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Fish Sampling and Analysis: A Guidance Document for Issuing Fish Advisories

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SECTION 5
FIELD PROCEDURES

The major objective of this section is to provide guidance to States on (1) sampling design for initial screening and intensive monitoring phases of fish contaminant monitoring programs and (2) field procedures for collecting, processing, preserving, and shipping samples to a central processing laboratory for pollutant analysis. This guidance emphasizes planning and documentation of all field procedures to ensure that collection activities are cost-effective in meeting sampling objectives and that sample integrity is preserved during all phases of the sampling process, from collection to delivery of samples to the central processing laboratory.

The format of the Work/QA Project Plan outlined in Appendix F is recommended for documenting the specific procedures used in State fish/shellfish contaminant monitoring programs. In addition, protocols for sample collection procedures should be prepared to document the methods used by each State and to allow assessment of final data quality and comparability.

5.1 SAMPLING DESIGN

Prior to making a field collection trip, the program manager and field sampling staff should meet to develop a detailed plan for sampling at the proposed sample collection sites. In preparation for these planning meetings, staff should review all pertinent information on the sites that have been selected for inclusion in the contaminant monitoring studies. Historic information on water and sediment quality and any previously conducted tissue contaminant monitoring data should be reviewed. Existing data on pollutant inputs to the waterbody from point and nonpoint sources should also be reviewed. In addition, personnel roles and responsibilities with respect to all phases of the fish/shellfish sampling effort should be clearly defined. All aspects of the final sampling design for a State's fish/shellfish contaminant monitoring program should be documented clearly by the program manager in the Work/QA Project Plan (see Appendix F).

In the recommended two-tiered monitoring strategy described in Section 2, there are six major parameters directly associated with sample collection that must be specified for each sampling site during the planning stage and prior to the initiation of any field collection

activities. The following parameters must be selected for each site:

- Site location
- Target species
- Target contaminants
- Sampling times
- Sample type
- Sample replication.

After reviewing the objectives of initial screening studies or intensive monitoring studies (Section 2) and all relevant information on the sites to be monitored, States should plan the specific aspects of field collection activities for each site by considering all of those parameters that influence sample collection procedures. The program manager should document specific aspects of each parameter in a sample request form (Figure 5-1) for each sampling site. (A copy of this form is available in Appendix G.) The sample request form should provide the field collection team with readily available information on the project objective, sample type to be collected, target contaminants to be evaluated, site name/number, site location, target species and alternate species to be collected, sampling date, sampling method to be used, number of replicates to be collected, and number of samples to be collected for each composite. The original sample request form should be maintained on file with the program manager and a copy taken into the field by the field sampling team and maintained with the field logbook. Each of the major parameters that influence sample collection procedures is discussed for initial screening studies in Section 5.1.1 and for intensive monitoring studies in Section 5.1.2.

5.1.1 Initial Screening Study

The primary objective of initial screening studies is to monitor probable worst-case exposure situations and some reference sites for a wide range of target contaminants to identify hot spots for more intensive followup monitoring. **Analyses of fish fillets (skin-on including belly flap tissue and edible portions of shellfish) are recommended for screening studies to estimate worst-case exposures of the general U.S. population.** Note: To provide an indication of potential exposures of the subpopulations of sport or subsistence fishermen (e.g., certain ethnic groups) who do consume whole fish or parts other than fillets, States may deem it necessary to collect whole fish and/or shellfish for analyses during the initial screening study and/or intensive study.

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 TVs *(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 (10 fish optimum)

_____ Shellfish per composite *(specify number to obtain 500 grams of tissue)*

Figure 5-1. Example of a sample request form.

[Reviewers, please comment on fillet type to use in both initial screening and in intensive studies. We are recommending skin on--but should bellyflap be included for worst case scenario?]

5.1.1.1 Site Selection--

The field collection staff should review historical data on each screening site using a recent hydrologic map of the site, of the appropriate scale, to ensure that the sampling site is

- Located downstream of target point source discharges such as
  - -- Industrial or municipal dischargers
  - -- Combined sewer overflows (CSOs)
  - -- Urban storm drains
- Located downstream of target nonpoint source inputs such as
  - -- Landfill, RCRA, or CERCLA sites
  - -- Areas of intensive agricultural activities, mining activities, or urban land development
  - -- Areas receiving inputs through multimedia mechanisms such as atmospheric deposition or hydrogeologic connections
- Located in an area acting as a potential pollutant sink where contaminated sediments accumulate and bioaccumulation potential might be enhanced
- Located in an unpolluted area that can serve as a reference site for subsequent intensive studies.

Although the procedures required to identify candidate hot spot sites in proximity to significant point source discharges are usually straightforward, it is often more difficult to identify clearly defined hot spot areas associated with nonpoint sources. In these instances, assessment information summarized in State Section 305(b) reports or Section 319 nonpoint source assessment reports should be reviewed before site locations are selected.

The ultimate selection of any sampling site location must be a site-specific decision based on the best professional judgment of the field sampling staff. Several site-specific considerations have been identified that should be evaluated (Versar, 1982):

- Proximity of sites for sampling water and sediments
- Availability of data on fish or shellfish community structure
- Bottom condition
- Type of sampling equipment available
- 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, spawning areas, and migration patterns of target species is a valuable asset in locating populations of the target species (Versar, 1982). Knowledge of habitat preference provided by fishery 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 at the site 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 fishermen familiar with the candidate site to identify localized 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 be located where there is good land access. When a site must be reached 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 (during the proposed sampling time) required to deploy the selected sampling gear efficiently and to operate the boat safely.

All of the factors described above should be given consideration when selecting sampling sites. Once the site has been selected, it should be plotted and numbered on the

most accurate, up-to-date map of appropriate scale available. Recent 7.5-minute (1:24,000 scale) maps from the U.S. Geologic Survey (USGS) or National Ocean Survey or blue line maps produced by the U.S. Army Corps of Engineers are of sufficient detail and accuracy for sample site positioning. The type of sampling to be conducted, water depth, and estimated time to the station from an access point should be noted. The availability of known targets for visual or range fixes should be determined for each sampling site. Biological trawl paths (or other sampling gear transects) and navigational hazards should also be indicated. Additional information on site-positioning methods are described in Battelle (1986), Tetra Tech (1986), and Puget Sound Estuary Program (1990a).

An accurate description of each sampling site is important since State fish/shellfish contaminant monitoring data will be stored in the Ocean Data Evaluation System (ODES) database available to a broad spectrum of users nationwide. Each sampler should provide a detailed description of each site and should refer to a 7.5-minute USGS map to determine the exact latitude and longitude coordinates for the site. This information should be documented in the sample request form and on the field record sheets (see Section 5.2.3).

5.1.1.2 Target Species Selection--

After reviewing information on each sampling site, the field collection staff should identify the target species that can be expected to be collected at the site. **The national target species recommended for initial screening studies in freshwater systems are shown in Table 5-1.** For bottom feeders, the order of preference is carp, channel catfish, and white sucker. No preferred order is given for predator species. **In estuarine/marine ecosystems, one of the three bivalve species or a finfish species listed in Table 5-1 should be collected.** If a recommended national target species is not available for collection, a contingency plan for species selection should be decided upon at the planning meeting. Field collection staff should select a second and third choice for the target species if none of the national target species are available at the site. The alternate species for collection should be selected from the regional target species lists presented in Table 3-6 for freshwater and Tables 3-7 through 3-13 for estuarine/marine systems.

[Reviewers, please recommend estuarine finfish species as national target species. These recommended species should be widely distributed geographically,

TABLE 5-1. NATIONAL TARGET SPECIES RECOMMENDED FOR INITIAL SCREENING STUDIES

Freshwater systems[a]	Estuarine/marine systems[b]
Bottom feeders:	
Carp (*Cyprinus carpio*)	Blue mussel (*Mytilus edulis*)
Channel catfish (*Ictalurus punctatus*)	California mussel (*Mytilus californianus*)
White suckers (*Catostomus commersoni*)	American oyster (*Crassostrea virginica*)
Predators:	
Largemouth bass (*Micropterus salmoides*)	
Smallmouth bass (*Micropterus dolomieui*)	
White crappie (*Pomoxis annularis*)	
Northern pike (*Esox lucius*)	
Flathead catfish (*Pylodictus olivarus*)	
Brown trout (*Salmo trutta*)	
Walleye (*Stizostedion vitreum*)	
White bass (*Morone chrysops*)	

[a] For freshwater systems, one bottom-feeder and one predator species should be collected at each site.

[b] For estuarine/marine systems, one bivalve species and one finfish species should be collected at each site.

preferably demersal, nonmigratory species with a known ability to bioconcentrate pollutants. The National Bioaccumulation Study recommended the following estuarine species: hardhead catfish (*Arius felis*), blue catfish (*Ictalurus furcatus*), freshwater drum (*Aplodinotus grunniens*), spot (*Leiostomus xanthurus*), southern flounder (*Paralictys lethostigma*), and black drum (*Pogonias cromis*). The NOAA Status and Trends Program recommended the following estuarine species: winter flounder (*Pseudopleuronectes americanus*), Atlantic croaker (*Micropogonias undulatus*), spot (*Leiostomus xanthurus*), in Atlantic and Gulf waters and starry flounder (*Platichthys stellatus*), English sole (*Parophrys vetulus*), white croaker (*Genyonemus lineatus*), barred sandbass (*Paralabrax nebulifer*), black croaker (*Cheilotrema saturnum*), hornyhead turbot (*Pleuronichthys verticalis*) in Pacific waters. Recommendations are needed for all estuarine waters.

5.1.1.3 Target Contaminant Selection--

For initial screening studies, all of the recommended target contaminants in Table 4-3 should be analyzed. During the planning meeting, State staff should consider whether additional contaminants should be analyzed. Historic data on water, sediment, and

tissue contamination should be reviewed. In addition, priority pollutant scans from known point source discharges should be examined to determine whether additional contaminant analysis is warranted.

5.1.1.4 Sampling Times--

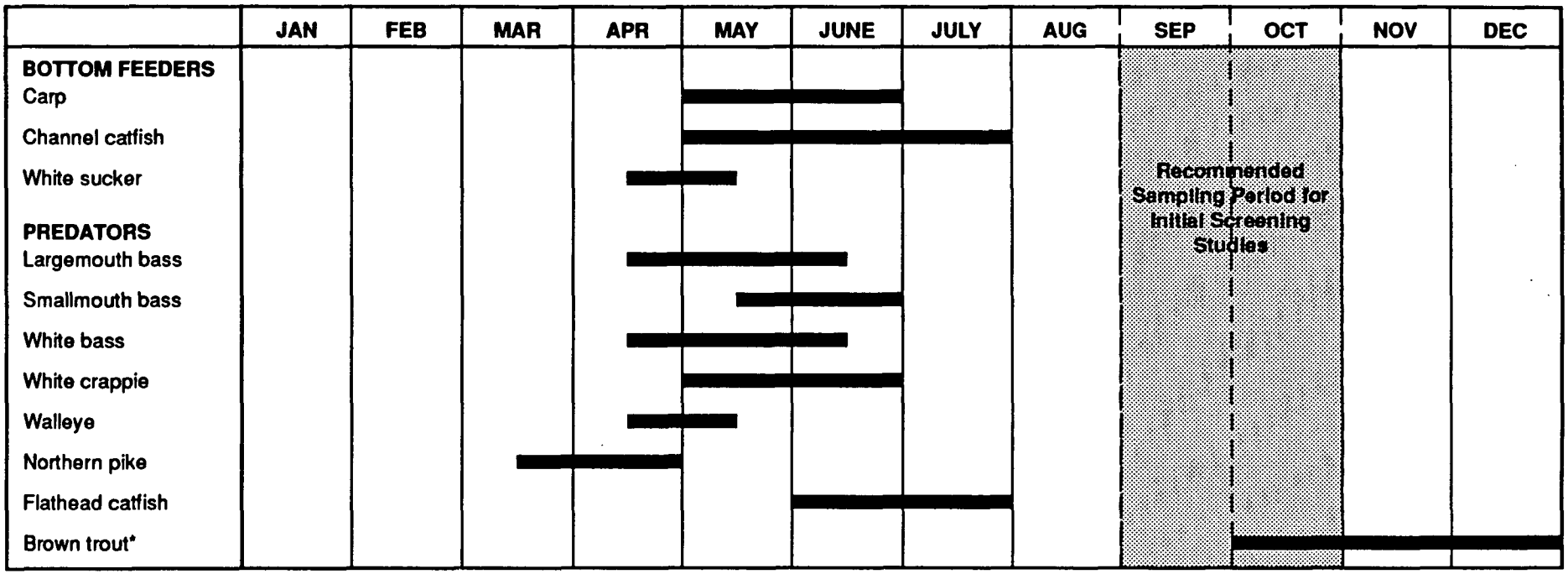
If program resources are sufficient, biennial screening of waterbodies where commercial, recreational, or subsistence harvesting is practiced (as identified by the State) is recommended. This recommended screening frequency will allow screening data to be used in the biennial State 305(b) reports to document the extent of support of Clean Water Act goals. At a minimum, these waterbodies should be screened once every 3 to 5 years.

Selection of the most appropriate sampling period is very important, particularly when screening sampling will be conducted no more often than biennially. **For initial screening studies, the recommended sampling period is from late summer to fall (i.e., September to October).** This sampling period avoids the spawning periods of most of the target species except the brown trout (Figure 5-2). Water levels in many waterbodies are typically lower during this time, which may simplify sampling procedures. In addition, this sampling period is recommended to simulate a worst-case exposure scenario for organic pollutants (see Section 2.1.6).

Exceptions to this recommended sampling period for national target species should be made only when important regional or site-specific factors favor alternative sampling periods. For many States, budgetary constraints may require that a major portion of their sampling efforts be carried out during July and August when they are able to employ temporary help or student interns. When sampling is not conducted during the recommended late summer to fall sampling period, the actual sampling period and the rationale for its selection should be documented fully and the final data evaluation should include an assessment of how the results may have been affected by sampling at a less than optimal time.

5.1.1.5 Sample Type--

Composite samples of homogenates of fish fillets (skin-on and including bellyflap tissue) or the edible portions of shellfish are recommended for the analysis of target contaminants in initial screening studies. Fish or shellfish collected for tissue analysis should satisfy any legal requirements for harvestable sizes or weights and at least be



Source: U.S. EPA, 1991. National Bioaccumulation Study (Draft Report). Office of Water Regulations and Standards, Washington, DC.

*Great Lakes only? Reviewers please comment on the use of brown trout as national target species and when they should be sampled. Please specify whether you are referring to Great Lake populations or other riverine populations of brown trout.

Figure 5-2. Spawning period of national freshwater target species.

of consumable size where no legal harvestable requirements are in effect. Given the aim of screening studies to identify worst-case exposure conditions, it is recommended that the largest available individuals of the target species be selected because larger (older) organisms generally show the highest bioaccumulation levels (Phillips, 1980).

It is extremely important that the individual organisms used in composite samples be of similar length or size (Wisconsin Department of Natural Resources, 1988). **For fish or shellfish, it is recommended that the total length (size) of the smallest individual in a composite sample be no less than 75 percent of the total length (size) of the largest individual in the composite sample (U.S. EPA, 1990b).**

A minimum of 500 grams of tissue homogenate is recommended for each composite sample so that sufficient material will be available for the number of analyses required for the recommended target contaminants (Versar, 1982; 1984). If, in addition to the recommended target contaminants, a State has included other pollutants for analysis to address regional or site-specific concerns, a larger composite sample mass may be required, and the estimated numbers of individuals required for each composite sample noted in the following paragraphs may also need to be increased.

The number of individual organisms from a given species required to prepare a 500-g composite whole-body sample will depend primarily on the target species and the age of the individuals in the sample. For this reason, only approximate ranges can be suggested for the number of individual organisms to collect (Versar, 1982; U.S. EPA, 1989d). **For fish, 6 to 10 individuals (10 is the preferred number for each composite) of legal harvestable size or at least of consumable size should be collected for a given target species, with preference given to the largest available individuals.**

For shellfish, composite samples should be prepared from 10 to 50 individuals, although for smaller shellfish (e.g., mussels, shrimp, crayfish) more than 50 individuals may be needed to obtain the required 500-g composite sample.

Whenever possible, the same number of individuals should be used to prepare each composite sample for a given target species for all sites. The number of individuals actually used to prepare each composite sample should be clearly documented. If this number is outside the recommended range, the reasons for this deviation should be recorded.

Recommended sample preparation procedures are discussed in Section 6.2.

5.1.1.6 Sample Replication--

Sample replication requires the collection of sufficient numbers of individual organisms from a target species at a target site to allow for the independent preparation of more than one composite sample. **Sample replication is optional in initial screening studies.** If resources are available, however, single replicate (i.e., duplicate) composite samples should be collected for QA/QC purposes at a minimum of 10 percent of the screening sites (U.S. EPA, 1990b). 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, the relative difference (in percent) between the overall mean length (size) of the replicate samples and mean length (size) of any individual replicate sample should be no greater than 10 percent (U.S. EPA, 1990b). **Note:** Additional replicates must be collected at each site for each target species if statistical comparisons to the target contaminant TVs are required in the State monitoring programs. The statistical advantages of replicate sampling are discussed in detail in Section 2.2.8 and Section 7.2.

5.1.2 Intensive Monitoring

The primary objective of intensive followup monitoring is to characterize the magnitude and geographic extent of contamination in a range of legal size classes of harvestable fish/shellfish species at those initial screening sites where concentrations of specific target contaminants in tissues were found to be above recommended TVs. **Intensive monitoring focuses on the edible tissues of shellfish and fish (fillets) in order to assess whether the contamination poses an unacceptable health risk to local fish/shellfish consumers and whether a consumption advisory should be issued.** Rather than discouraging all fish consumption, intensive monitoring studies should be designed to identify those specific fish and shellfish species or age classes for which advisories should be issued. In addition, intensive monitoring studies should be designed to tailor advisories to the consumption habits or sensitivities of specific local human subpopulations.

Fillets (skin-on and including belly flap tissue) are recommended for analyses because they are most representative of what the general U.S. population consumes. However, if local subpopulations are known to consume whole fish or other specific parts (e.g., heads, livers) of certain target species, the screening and intensive monitoring programs should be expanded to include composites of those portions consumed in addition to fillet

composites. The specific tissue type(s) to be collected should be noted on the sample request form.

After reviewing the objectives of intensive monitoring studies (Section 2.2) and reviewing the fish contaminant data obtained in the initial screening studies, State staff should plan the specific aspects of field collection activities for each intensive monitoring site by considering all the parameters that influence sample collection activities. Specific aspects of each parameter should be documented clearly by the program manager for each site on a sample request form.

