab and lobsters, however, movements both into and out of estuaries as well as into deeper water offshore are possible. As the complexity of the hydrodynamics of an ecosystem increases and the mobility of the target species increases, so too does the number of samples and the number of sampling stations required to delineate the area where contaminated individuals may be encountered by the fishing public.

6.1.2.2 Target Species and Size Class Selection—

Whenever possible, the target species found in the screening study to have elevated tissue concentrations of one or more of the target analytes should be resampled in the intensive study. Recommended target species for freshwater sites are listed in Tables 3-1, 3-2, and 3-4; target species for estuarine/marine waters are listed in Tables 3-10 through 3-12 for Atlantic Coast estuaries, in Table 3-13 for Gulf Coast estuaries, and in Tables 3-14 through 3-16 for Pacific Coast estuaries. If the target species used in the screening study are not collected in sufficient numbers, alternative target species should be selected using criteria provided in Section 3.2. The alternative target species should be specified on the sample request form.

For Phase I intensive studies, states should collect replicate composite samples of one size class for each target species and focus sampling on larger individuals commonly harvested by the local population (as appropriate). If contamination of this target size class is high, Phase II studies should include collection of replicate composite samples of three size classes within each target species.

EPA recognizes that resource limitations may influence the sampling strategy selected by a state. If monitoring resources are limited for intensive studies, states may determine that it is more resource-efficient to collect replicate composite samples of three size classes (as recommended for Phase II studies) during Phase I sampling rather than revisit the site at a later time to conduct Phase II intensive studies. In this way, the state may save resources by reducing field sampling costs associated with Phase II intensive studies.

By sampling three size (age) classes, states collect data on the target species that may provide them with additional risk management options. If contaminant concentrations are positively correlated with fish and shellfish size, frequent consumption of smaller (less contaminated) individuals may be acceptable even though consumption of larger individuals may be restricted by a consumption advisory. In this way, states can tailor an advisory to protect human health and still allow restricted use of the fishery resource. Many Great Lakes states have used size (age) class data to allow smaller individuals within a given target species to remain fishable while larger individuals are placed under an advisory.

6.1.2.3 Target Analyte Selection—

Ideally, Phase I intensive studies should include only those target analytes found in the screening study to be present in fish and shellfish tissue at concentrations exceeding selected SVs (Section 5.2). Phase II studies should include only those target analytes found in Phase I intensive studies to be present at concentrations exceeding SVs. In most cases, the number of target analytes evaluated in Phase I and II intensive studies will be significantly smaller than the number evaluated in screening studies.

6.1.2.4 Target Analyte Screening Values—

Target analyte SVs used in screening studies should also be used in Phase I and II intensive studies. Specific methods used to calculate SVs for noncarcinogenic and carcinogenic target analytes, including examples of SVs calculated for various exposure scenarios, are given in Section 5.1.

6.1.2.5 Sampling Times—

To the extent that program resources allow, sampling in intensive studies should be conducted during the same period or periods during which screening studies were conducted (i.e., when the target species are most frequently harvested for consumption) and should be conducted preferably within 1 year of the screening

studies. In some cases, it may be best to combine Phase I and Phase II sampling to decrease both the time required to obtain adequate data for issuance of specific advice relative to species, size classes, and geographic extent and/or the monitoring costs entailed in revisiting the site (see Section 6.1.2.2).

States should follow the general guidance provided in Section 6.1.1.5 for recommended sampling times. The actual sampling period and rationale for its selection should be documented fully for Phase I and II studies.

6.1.2.6 Sample Type—

Composite samples of fish fillets or the edible portions of shellfish are recommended for analysis of target analytes in intensive studies. The general guidance in Section 6.1.1.6 should be followed to prepare composite samples for each target species. In addition, separate composite samples may be prepared for selected size (age) classes within each target species, particularly in Phase II studies after tissue contamination has been verified in Phase I studies. Because the number of replicate composite samples and the number of fish and shellfish per composite required to test whether the site-specific mean contaminant concentration exceeds the selected SV are intimately related, both will be discussed in the next section.

Note: The same number of individual organisms should be used to prepare all replicate composite samples for a given target species at a given site. If this number is outside the recommended range, documentation should be provided.

Recommended sample preparation procedures are discussed in Section 7.2.

States interested in analyzing target analyte residues in individual fish or shellfish samples should review information presented in Appendix C.

6.1.2.7 Replicate Samples—

In intensive studies (Phases I and II), EPA recommends that states analyze replicate composite samples of each target species at each sampling site.

Replicate composite samples should be as similar to each other as possible. In addition to being members of the same species, individuals within each composite should be of similar length (size) (see Section 6.1.1.6). The relative difference between the average length (size) of individuals within any composite sample from a given site and the average of the average lengths (sizes) of individuals in all composite samples from that site should not exceed 10 percent (U.S. EPA, 1990d). To determine this, states should first calculate the average length of the target species fish constituting each composite replicate sample from a site. Then, states should take the average of these averages for the site. In the following example, the average of the average lengths of individuals (±10 percent) in five replicate composite samples is calculated to be 310 (±31) mm.