5.1.2.1 Site Selection--

In planning the intensive followup monitoring that is required at all sites where TVs for one or more target contaminants are exceeded, the field collection staff should review a 7.5-minute (1:24,000 scale) USGS hydrologic map of the potential hot spot and all relevant water, sediment, and tissue contaminant data related to the site. Many of the same considerations of site selection evaluated in the initial screening must be reevaluated before sampling is initiated in the intensive study, including

- Bottom conditions
- Type of sampling equipment available
- Accessibility of the screening site used in the initial screening study and Phase I intensive study as well as additional sites where sampling efforts may be conducted to bracket the geographic extent of the contamination as part of the Phase II intensive study.

To the extent that program resources allow, intensive monitoring studies should be conducted in two phases. Phase I of the intensive monitoring study should be designed to identify the magnitude of tissue contamination (in edible tissues) in key species and size classes of fish/shellfish of commercial, recreational, or subsistence fishing value at the site sampled in the initial screening study. Phase II of the intensive monitoring study should be designed to define clearly the geographic extent of the suspected contamination at the targeted site and should include the Phase I intensive study site and additional sites located in the waterbody under study. This may be quite straightforward where the sources of pollutant introduction are highly localized or if site-specific hydrologic features create a significant pollutant sink where contaminated sediments accumulate and the bioaccumulation potential

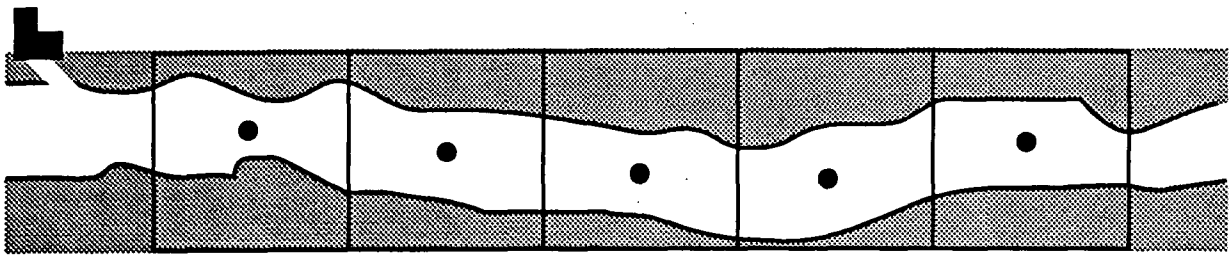
might be enhanced (U.S. EPA, 1986b). For example, upstream and downstream monitoring to bracket point source discharges, outfalls, and regulated disposal sites showing contaminants from surface runoff or leachate can often be used to characterize the geographic extent of the contaminated area. Within coves or small embayments where streams enter large lakes, estuaries, or harbors, 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.

Although bracketing approaches work best where the ultimate sources of contamination can be associated with spatially well-defined hot spots, alternative sampling designs are usually required where hot spots are not suspected. In the absence of historic data, other appropriate sampling designs may be used to determine the geographic extent of contamination in monitoring larger reservoirs, estuaries, or near-coastal areas. Several of the more common recommended sampling designs are discussed in Gilbert (1987). Guidelines for selecting appropriate sampling designs are summarized briefly here.

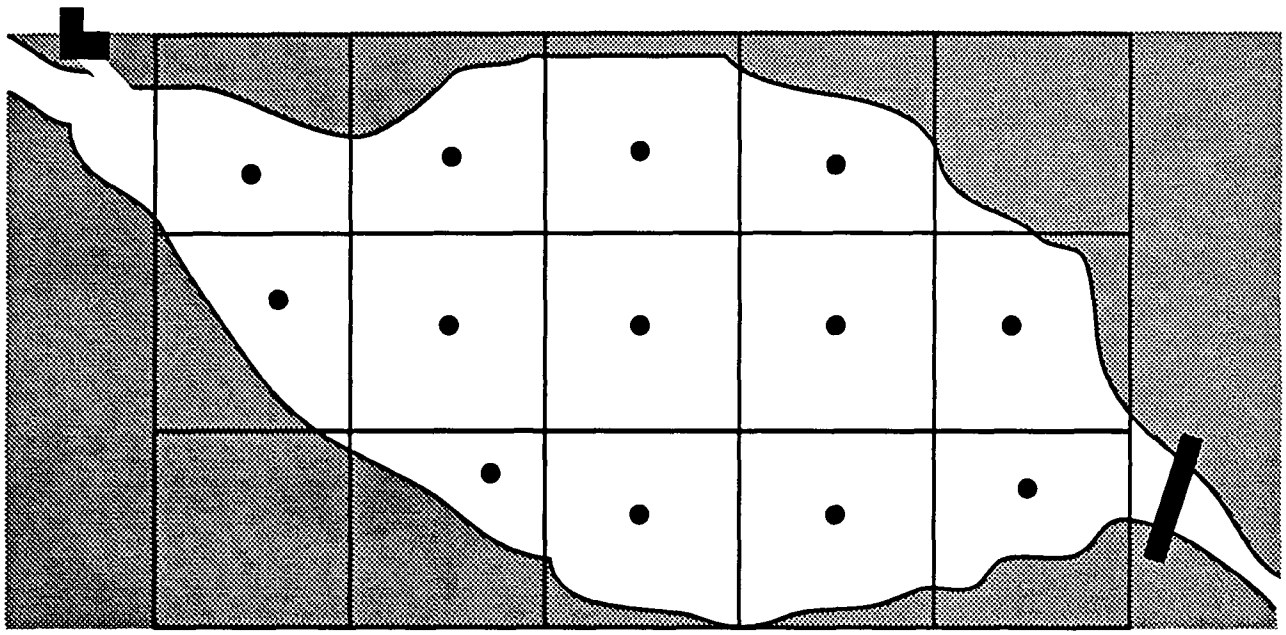
Although Gilbert (1987) discusses several sampling designs, systematic and two-stage sampling appear to be the approaches most applicable for fish and shellfish contaminant monitoring programs and the easiest to implement in the field. Where the target species are widely and homogeneously distributed throughout the study area, a systematic sampling design is often appropriate. This approach, which consists of sampling target species at locations using a spatial pattern, is appropriate only for species with limited mobility (i.e., shellfish and fish with limited home ranges) so that the contaminant concentration in their tissues is characteristic of the sampling site. For example, a State may select locations at equidistant intervals in a river downstream from a suspected point source of contamination as shown in Figure 5-3A. Or, the State may overlay a grid on a map of a large reservoir and then systematically sample locations for collecting the target species (Figure 5-3B).

If habitat requirements or other life history features indicate that target species are restricted to specific identifiable habitats within the study areas (e.g., the target species are found in shallow water areas only), a two-stage sampling design may be considered. Using this design, the State would first identify the shallow areas of a waterbody (e.g., estuary) on a hydrologic map (see Figure 5-3C). Then, a probability sample of these shallow water areas (first-stage units) would be selected prior to the initiation of field collection activities. In the

A) Systematic Sampling



B) Grid Sampling



C) Two-stage Sampling

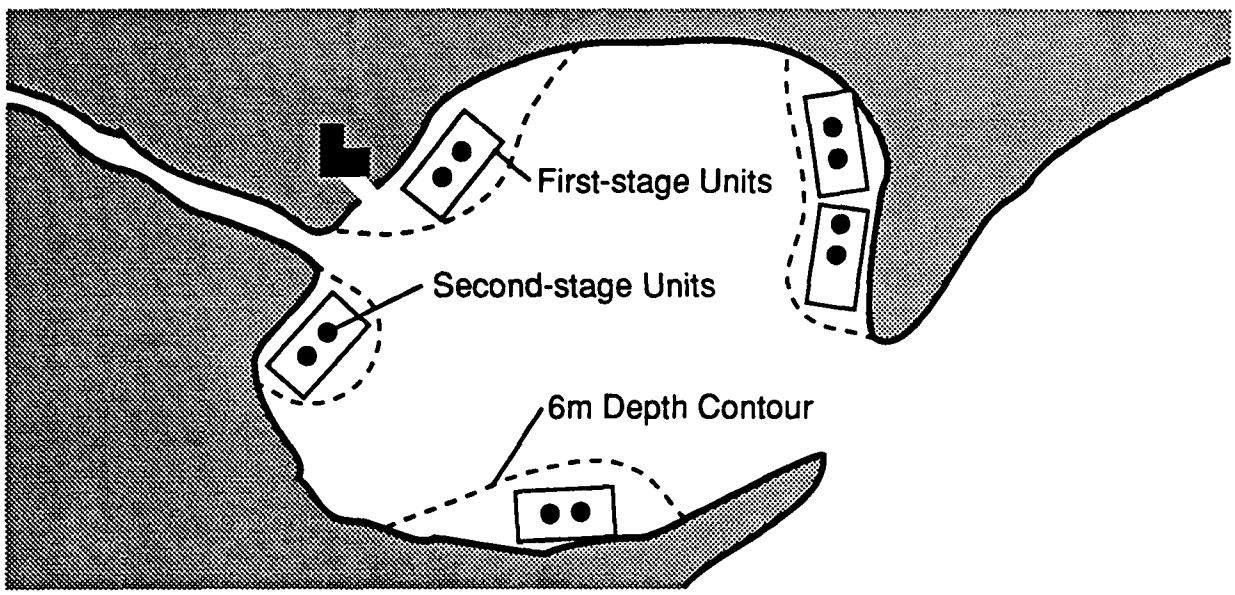


Figure 5-3. Sampling station layouts for probability sampling in two dimensions.

second stage, the target species would be sampled within the selected shallow water habitats (second-stage units) (see Figure 5-3C).

Data collected from such complex sampling designs must be analyzed with statistical estimation procedures that incorporate the complex design. The reader is referred to Gilbert (1987) for details on proper estimation procedures. In all cases where intensive monitoring studies are conducted, the program manager should enlist the assistance of a qualified statistician in the initial sampling design phase through to the final data analysis and interpretation phase.

5.1.2.2 Target Species Selection--

The main goal of intensive monitoring is to expand the range of fish and shellfish species examined in initial screening studies to include as target species those species most frequently consumed by the local population or specific subpopulations. **The regional target species recommended for sampling in intensive monitoring studies in freshwater systems are listed in Table 3-6. The recommended regional target species that should be considered for sampling in estuarine/marine waters are listed in Tables 3-7 through 3-9 for Atlantic Coast estuaries, in Table 3-10 for Gulf Coast estuaries, and in Tables 3-11 through 3-13 for Pacific Coast estuaries.**

Final selection of regional target species must be the responsibility of State fisheries personnel who have the expertise to identify local fish/shellfish species of commercial, recreational, or subsistence value as a human food source in the study area and who are also most familiar with local consumption patterns. In the event that the selected target species are not available for collection, a contingency plan for collecting alternative species should be decided upon at the planning meeting and the selection of species documented by the program manager on the sample request form.

5.1.2.3 Target Contaminants--

Intensive monitoring at a given site should focus on those target contaminants found in the initial screening study to be present in fish/shellfish tissue at concentrations exceeding EPA-recommended TVs (Sections 2.1.4 and 4.2). Thus, in general, the number of target contaminants evaluated in intensive followup monitoring studies will be significantly smaller than the number evaluated in initial screening studies.

5.1.2.4 Sampling Times--

To the extent that program resources allow, **sampling times in intensive monitoring studies should cover the principal period or periods when the target species is most frequently harvested for human consumption and should ensure the collection of appropriate samples of size and/or age classes over the legal harvestable size.**

5.1.2.5 Sample Type--

The type of sample required for analysis of target contaminants in intensive monitoring studies should be prepared from edible fish and/or shellfish tissue. For finfish, edible tissue is defined as the fillet portion (skin-on and bellyflap tissue included). **[Reviewers: Please indicate whether belly flap should be included in the intensive study design.]** It is extremely important that the individual specimens used in the composite sample be of the same species and that the total length (size) of the smallest individual in the composite be ≥75 percent of the total length (size) of the largest individual (U.S. EPA, 1990b). Composite samples should be prepared for each target fish species from equal weights of individual homogenates of fillets from 6 to 10 fish.

For shellfish, the tissues considered to be edible will vary depending on the target species used and regional or local dietary preferences. For each target shellfish species, a clear description of the edible tissue selected for analysis and the rationale for selection should be provided in the Work/QA Project Plan. Because of the small size of shellfish, it is not practical to prepare homogenates of individual organisms. Composite samples should be prepared for each regional target shellfish species from the homogenization of the combined edible tissue from enough organisms to produce a 500-g-minimum composite sample.

Separate composite samples are required for all subgroups (e.g., size or age class) within a target species population that have been selected for evaluation in the intensive monitoring study. For example, if three size classes of a specific target species are of interest, then the sampling design should allow for the collection of a sufficient number of individuals in each size class to allow for the preparation of composite samples for each class.

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, the reasons for this deviation should be documented clearly.

Recommended sample preparation procedures are discussed in Section 6.2.

5.1.2.6 Sample Replication--

A minimum of five replicate composite samples (each composed of equal numbers of 6 to 10 individual fish) for each selected size or age class of each target species is recommended for both Phase I and Phase II of intensive monitoring studies. For shellfish, five replicate composite samples (each composed of the same number of individuals) should be used. Each composite may contain from 10 to 50 individuals depending on the species and size class being sampled. The relative difference (in percent) between the overall mean length (size) of the replicate samples and the mean length (size) of any individual replicate should be no greater than 10 percent (U.S. EPA, 1990b).

Previous EPA guidance (U.S. EPA, 1987c; 1987g; 1989d) has emphasized the importance of using replicate samples to permit the analysis of the data by statistical methods (e.g., ANOVA, power analysis, and trend analysis techniques) to detect differences in mean concentrations among sites. These types of statistical analyses are essential in characterizing the geographic extent of fish consumption advisories and in assessing the effectiveness of management efforts to protect fishery resources from contaminants or to mitigate existing pollution problems.

Selection of the appropriate number of replicate composites for the intensive monitoring study depends on site-specific levels of sample variability in target contaminant tissue concentration and is discussed in detail in Sections 2.2.8 and 7.2.3. Replicate composite sampling is most appropriate for intensive monitoring studies that have as a primary objective the determination of differences in contaminant tissue concentrations among sampling locations (e.g., using multilocational sites to determine the geographic extent of contamination).

5.2 SAMPLE COLLECTION

After all sampling parameters have been reviewed and specified, sample collection activities can be initiated in the field. 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.

5.2.1 Sampling Equipment and Use

In response to the variations in environmental conditions and target species of interest, fisheries biologists have had to devise 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 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 5-2 for fish and in Table 5-3 for shellfish.

A basic checklist of field sampling equipment and supplies appropriate to field collection activities is shown in Table 5-4. Safety considerations associated with the use of a boat in sample collection activities are summarized in Table 5-5.

5.2.1.1 Active Collection--

Active collection methods encompass a wide variety of fish sampling devices, including

- Electroshocking units
- Seines
- Trawls
- Angling equipment (hook and line),

and shellfish (e.g., bivalves and crustaceans) sampling devices, including

- Seines
- Trawls
- Mechanical grabs (e.g., pole-operated grab buckets and tongs and line- or cable-operated grab buckets)
- Biological dredges
- Scoops and shovels
- Rakes
- Dip nets
- Manual collection by SCUBA divers.

TABLE 5-2. SUMMARY OF FISH SAMPLING EQUIPMENT

Device	Use	Advantages	Disadvantages
ACTIVE METHODS			
Electrofishing	Shallow rivers, lakes, and streams.	Most efficient nonselective 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 get over, around, and under 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 personnel with operator training.
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 fishermen	Can be used where sampling sites are located in areas where target species are commercially harvested	Most cost-effective and efficient means of obtaining commercially valuable species from harvested waters	Commercially harvested areas may not include sampling sites chosen for fish contaminant monitoring. The field collection staff **must** accompany the commercial fishermen 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. Not particularly difficult 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.

TABLE 5-2. (continued)

Device	Use	Advantages	Disadvantages
PASSIVE METHODS			
Hoop, Fyke and Pound Nets	Shallow rivers, lakes, and estuaries where 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 valuable as backup for other methods.

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

TABLE 5-3. 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 get over, around, and under 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 personnel with operator training.
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 efficient 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 (clams 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 winch for deployment. Possible incomplete closure of jaws can result in 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).

TABLE 5-3. (continued)

Device	Use	Advantages	Disadvantages
Biological dredge	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.
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 fishermen	Can be used where sampling sites are located in areas where target species are commercially harvested.	Most cost-effective and efficient means of obtaining bivalves for pollutant analysis from commercially harvested waters.	Commercially harvested areas may not include sampling sites chosen for shellfish contaminant monitoring. The field collection staff **must** accompany the commercial fishermen 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.

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

TABLE 5-4. CHECKLIST OF FIELD SAMPLING EQUIPMENT AND SUPPLIES FOR FISH/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)
  - Balance to weigh representative specimens for estimating tissue weight (metric units)
  - Aluminum foil (extra heavy duty)
  - Freezer tape
  - String
  - Large plastic bags for holding 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 5-5. 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 onboard boats.
- If electrofishing is the sampling method used, there must be two shut-off 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.
- Minimize or eliminate all sampling during nondaylight hours, during severe weather conditions, or during periods of high water when the safety of field collection personnel might be jeopardized.
- All field collection personnel should be trained in first aid procedures to allow 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.

For fish/shellfish contaminant monitoring programs, EPA recommends that active collection methods be used whenever possible. 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, along lake shorelines, and shallow coastal areas of estuaries).

When sampling must be conducted in deep water, however, active collection methods have distinct disadvantages because they are more resource-intensive, requiring larger numbers of field personnel and expensive equipment. This problem may be overcome by coordinating sampling efforts with commercial collection efforts. **Purchasing fish/shellfish from commercial fishermen using active collection devices is acceptable only when field sampling staff accompany the commercial fishermen during the collection operation to ensure that proper collection and handling techniques are observed.** Thus, trained personnel can remove the target species directly from the nets 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).

One active collection method that is not recommended by EPA involves the use of chemical poisons to stun or kill fish. **EPA strongly advises against the use of chemical poisons as a technique for collecting fish and shellfish for contaminant monitoring programs** because these toxicants may induce physiological changes that could alter contaminant concentrations in the tissues.

A more detailed description of active sampling devices and their use is provided in Bennett (1970); Weber (1973); Battelle (1975); Mearns and Allen (1978); Pitt, Wells and McKrone (1981); Versar (1982); Hayes (1983); Gunderson and Ellis (1986); and Puget Sound Estuary Program (1990b).

5.2.1.2 Passive Collection--

Passive collection methods encompass a wide array of sampling gears for fish and shellfish including

- Gill nets
- Fyke nets
- Trammel nets
- Hoop nets
- Pound nets
- D-traps.

Passive methods of fish and shellfish collection 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 techniques are generally more efficient than active methods. A more detailed description of passive sampling devices and their use is provided in Versar (1982 and 1984) and Hubert (1983).

The following procedures should be observed when passive collection devices must be deployed:

- Target fish and shellfish must be removed from the passive collection device (i.e., nets) at frequent intervals (<1 hour) during daylight sample collection hours to avoid physiological stress associated with capture (U.S. EPA, 1991c).

- Target fish and shellfish captured during the night in nets must be discarded before daylight sample collection activities are initiated because there is no way to determine the length of time the specimen was in the collection device (U.S. EPA, 1991c).

- Target shellfish species (lobster, crabs, crayfish) captured using D-traps must be removed at an interval not to exceed 48 hours.

- All target species captured using passive collection devices must be alive at the time of retrieval of the sampling equipment. If they are not alive, they must be discarded.

Purchasing fish/shellfish from commercial fishermen using passive collection methods is acceptable only when field sampling staff accompany the fishermen during both the deployment and collection operations. Thus, the field sampling staff can verify that proper collection processing and preservation techniques were used and that specimens were alive at the time of collection.

Although passive methods for sample collection may be needed in some environmental situations for some target species, **EPA recommends that passive methods be used only as a last resort.**

5.2.2 Preservation of Sample Integrity

The primary QA/QC consideration when defining sample collection, processing, preservation, and shipping procedures is the preservation of sample integrity to ensure the accuracy of target contaminant analyses. Sample integrity is preserved by

- Prevention of extraneous tissue contamination
- Prevention of loss of contaminants already present in the tissues (Smith, 1985).

In the field, sources of contamination include sampling gear, boats and motors, grease from ship winches or cables, spilled engine fuel (gasoline or diesel), engine exhaust, dust, ice chests, and ice used for cooling. Care must be taken during handling to avoid these and any other sources of contamination. 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 watertight plastic bags (Stober, 1991). Sampling equipment that has been obviously 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 stations, the field collection team should clean each measurement device by rinsing it with ambient water and rewrapping it in aluminum foil to prevent contamination. All potential sources of contamination in the field should be identified and steps taken to minimize or eliminate them.

In addition to controlling sources of contamination during the sample collection process, many sources of contamination can be avoided by resecting (i.e., surgically removing) tissues in a controlled laboratory environment. **EPA recommends that all resecting of fish fillets or of shellfish edible portions be conducted in a clean area of the central processing laboratory to reduce contamination of specimens (Stober, 1991).** Procedures for laboratory processing and resection are described in Section 6.2. Procedures for assessing sources of sample contamination through the analyses of field and processing blanks are described in Section 6.4.3.5.

5.2.3 Field Recordkeeping

Thorough documentation of the sample collection and processing work done in the field is necessary for interpretation of the results of a field survey. For fish and shellfish contaminant monitoring studies, it is advisable to have preprinted, waterproof data forms and writing implements that produce indelible markings and 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 while allowing retention of the data for the project file.

Four distinct 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 central processing laboratory. These are

- Field record form
- Sample identification label

- Chain-of-custody (COC) label or tag
- Chain-of-custody (COC) form.

Full-sized copies of each of these forms for use in both the initial screening and intensive studies are included in Appendix G for use by the States.

5.2.3.1 Field Record Form--

The following information is recommended for inclusion on the field record for each sampling site in both the initial screening (Figures 5-4 and 5-5) and intensive followup studies (Figures 5-6 and 5-7):

- Project number
- Sampling date and time
- Sampling site location (including site name and number, county/parish, latitude/longitude, State waterbody segment number, waterbody type, and site description)
- Collection method
- Collectors' names and signatures
- Agency (including telephone number and address)
- Species collected (including species scientific name, composite sample number [5 digits] and individual specimen suffix number [3 digits], number of individuals per composite, number of replicate samples, total length/size [cm], sex [male, female, indeterminate])
- Compute 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 $\geq$ 75 percent) and compute mean composite length or size.
- Notes (including visible morphological abnormalities, e.g., fin erosion, skin ulcers, cataracts, skeletal and exoskeletal anomalies, neoplasms, or parasites).

5.2.3.2 Sample Identification Label--

The following information should be included on the sample identification label:

- Species scientific name or code number
- Total length/size of specimen (cm)
- Composite number (5 digits) and individual specimen suffix number (3 digits)

Field Record for Fish Contaminant Monitoring Program — Screening Study

Project Number: _____ Sampling Date and Time: _____

SITE LOCATION

Site Name/Number: _____

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

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

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

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

_____

Predator—Species Name: _____

Composite Sample #: _____ Number of Individuals: _____

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

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

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

_____

Figure 5-4.

Field Record for Shellfish Contaminant Monitoring Program — Screening Study

Project Number: _____ Sampling Date and Time: _____

SITE LOCATION

Site Name/Number: _____

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

State Waterbody 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 (cm)	Bivalve #	Size (cm)	Bivalve #	Size (cm)
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 _____ cm

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

_____

Figure 5-5.

Field Record for Fish Contaminant Monitoring Program — Intensive Study

Project Number: _____ Sampling Date and Time: _____

SITE LOCATION

Site Name/Number: _____

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

State Waterbody 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 (cm)	Sex (M, F, or I)	Fish #	Length (cm)	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 _____ cm

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

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

Composite Sample #: _____ Number of Individuals: _____

Fish #	Length (cm)	Sex (M, F, or I)	Fish #	Length (cm)	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 _____ cm

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

_____

Figure 5-6.

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 (cm)	Sex (M, F, or I)	Fish #	Length (cm)	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 _____ cm

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

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

Composite Sample #: _____ Number of Individuals: _____

Fish #	Length (cm)	Sex (M, F, or I)	Fish #	Length (cm)	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 _____ cm

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

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

Composite Sample #: _____ Number of Individuals: _____

Fish #	Length (cm)	Sex (M, F, or I)	Fish #	Length (cm)	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 _____ cm

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

Figure 5-6. (con.)

Field Record for Shellfish Contaminant Monitoring Program — Intensive Study

Project Number: _____ Sampling Date and Time: _____

SITE LOCATION

Site Name/Number: _____

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

State Waterbody 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 (cm)	Sex	Shellfish #	Size (cm)	Sex	Shellfish #	Size (cm)	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					

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

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

_____

Figure 5-7.

- Sample type: F (fish fillet analysis only)
  S (shellfish edible portion analysis only)
  W (whole fish analysis)
  O (other fish tissue analysis)
- Sampling site–name and/or identification number
- Sampling date/time (24-h clock).

Information on this label should be completed in indelible ink after each individual fish or shellfish specimen is processed to identify each sample uniquely (Figure 5-8). The sample identification label should then be taped to each aluminum-foil-wrapped specimen.

5.2.3.3 Chain-of-Custody Label or Tag--

The information to be completed for each composite fish or shellfish sample on the chain-of-custody (COC) label or tag is shown in Figure 5-9. After all information on a specific composite sample has been completed, the COC label or tag should be taped or attached with string to the outside of the water-proof plastic bag containing the composite sample. Information on the COC tag/label also should be recorded on the COC form.

5.2.3.4 Chain-of-Custody Form--

Information recommended for documentation on the chain-of-custody form (Figure 5-10) is necessary to track all composite samples from field collection to receipt at the central processing laboratory. In addition, this form can be used for tracking samples through initial laboratory processing (e.g., resection) as described in Section 6.2.

One copy of the COC form and a copy of the field record sheet should be sealed in a resealable watertight plastic bag and placed in the ice chest with the samples being tracked prior to sealing the ice chests. Ice chests should be sealed with reinforced tape for shipment.

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 problems encountered that would be useful to the program manager in evaluating the quality of the fish/shellfish contaminant monitoring data.

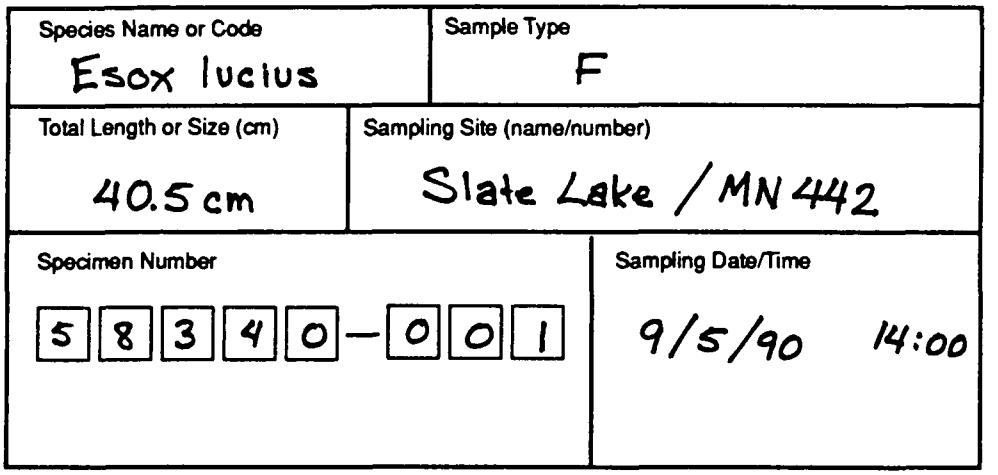


Figure 5-8. Sample identification label.

Project Number	Collecting Agency (name, address, phone)		
Sampling Site (name and/or ID number)	Sampler (name and signature)		
Composite Number	Chemical Analyses ☐ All target contaminants ☐ Others (specify) _____	Study Type	
		Screening	Intensive
Sampling Date/Time			
Species Name or Code	Processing	Type of Ice	
	Whole Body / Resection	Wet	Dry
Comments			

Figure 5-9. Example of a chain-of-custody tag or label.

Chain-of-Custody Record

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

Composite Number	Sample Nos.	Sampling Time	Study Type: Scr	Study Type: Int	Sampling Site (name/number)	All Target Contaminants	Specific Contaminants Only (Specify)	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 5-10. Example of a chain-of-custody form.

5.3 SAMPLE PROCESSING, PRESERVATION, AND SHIPPING

5.3.1 Sample Processing

As soon as individual fish specimens are removed from the collection device or water, they should be stunned by a sharp blow to the base of the skull with a wooden stick or metal rod. This 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). Each fish should then be rinsed in ambient water to remove any foreign material. Individual specimens of the target species should be grouped by species and general size class and placed in clean holding trays to prevent contamination.

As soon as shellfish are removed from the collection device, they should be rinsed in ambient water to remove any sediment deposits. Bivalves (oysters and mussels) should be separated when found to be adhering to one another and scrubbed with a nylon or natural fiber brush to remove any adhering detritus or fouling organisms from the exterior shell surface (NOAA, 1987). All bivalves should be inspected carefully to ensure that the shells have not been cracked or damaged by the sampling equipment; damaged specimens should be discarded (Versar, 1982). Bivalves should never be removed from their shells in the field. A few specimens may be shucked to determine the wet weight of the edible portion (meats). This will provide an estimate of the number of individuals required to ensure that the minimum sample weight (500 g) can be attained.

Crustaceans, including shrimp, crabs, crayfish, and lobsters, should be rinsed in ambient water to remove any foreign material from their external surface. All crustaceans should be inspected to ensure that their exoskeletons have not been cracked or damaged during the sampling process; damaged specimens should be discarded.

After they have been rinsed, individual shellfish specimens should be grouped by species and general size class and placed in a clean holding tray to prevent contamination. A few specimens may be resectioned (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 minimum sample weight (500 g) can be attained. For blue crabs (*Callinectes sapidus*), the edible meat (claw and back fin meat) constitute approximately 10 percent of the overall body weight including the carapace (Sean McKenna, North Carolina Division of Marine

Fisheries, personal communication). Thus, a 100-g adult crab will yield approximately 10 g of edible tissue and 50 crabs would be required to obtain the minimum sample weight (500 g).

5.3.1.1 Species Identification--

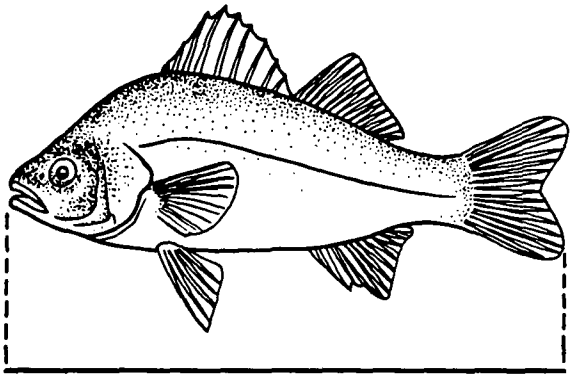
Species identification should be conducted only by experienced personnel knowledgeable of the taxonomy of species in the waterbodies included in the fish/shellfish contaminant monitoring program. Taxonomic keys, appropriate for the waters being sampled, should be consulted for species identification. Because the objective of both the screening and intensive monitoring studies is to determine the magnitude of contamination in specific fish and shellfish species, it is necessary that all individuals used in a composite sample be of a single species. **Correct species identification is important and different species should never be combined in a single composite sample for any reason.**

When sufficient numbers of the target species have been identified to make up a composite sample, a member of the field collection team should record the species name and all other appropriate information on the field record sheet (Figures 5-4 through 5-7).

5.3.1.2 Length or Size Measurements--

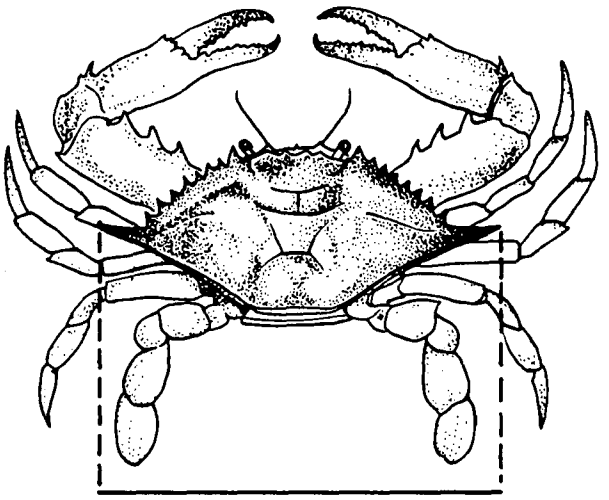
Each individual fish within the target species selected for analysis should be measured to determine total body length (cm). To be consistent with the convention used by most fisheries biologists in the United States, maximum total length should be measured as shown in Figure 5-11. The maximum body length 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).

For shellfish, after initial processing, each individual specimen selected for analysis should be measured to determine total body size (cm). As shown in Figure 5-11, 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 (Galtsoff, 1964; Abbott, 1974). The height is the distance from the umbo to the anterior shell margin. For crabs, the lateral width of the carapace is a standard size measurement (U.S. EPA, 1990c), and for shrimp, lobster and crayfish, a standard measurement of body size is the length from the rostrum to the tip of the telson (Texas Water Commission, 1990).



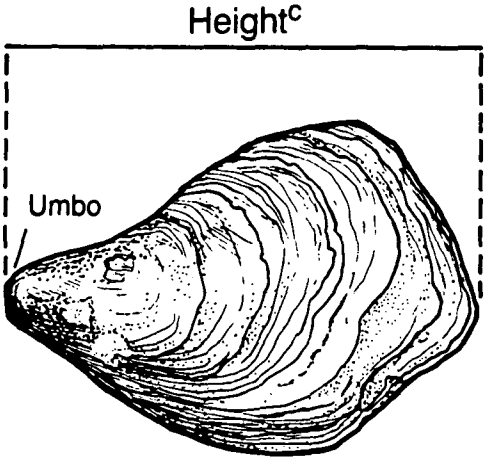
Maximum body length[a]

Fish

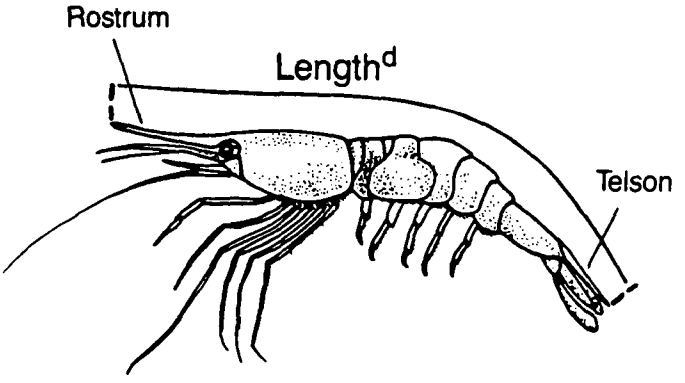


Carapace width[b]

Crab



Bivalves



Shrimp, Lobster, Crayfish

[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 dorso ventrally) (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 shell margin (Galtsoff, 1964).

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

Figure 5-11. Recommended measurements of body length and size for fish and shellfish.

[Reviewers, please comment on acceptability of shellfish size measurements. If different from above, please provide complete literature citation.]

5.3.1.3 Sex Determination (Optional)--

An experienced fisheries biologist can often make a preliminary sex determination for fish by visual inspection. **Under no circumstances, however, should the body of the fish be dissected in the field** to determine sex; sex can be determined through internal examination of the gonads during laboratory processing (Section 6.2).

For shellfish, a preliminary sex determination can be made by visual inspection only for crustaceans. Sex determination cannot be made in bivalve molluscs without shucking the bivalves and microscopically examining gonadal material. **Under no circumstances should bivalves be shucked in the field to determine sex; sex determination through examination of the gonads can be performed during laboratory processing** (Section 6.2).

5.3.1.4 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 (Sinderman et al., 1980; Sinderman, 1983; Malins et al., 1984 and 1985; Mix, 1986; Krahn et al., 1986).

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 the fins 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 involve the spinal column and include fusions, flexures, and vertebral compressions. Skeletal anomalies also include abnormalities of the head, fins, and gills.

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.

The occurrence of fish parasites and other gross morphological abnormalities that are suspected at a specific site location should be noted on the field record sheet. States interested in documenting morphological abnormalities in fish should review the recommended protocols for fish pathology studies used in the Puget Sound Estuary Program (1990c).

Although gross morphological observations generally are not definitive evaluations of fish health, they may be very useful in uncovering previously unknown pathological conditions in fishes from polluted areas (Puget Sound Estuary Program, 1990c). These relatively quick examinations are very cost-effective because they do not require specialized equipment or preparation techniques and can be made as the specimens are sorted from the catch. In addition, gross external observations generally do not require that a trained pathologist be aboard the sampling boat. However, it is extremely important that at least one member of the collecting team be trained by a qualified pathologist to identify the various kinds of pathological conditions that may be encountered, because at least two pathological conditions (fin erosion and skin ulcers) can easily be confused with the external damage that fishes may suffer as they are dragged along the seafloor in an otter trawl (Puget Sound Estuary Program, 1990c).

Given the potential usefulness of gross observations and the need for accurate and verifiable determinations, it is recommended that representative fishes having each kind of pathological condition be archived for each major sampling survey, and that the conditions be confirmed by a qualified pathologist. This verification step is especially important if different personnel make the gross observations during different surveys. For all suspected pathological conditions that cannot be identified in the field, representative specimens should

be archived for later evaluation by a qualified pathologist (Puget Sound Estuary Program, 1990c).