Replicate	Average Length of Individual Fish in Composite Sample (mm)
1	300
2	320
3	330
4	280
5	320

Average of the average length (±10%) = 310 (±31) mm.

Therefore, the acceptable range for the average length of individual composite samples is 279 to 341 mm, and the average length of individual fish in each of the five replicate composites shown above falls within the acceptable average size range.

All replicate composite samples for a given sampling site should be collected within no more than 1 week of each other so that temporal changes in target analyte concentrations associated with the reproductive cycle of the target species are minimized.

6.1.2.7.1 Guidelines for Determining Sample Sizes—This section provides general guidelines for estimating the number of replicate composite samples per site (n) and the number of individuals per composite (m) required to test the null hypothesis that the mean target analyte concentration of replicate composite samples at a site is equal to the SV versus the alternative hypothesis that the mean target analyte concentration is greater than the SV. These guidelines are applicable to any target species and any target analyte.

Note: It is not possible to recommend a single set of sample size requirements (e.g., number of replicate composite samples per site and the number of individuals per composite sample) for all fish and shellfish contaminant monitoring studies. Rather, EPA presents a more general approach to sample size determination that is both scientifically defensible and cost-effective. At each site, states must determine the appropriate number of replicate composite samples and of individuals per composite sample based on

- Site-specific estimations of the population variance of the target analyte concentration
- Fisheries management considerations
- Statistical power consideration.

If the population variance of the target analyte concentrations at a site is small, fewer replicate composite samples and/or fewer individuals per composite sample may be required to test the null hypothesis of interest with the desired statistical

power. In this case, using sample sizes that are larger than required to achieve the desired statistical power would not be cost-effective.

Alternatively, suppose EPA recommended sample sizes based on an analyte concentration with a population variance that is smaller than that of the target analyte. In this case, the EPA-recommended sample size requirements may be inadequate to test the null hypothesis of interest at the statistical power level selected by the state. Therefore, EPA recommends an approach that provides the flexibility to sample less in those waters where the target analyte concentrations are less variable, thereby reserving sampling resources for those site-specific situations where the population variance of the target analyte tissue concentration is greater.

EPA recommends the following statistical model, which assumes that z_i is the contaminant concentration of the ith replicate composite sample at the site of interest where i=1,2,3,...,n and, furthermore, that each replicate composite sample is comprised of m individual fish fillets of equal mass. Let $\bar{z}$ be the mean target analyte concentration of observed replicate composite samples at a site. Ignoring measurement error, the variance of $\bar{z}$ is

$$\text{Var}(\bar{z}) = \sigma^2/(nm) \qquad (6\text{-}1)$$

where

σ^2 = Population variance
n = Number of replicate composite samples
m = Number of individual samples in each composite sample.

To test the null hypothesis that the mean target analyte concentration across the n replicate composite samples is equal to the SV versus the alternative hypothesis that the mean target analyte concentration is greater than the SV, the estimate of the $\text{Var}(\bar{z})$, s^2, is

$$s^2 = [\Sigma(z_i - \bar{z})^2] / [n(n - 1)] \qquad (6\text{-}2)$$

where the summation occurs over the n composite samples. Under the null hypothesis, the following statistic

$$(\bar{z} - SV) / s \qquad (6\text{-}3)$$

has a Student-t distribution with (n – 1) degrees of freedom (Cochran, 1977; Kish, 1965). The degrees of freedom are one less than the number of composite samples.

Note: Use of a single composite sample precludes estimating the variability of the mean target analyte concentration. The estimator s^2 can only be calculated with at least two (but preferably three or more) replicate composite samples.

An optimal sampling design would specify the minimum number of replicate composite samples (n) and of individuals per composite (m) required to detect a minimum difference between the selected SV and the mean target analyte concentration of replicate composite samples at a site. Design characteristics necessary to estimate the optimal sampling design include

- Minimum detectable difference between the site-specific mean target analyte concentration and the selected SV
- Power of the hypothesis test (i.e., the probability of detecting a true difference when one exists)
- Level of significance (i.e., the probability of rejecting the null hypothesis of no difference between the site-specific mean target analyte concentration and the SV when a difference does not exist)
- Population variance, σ^2 (i.e., the variance in target analyte concentrations among individuals from the same species, which the statistician often must estimate from prior information)
- Cost components (including fixed costs and variable sample collection, preparation, and analysis costs).

In the absence of such design specifications, guidance for selecting the number of replicate composite samples at each site and the number of fish per composite sample is provided. This guidance is based on an investigation of the precision of the estimate of σ^2/nm and of statistical power.

Note: Under optimal field and laboratory conditions, at least two replicate composite samples are required at each site for variance estimation. To minimize the risk of a destroyed or contaminated composite sample precluding the site-specific statistical analysis, a **minimum** of three replicate composite samples should be collected at each site if possible. Because three replicate composite samples provide only two degrees of freedom for hypothesis testing, additional replicate composite samples are recommended.

The stability of the estimated standard error of $\bar{z}$ must also be considered because this estimated standard error is the denominator of the statistic for testing the null hypothesis of interest. A measure of the stability of an estimate is its statistical precision. The assumption is made that the z_i's come from a normal distribution, and then the standard error of $\hat{\sigma}^2/nm$ is defined as a product of $\hat{\sigma}^2$ and a function of n (the number of replicate composite samples) and m (the number of fish per composite). A fortunate aspect of composite sampling is that the composite target analyte concentrations tend to be normally distributed via the Central Limit Theorem. This formulation is used to determine which combinations of n and m are associated with a more precise estimate of σ^2/nm.

Modifying Cochran (1963) to reflect the normality assumption and the sampling design of n replicate composite samples and m fish per composite sample, the function of n and m of interest is shown in square brackets:

$$se\left(\frac{\hat{\sigma}}{nm}\right) = \sigma^2\left[\frac{2}{n^2m^2(n-1)}\right]^{1/2} \qquad (6\text{-}4)$$

Table 6-1 provides values of this function for various combinations of m and n. The data presented in Table 6-1 suggest that, as either n or m increases, the standard error of $\hat{\sigma}^2/nm$ decreases. The advantage of increasing the number of replicate composite samples can be described in terms of this standard error. For example, the standard error of $\hat{\sigma}^2/nm$ from a sample design of five replicate composite samples and six fish per composite (0.024) will be more than 50 percent smaller than that from a sample design of three replicate composite samples and six fish per composite (0.056). In general, holding the number of fish per composite fixed, the standard error of $\hat{\sigma}^2/nm$ estimated from five replicate samples will be about 50 percent smaller than that estimated from three replicate samples.

Table 6-1. Values of $\left[\frac{2}{n^2m^2(n-1).}\right]^{1/2}$ for Various Combinations of n and m

No. of replicate composite samples (n)	Number of fish per composite sample (m)									
	3	4	5	6	7	8	9	10	12	15
3	0.111	0.083	0.067	0.056	0.048	0.042	0.037	0.033	0.028	0.022
4	0.068	0.051	0.041	0.034	0.029	0.026	0.023	0.020	0.017	0.014
5	0.047	0.035	0.028	0.024	0.020	0.018	0.016	0.014	0.012	0.009
6	0.035	0.026	0.021	0.018	0.015	0.013	0.012	0.011	0.009	0.007
7	0.027	0.021	0.016	0.014	0.012	0.010	0.009	0.008	0.007	0.005
10	0.016	0.012	0.009	0.008	0.007	0.006	0.005	0.005	0.004	0.003
15	0.008	0.006	0.005	0.004	0.004	0.003	0.003	0.003	0.002	0.002

The data in Table 6-1 also suggest that greater precision in the estimated standard error of $\bar{z}$ is gained by increasing the number of replicate samples (n) than by increasing the number of fish per composite (m). If the total number of individual fish caught at a site, for example, is fixed at 50 fish, then, with a design of 10 replicate samples of 5 fish each, the value of the function of n and m in Table 6-1 is 0.009; with 5 replicate samples of 10 fish each, the value is 0.014. Thus, there is greater precision in the estimated standard error of $\bar{z}$ associated with the first design as compared with the second design.

Two assumptions are made to examine the statistical power of the test of the null hypothesis of interest. First, it is assumed that the true mean of the site-specific composite target analyte concentrations (μ) is either 10 percent, 25 percent, or 50 percent higher than the screening value. Second, it is presumed that a factor similar to a coefficient of variation, the ratio of the estimated population standard deviation to the screening value (i.e., σ/SV), is 50, 75 or 100 percent. Nine

scenarios result from joint consideration of these two assumptions. The power of the test of the null hypothesis that the mean composite target analyte concentration at a site is equal to the SV versus the alternative hypothesis that the mean target analyte concentration is greater than the SV is estimated under each set of assumptions. Estimates of the statistical power for six of the nine scenarios are shown in Table 6-2.

Power estimates for the three scenarios where the true mean of the site-specific composite target analyte concentration was assumed to be only 10 percent higher than the screening value are not presented. The power to detect this small difference was very poor: for 242 of the resulting 270 combinations of n and m, the power was less than 50 percent.

Several observations can be made concerning the data in Table 6-2. **Note:** The statistical power increases as either n (number of replicate composite samples) or m (number of fish per composite) increases. However, greater power is achieved by increasing the number of replicate composite samples as opposed to increasing the number of fish per composite. Furthermore, if the number of replicate composite samples per site and the number of fish per composite are held constant, then, as the ratio of the estimated population standard deviation to the SV increases (i.e., σ/SV), the statistical power decreases. Higher variability in the true population of target analyte concentration in fish will require more samples to detect a difference between the mean target analyte concentration and the SV.

States may use these tables as a starting point for setting the number of replicate composite samples per site and the number of fish per composite in their fish and shellfish contaminant monitoring studies. The assumption regarding the ratio of the estimated population standard deviation to the SV presented in Sections A and D of Table 6-2 is unrealistic for some fish and shellfish populations. Data in Sections C through F, which reflect more realistic assumptions concerning the estimated population standard deviation, show that states will be able to detect only large differences between the site-specific mean target analyte concentrations and the selected SV. Specifically, if the assumed ratio of the estimated population standard deviation to the SV is 1.0, using five replicate composite samples and six to seven fish per composite sample, the power to detect a 50 percent increase over the SV is between 70 and 80 percent. However, when the number of fish per composite increases to 8 to 10, the power increases by about 10 percentage points. In comparison, the power to detect a 25 percent increase over the SV is less than 50 percent.