5.3.1.5 Composite Samples--

For each target fish species (or age class of target fish species) sampled, 10 individual fish of the same species and similar size should be composited. However, samples containing 6 fish are minimally acceptable. The smallest individual fish used in a target species composite sample should be no less than 75 percent of the total length of the largest individual. For example, if the largest fish is 40 cm, then the smallest individual included in the composite sample should be no smaller than 30 cm (U.S. EPA, 1990b).

For each shellfish species (or age class of shellfish species) sampled, 10 to 50 individual specimens of the same species and similar size should be composited. The number of specimens to be composited cannot be specified for shellfish because the number will depend on the size of the specimens collected and the weight of the edible portion. For small shellfish, larger numbers of specimens (>50) may have to be composited to achieve the minimum tissue mass of 500 g (excluding bivalve shell weight). For shellfish, the smallest individual specimen used in the composite should be no less than 75 percent of the total size of the largest individual. In some State sampling programs such as 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).

5.3.1.6 Replicate Samples--

If replicate field samples for target fish or shellfish species are to be collected, the relative difference between the overall mean length of the replicate samples and the mean length of any individual replicate sample should be no greater than 10 percent. In the following example, the overall mean length (±10 percent) of five replicate composite samples is calculated to be 31 (±3.1) cm.

Replicates	Mean Length of Composite Fish Sample (cm)
1	30
2	32
3	33
4	28
5	32

Overall mean length (± 10%) = 31 (± 3.1) cm.

Therefore, the acceptable range for the mean length of individual composite samples is 27.9 to 34.1 cm and the five replicate composite samples listed above all fall within this acceptable size range.

5.3.2 Sample Preservation

After initial processing, 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 5-8 should be taped to the outside of each aluminum foil package.

After wrapping and labeling each individual fish in the composite sample, all of the wrapped specimens in the composite sample should be secured with string or tape. If tape is used, care should be taken not to tape over any of the individual sample identification labels. The COC tag or label (Figure 5-9) should be completed for the composite sample and the appropriate information should be recorded on both the field record sheet and COC form (Figure 5-10). The composite fish sample should be placed into a watertight plastic bag and sealed, and the COC tag should be attached to the outside of the plastic bag with string or tape. Once packaged, the composite sample should be cooled on ice immediately.

After processing, each shellfish specimen should be wrapped individually in extra heavy duty aluminum foil. A completed sample identification label 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 carapaces that might puncture the aluminum foil wrapping. For such species, samplers may use one of the following procedures 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.
- Selectively wrap the spines with multiple layers of foil prior to wrapping the entire specimen in aluminum foil.

Carapace spines should never be sheared off as this would destroy the integrity of the carapace. A COC tag or label should be completed for the composite sample and appropriate information should be recorded on the field record sheet and COC form. After wrapping and labeling each shellfish specimen in the composite sample, all of the wrapped

specimens in the composite sample should be placed in a plastic watertight bag. The COC label or tag should be completed for the composite sample and appropriate information should be recorded on both the field record sheet and COC form. The COC label or tag should be attached to the outside of the plastic bag with string or tape. Once packaged, the composite sample should be cooled on ice immediately.

5.3.2.1 Preservation of Fish or Shellfish for Resection--

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 central processing laboratory and the sample type to be analyzed (Table 5-6). Fish and shellfish specimens should not be frozen prior to resection if analyses will include internal tissue (e.g., fillets or edible tissues) because freezing may cause some internal organs to rupture and contaminate fillets or other edible tissues (Tetra Tech, 1989). If fish fillet samples or edible portions of shellfish are to be analyzed, wet ice or blue ice (sealed prefrozen ice packets) should be used and samples should be delivered to the processing laboratory within 24 hours. **Wet ice or blue ice is recommended as the preservative of choice when the fish fillet or shellfish edible portions are the primary tissues to be analyzed.**

5.3.2.2 Fish or Shellfish for Whole-Body Analysis--

At some sites, States may deem it necessary to collect fish for whole-body analysis, if a specific human subpopulation typically consumes whole fish or shellfish. **If whole fish or shellfish samples are to be analyzed, either wet ice, blue ice, or dry ice is recommended. If shipping time to the laboratory will take more than 24 hours, dry ice must be used.**

Dry ice requires special packaging precautions before shipping to comply with U.S. Department of Transportation (DOT) regulations. The *Code of Federal Regulations* classifies dry ice as ORM-A (Other Regulated Material). These regulations specify the amount of dry ice that may be shipped by air transport and the type of packaging required. For any amount of dry ice to be shipped by air, advance arrangements must be made with the carrier and not more than 440 pounds of dry ice may be shipped by air freight unless the shipper has made special arrangements with the aircraft operator. Quantities of dry ice for tissue preservation are usually considerably less than 440 pounds (Versar, 1982).

TABLE 5-6
RECOMMENDATIONS FOR PRESERVATION OF FISH/SHELLFISH SAMPLES FROM TIME OF COLLECTION TO DELIVERY AT CENTRAL PROCESSING LABORATORY

Sample Type	Number per Composite	Container	Preservation	Maximum Holding Time
FISH*				
Whole fish (to be filleted)	6-10	Same as above	Cool on wet ice or blue ice packets	24 hours
Whole fish	6-10	Extra heavy duty aluminum foil wrap of each fish. All fish in a composite taped together and placed in a watertight plastic bag	Cool on wet ice, or blue ice packets,	24 hours
			or on dry ice for transport to the processing laboratory	48 hours
SHELLFISH*				
Whole shellfish (to be resected for edible portion)	10-50 (species and size dependent)	Same as above	Cool on wet ice or blue ice packets	24 hours
Whole shellfish	10-50 (species and size dependent)	Extra heavy duty aluminum foil wrap of each specimen. All shellfish in a composite placed in a watertight plastic bag	Cool on wet ice or blue ice packets,	24 hours
			or on dry ice for transport to the processing laboratory	48 hours

*Use only individuals that have attained at least legal or consumable size.

The regulations further specify that the packaging must be constructed in a manner to permit the release of carbon dioxide gas which, if restricted, could cause rupture of the package. If samples are being 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. Furthermore, wire screen or cheesecloth should be installed to help keep foreign materials from entering the vents. 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 paper and certification requirements if the amount is less than 440 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).

5.3.3 Sample Shipping

The fish/shellfish samples should be hand-delivered or shipped to the central processing laboratory as soon as possible after collection. The time of collection and time of arrival at the processing laboratory should be recorded on the COC form (Figure 5-10).

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 call ahead to the central 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 central processing laboratory unless prior plans for such a delivery have been agreed upon with the central processing laboratory staff.

SECTION 6
LABORATORY PROCEDURES

This section provides guidance to States on laboratory procedures followed from the time a field sample is received at the central processing facility, through sample analysis for target analytes, to final archiving. It includes recommended procedures for chain-of-custody, sample processing, sample distribution, and sample analyses. Planning, documentation, and quality assurance/quality control (QA/QC) of all laboratory activities are emphasized to ensure that the integrity of samples is preserved during all phases of sample preparation and chemical analyses, that chemical analyses are performed cost-effectively and meet program data quality objectives, and that the data produced by different States and Regions are comparable.

Laboratory procedures used in State fish/shellfish contaminant monitoring programs should be documented in a Work/QA Project Plan as described in Appendix F and all routine sample processing and analysis procedures should be prepared as standard operating procedures (SOPs) (U.S. EPA, 1984b).

6.1 SAMPLE RECEIPT AND CHAIN-OF-CUSTODY

Collected samples are shipped or hand-carried from the field according to one or more of the following pathways:

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

In each case, sample processing and distribution for analysis, if necessary, must be performed by one central processing laboratory. Because EPA recommends that dioxin analyses be performed by a contract laboratory (see Section 6.4.2), aliquots of each composite sample designated for dioxin analyses must be shipped from the sample processing laboratory to a contract laboratory.

Transportation of the samples from the field must be coordinated by the sampling team supervisor and the laboratory responsible for sample processing (see Section 5.3.3). An

accurate written record must be maintained so that possession and treatment of each sample can be traced from the time of collection through analysis and final archiving, if applicable.

The fish and shellfish samples should be brought to or shipped to the sample processing laboratory in sealed containers accompanied by a copy of the sample request form (Figure 5-1), a chain-of-custody (COC) form (Figure 5-10), and the field records (Figures 5-4 through 5-7). Each time a sample or group of samples changes hands, the Personnel Custody Record of the COC form must be completed and signed by both parties. Corrections to the COC form should be made by drawing a line through and initialing and dating the error and then entering the correct information.

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

- Check that each shipping container has arrived undamaged and that the seal is intact.
- Open each shipping container and remove the copy of the sample request form, the COC form, and the field records.
- Note the general condition of the shipping container (samples iced properly with no leaks, etc.) and the accompanying documentation (dry, legible, etc.).
- Locate each composite sample listed on the COC form and note the condition of its container. Composite sample containers should be properly sealed and labeled. Note any problems (container punctured, illegible labels, etc.) on the COC form.
- Check the contents of each composite sample container against the field record for that sample to ensure that the individual specimens are properly wrapped and labeled. Note any discrepancies or missing information.
- Initial the COC form and record the date and time of sample receipt.
- Enter the following information for each composite sample into a permanent laboratory record book and, if applicable, a computer database:
  - -- Sample identification number (5-digit composite sample number and 3-digit sample suffix)
  - -- Collection date
  - -- Collection site (name and number)
  - -- Fish species (scientific name or code number)

-- Total length of each fish (cm) or size of each shellfish (cm)

- Store samples according to the procedures described in Section 6.2 and in Table 6-1. If the fish are on wet ice or blue ice and fillets are to be resected, distribute the samples immediately to the biologist responsible for resection. Note: Samples must remain iced until they are placed in a freezer for longer term storage.

TABLE 6-1. RECOMMENDATIONS FOR CONTAINER MATERIALS, PRESERVATION, AND HOLDING TIMES FOR FISH/SHELLFISH TISSUES FROM DELIVERY AT CENTRAL PROCESSING LABORATORY TO ANALYSIS

Analyte	Matrix	Sample container	Storage	
			Preservation	Holding time
Trace metals (except Hg)	Tissue (whole specimens, edible portions, homogenates)	Plastic, glass	Freeze at $\leq$-20 °C	1 year
Hg	Tissue (whole specimens, edible portions, homogenates)	Plastic, glass	Freeze at $\leq$-20 °C	28 days
Organics	Tissue (whole specimens, edible portions, homogenates)	Glass, teflon	Freeze at $\leq$-20 °C	1 year

6.2 SAMPLE PROCESSING

This section describes recommended procedures for preparing composite samples of fish fillets (skin on and belly flap included) and edible portions of shellfish as required in initial screening studies and intensive followup monitoring studies (Phases I and II, see Section 7). Recommended procedures for preparing whole fish/shellfish composite samples are included in Appendix GG for use when States determine that it is necessary to assess the potential risk to local subpopulations that are known to consume whole fish or shellfish.

6.2.1 General Considerations

Avoiding contamination is one of the most important considerations in sample processing. All instruments, work surfaces, and containers used in processing a sample must be composed of materials that can be cleaned easily and that are not themselves potential sources of contamination. Sources of contamination by organics are different from sources of

contamination by trace metals. Therefore, if time and funding permit, it is recommended that duplicate samples be collected for the initial screening; one sample to be processed and analyzed for organics and the other to be processed independently and analyzed for trace metals. Alternatively, for fish of adequate size, separate composites of right and left fillets may be prepared and analyzed independently for trace metals and organics. If only one composite sample is prepared for screening analysis, the processing equipment must be chosen and cleaned carefully to avoid contamination by both organics and trace metals.

Intensive monitoring focuses on target contaminants identified in the initial screening study. If intensive monitoring samples are to be analyzed only for organics or trace metals, processing equipment and procedures should be chosen accordingly. If intensive monitoring samples are to be analyzed for both organics and trace metals, fish may be filleted and the left fillet processed and analyzed for organics and the right fillet processed and analyzed for trace metals.

Suggested sample processing equipment and cleaning procedures by analysis type are discussed in more detail in Sections 6.2.1.1, 6.2.1.2, and 6.2.1.3. Variations of these procedures may be used if it can be demonstrated, through the analysis of sample blanks, that no contamination is introduced (see Section 6.4.3.5). To avoid cross-contamination, all equipment used in sample handling should be thoroughly cleaned between samples.

6.2.1.1 Samples for Organic Analysis--

Equipment used in processing samples for organic analysis should be constructed of stainless steel, anodized aluminum, borosilicate glass, and/or quartz. Polypropylene and polyethylene (plastic) surfaces and implements are a potential source of contamination by organics and should not be used.

A suggested cleaning procedure is to wash with detergent solution, rinse with tap water, soak in isopropanol (distilled in glass or pesticide grade), and rinse with organic-free, distilled, deionized water. Work surfaces should be cleaned with isopropanol, washed with distilled water, and allowed to dry completely (Stober, 1991). Alternative washing procedures may be used if it can be demonstrated, through the analysis of appropriate processing blanks, that all surfaces and equipment are free of organic contaminants (see Section 6.4.3.5).

Filleting should be done on cutting boards covered with heavy duty aluminum foil, which is changed between each composite sample. Tissue removal should be done with clean stainless steel or quartz instruments. Knives, fish scalers, measurement boards, etc.,

should be cleaned with pesticide-grade isopropanol followed by a rinse with distilled water between each composite sample (Stober, 1991).

Samples may be stored in glass or Teflon containers with Teflon-lined lids.

6.2.1.2 Samples for Trace Metals Analysis--

Equipment used in processing samples for trace metal analyses should be made of quartz, TFE (tetrafluoroethylene), polypropylene, or polyethylene. Stainless steel that is resistant to corrosion may be used if necessary. Stainless steel scalpels have been found not to contaminate mussel samples (Stephenson et al., 1979). However, other biological tissues (e.g., fish muscle) containing low concentrations of heavy metals may be contaminated significantly by any exposure to stainless steel. The predominant metal contaminants from stainless steel are chromium and nickel. If these metals are not of concern, the use of stainless steel for sample processing is acceptable. Quartz utensils are ideal but expensive. To control contamination when resecting tissue, separate sets of utensils should be used for removing outer tissue and for removing tissue for analysis. For bench liners and bottles, borosilicate glass is preferred over plastic (Stober, 1991).

Prior to use, utensils and bottles should be cleaned thoroughly with a detergent solution, rinsed with tap water, soaked in acid, and then rinsed with metal-free water. For quartz, TFE, or glass containers, 50% $HN0_3$, 50% HC1, or aqua regia (3 parts conc HC1 + 1 part conc $HN0_3$) should be used for soaking. For plastic material, 50% $HN0_3$ or 50% HC1 is appropriate. Reliable soaking conditions are 24 h at 70 °C (Greenburg et al., 1985). Chromic acid should not be used for cleaning any materials. Acids used should be at least reagent grade. Metal parts may be cleaned as stated for glass or plastic, omitting the acid soaking step (Stober, 1991).

6.2.1.3 Samples for Organics and Trace Metals Analyses--

Several established monitoring programs, including the Puget Sound Estuary Program (1990c,d), the NOAA Mussel Watch Program (Battelle, 1989), and the California Mussel Watch Program (California, 1990) recommend that different procedures be used to process samples for organics analysis and for trace metals analysis. However, this may not always be feasible, especially in a screening program where only one shellfish composite is collected and processed or where fish are not of adequate size to allow the preparation of separate composites from right and left fillets. In these cases, precautions must be taken to use

materials and cleaning procedures that are noncontaminating for both organics and trace metals. (Corrosion-resistant stainless steel, quartz, and Teflon are recommended materials.)

A suggested procedure for cleaning sample processing instruments is to wash them with a detergent solution, rinse with tap water, and rinse with organics- and metal-free water. Work surfaces may be cleaned with isopropanol, washed with distilled water, and allowed to dry. Borosilicate glass bench liners are recommended.

Homogenates and composites should be stored in clean glass, quartz, or Teflon containers with Teflon-lined lids. All containers should be thoroughly cleaned with a detergent solution, rinsed with tap water, soaked in acid (50% HNO_3, 50% HCl, or aqua regia), and then rinsed with organics- and metal-free water. Reliable soaking conditions are 24 h at 70 °C (Greenburg et al., 1985).

Composite sample aliquots taken for metals analysis may be stored in plastic containers that have been cleaned according to the procedure given above for glass, with the exception that aqua regia must not be used for the acid soaking step.

6.2.2 Fish Samples

Processing in the laboratory to prepare fish fillet composite samples (diagrammed in Figure 6-1) involves

- Weighing individual fish
- Removing scales and/or otoliths for age determination
- Determining the sex of each fish (optional)
- Removing skin of catfish, bullheads, and sturgeons and scaling all other fish (leaving belly flap on)
- Filleting the fish
- Weighing individual fillets
- Homogenizing individual fillets
- Preparing a composite homogenate
- Aliquotting the composite homogenate for analysis
- Shipping frozen aliquots to one or more contract laboratories for analysis as necessary.

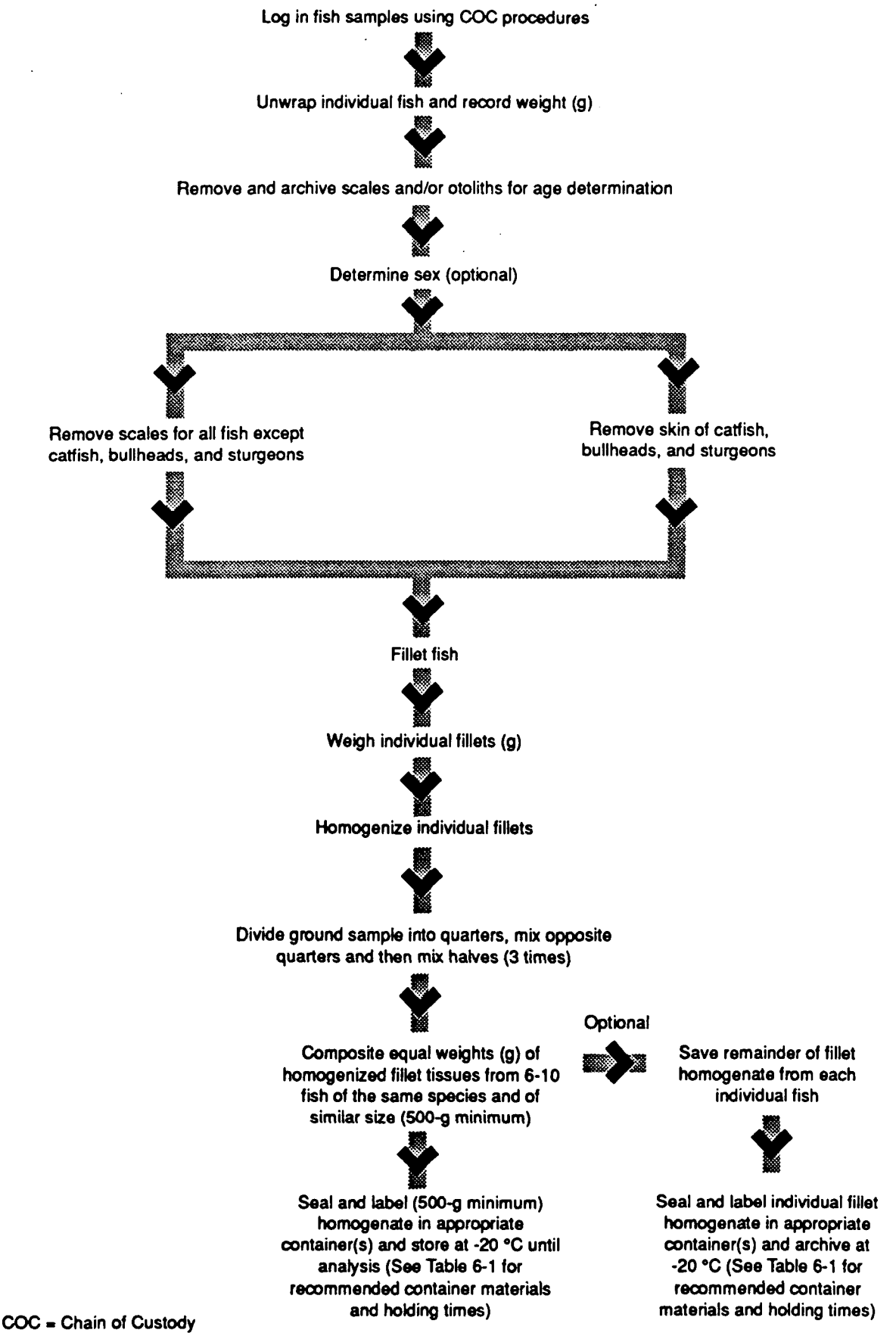


Figure 6-1. Laboratory sample preparation and handling for fish fillet composite samples.

Whole fish samples should be shipped or brought to the processing laboratory on wet or blue ice and fillets resected within 48 hours of sample collection. Fish should not be frozen prior to resection because freezing may cause internal organs to rupture and contaminate edible tissue (Stober, 1991). Fish arriving in the laboratory should be weighed, scales and/or otoliths removed, the sex of each fish determined, and fillets (with belly flap) taken within 48 hours of sample collection. Individual fillets then should be frozen at ≤-20 °C in the laboratory prior to being homogenized. The grinding/homogenization procedure can be carried out more easily if the sample is frozen (Stober, 1991). If resection cannot be performed within 48 hours, the samples should be frozen at the sample site and shipped to the central sample processing laboratory on dry ice. The fish should then be partially thawed prior to resection. If rupture of organs is noted for an individual fish, the specimen should be eliminated from the composite sample.

The thawed or partially thawed fillets should be homogenized individually, and portions of each homogenate should be combined and mixed to form the composite sample. Individual homogenates and/or composite homogenates may be refrozen; however, frozen individual homogenates must be rehomogenized before compositing, and frozen composite homogenates must be rehomogenized before aliquotting, extraction, and analysis. The maximum holding time from sample collection to analysis for mercury is 28 days at ≤-20 °C; for all other analytes, the holding time is 6 months to 1 year at ≤-20 °C (Stober, 1991). Sample processing procedures are discussed in more detail in the following sections. Data from each procedure should be recorded directly in a bound laboratory notebook or on forms that can be taped or pasted into the laboratory notebook. A sample processing record for fish fillet composites is shown in Figure 6-2.

6.2.2.1 Sample Weighing--

A wet weight should be determined for each fish collected. If the fish has been shipped on wet or blue ice, it should be unwrapped and placed on a foil-lined balance tray and the weight recorded to the nearest gram on the sample processing record and/or in the laboratory notebook. To avoid contamination, the foil lining should be replaced between each weighing. Frozen fish should be weighed in clean, tared containers if thawing is expected before the weighing can be completed. Liquid associated with the sample when thawed must be maintained in the container as part of the sample because it will contain lipid material that has separated from the tissue (Stober, 1991).

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

Project Number: _____ Sampling Date and Time: _____

STUDY PHASE: Initial Screening ☐; Intensive Monitoring: Phase I ☐ Phase II ☐

SITE LOCATION

Site Name/Number: _____

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

State Waterbody Segment Number: _____ Waterbody Type: _____

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

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

					Left Fillet			Right Fillet		
Fish #	**Weight (g)**	**Scales/Otoliths Removed (✓)**	**Sex (M,F)**	**Resection Performed (✓)**	**Weight (g)**	**Homogenate Prepared (✓)**	**Wt. of Homog. for Composite**	**Weight (g)**	**Homogenate Prepared (✓)**	**Wt. of Homog. for Composite**
001										
002										
003										
004										
005										
006										
007										
008										
009										
010										
Analyst										
Date										

Total Composite Weight (g) **(left)** _____ **(right)** _____

Notes: _____

Figure 6-2.

6.2.2.2 Removal of Scales and/or Otoliths for Aging--

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

6.2.2.3 Sex Determination--

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

6.2.2.4 Sample Resection (Filleting)--

Resection should be carried out by or under the supervision of an experienced fisheries biologist. Tissue should be removed with carefully cleaned instruments (see Section 6.2.1), and the specimens should come into contact with noncontaminating surfaces only. To control contamination when resecting tissue, technicians should use separate sets of utensils for removing outer tissue and for resecting tissue for analysis.

Special care must be taken to avoid contaminating targeted tissues with material adhering to the fish exterior. The proper handling of fish tissue to prevent contamination during laboratory processing cannot be overemphasized. Filleting should be conducted on

cutting boards covered with heavy duty aluminum foil that is changed between samples (Puget Sound Estuary Program, 1990d,e). For catfish, bullheads, and sturgeon, the skin should be removed before filleting. Belly flaps should be included with all fillets.

The FDA method (1990) for filleting fish is as follows:

> Remove and discard heads, scales, tails, fins, guts, and inedible bones; do not remove skin; fillet and obtain all flesh and skin from head to tail and from top of back to belly on both sides.

A comparable fillet can be obtained from the other side of the fish and can be composited with the first fillet, kept separate for duplicate quality assurance analysis, analyzed for different analytes, or archived.

Large fish should be sectioned according to the following FDA (1990) method:

> Clean, scale, and eviscerate fish. Take 1-inch thick slices, one from behind the pectoral fins, one from halfway between the first slice and the vent, and one from behind the vent. Remove bones from each slice before combining.

Care must be exercised not to puncture any of the internal organs. If the body cavity is inadvertently penetrated, the fillet should be rinsed with distilled water. This skin-on fillet deviates from the skin-off fillets analyzed in the National Bioaccumulation Study (U.S. EPA, 1991c); however, skin-on is recommended because that is the way most sport anglers prepare their fillets.

Each fillet should be weighed and the weight recorded to the nearest gram on the sample processing record. If the fillets are to be homogenized later, they should be wrapped individually in aluminum foil and labeled with the sample identification number, the weight (g), and the date of resection. The designation "L" (for left fillet) or "R" (for right fillet) should be added to the composite sample identification number at this time. The right and left fillets from each fish should be kept together, all fillets from a composite should be placed in a labeled plastic bag, and the bag stored at $\leq$-20 °C until homogenization.

6.2.2.5 Preparation of Individual Homogenates--

Small fish fillets (<300 g) should be ground in a hand crank meat grinder and fillets (300 to 1,000 g) should be ground in a food processor. Larger fillets may be cut into 2.5-cm cubes with a food service band saw (e.g., Hobart Model 5212) and then ground in either a small (e.g., Hobart, 1/4 hp, Model 4616) or large (e.g., Hobart, 1 hp, Model 4822) meat grinder. Homogenizers used to grind tissue should have tantalum or titanium parts if possible.

The ground sample should be divided into quarters, opposite quarters mixed together by hand, and the two halves mixed back together. The grinding, quartering, and hand-mixing steps should be repeated two more times. If chunks of tissue are present at this point, grinding/homogenizing should be repeated. No chunks should be discarded because they will not be extracted efficiently. If the sample is to be analyzed for trace metals only, the ground tissue may be mixed by hand in a polyethylene bag. As each individual fish is homogenized, it should be noted (marked with a check) on the sample processing record.

Individual fish fillet homogenates may be either composited or frozen individually and stored at ≤-20 °C.

6.2.2.6 Preparation of Composite Homogenates--

If individual fish fillet homogenates are frozen they should be thawed partially and rehomogenized prior to compositing. Any associated liquid should be maintained as a part of the sample. Equal weights from each individual homogenate should be removed and blended to provide a composite sample of sufficient size (500 g minimum) to perform all necessary analyses. Weights of individual homogenates required for a composite sample, based on the total number of fish per composite and the quantity of composite prepared, are given in Table 6-2. The actual weight of each individual homogenate that is used in the composite sample should be recorded, to the nearest gram, on the sample processing record. The remaining individual homogenates should be archived at ≤-20 °C with the designation "Archive" and the expiration date added to each sample label. Location of the archived samples should be indicated on the sample processing record under "Notes." Each composite sample should be divided into quarters, opposite quarters mixed together by hand, and the two halves mixed together. The quartering and mixing should be repeated two more times. If the sample is to be analyzed only for trace metals, the composite sample may be mixed by hand in a polyethylene bag. At this point, the composite sample may be frozen and stored at ≤-20 °C or processed for analysis.

6.2.3 Shellfish Samples

Laboratory processing of shellfish to prepare edible tissue composites (diagrammed in Figure 6-3) involves

- Removing the edible parts from each shellfish in the composite sample (10 to 50 individuals, depending upon the species)
- Combining the edible parts in an appropriate noncontaminating container

TABLE 6-2. INDIVIDUAL WEIGHTS (g) OF HOMOGENATE REQUIRED FOR A COMPOSITE SAMPLE[a]

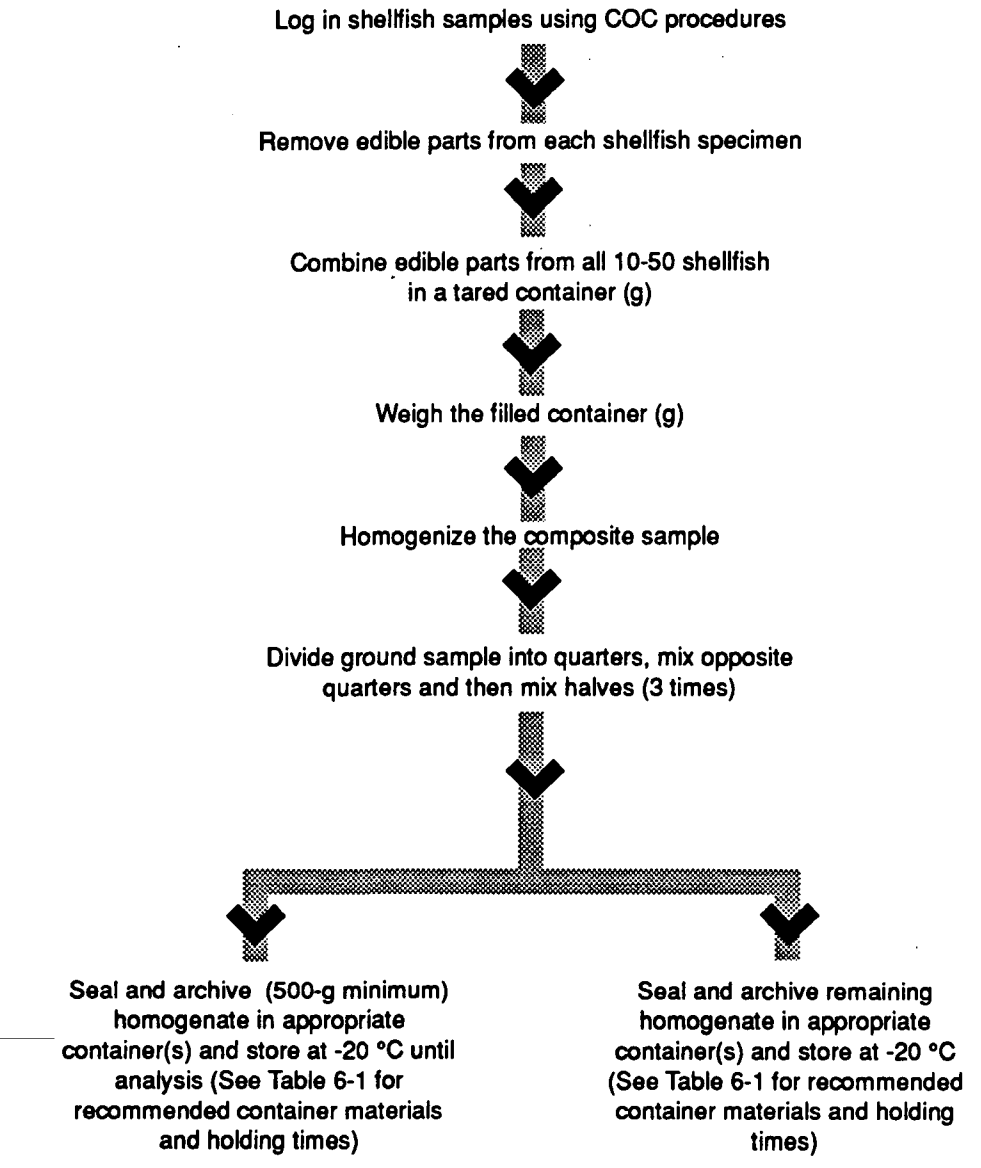
Total number of fish per sample	Total homogenate weight		
	500 g (minimum)	1,000 g (average)	2,000 g
6	84	167	334
7	72	143	286
8	63	125	250
9	56	112	223
10	50	100	200

[a] Based on total number of fish per composite and the total homogenate weight required for analysis.

- Homogenizing the composite sample
- Aliquotting the composite homogenate for analysis
- Shipping frozen aliquots to one or more contract laboratories for analysis as necessary.

Sample aliquotting and shipping are discussed in Section 6.3; all other processing steps are discussed in this section. A sample processing record for shellfish edible tissue composite samples is shown in Figure 6-4.

Shellfish samples collected for intensive monitoring studies should be shipped to the sample processing laboratory either on wet ice or blue ice (if next-day delivery is assured) or on dry ice (see Section 5.3.2). Shellfish samples arriving on wet ice or blue ice should have edible tissue removed and should be frozen to ≤-20 °C within 48 hours after collection. Shellfish samples that arrive frozen at the central processing laboratory should be placed in a freezer for storage until edible tissue is removed. Thawing of frozen shellfish samples should be kept at a minimum during tissue removal procedures to avoid loss of liquids. Shellfish should be rinsed well with organic- and metal-free water to remove any loose external debris.



COC = Chain of Custody

Figure 6-3. Laboratory sample preparation and handling for shellfish edible tissue composite samples.

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

Project Number: _____ Sampling Date and Time: _____

STUDY PHASE: Initial Screening ☐ ; Intensive Monitoring Phase I ☐ Phase II ☐

SITE LOCATION

Site Name/Number: _____

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

State Waterbody Segment Number: _____ Waterbody Type: _____

SHELLFISH COLLECTED

Species Name: _____

Composite Sample #: _____ Number of Individuals: _____

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

Preparation of Composite:

Weight of container + shellfish _____ g

Weight of container _____ g

Total weight of composite _____ g ÷ _____ = _____

(÷ # of specimens; = Average weight of specimen)

Analyst _____ Date _____

Figure 6-4.

Edible parts from all shellfish constituting a composite (10-50 individuals) should be placed in an appropriate preweighed and labeled noncontaminating container. The weight ofthe empty container should be recorded on the sample processing record. All fluids accumulated during removal of edible tissue are considered part of the sample. As the edible portion of each shellfish is placed in the container, it should be noted on the sample processing record. When the edible tissue has been removed from all shellfish in the composite, the container should be reweighed and the weight recorded on the sample processing record. At this point, the composite sample may be frozen and stored at ≤-20 °C or processed for analysis.

Each composite sample should be homogenized to a paste-like consistency in a Polytron or blender before aliquots are taken for analysis. Composite homogenates may be refrozen; however, they must be rehomogenized before aliquotting. The maximum holding time from sample collection to analysis for mercury is 28 days at ≤-20 °C. For all other analytes, the holding time is 6 months to 1 year at ≤-20 °C (Stober, 1991). Bivalve sample processing procedures are discussed in more detail in the section below. Performance of each procedure should be documented in the laboratory notebook or on an appropriate form that can be taped or pasted in the laboratory notebook (see Figure 6-4).

6.2.3.1 Removal of Edible Parts--

For the intensive study, analysis of shellfish is restricted to tissues that consumers might reasonably be expected to eat. Edible portions should be clearly defined in sample processing protocols by each State because the definition of edible parts may be site- or region-specific. **Bivalve molluscs** (oysters, clams, mussels, and scallops) typically are prepared by severing the adductor muscle, prying open the shell, and removing the soft tissue. The soft tissue includes viscera, meat, and body fluids (U.S. EPA, 1985c). Byssal threads from mussels should be removed with a knife before shucking and should not be included in the composite sample. Edible tissue for **crabs** typically includes all leg and claw meat, back shell meat, and body cavity meat. Internal organs generally are removed. A decision on inclusion of the hepatopancreas should be based upon the eating habits of the local population or subpopulations of concern. If the crab is soft-shelled, the entire crab should be used in the sample. Hard- and soft-shelled crabs must not be combined in the same composite (U.S. EPA, 1985c). Typically, **shrimp** and **crayfish** are prepared by removing the cephalothorax and removing the tail meat from the shell. Only the tail meat with the

section of intestine passing through the tail muscle is retained for analysis (U.S. EPA, 1985c). Edible tissue for **lobsters** may include tail meat, claw meat, tomalley (hepatopancreas), and gonad or ovaries (Duston, 1990).

6.2.3.2 Preparation of Composite Homogenate--

Grinding of tissue is easier when the tissue is partially frozen (Stober, 1991). Chilling the grinder briefly with a few chips of dry ice will reduce the tendency of the tissue to stick to the grinder. However, do not freeze the grinder because it will make it difficult to force frozen tissue through the chopper plate.

Tissue for trace metals analysis may be homogenized in 4-oz polyethylene jars (California, 1990) using a Polytron (e.g., Brinkman Model PT10-35) equipped with a titanium generator (e.g., Brinkman Model PTA 20). If the tissue is to be analyzed for organics only, or if chromium and nickel contamination are not of concern, a commercial food chopper with stainless steel blades and glass container may be used. The edible parts of all samples in the composite should be ground together to a paste-like consistency. Larger samples may be cut into 2.5-cm cubes before grinding. If samples were frozen after dissection, they can be cut without thawing with either a knife-and-mallet or a clean bandsaw. Samples should be homogenized in a grinder, blender, or chopper that has been cooled briefly with dry ice (U.S. EPA, 1985c). The ground sample should be divided into quarters, opposite quarters mixed together by hand, and the two halves mixed back together. The quartering and mixing should be repeated two more times. At this point, the composite sample may be frozen and stored at $\leq$-20 °C (see Table 6-2) or processed for analysis.