Table 6-2 shows that a statistical power level of (at least) 70 percent is attainable for moderate values of m and n, as long as the ratio σ/SV is not large and/or the desired detectable difference between the target analyte concentration and the SV is not too small.

Table 6-2. Estimates of Statistical Power of Hypothesis of Interest Under Specified Assumptions

No. of Replicate Composite Samples (n)	Number of Fish Per Composite (m)									
	3	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**12**	**15**
A. Ratio of σ/SV = 0.5 and μ = 1.5 x SV:										
3	5	6	7	8	9	9	9	9	9	9
4	8	9	9	9	9	9	9	9	9	9
5	9	9	9	9	9	9	9	9	9	9
6	9	9	9	9	9	9	9	9	9	9
7	9	9	9	9	9	9	9	9	9	9
8	9	9	9	9	9	9	9	9	9	9
9	9	9	9	9	9	9	9	9	9	9
10	9	9	9	9	9	9	9	9	9	9
15	9	9	9	9	9	9	9	9	9	9
B. Ratio of σ/SV = 0.75 and μ = 1.5 x SV:										
3	–	–	–	–	5	6	6	7	7	8
4	–	6	7	7	8	8	9	9	9	9
5	6	7	8	8	9	9	9	9	9	9
6	7	8	9	9	9	9	9	9	9	9
7	8	9	9	9	9	9	9	9	9	9
8	8	9	9	9	9	9	9	9	9	9
9	9	9	9	9	9	9	9	9	9	9
10	9	9	9	9	9	9	9	9	9	9
15	9	9	9	9	9	9	9	9	9	9
C. Ratio of σ/SV = 1.0 and μ = 1.5 x SV:										
3	–	–	–	–	–	–	–	–	5	6
4	–	–	–	5	6	6	7	7	8	8
5	–	5	6	7	7	8	8	8	9	9
6	5	6	7	8	8	8	9	9	9	9
7	6	7	8	8	9	9	9	9	9	9
8	7	8	8	9	9	9	9	9	9	9
9	7	8	9	9	9	9	9	9	9	9
10	8	8	9	9	9	9	9	9	9	9
15	9	9	9	9	9	9	9	9	9	9
D. Ratio of σ/SV = 0.5 and μ = 1.25 x SV:										
3	–	–	–	–	–	–	–	–	5	6
4	–	–	–	5	6	6	7	7	8	8
5	–	5	6	7	7	8	8	8	9	9
6	5	6	7	8	8	8	9	9	9	9
7	6	7	8	8	9	9	9	9	9	9
8	7	8	8	9	9	9	9	9	9	9
9	7	8	9	9	9	9	9	9	9	9
10	8	8	9	9	9	9	9	9	9	9
15	9	9	9	9	9	9	9	9	9	9
E. Ratio of σ/SV =0.75 and μ = 1.25 x SV:										
3	–	–	–	–	–	–	–	–	–	–
4	–	–	–	–	–	–	–	–	–	6
5	–	–	–	–	–	–	5	5	6	7
6	–	–	–	–	5	6	6	7	7	8
7	–	–	5	5	6	6	7	7	8	9
8	–	–	5	6	7	7	8	8	8	9
9	–	5	6	7	7	8	8	8	9	9
10	–	6	6	7	8	8	8	9	9	9
15	6	7	8	9	9	9	9	9	9	9

Table 6-2. (continued)

No. of Replicate Composite Samples (n)	Number of Fish Per Composite (m)									
	3	4	5	6	7	8	9	10	12	15
F. Ratio of σ/SV = 1.0 and μ = 1.25 x SV:										
3	–	–	–	–	–	–	–	–	–	–
4	–	–	–	–	–	–	–	–	–	–
5	–	–	–	–	–	–	–	–	–	5
6	–	–	–	–	–	–	–	–	5	6
7	–	–	–	–	–	–	5	5	6	7
8	–	–	–	–	–	5	5	6	7	7
9	–	–	–	–	5	5	6	6	7	8
10	–	–	–	5	5	6	6	7	8	8
15	–	5	6	5	7	8	8	8	9	9

–: Power less than 50 percent.
5: Power between 50 and 60 percent.
6: Power between 60 and 70 percent.
7: Power between 70 and 80 percent.
8: Power between 80 and 90 percent
9: Power greater than 90 percent

One final note on determining the number of replicate composite samples per site and the number of fish per composite should be emphasized. According to Section 6.1.2.3, Phase I intensive studies will focus on those target analytes that exceeded the selected SV used in the screening study. Thus, multiple target analytes may be under investigation during Phase I intensive studies, and the population variances of these analytes are likely to differ. **Note:** States should use the target analyte that exhibits the largest population variance when selecting the number of replicate composite samples per site and the number of fish per composite. This conservative approach supports use of the data in Section B of Table 6-2 where the ratio of σ/SV is twice that of the data in Section A. States may estimate population variances from historic fish contaminant data or from composite data as described by U.S. EPA (1989d). This estimate of σ^2 can be used to determine whether the sampling design (i.e., number of replicate composite samples [n] and number of individuals per composite [m]) should be modified to achieve a desired statistical power.