6.3 SAMPLE DISTRIBUTION

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

6.3.1 Sample Aliquotting

If composite homogenate tissue samples have been frozen, they must be thawed and rehomogenized before aliquots are prepared. Samples may be thawed overnight in an insulated cooler or refrigerator and then homogenized. Suggested aliquot weights and appropriate containers are as follows:

Analysis	Aliquot Weight	Shipping/Storage Container
Trace metals	1-5 g	Polystyrene jar
Organics	20-50 g	Glass or Teflon jar with Teflon-lined lid
Dioxins	20-50 g	Glass or Teflon jar with Teflon-lined lid

It has been recommended (Stober, 1991) that the exact quantity of tissue required for extraction and analysis be weighed and placed in an appropriate container that has been labeled with the sample ID and the exact tissue weight. The analytical laboratory can then recover the entire sample, including any liquid from thawing, by rinsing the container directly into the digestion or extraction vessel with the appropriate solvent. If this procedure is used, it is the responsibility of the central processing laboratory to provide a sufficient number of duplicate aliquots and aliquots for matrix spikes so that the QA/QC requirements of the program can be met. It is extremely important that accurate records be maintained when samples are aliquotted for analysis (see Section 6.4.3). It is recommended that a carefully designed form be used to ensure that all the necessary information is recorded. Several programs have designed sample aliquotting forms to fit particular needs. An example of a sample aliquotting record for a fish/shellfish monitoring program is presented in Figure 6-5.

The composite sample identification number is assigned to the composite sample at the time of collection and carried through sample processing (plus "L" or "R," if the composite represents a left fillet or right fillet, respectively). The aliquot identification number should indicate analyte class (e.g., TM for trace metals, OR for organics, DX for dioxin, etc.) and the sample type (e.g., R for routine sample; RS for a routine sample that is split for analysis by a second laboratory; MS1 and MS2 for sample pairs, one of which will be prepared as a matrix spike). The composite sample identification number may be of the form WWWWWX-YY-ZZZ, where WWWWW is the sample composite identification number, X indicates the left or right fillet, if applicable, YY is the analyte code, and Z is the sample type.

"Blind" duplicates may be introduced by preparing two separate aliquots of the same composite homogenate andlabeling one aliquot with a "dummy" composite sample identification. However, the analyst who prepares the sample aliquots must be careful to assign a "dummy" identification number that has not been used for an actual sample and to indicate clearly on the processing records that the samples are blind duplicates. **The analytical laboratory should not receive this information.**

Fish/Shellfish Monitoring Program
Sample Aliquotting Record

Aliquotted by _____ Date _____ Time _____
(name)

Comments _____

Samples from:

Project No. _____ Site # _____ ☐ Screening study ☐ Intensive study

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

Page ____ of ____

Figure 6-5.

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

6.3.2 Sample Transfer

When all composite homogenates have been aliquotted for analysis, the frozen aliquots should be transferred on dry ice to the analytical laboratory (or laboratories) accompanied by a sample transfer record such as the one shown in Figure 6-6. Further details on Federal regulations for shipping biological specimens in dry ice are given in Section 5.3.2.1. The sample transfer record may include a section to serve as the analytical laboratory COC record. The COC record must be signed each time the samples change hands for preparation and analysis.

6.4 SAMPLE ANALYSES

6.4.1 Target Analytes

In initial screening studies, composite samples of fish fillets or edible portions of shellfish should be analyzed for all target contaminants listed in Table 4-3 and for any additional site-specific target contaminants that have been identified by States or Regions. In intensive monitoring studies, composite samples of edible portions of fish or shellfish should be analyzed only for those target contaminants that were found to exceed recommended trigger values (TVs) in initial screening studies (see Section 4.2).

All samples analyzed for organic target contaminants in initial screening studies and intensive monitoring studies should also be analyzed for percent lipid to allow data users to normalize organic target contaminant data if desired (e.g., for trend analysis or model validation) (see Sections 2.1.9 and 2.2.9).

6.4.2 Analytical Methods

A recommended procedure for lipid analysis is given in Appendix H.

Fish/Shellfish Monitoring Program
Sample Transfer Record

Date ____ ____ ____ Time ____ : ____ (24-h clock)
DD MM YY HH MM

Released by: _____
(name)

At: _____
(location)

Shipment Method _____

Shipment Destination _____

Date ____ ____ ____ Time ____ : ____ (24-h clock)
DD MM YY HH MM

Received by: _____
(name)

At: _____
(location)

Comments _____

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

☐ Intensive—Analyze for *(specify)* _____

Sample IDs:

Laboratory Chain of Custody

Relinquished by	Received by	Purpose	Location

Figure 6-6.

[Reviewers' comments are requested regarding this procedure. If modifications or alternative methods are recommended, please be specific and include full literature citations.]

At present, no procedures have been approved officially by the EPA or other regulatory agencies for the analysis of low parts-per-billion concentrations of organic contaminants in fish and shellfish tissues (Puget Sound Estuary Program, 1990d), and only interim procedures have been proposed for the analysis of metals in tissue samples (U.S. EPA, 1981). However, based on a review of EPA guidance for bioaccumulation monitoring programs (U.S. EPA, 1986a) and of analytical methods currently used or recommended in a variety of these programs (Puget Sound Estuary Program 1990d,e; California, 1990; U.S. EPA, 1989a-c; U.S. FDA, 1990; Krahn et al., 1988; MacLeod et al., 1985), **it is recommended that organic target contaminants be analyzed by gas chromatography/mass spectrometry (GC/MS) or gas chromatography/electron capture detection (GC/ECD) methods using the sample preparation techniques shown in Table 6-3.**

Because of the relatively poor sensitivity of GC/MS for analysis of chlorinated compounds, PCBs and chlorinated pesticides should be quantified by GC/ECD. However, analysis by GC/ECD does not provide definitive compound identification, and false positives due to interferences have been commonly reported. Therefore, confirmation by GC/MS using selected ion monitoring or by using an alternative GC column phase (with ECD) is required for positive identification of chlorinated pesticides and PCBs. The large number of congeners of PCBs and their chemical nature present serious analytical difficulties. Quantitation of individual congeners, or even individual aroclors, is tedious and expensive. It is therefore recommended that total PCB analysis be performed routinely, especially in initial screening studies. If initial screening study results indicate significant PCB contamination, more detailed analyses of PCB isomer distributions may be performed during intensive followup monitoring studies.

[Reviewers are asked to provide recommendations as to which chemical analysis procedures to use for the analysis of PCB congeners and which PCB congeners are most important to monitor.]

All other organic compounds should be analyzed by GC/MS (U.S. EPA, 1985b). The determination of individual PAHs is not recommended in initial screening studies. However, if initial screening study results indicate a high level of PAH contamination in the target species, identification and quantitation of individual PAH compounds should be performed with

TABLE 6-3. SUMMARY OF BASIC SAMPLE PREPARATION AND ANALYTICAL TECHNIQUES FOR ORGANIC TARGET CONTAMINATION

Procedural step	Recommended technique
Sample drying	Centrifugation or sodium sulfate
Extraction	Shaker/roller; Soxhlet, sonication
Extract drying	Separatory funnel partitioning as needed to remove water (pH must be controlled); sodium sulfate for all other extract drying. Kuderna-Danish apparatus (to *ca.* 1 mL), rotary evaporation (to 2 mL) or comparable technique; purified nitrogen gas for concentration to smaller volumes
Extract cleanup	Removal of organic interferents with GPC, size exclusion chromatography (e.g., phenogel, Sephadex), bonded octadecyl columns, HPLC, silica gel, or alumina
Extract analysis	GC/MS for volatiles and semivolatiles, GC/ECD for chlorinated pesticides, PCBs, and aroclor mixtures

GPC = Gel permeation chromatography.
HPLC = High performance liquid chromatography.
GC/MS = Gas chromatography/mass spectrometry.
GC/ECD = Gas chromatography/electron capture detection.
PCB = Polychlorinated biphenyls.

Source: Puget Sound Estuary Program (1990a).

particular attention given to benzo[a]pyrene and related compounds (e.g., 1,2-benzanthracene; 3,4-benzpyrene; 3-methylcholanthrene; 5,6-dimethylphenanthrene).

[Reviewers are asked to provide recommendations as to which chemical analysis procedures to use for the analysis of individual PAHs and which PAH compounds (in addition to benzo[a]pyrene) are most important to monitor.]

Because of the toxicity of dioxins and the difficulty and cost of analysis for dioxins and furans (U.S. EPA, 1989b), it is recommended that tetra- through octa-chlorinated dibenzo-p-dioxins and dibenzofurans be analyzed by a contract laboratory with demonstrated expertise in these analyses. If resources are limited, the 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) and 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF) congeners should be analyzed for at a minimum. Contract laboratories currently performing dioxin/furan analyses are listed in Table 6-4. This list is included for information purposes only and should not be construed as an endorsement of laboratories.

It is recommended that all metal target contaminants except mercury be analyzed by graphite furnace atomic absorption (GFAA) spectrophotometric methods. Mercury analysis should be performed by cold vapor atomic absorption (CVAA) spectrophotometric methods (U.S. EPA, 1989a). GFAA requires a separate determination for each analyte, which increases the time and cost relative to broad-scan methods such as inductively coupled plasma emission spectrometry (ICP). However, because detection limits typically achieved with GFAA are significantly lower than those achieved with ICP, GFAA is recommended for the analysis of target metal contaminants (U.S. EPA, 1985b).

Recommended methods for the analysis of target contaminants are summarized in Table 6-5. As shown in Tables 6-6 and 6-7, these methods have demonstrated detection limits in the low parts-per-billion range, which is well below the screening study target contaminant TVs (see Section 4.2). Alternative methods of analysis may be used if comparable detection limits and acceptable accuracy and precision can be demonstrated (see Sections 6.4.3.3 and 6.4.3.4). If lower TVs are used (e.g., for susceptible populations in intensive monitoring studies), it is the responsibility of the program manager to ensure that the detection and quantitation limits of the analytical methods are sufficiently low to allow reliable quantitation of target analytes at or below these TVs (see Section 6.4.3.3).

Because of the lack of official EPA-approved methods and to allow States and Regions flexibility in developing their analytical programs, specific step-by-step procedures for the

TABLE 6-4. CONTRACT LABORATORIES CONDUCTING DIOXIN/FURAN ANALYSES IN FISH/SHELLFISH TISSUES[a]

Alta Analytical Laboratory[b]
5070 Robert J. Matthews Parkway, Suite 2
Eldorado Hills, CA 95630
916/933-1640
FAX: 916/933-0940
Bill Luksemburg

Battelle-Columbus Laboratories[b]
505 King Avenue
Columbus, OH 43201
614/424-7379
Karen Riggs/Gerry Pitts

Enseco-California Analytical Labs[b]
2544 Industrial Blvd.
West Sacramento, CA 95691
916/372-1393
916/372-1059
Kathy Gill/Michael Filigenzi/Mike Miille

IT Corporation
Technology Development Laboratory[b]
304 Directors Drive
Knoxville, TN 37923
615/690-3211
Duane Root/Nancy Conrad/Bruce Wagner

Midwest Research Institute[b]
425 Volker Boulevard
Kansas City, MO 64110
816/753-7600 ext. 190/ext. 160
Paul Kramer/John Stanley

New York State Department of Health[b]
Wadsworth Laboratories
Empire State Plaza
P.O. Box 509
Albany, NY 12201-0509
518/474-4151
Arthur Richards/Kenneth Aldous

Pacific Analytical Inc.[b]
1989-B Palomar Oaks Way
Carlsbad, CA 92009
619/931-1766
Phil Ryan/Bruce Colby

Seakem Analytical Services[b]
P.O. Box 2219
2045 Mills Road
Sidney, BC V8L 351
Canada
604/656-0881
Valerie Scott/Allison Peacock/Coreen Hamilton

TMS Analytical Services[b]
7726 Moller Road
Indianapolis, IN 46268
317/875-5894
FAX: 317/872-6189
Dan Denlinger/Don Eickhoff/
Kelly Mills/Janet Sachs

Triangle Laboratories[b]
Alston Technical Park
801 Capitola Drive, Suite 10
Research Triangle Park, NC 27713
919/544-5729
Steve Guyan/Diane Williford/
Bill Hurst/Mary Collins

Twin City Testing Corporation[b]
662 Cromwell Avenue
St. Paul, MN 55114
612/649-5502
Chuck Sueper/Fred DeRoos

University of Nebraska
Mid-West Center for Mass Spectrometry
12th and T Street
Lincoln, NE 68588
402/472-3507
Michael Gross

Wellington Environmental Consultants[b]
395 Laird Road
Guelph, Ontario N1G 3X7
Canada
519/822-2436
Judy Sparling/Brock Chittin

Wright State University[b]
175 Brehm Laboratory
3640 Colonel Glen Road
Dayton, OH 45435
513/873-2202
Thomas Tiernan/Garrett Van Ness

[a]**This list should not be construed as an endorsement of these laboratories, but is provided for information purposes only.**
[b]Laboratory participating in Method 1613 interlaboratory (round- robin) dioxin study (May 1991).

TABLE 6-5. RECOMMENDED METHODS FOR ANALYSIS OF TARGET CONTAMINANTS

Analyte type	Recommended analytical method
Metals (except mercury)	GFAA
Mercury	CVAA
Semivolatile organics (PAHs, chlorinated aromatics, phenols)	GC/MS
PCBs	GC/ECD
Pesticides	GC/ECD
Dioxins/furans	GC/MS[a,b]

GFAA = Graphite furnace atomic absorption spectrophotometry.
CVAA = Cold vapor atomic absorption spectrophotometry.
GC/MS = Gas chromatography/mass spectrometry.
GC/ECD = Gas chromatography/electron capture detection.
PAH = Polycyclic aromatic hydrocarbons.
PCB = Polychlorinated biphenyls.

[a] For the analysis of tetra- through octa-chlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) using isotope dilution. **Note: If resources are limited, 2,3,7,8-TCDD and 2,3,7,8-TCDF should be analyzed for at a minimum.**

[b] Because of the difficulty and cost of the analysis, and human health considerations, it is recommended that dioxins and furans be analyzed by a contract laboratory expert in conducting dioxin/furan analyses (see Table 6-4; **this list is provided for information purposes only and is not to be construed as an endorsement of laboratories**).

TABLE 6-6. COMPARISON OF TARGET CONTAMINANT TRIGGER VALUES (TVs)[a] WITH TYPICAL DETECTION LIMITS[b] FOR ORGANIC COMPOUNDS IN TISSUE SAMPLES

Compound type (target contaminant)	TV (ppm; µg/g wet weight)	Detection limits (ppm; µg/g wet weight)[b,c]	
		GC/MS	GC/ECD[d]
Phenols		**0.02**[e]	
• Pentachlorophenol	320	**0.08**	[f]
Aromatic hydrocarbons (low and high molecular weight)		**0.01**	[f]
• PAHs	0.095		
• 1,2-Dichlorobenzene	970		
• 1,4-Dichlorobenzene	143		
• Hexachlorobenzene	8.6		
• Pentachlorobenzene	8.6		
• 1,2,4,5-Tetrachlorobenzene	3.2		
• 1,2,4-Trichlorobenzene	215		
PCBs	0.14	[f]	**0.02**
Pesticides		0.05	**0.0001-0.005**[g]
• Aldrin	0.063		
• Chlordane	0.65		
• DDT	3.2		
• Dieldrin	0.067		
• Endosulfan	0.54		
• Endrin	3.2		
• Heptachlor	0.23		
• Heptachlor epoxide	0.12		
• Lindane	0.82		
• Mirex	0.02		
• Toxaphene	0.98		

GC/MS = Gas chromatography/mass spectrometry.
GC/ECD = Gas chromatography/electron capture detection.
PAH = Polycyclic aromatic hydrocarbons.
PCB = Polychlorinated biphenyls.

[a] From Table 4-6.
[b] From U.S. EPA (1985b). Values in boldface type are typically achievable detection limits for methods recommended in this guidance document for the analysis of organic compounds in tissue samples.
[c] Detection limits are based on a 25-g (wet weight) tissue sample extracted, concentrated to 0.5 mL after gel permeation chromatography cleanup, and 1 uL injected. Bonded, fused silica capillary GC columns, which provide better resolution than packed columns, are assumed for analyses of semivolatile compounds.
[d] Extract cleanup (e.g., removal of polar interferences by alumina column chromatography) is assumed.
[e] Substantially increased detection limits (ppm) are observed for 4-nitrophenol (0.1), 2,4-nitrophenol (0.1), and **pentachlorophenol (0.08)**.
[f] No detection limits provided because methodology does not allow adequate recovery and/or detection.
[g] The higher range of detection limits are appropriate for pesticides such as mirex, methoxychlor, the DDTs, and endosulfans, and for chlorinated butadienes. Compounds such as lindane, aldrin, heptachlor, and hexachlorobenzene can be detected at the lower limit. Toxaphene (a mixture) may require a higher detection limit than the other organochlorine pesticides.

TABLE 6-7. COMPARISON OF TARGET CONTAMINANT TRIGGER VALUES (TVs)[a] WITH TYPICAL DETECTION LIMITS FOR TRACE METALS IN TISSUE SAMPLES[b]

Element	TV (ppm; µg/g wet weight)	Recommended detection limit[c] (ppm; µg/g wet weight)
Arsenic	0.61	0.02
Cadmium	11	0.01
Lead	[d]	0.03
Mercury	3.2	0.01
Selenium	43	0.02

[a] From Table 4-6.
[b] From U.S. EPA (1985b). Based on detection levels normally achieved in methods commonly used for tissue analyses in environmental laboratories: Graphite furnace atomic absorption (GFAA) analysis for arsenic, cadmium, lead, selenium; cold vapor atomic absorption (CVAA) analysis for mercury. Lower detection limits may be achieved by experienced analysts with state-of-the-art equipment.
[c] Detection limits are based on 5 g (wet weight) of muscle tissue, digested and diluted to 50 mL.
[d] No reference dose (RfD) available at this time for calculating the TV (see Section 4.2).

analysis of target contaminants in fish/shellfish monitoring programs are not included in this guidance document. Instead, a performance-driven analytical program is recommended. This recommendation is based on the assumption that the analytical results produced by different laboratories and/or different methods will be comparable if appropriate minimum QA/QC procedures are implemented within each laboratory and if comparable analytical performance on round-robin comparative analyses of standard reference materials or split sample analyses of field samples can be demonstrated. Performance-based analytical programs currently are used in several fish/shellfish monitoring programs (e.g., NOAA Status and Trends Program [NOAA, 1987; Battelle, 1989; Cantillo, 1991], E-MAP Program [REF], Puget Sound Estuary Program [1990a-e]).

Analytical methods and QA/QC procedures described in the following documents are recommended as guidelines for methods used by State or Regional laboratories or by selected contract laboratories for the analyses of target contaminants in fish or shellfish tissues:

- Bioaccumulation Monitoring Guidance: 4. Analytical Methods for U.S. EPA Priority Pollutants and 301(h) Pesticides in Tissues from Marine and Estuarine Organisms (U.S. EPA, 1986a)

- Quality Assurance/Quality Control (QA/QC) for 301(h) Monitoring Programs: Guidance on Field and Laboratory Methods (U.S. EPA, 1987e)

- U.S. EPA Method 1624: Volatile Organic Compounds by Isotope Dilution GC/MS. Method 1625: Semivolatile Organic Compounds by Isotope Dilution GC/MS (U.S. EPA, 1989c)

- U.S. EPA Interim Methods for the Sampling and Analysis of Priority Pollutants in Sediments and Fish Tissue (U.S. EPA, 1981)

- Puget Sound Estuary Program Plan (1990d,e)

- U.S. EPA Contract Laboratory Program Statement of Work for Inorganic Analysis (U.S. EPA, 1991a)

- U.S. EPA Contract Laboratory Program Statement of Work for Organic Analysis (U.S. EPA, 1991b)

- U.S. Food and Drug Administration Pesticide Analytical Manual (PAM Vols. I and II) (U.S. FDA, 1990)

- Standard Analytical Procedures of the NOAA National Analytical Facility (Krahn et al., 1988; MacLeod et al., 1985)

- Official Methods of Analysis of the Association of Official Analytical Chemists (Williams, 1984)

- Analytical Procedures and Quality Assurance Plan for the Determination of Mercury in Fish (U.S. EPA, 1989a).

- Analytical Procedures and Quality Assurance Plan for the Determination of PCDD/PCDF in Fish (U.S. EPA, 1989b)

- Analytical Procedures and Quality Assurance Plan for the Determination of Xenobiotic Chemical Contaminants in Fish (U.S. EPA, 1989c)

- U.S. EPA Test Methods for the Evaluation of Solid Waste, Physical/Chemical Methods (U.S. EPA, 1986b)

- Standard Methods for the Examination of Water and Wastewater (Greenburg et al., 1985)

- U.S. EPA Test Methods for the Chemical Analysis of Municipal and Industrial Wastewater (U.S. EPA, 1982b)

- U.S. EPA Methods for the Chemical Analysis of Water and Wastes (U.S. EPA, 1979b)

- State of California, Department of Fish and Game, Laboratory Quality Assurance Program Plan (California, 1990)

A recent evaluation of current methods for the analyses of organic and trace metal target contaminants in fish tissue (Capuzzo et al., 1990) provides useful guidance on method selection, validation, and data reporting procedures. Laboratories should select or develop analytical procedures for routine analyses of target contaminants that are most appropriate for their programs based on available resources, experience, program objectives, and data quality requirements.

All methods used by a laboratory for the analyses of target contaminants and lipid content must be validated by the laboratory prior to routine sample analysis. That is, the detection and quantitation limits and accuracy and precision of each method must be assessed and documented to be sufficient for reliable quantitation of all target contaminants at or below their estimated TVs (see Sections 6.4.3.3 and 6.4.3.4).

All analytical methods used routinely for the analyses of fish and shellfish tissues should be documented thoroughly, preferably as formal standard operating procedures (SOPs) (U.S. EPA, 1984b). Analytical SOPs should include the following information:

- Scope and application
- Method performance characteristics (accuracy, precision, and method detection and quantitation limits) for each analyte
- Interferences
- Equipment, supplies, and materials
- Sample preservation and handling
- Instrument calibration procedures
- Sample preparation procedures
- Sample analysis procedures
- Quality control procedures
- Data reduction and analysis procedures (with example calculations)
- Recordkeeping procedures (with standard data forms, if applicable)
- Safety procedures and/or cautionary notes
- References.

A published method may serve as an analytical SOP only if the analysis is performed **exactly** as described.

Analytical SOPs must be followed exactly as written. Any deviations should be documented in the laboratory records (signed and dated by the responsible person) and noted in the final data report. Adequate evidence must be provided to demonstrate that SOP deviations did not adversely affect method performance (i.e., detection or quantitation limits, accuracy, precision), or the effect on data quality must be assessed and documented and all suspect data identified.

Examples of SOPs for the analysis of cadmium by GFAA (California, 1990) are included in Appendix I as a guide to laboratories for developing their own analytical SOPs. They are intended to illustrate the kind of information and level of detail that is required in an SOP to permit a suitably trained person to conduct the analysis accurately and reproducibly.

6.4.3 General QA/QC Considerations for Sample Analysis

Definitions of QA/QC terminology (including QA/QC samples) used in this section are included in the Glossary. **[Note to reviewers: The Glossary will be included in the next iteration of this document.]**

Each laboratory performing target contaminant analyses for fish consumption advisory programs should have a formal QA/QC program as described in Appendix F (U.S. EPA, 1984b). It is the responsibility of each program manager, in consultation with the analytical laboratory staff, to ensure that appropriate detection and quantitation limits and QA/QC requirements have been established for each analytical method prior to beginning routine sampling and analysis. In particular, the QA/QC guidelines in the EPA Contract Laboratory Program (CLP) (U.S. EPA, 1991a,b), the Puget Sound Estuary Program (1990d,e), the NOAA Status and Trends Program (NOAA, 1987; Battelle, 1989; Cantillo, 1991), and the EPA 301(h) Monitoring Programs (U.S. EPA, 1987e) are recommended as a basis for developing program-specific QA/QC programs. The Puget Sound Estuary Program QA/QC requirements for organic and metal analyses are included in Appendixes J and K, respectively, as specific examples of the application of EPA CLP QA/QC requirements to a bioaccumulation monitoring program.

The QA/QC program for each analytical laboratory should be documented fully in a QA/QC plan or in a combined Work/QA Project Plan (U.S. EPA, 1980c). (See Appendix F.) Each QA/QC requirement or procedure should be described clearly and

the rationale for each provided. Documentation should clearly demonstrate that the QA/QC program meets overall program objectives and data quality requirements.

For sample analyses, minimum QA/QC requirements consist of initial demonstration of laboratory capability and routine analyses of appropriate QA/QC samples to document data quality and to demonstrate continued acceptable performance. The QA/QC requirements for the analyses of target contaminants in tissues should be based on specific performance criteria, or control limits, for data quality indicators such as accuracy and precision.

Typically, control limits for accuracy are based on the historical mean recovery plus or minus three standard deviation units, and control limits for precision are based on the historical standard deviation or coefficient of variation (or mean relative percent difference for duplicate samples) plus three standard deviation units. Procedures should be in place for monitoring historical performance and should include control charts (Taylor, 1985; ASTM, 1976) and/or tabular presentations of the data. When established control limits are not met, appropriate corrective action should be taken and, if possible, all suspect samples reanalyzed. If reanalyses cannot be performed, all suspect data should be identified clearly.

Recommended QA/QC samples, suggested frequencies of analyses, example control limits (performance criteria), and appropriate corrective actions are summarized in Table 6-8. **It is the responsibility of program managers to ensure that appropriate QA/QC programs are developed for all participating analytical laboratories to ensure the quality and comparability of reported data.**

The following QA/QC procedures are necessary to ensure the quality and intra- and interlaboratory comparability of the data obtained by various analytical methods used for analyzing target contaminants in fish by consumption advisory programs (Battelle, 1989):

- Instrument calibration and calibration checks
- Assessment of method detection and quantitation limits
- Assessment of method accuracy and precision
- Routine monitoring of interferences and contamination
- Regular external QA assessment of analytical performance--interlaboratory comparison programs
- Appropriate documentation and reporting of data (including QA/QC data).

TABLE 6-8. RECOMMENDED QUALITY ASSURANCE/QUALITY CONTROL (QA/QC) SAMPLES

Sample type[a]	Objective	Suggested frequency of analysis[b]	Example control limits[c]	Corrective action
Calibration Standards (3-5 standards over the expected range of sample concentrations, with the lowest concentration standard at or near the MDL).	Full calibration: Establish relationship between instrument response and analyte concentration (i.e., r^2, slope, or relative response factor [RRF]).	Instrument/method dependent; follows manufacturers recommendations or procedures in specific analytical protocols. At a minimum, perform a 3-point calibration at beginning of project, after each major equipment change or disruption, and when routine calibration check exceeds specific action limits.	*Organics*: RSD of RRFs >30%. *Metals*: %R of all standards = 95-105.	Recalibrate; prepare new calibration standards if necessary. Reanalyze all samples from last acceptable calibration or calibration check, or flag all suspect data.
Calibration Check Standards (minimum of one mid-range standard prepared independently from initial calibration standards, or a mid-range laboratory control sample [see below])	Verify initial calibration.	*Organics (GC/MS)*: At beginning and end of each work shift, and once every 12 hours (or every 10-12 analyses, whichever is more frequent). *Organics (GC/ECD)*: At beginning and end of each work shift, and once every 6 hours (or every 6 samples, whichever is less frequent). *Metals*: Every 10 samples or every 2 hours, whichever is more frequent.	*Organics*: Percent difference between the average RRF from initial calibration and the RRF from the calibration check >25%. *Mercury*: %R = 80-120 *Other Metals*: %R = 90-110	Recalibrate; prepare new calibration standards if necessary. Reanalyze all samples from last acceptable calibration or calibration check, or flag all suspect data.
Matrix Spikes (one spike for each analyte at 3-5 times the estimated MDL)	Establish or confirm MDL for analyte of interest.	Seven replicate analyses prior to use of method for routine analyses.	Determined by program manager.	Redetermine MDL.
(0.5 to 5 times the concentration of the analyte of interest or 5 times the PQL)	Assess matrix effects and accuracy (percent recovery).	One per 20 samples or one per batch, whichever is more frequent.	*Organics:* Determined by program manager. % R ≥ 50 with good precision is acceptable. *Metals*: %R = 75-125	Determine cause of problem (e.g., incomplete extraction or digestion, contamination), take appropriate corrective action, and reanalyze all suspect samples or flag all suspect data. Zero percent recovery requires rejection of all suspect data.

(continued)

TABLE 6-8. (continued)

Sample type[a]	Objective	Suggested frequency of analysis[b]	Example control limits[c]	Corrective action
Matrix Spike Duplicates (0.5 to 5 times the concentration of the analyte of interest or 5 times the PQL)	Assess method precision.	One per 20 samples or one per batch, whichever is more frequent.	*Organics*: A difference of no more than a factor of 2 among replicates (i.e., approximately 50% coefficient of variation). NOTE: pooling of variances in duplicate analyses from different sample batches is recommended for estimating the standard deviation or coefficient of variation of replicate analyses. *Metals*: ±20 RPD for duplicates.	Determine cause of problem (e.g., incomplete extraction or digestion, contamination, instrument instability or malfunction), take appropriate corrective action, and reanalyze all suspect samples or flag all suspect data.
Blanks (Method, Field, Processing, Bottle)	Assess contamination from equipment, reagents, etc.	One method blank and one field blank per 20 samples or one per batch, whichever is more frequent. At least one processing blank per study. At least one bottle blank per lot or per study, whichever is more frequent.	Concentration of any analyte ≥MDL or PQL, or ≤10-30 % of sample concentration as determined by program manager.[d]	Determine cause of problem (e.g., contaminated reagents, equipment), take appropriate corrective action, and reanalyze all suspect samples or flag all suspect data.
Reagent Blanks	Check purity of reagents.	Prior to use of a new batch of reagent and whenever method blank exceeds action limits.	Concentration of any target analyte ≥ MDL or PQL.	Discard and use new batch of reagent, or purify.
Surrogate Spikes	Assess method performance and estimate the recovery of target analytes.	In every sample analyzed for organics, unless isotope dilution technique is used: *Semivolatiles*: 3 for neutral fraction +2 for acid fraction *Volatiles*: 3 *Pesticides/PCBs*: 1	Determined by program manager according to EPA CLP guidelines[e].	Determine cause of problem (e.g., incomplete extraction or digestion, contamination, inaccurate preparation of surrogates), take appropriate corrective action, and reanalyze all suspect samples or flag all suspect data.
Laboratory Control Samples (Spiked method blanks or QC check smples)	Assess method performance (initial method validation and ongoing assessment); check calibration.	*Method validation*: as many as required to establish confidence in method before routine analysis of samples (i.e., when using a method for the first time or after any method modification).	Determined by program manager.	

(continued)

TABLE 6-8. (continued)

Sample type[a]	Objective	Suggested frequency of analysis[b]	Example control limits[c]	Corrective action
Laboratory Control Samples (continued)		*Routine assessment and calibration check*: one per 20 samples or one per batch, whichever is more frequent.	*Organics:* determined by program manager. *Metals:* 80% to 120% recovery.	Determine cause of problems (e.g., inaccurate calibration, inaccurate preparation of control samples), take appropriate corrective action, and reanalyze all suspect samples or flag all suspect data. Zero percent recovery requires rejection of al suspect data.
Reference Materials[f]	Assess method performance (initial method validation and ongoing assessment).	*Method validation*: as many as required to assess accuracy (and precision) of method before routine analysis of samples (i.e., when using a method for the first time or after any method modification)	*Organics:* <95% confidence intervals, if certified, or determined by program manager. *Metals:* 80% to 120% accuracy.	
		Routine assessment: one (preferably blind) per 20 samples or one per batch, whichever is more frequent.	*Organics:* <95% confidence interval, if certified, or determined by the program manager. *Metals:* 80% to 120% accuracy.	Determine cause of problem (e.g., inaccurate calibration, contamination), take appropriate corrective action, and reanalyze all suspect samples or flag all suspect data.
Laboratory Replicates[g]	Assess method precision.	One blind duplicate sample per 20 samples or one per batch, whichever is more frequent.	*Organics*: A difference of no more than a factor of 2 among replicates (i.e., approximately 50% coefficient of variation). NOTE: pooling of variances in duplicate analyses from different sample batches is recommended for estimating the standard deviation or coefficient of variation of replicate analyses. *Metals*: ±20 RPD for duplicates.	Determine cause of problem (e.g., composite sample not homogeneous, instrument instability or malfunction), take appropriate corrective action, and reanalyze all suspect samples or flag all suspect data.
Analytical Replicates	Assess analytical precision.	Duplicate injections for all metal analyses.[d]	Determined by program manager.[d]	Determine cause of problem (e.g., instrument instability or malfunction), take appropriate corrective action, and reanalyze sample.

(continued)

TABLE 6-8. (continued)

Sample type[a]	Objective	Suggested frequency of analysis[b]	Example control limits[c]	Corrective action
Field Replicates	Assess total sample variability (i.e., population variability, field or sampling variability, and analytical method variability).	*Initial screening*: **OPTIONAL**; if program resources allow, a minimum of one replicate (i.e., duplicate) for each primary target species at 10 percent of screening sites.	Determined by program manager.	Determined by program manager.
		Intensive monitoring: five blind replicate samples for each target species (and size, age or sex class, if appropriate) at each sampling location.	Determined by program manager.	Determined by program manager.
Field Blanks	Assess contamination in the field.	One field blank per sampling location.	Concentration of any target analyte ≥ MDL or PQL.	Identify and remove sources of field contamination. Flag all suspect data.
Split Samples	Assess interlaboratory comparability.	5-10 percent of field samples split between States and/or Regions that routinely share monitoring results, or as determined by program managers.[h]	Determined by program managers.	Review sampling and analytical methods. Identify sources of noncomparability. Standardize and validate methods to document comparability.

RSD = Relative standard deviation (see Section 6.4.3.4.2 and Glossary).
%R = Percent recovery (see Section 6.4.3.4.1 and Glossary).
RRF = Relative response factor (see Section 6.4.3.2.2 and Glossary).
MDL = Method detection limit (see Section 6.4.3.3.1 and Glossary).
RPD = Relative percent difference (see Section 6.4.3.4.2 and Glossary).
PQL = Practical quantitation limit (see Section 6.4.3.3.2 and Glossary).

[a] Definitions of QA/QC samples are given in the Glossary. **[Note: A Glossary of Terms will be included in the next iteration of this document].**

[b] Suggested frequencies are based primarily on recommendations in U.S. EPA, 1986b, 1987e, 1991a,b, 1989c; Puget Sound Estuary Program, 1990d,e; and Batelle, 1989. **It is the responsibility of each program manager to determine the appropriate level of QA/QC needed to meet program objectives.**

[c] From Puget Sound Estuary Program (1990 d,e) control (action) limits, except where otherwise noted. Individual programs may require different control limits. **It is the responsibility of each program manager to set control limits that will ensure that the measurement data meet program data quality objectives.**

[d] From U.S. EPA, 1987e.

[e] From U.S. EPA, 1991a,b.

[f] As available (see Table 6-9). If available, SRMs or CRMs should be used.

[g] Sometimes referred to as Analytical Replicates (e.g., in Puget Sound Estuary Program [1990d]).

[h] Recommended in this guidance document.

These procedures should be documented thoroughly (e.g., as part of the analytical SOPs or as separate SOPs) and approved by appropriate supervisory personnel **prior to initiation of sample analyses.** A more detailed discussion of recommended QA/QC procedures and the use of appropriate QA/QC samples is provided in Sections 6.4.3.2 through 6.4.3.6. Recommended procedures for documenting and reporting analytical and QA/QC data are given in Section 6.4.3.7. Because of their importance in assessing data quality and interlaboratory comparability, reference materials are discussed separately in Section 6.4.3.1.

6.4.3.1 Reference Materials--

The appropriate use of reference materials is an important part of good QA/QC practices for analytical chemistry. The following definitions of reference materials, taken from the Puget Sound Estuary Program (1990d), are used in this guidance document:

- A **reference material** is any material or substance of which one or more properties have been sufficiently well established to allow its use for instrument calibration, method evaluation, or characterization of other materials.

- A **certified reference material (CRM)** is a reference material of which the value(s) of one or more properties have been certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation that is issued by the certifying organization (e.g., U.S. EPA; National Institute of Standards and Technology [NIST]; National Research Council of Canada [NRCC]).

- A **standard reference material (SRM)** is a CRM issued by the NIST.

Reference materials may be used to (1) provide information on method accuracy and, when analyzed in replicate, on precision, and (2) obtain estimates of intermethod and/or interlaboratory comparability. An excellent discussion of the use of reference materials in QA/QC procedures is given in Taylor (1985). The following general guidelines should be followed to ensure proper use of reference materials (UNESCO, 1990):

- When used to assess the accuracy of an analytical method, the matrix of the reference material should be as similar as possible to that of the samples of interest. If reference materials in matrices other than fish or shellfish tissue are used, possible matrix effects should be addressed in the final data analysis or interpretation.

- Concentrations of reference materials should cover the range of possible concentrations in the samples of interest. However, because there is a lack of low- and high-concentration reference materials for most analytes in tissue matrices, potential problems at low or high concentrations often cannot be documented.