Table 6-3 summarizes some observed ratios (σ/SV) of selected target analytes. These values were estimated from composite samples of siscowet trout and lake trout collected and analyzed by the Great Lakes Indian Fish and Wildlife Commission in a study funded by the Administration for Native Americans.

Table 6-3. Observed Ratios (σ/SV) of Selected Target Analytes

		Observed σ/SV (Mean)	
Target Species	Total PCB SV=0.02 ppm	Toxaphene SV=0.0363 ppm	Heptachlor Epoxide SV=0.00439 ppm
Siscowet trout	4.08 (1.01)	7.07 (2.18)	0.68 (0.01)
Lake trout	10.70 (0.47)	3.01 (0.38)	0.93 (0.007)

Source: Personal communication, Kory Groetsch, Great Lakes Indian Fish and Wildlife Commission, Odana, WI, with Elvessa Aragon, Research Triangle Institute, Research Triangle Park, NC, May 10, 2000.
SV = EPA default value for recreational fishers.

Consider a study of heptachlor epoxide concentrations in lake trout. The observed ratio (σ/SV) is close to 1.0 and the observed mean is approximately 1.5 x SV. To determine the appropriate values of n and m, we look at Section C of Table 6-2. To achieve statistical power between 80 and 90 percent, the combination of n and m that requires the smallest number of individual fish is n=10 and m=3. Ten replicate composite samples, each with three fish, will provide between 80 and 90 percent power for detecting a mean heptachlor epoxide concentration that is higher than the SV, if the difference truly exists. Other combinations of n and m might be more desirable. For instance, if the cost of analyzing composite samples is much higher than the cost of compositing individual fish, a combination that yields fewer replicate composite samples (say, n=5 and m=8, or n=6 and m=6) may be chosen. For siscowet trout, the observed ratio (σ/SV) is close to 0.75 while the observed mean is approximately 2.25 x SV. A comparison of the combinations of n and m in Sections B and E (for σ/SV = 0.75) shows that higher values of n and m are required to detect a difference at the same level of statistical power. For instance, in Section B, where μ = 1.5 x SV, the smallest number of individual fish needed to achieve 80 to 90 percent power is given by n=7 and m=3. In Section E, where μ=1.25 x SV, the combination of n=15 and m=5 achieves 80 to 90 percent power. For the same level of power and the same σ/SV, detecting a larger difference between the SV and the true mean concentration requires larger sample sizes (n or m or both).

After states have implemented their fish and shellfish contaminant monitoring program, collected data on cost and variance components, and addressed other design considerations, they may want to consider using an optimal composite sampling protocol as described in Rohlf et al. (1991) for refining their sampling design. An optimal sampling design is desirable because it detects a specified minimum difference between the site-specific mean contaminant concentration and the SV at minimum cost.

6.1.2.7.2 Comparison of Target Analyte Concentrations with Screening Values for Issuing Fish Advisories—Using the statistical model described in Section 6.1.2.7.1, target analyte concentrations from replicate composite samples at a particular site can be compared to screening values using a t-test. Assume that z_i is the contaminant concentration of the ith replicate composite sample at the site of interest where i=1,2,3,...,n and, furthermore, that each replicate composite sample comprises m individual fish fillets of equal mass. To test the null hypothesis that the mean target analyte concentration across the n replicate composite samples is equal to the SV versus the alternative hypothesis that the mean target analyte concentration is greater than the SV, perform the following steps:

1. Calculate $\bar{z}$, the mean target analyte concentration of observed replicate composite samples at a site:

$$\bar{z} = \Sigma z_i / n$$

where the summation occurs over the n composite samples.

2. Calculate the estimate of the Var($\bar{z}$), s^2 :

$$s^2 = [\Sigma(z_i - \bar{z})^2] / [n(n - 1)]$$

where the summation occurs over the n composite samples.

3. Calculate the test statistic:

$$t_c = (\bar{z} - SV) / s$$

4. The null hypothesis of no difference is rejected in favor of the alternative hypothesis of exceedance if

$$t_c > t_{\alpha,n-1}$$

where $t_{\alpha,n-1}$ is the tabulated value of the Student-t distribution corresponding to level of significance α and n-1 degrees of freedom. Note that the inequality is in one direction (>) since it is **exceedance** of the SV that is of interest.

When several sites are sampled and/or fish of different size ranges are collected, it is important to conduct the test separately at each site and for each size range. Combining sites or size ranges introduces variance components that are not accounted for in this procedure. The variance estimate may be larger with the additional sources of variability, and more replicate samples may be needed to detect a significant overall exceedance of the SV.

Example

Samples of siscowet trout were collected by the Great Lakes Indian Fish and Wildlife Commission and composited according to the guidelines discussed in this document. Composites of 12 fish were prepared, and four replicate samples of each of four size classes were analyzed for total mercury, PCBs, and a suite of chlorinated pesticides. Following is a summary of the test for exceedance of the SV for hexachlorobenzene (SV=0.025 ppm) based on the recreational fish consumption default value.

At the 5 percent level of significance the critical value of the Student-t distribution with three degrees of freedom is 2.353. All of the test statistic values are less than the critical value. The mean levels of hexachlorobenzene in the four size ranges of siscowet trout are less than the SV, so no fish advisory is needed.

Size Range (in.)	No. of Replicate Samples (n)	No. of Fish per Composite (m)	Composite Measurements of HCB (ppm)	Mean (Estimated Standard Deviation)	Test Statistic
17.0-18.0	4	12	0.00419 0.00507 0.00483 0.00405	4.53×10^{-3} (2.46×10^{-4})	-83.21
19.5-20.5	4	12	0.00604 0.00780 0.00925 0.00990	8.25×10^{-3} (8.57×10^{-4})	-19.54
22.0-23.0	4	12	0.01800 0.01808 0.01868 0.02389	1.97×10^{-2}) (1.42×10^{-3})	-3.73
24.5-25.5	4	12	0.01050 0.00960 0.00850 0.01090	9.88×10^{-3} (5.33×10^{-4})	-28.37

HCB=Hexachlorobenzene.

6.1.2.7.3 Comparison of Target Analyte Concentrations with Screening Values for Rescinding Fish Advisories—The comparison of mean target analyte concentrations to the screening values must be statistically based when considering rescinding a fish advisory. Statistical tests are constructed to control the Type I and Type II errors. The Type I error is defined as rejecting the null hypothesis (based on the evidence from the data) even though it is really true. The Type II error is defined as failing to reject the null hypothesis even though it is really false. In the context of the null and alternative hypotheses presented in the previous section, the Type I error is concluding that the mean target analyte concentration exceeds the SV when in fact it does not. The state concludes that there is a need to issue a fish advisory and proceeds to issue one, albeit unnecessarily. The Type II error is concluding that the mean target analyte concentration tissue residue level does not exceed the SV when in fact it does. The state decides that the mean target analyte concentration is no longer endangering the public health, so the fish advisory is rescinded. The implications of such errors may be costly; a Type II error in this case will put the public at risk without their knowledge. The Type I error is controlled by setting the level of significance to a small value, and the Type II error is controlled by increasing the power of the test. Both error types can be controlled simultaneously by increasing the sample sizes (n or m or both).

There are two basic statistical questions that must be answered before a fish advisory is rescinded:

- Is the screening value still being exceeded?
- If the screening value is no longer being exceeded, can the target analyte concentrations be expected to remain below the screening value?

The first question may be answered with the t-test described in the previous section. The second question may be answered by monitoring the target analyte concentrations long enough to observe a downward trend or a constant trend below the screening value. The simple approach would be to obtain replicate composite samples each year and test for exceedance of the screening value. (Section 6.1.1.5 recommends that screening be done biennially or at least once every 5 years. "Year" then signifies the years when screening is performed.) If the screening value is no longer being exceeded in year X, the state should continue obtaining replicate samples for at least one more year. The state should then test the differences between the tissue residue levels at years X-1, X, and X+1. Significant differences between the levels, especially between years X-1 and X, as well as between years X-1 and X+1, allows verification that the decrease in the target analyte concentration below the screening value at year X was not by chance. Appendix N discusses some statistical methods for comparing samples at different time points.

It is recommended that the yearly studies be as similar in study design as possible. Introducing changes in the study design will add more sources of variability and may necessitate increasing the number of replicate samples or accounting for the additional variance components in the statistical methods used.

6.1.2.7.4 Issuing Statewide Advisories—In addition to issuing fish consumption advisories for individual waterbodies, 18 states have also issued blanket statewide advisories for certain types of waterbodies within their jurisdictions (U.S. EPA, 1999c). States have issued statewide advisories for their freshwater lakes and/or rivers and their coastal waters, which can include estuaries and/or coastal marine waters. States often issue statewide advisories for certain waterbody types to warn the public of the potential for widespread contamination of certain species of fish or shellfish in these waterbodies. In these cases, the state has typically found a level of contamination of a specific pollutant in a particular fish species over a relatively wide geographic area that warrants advising the public of the situation. A state often issues a statewide advisory when, for example, it has many lakes that need to be monitored but has limited resources to collect fish (can sample only four or five lakes per year). If the state has even 100 lakes that need monitoring at the level of resources available, it could take 10 to 20 years to adequately monitor all 100 lakes. As an alternative, some states monitor a small percentage of their lakes and, based on the level of contamination found, many have determined that a statewide advisory should be issued to be conservative with respect to protection of public health. Methylmercury, because it is dispersed and transported via the atmosphere, is the leading pollutant responsible for the issuance of statewide advisories in 15 states, although PCBs, dioxins/furans, cadmium, chlordane, mirex, and DDT are also responsible for statewide advisories in a smaller number of states. Assuming that the levels of contamination are determined based on the fish compositing guidelines in this document, the biggest question is determining which waterbodies to monitor. Finding a "representative" sample of waterbodies is a daunting task since there are many different ways to determine representativeness: size of waterbody,

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): _____

_____

page 1 of 2

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): _____

page 2 of 2

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)

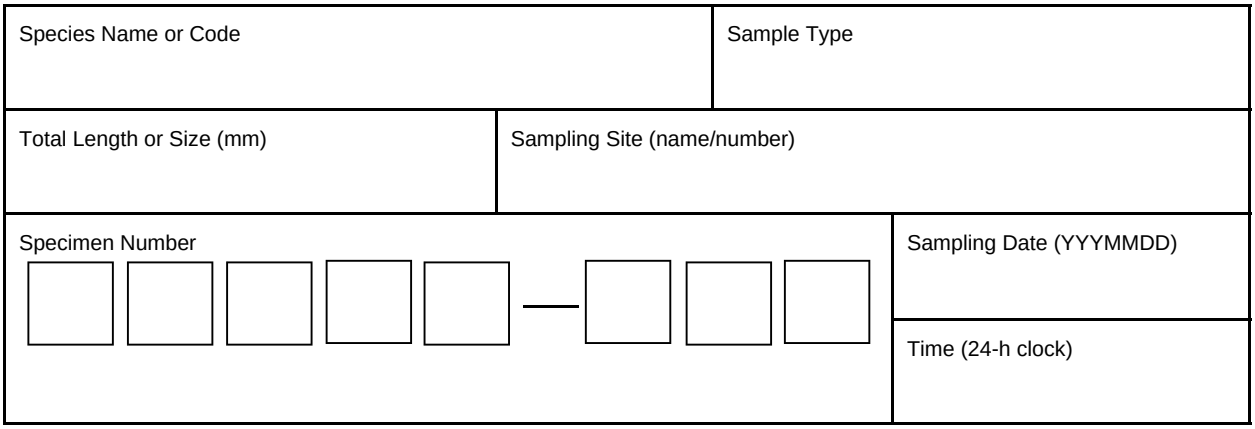


Figure 6-7. Example of a sample identification label.

- 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.

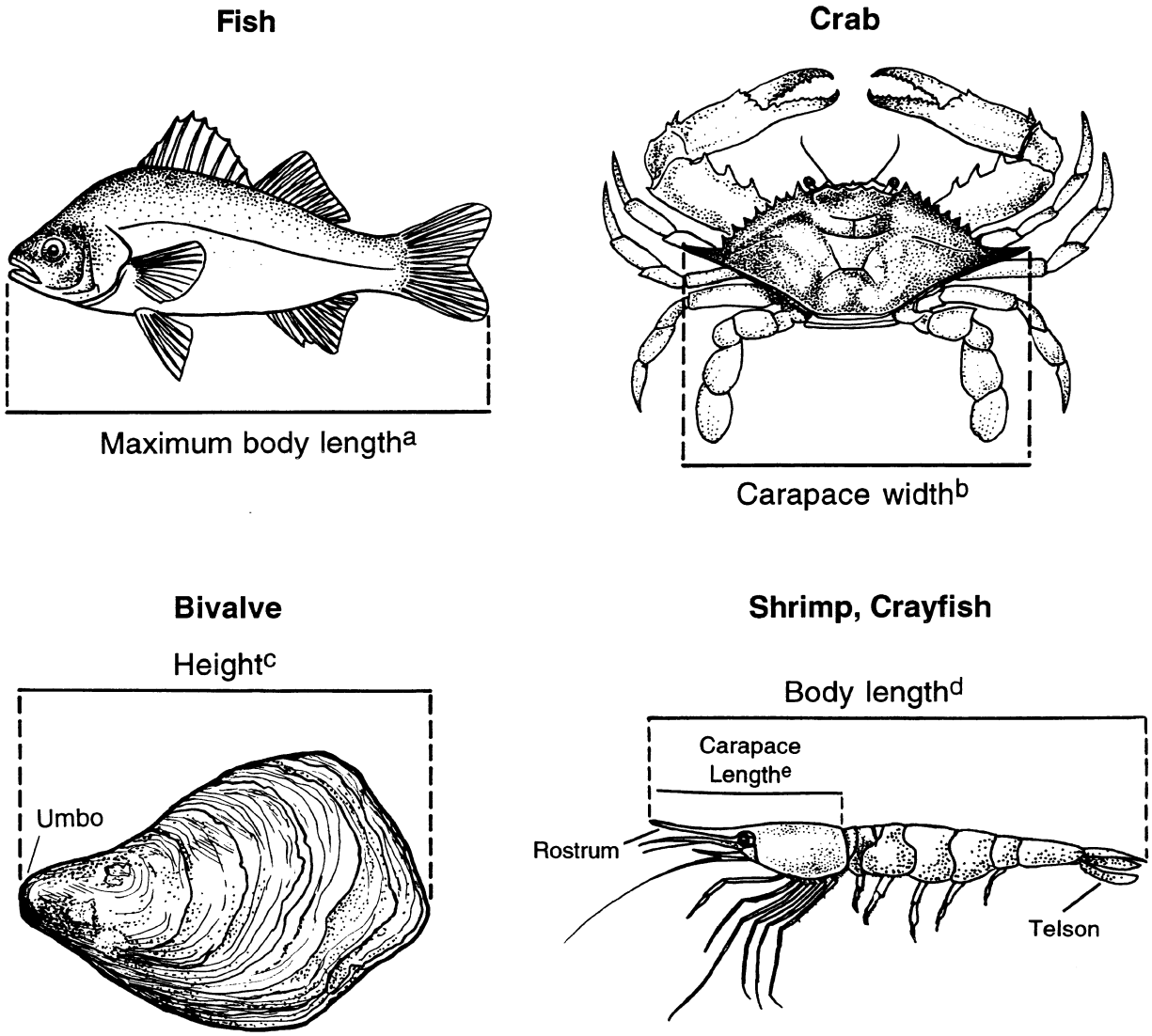
Stone et al. (1980) reported extremely high concentrations of PCBs in various tissues of snapping turtles from a highly contaminated site on the Hudson River. Contaminant analysis of various turtle tissues showed mean PCB levels of 2,991 ppm in fatty tissue, 66 ppm in liver tissue, and 29 ppm in eggs 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).

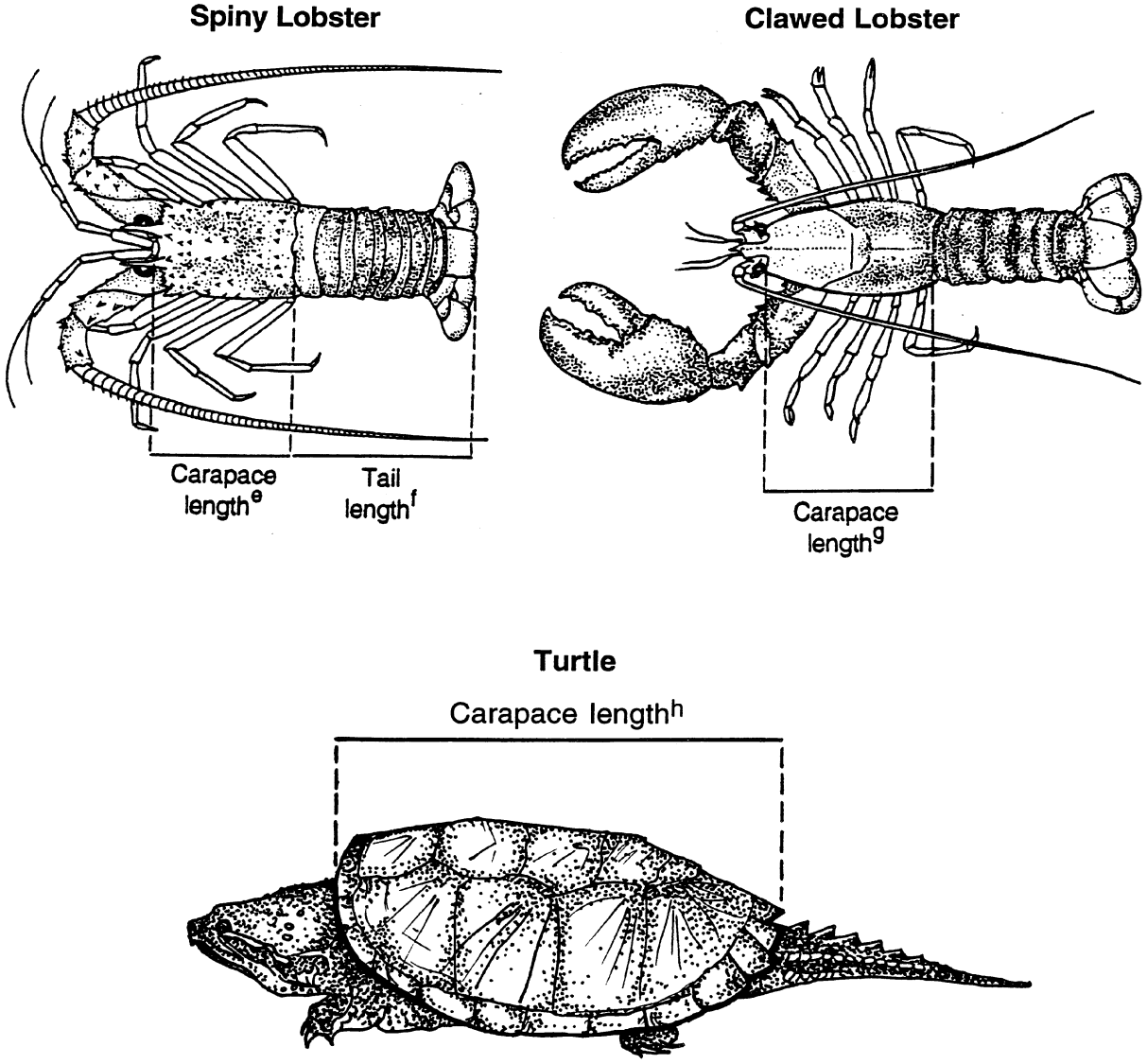
[b] Carapace width is the lateral distance across the carapace (from tip of spine to tip of spine (U.S. EPA, 1990c).

[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.