- Reference materials should be analyzed regularly to detect and document any changes in the analytical procedure over time. Appropriate corrective action should be taken whenever changes are observed outside specified performance limits (e.g., accuracy, precision, detection limit). Note: Because of the limited number of certified marine/estuarine tissue reference materials available, the results of analyses of these materials may be biased by an analyst's increasing ability to recognize these materials with increased use. If possible, reference material samples should be introduced into the sample stream as double blinds, that is, with identity and concentration unknown to the analyst.

- Results of reference material analyses are essential to assess the comparability of data from different laboratories and/or from different methods. However, the results of sample analyses should not be corrected based on percent recoveries of reference materials. Final reported results should include both uncorrected sample results and percent recoveries of reference materials.

Sources of EPA-certified analytical reference materials for priority pollutants and selected related compounds are given in Appendix L. In addition, the following comprehensive publications on certified standards and reference materials are recommended:

- **Standard and Reference Materials for Marine Science** (UNESCO, 1990). Available from

  Dr. Adrianna Cantillo
  National Ocean Service
  National Oceanic and Atmospheric Administration
  U.S. Department of Commerce
  6001 Executive Blvd., Room 323
  Rockville, MD 20852

  This catalog lists over 900 reference materials and includes information on their producers, sources, matrix type, analyte concentrations, proper use, availability, and costs. Reference materials are categorized as follows: ashes, gases, instrumental performance, oils, physical properties, rocks, sediments, sludges, tissues, and waters.

- **Biological and Environmental Reference Materials for Trace Elements, Nuclides and Organic Microcontaminants** (Toro et al., 1990). Available from

  Dr. R.M. Parr
  Section of Nutritional and Health-Related Environmental Studies
  International Atomic Energy Agency
  P.O. Box 100
  A-1400 Vienna, Austria

  This report contains approximately 2,700 analyte values for 117 analytes in 116 biological and 77 nonbiological environmental reference materials from more

than 20 sources. Additional information on cost, sample size available, and minimum amount of material recommended for analysis is also provided.

Currently available marine or estuarine tissue reference materials that may be appropriate for use by analytical laboratories in fish and shellfish consumption advisory programs are listed in Table 6-9.

6.4.3.2 Instrument Calibration and Calibration Checks--

Specific calibration procedures and requirements for recommended analytical methods (i.e., GFAA, CVAA, GC/MS, GC/ECD) are included in the methods referenced in Section 6.4.2. **It is the responsibility of each program manager to ensure that proper calibration procedures are developed and followed for each analytical procedure to ensure the accuracy of the measurement data.**

6.4.3.2.1 General Guidelines--The following general guidelines should be followed in developing calibration procedures and requirements.

All analytical instruments and equipment should be maintained properly and calibrated to ensure optimum operating conditions throughout a measurement program. Calibration and maintenance procedures should be performed according to SOPs based on the manufacturers' specifications and the requirements of specific analytical procedures. Calibration procedures must include provisions for documenting calibration frequencies, conditions, standards, and results to describe adequately the calibration history of each measurement system.

An established schedule for the routine calibration and maintenance of analytical instruments should be followed, based on manufacturers' specifications, historical data, and specific procedural requirements. At a minimum, calibration should be performed each time an instrument is set up for analysis, after any major disruption or failure, and after any unacceptable calibration check.

Calibration standards of known and documented accuracy must be used to ensure the accuracy of the analytical data. Each laboratory should have a program for verifying the accuracy and traceability of calibration standards against the highest quality standards available. If possible, EPA-certified standards should be used for calibration standards (see Appendix L). A log of all calibration materials and standard solutions should be maintained. Appropriate storage conditions (i.e., container specifications, shelf-life, temperature, humidity, light condition) should be documented and maintained.

TABLE 6-9. MARINE/ESTUARINE TISSUE REFERENCE MATERIALS

Identification code	Analyte type	Source	Matrix
DOLT-1	Elements	NRCC	Dogfish liver (freeze-dried)
DORM-1	Elements	NRCC	Dogfish muscle (freeze-dried)
LUTS-1	Elements	NRCC	Non-defatted lobster hepatopancreas
TORT-1	Elements	NRCC	Lobster hepatopancreas
MA-A-1/OC	Organic compounds	IAEA	Copepod homogenate
MA-A-3/OC	Organic compounds	IAEA	Shrimp homogenate
MA-B-3/OC	Organic compounds	IAEA	Fish tissue
MA-M-2/OC	Organic compounds	IAEA	Mussel tissue
MA-A-1/TM	Elements	IAEA	Copepod homogenate
MA-A-2/TM	Elements	IAEA	Fish flesh homogenate
MA-B-3/TM	Elements	IAEA	Fish tissue
MA-B-3/RN	Isotopes	IAEA	Fish tissue
CRM-278	Elements	BCR	Mussel tissue
EPA-FISH	Pesticides	EPA	Fish tissue
RM-50	Elements	NIST	Albacore tuna (freeze-dried)
SRM-1566	Elements	NIST	Oyster tissue (freeze-dried)
NIES No. 6	Elements	NIES	Mussel tissue

Sources:

NRCC = National Research Council of Canada, Institute for Environmental Chemistry, Marine Analytical Chemistry Standards Program, Division of Chemistry, Montreal Road, Ottawa, Ontario K1A 0R9, Canada.

IAEA = International Atomic Energy Agency, Analytical Quality Control Service, Laboratory Seibersdorf, P. O. Box 100, A-1400 Vienna, Austria.

BCR = Community Bureau of Reference, Commission of the European Communities, Directorate General for Science, Research and Development, 200 rue de la Loi, B-1049 Brussels, Belgium.

EPA = U.S. Environmental Protection Agency, Quality Assurance Branch, EMSL-Cincinnati, Cincinnati, Ohio, 45268, USA. (Material now available from Supelco, Inc., Supelco Park, Bellefonte, Pennsylvania, 16823-0048, USA.)

NIST = National Institute of Standards and Technology, Office of Standard Reference Materials, Gaithersburg, Maryland, 20899, USA.

NIES = National Institute for Environmental Studies, Yatabe-machi, Tsukuba, Ibaraki, 305, Japan.

A minimum of three (and preferably five) calibration standards should be used to construct a calibration curve covering the normal working range of the instrument or bracketing the concentration range of the samples to be analyzed. The lowest-concentration calibration standard should be at or near the estimated detection limit (see Section 6.4.3.3.1). Calibration standards should be prepared in the same matrix as the prepared sample extract or digestate. Criteria for acceptable calibration (e.g., acceptable limits for r^2, slope, intercept, response factors) should be established for each analytical procedure. If these criteria (control limits) are exceeded, the source of the problem should be identified (e.g., inaccurate standards, instrument instability or malfunction) and appropriate corrective action taken. No analyses should be performed until acceptable calibration has been achieved and documented.

After initial calibration has been achieved and prior to the routine analyses of samples, the accuracy of the calibration should be verified by the analysis of a mid-range calibration standard that has been prepared independently (i.e., using a different stock) from the initial calibration standards, or by the analysis of a mid-calibration-range laboratory control sample (i.e., a sample consisting of a known matrix spiked with compounds representative of the target analytes). Thereafter, routine calibration checks should be performed using a mid-range calibration check standard or laboratory control sample at a frequency that has been documented to provide adequate assurance of maintaining instrument calibration (e.g., once every 10 samples or every 2 hours during an analysis run, whichever is more frequent [U.S. EPA, 1991a,b]; or once every 20 samples or once every sample batch, whichever is more frequent [California, 1990]). A calibration check should always be performed after analyzing the last sample in a batch.

If a calibration check does not fall within the calibration control limits specified in the method, the source of the problem should be determined and appropriate corrective action taken. After acceptable calibration has been achieved, all suspect analyses should be reperformed. If reanalysis is not possible, all suspect data should be identified clearly.

All reported data should be within the calibration range. That is, data above or below the range of calibration standards should not be reported. If a sample concentration occurs outside the calibration range, the sample volume must be adjusted appropriately and the sample reanalyzed, or the calibration range must be extended. Extremely high concentrations of organic compounds may indicate that the extraction capabilities of the method have been saturated and extraction of a smaller sample size or

modification of the extraction procedure may be required (U.S. EPA, 1982b). **If, for any reason, data outside the calibration range are reported, they must be clearly qualified (e.g., as greater than the concentration of the highest calibration standard).**

All calibration and maintenance procedures and results should be documented clearly in the laboratory records. Calibration and maintenance records should be inspected regularly to ensure that these procedures are being performed at the required frequency and according to established SOPs. Any deficiencies in the records or deviations from established procedures should be documented and appropriate corrective action taken.

6.4.3.2.2 Calibration and Performance Evaluation of GC/MS Systems--The general guidelines presented above pertain to external calibration procedures, which involve the analysis of standard solutions, independent of the samples, to determine the relationship between instrument response and the concentration of the analyte being measured. Internal standard calibration involves the determination of relative response factors (RRFs), that is, of instrument responses from target analytes relative to the responses from one or more internal standards added to every sample prior to sample preparation.

Both external-standard and internal-standard calibration procedures are used for the analysis of organic compounds by GC/MS. Ideally, the chemical and physical properties of an internal standard should be as similar as possible to those of the target analyte. A stable isotope-labeled analog of the target analyte is an ideal internal standard, and, if resources permit, an isotope dilution technique is recommended for the analysis of organic compounds for which isotope-labeled analogs are available (Puget Sound Estuary Program, 1990d; U.S. EPA, 1987e,f; U.S. EPA, 1991a,b; U.S. EPA, 1989c,e). **Acceptance criteria for the RRF of each target analyte should be established consistent with program data quality requirements.**

When an isotope dilution technique is used for the analysis of organic target contaminants, an instrument internal standard (e.g., 2,2'-difluorobiphenyl) must be added to the final sample extract prior to actual analysis to determine the physical percent recoveries of isotopically labeled internal standards added prior to extraction. Instrument internal standards are used only for QA/QC purposes (i.e., to assess the quality of data) and not to quantify analytes. Acceptance limits for percent recovery and recommended corrective actions are given in EPA Method 1625c (U.S. EPA, 1987f, 1989e).

If the isotope dilution technique cannot be used (e.g., for chlorinated pesticides and PCBs analyzed by GC/ECD), surrogate spikes must be added as internal standards to each

sample prior to extraction. As noted above, surrogate compounds should have chemical and physical properties similar to the target analytes. In addition, surrogates should be compounds not expected to be present in the original samples. The percent recovery (% R_s) of each surrogate spike should be determined for all samples as follows:

$$\% R_s = 100 (C_m/C_a)$$

where

$\% R_s$ = surrogate percent recovery
C_m = measured concentration of surrogate
C_a = actual concentration of surrogate added to the sample.

Acceptance criteria for the percent recovery of each surrogate compound should be established consistent with program data quality requirements.

The following additional procedures are required to evaluate the performance of GC/MS systems. In the discussion below, procedural details and performance criteria are those recommended for Phase II of the National Dioxin Study (U.S. EPA, 1989c), unless otherwise noted. **It is the responsibility of each program manager to determine specific GC/MS evaluation procedures and criteria appropriate for their data quality requirements.**

- <u>Evaluation of the GC System</u>

  The GC performance should be evaluated by determination of the number of theoretical plates of resolution and by the relative retention times of the internal standards.

  <u>Column Resolution</u>: The number of theoretical plates of resolution, N, should be determined at the time the calibration curve is generated (using chrysene-d_{10}) and monitored with each sample set. The value of N should not decrease by more than 20percent during an analysis session. The equation for N is given as follows:

$$N = 16 (RT/W)^2$$

  where

  RT = retention time of chrysene-d_{10} (s)
  W = peak width of chrysene-d_{10} (s).

  <u>Relative Retention Time</u>: Relative retention times of the internal standards should not deviate by more than ±3 percent from the values calculated at the time the calibration curve was generated.

If the column resolution or relative retention times are not within the specified performance criteria, appropriate corrective action (e.g., adjust GC parameters, flush GC column, replace GC column) should be taken.

- **Evaluation of the MS System**

The performance of the mass spectrometer should be evaluated for sensitivity and spectral quality.

Sensitivity: The signal-to-noise value must be at least 3.0 or greater for m/z 198 from an injection of 10 ng decafluorotriphenylphosphine (DFTPP).

Spectral Quality: The intensity of ions in the spectrum of DFTPP must meet the criteria listed below (U.S. EPA, 1987f):

m/z	Criteria
51	30-60% mass 198
68	<2% mass 69
70	<2% mass 69
127	40-60% mass 198
197	<1% mass 198
199	5-9% mass 198
275	10-30% mass 198
365	>1% mass 198
441	present and <mass 443
442	>40% mass 198
443	17-23% mass 442

If the performance criteria for MS sensitivity or spectral quality are not met, appropriate corrective action (e.g., clean MS, retune MS) should be taken.

- **Evaluation of Cleanup Columns**

Because the fatty content of many tissue samples may overload the cleanup columns, these columns should be calibrated and monitored regularly to ensure that target contaminants are consistently collected in the proper fraction. The gel permeation columns should be monitored by visual inspection (for column discoloration, leaks, cracks, etc.) and by measurement of flow rate, column resolution, collection cycle, and method blanks (see Section 6.4.3.5). Silica gel columns should be evaluated by their ability to resolve cholesterol from a selected target analyte.

6.4.3.3 Assessment of Detection and Quantitation Limits--

EPA has previously issued guidance on recommended detection limits for trace metal and organic compound analytical methods used in bioaccumulation monitoring programs (U.S. EPA, 1985b). These recommended detection limits are summarized in Tables 6-6 and 6-7. Several factors influence achievable detection and quantitation limits regardless of the specific analytical procedure. These include amount of sample available, matrix interferences, and stability of the instrumentation (measurement precision). The limits of detection given in Tables 6-6 and 6-7 are representative of typically attainable values.

It is the responsibility of each laboratory to determine appropriate detection and quantitation limits for each analytical method for each target analyte in a fish or shellfish tissue matrix and to ensure that these limits are sufficiently low to allow reliable quantitation of the analyte at or below the recommended TVs (see Section 4.2). Detection and quantitation limits must be determined prior to use of a new method for routine analyses and after any significant changes are made to an existing method.

At present there is no clear consensus among analytical chemists on a standard procedure for determining and reporting the limits of detection and quantitation of analytical procedures. Furthermore, the bases for detection and quantitation limits reported in the literature are seldom given. Reported detection limits may be based on instrument sensitivity or determined from the analyses of method blanks or low-level matrix spikes; quantitation limits may be determined from the analyses of method blanks or low-level matrix spikes (Puget Sound Estuary Program, 1990d).

6.4.3.3.1 Detection Limits--Three types of detection limits have been defined by the American Chemical Society Committee on Environmental Improvement (Keith et al., 1983):

- **Instrument Detection Limit (IDL)**: The smallest signal above background noise that an instrument can detect reliably.

- **Limit of Detection (LOD)**: The lowest concentration that can be determined to be statistically different from a method blank. The recommended value for the LOD is 3 times the standard deviation of the blank in replicate analyses, corresponding to a 99 percent confidence level.

- **Method Detection Limit (MDL)**: The minimum concentration of an analyte in a given matrix that can be measured and reported with 99 percent confidence that the concentration is greater than zero. The MDL is determined by multiplying the appropriate (i.e., n-1 degrees of freedom) one-sided 99 percent student's t-statistic ($t_{0.99}$) by the standard deviation (S) obtained from a minimum of seven replicate analyses of a spiked matrix sample containing the analyte of interest at a

concentration 3 to 5 times the estimated MDL (Glaser et al., 1981; 40 CFR, Part 136, App. B, 1987):

$$MDL = (t_{0.99})\ (S).$$

It is important to emphasize that all sample processing steps of the analytical method (e.g., digestion, extraction, cleanup) must be included in the determination of the MDL.

Each of these estimates has its practical limitations. The IDL does not account for possible blank contaminants or matrix interferences. The LOD accounts for blank contaminants but not for matrix effects or interferences. In some instances, the relatively high value of the MDL may be too stringent and result in the rejection of valid data; however, it is the only detection limit estimate that accounts for matrix effects and interferences and provides a high level of statistical confidence in sample results. Therefore, **it is recommended that the MDL be used to define the limits of detection for the analytical methods used for routine analyses of all target contaminants. An EPA-recommended procedure for determining and reporting the MDL (U.S. EPA, 1982a) is given in Appendix M.**

The MDL, expressed as the concentration of target contaminant fish tissue, should be calculated from the measured MDL of the target analyte in the sample extract or digestate according to the following equation:

$$MDL_{tissue}\ (\text{ppm or ppb}) = [MDL_{extract}\ (\text{ppm or ppb}) \times V]/W$$

where

V = final extract or digestate volume, after dilution or concentration (mL)

W = weight of sample digested or extracted (g).

This equation clearly indicates that the MDL in tissue may be improved (lowered) by increasing the sample weight (W) and/or decreasing the final extract or digestate volume (V).

Experienced analysts may use their best professional judgment to adjust the measured MDL to a lower "typically achievable" detection limit (U.S. EPA, 1985b; Puget Sound Estuary Program, 1990e) **or to derive other estimates of detection limits.** For example, EPA recommends the use of **lower limits of detection (LLDs)** for methods used to analyze organic pollutants in bioaccumulation monitoring programs (U.S. EPA, 1986a). Estimation of the LLD for a given analyte involves determining the noise level in the retention window for the quantitation mass of the analyte for at least three field samples in the sample

set being analyzed. The LLD is then estimated as the concentration corresponding to the signal required to exceed the average noise level observed by at least a factor of 2. Based on the best professional judgment of the analyst, this LLD is applied to samples in the set with comparable or lower interference; samples with significantly higher interferences (i.e., by at least a factor of 2) are assigned correspondingly higher LLDs. LLDs are greater than IDLs, but usually less than the more rigorously defined MDLs. Thus, data quantified between the LLD and the MDL have a lower statistical confidence associated with them than data quantified above the MDL. However, these data are considered valid and useful in assessing low-level environmental contamination.

Similarly, in EPA 1600 series methods (e.g., U.S. EPA 1987f, 1989e), EPA recommends the use of a **minimum level of detection,** which is defined as the minimum concentration of the analyte of interest at which the entire GC/MS system must give a recognizable (background corrected) mass spectrum and acceptable calibration points. Thus, a minimum level of detection is the concentration of a target contaminant in a sample that is equivalent to the concentration of the lowest acceptable calibration standard.

If estimates of detection limits other than the MDL are developed and used to qualify reported data, they should be clearly defined in the analytical SOPs and in all data reports, and their relationship to the MDL should be clearly described.

6.4.3.3.2 Quantitation Limits--In addition to the method detection limits (e.g., MDL or LLD), a method limit of quantitation (MLQ), or minimum concentration allowed to be reported at a specified level of confidence without qualifications, should be derived for each analyte. Ideally, MLQs should account for matrix effects and interferences. The MLQ can be greater than or equal to the MDL (or LLD). No consistent guidance for determining MLQs has been found in the recent literature; therefore, it is not possible to provide specific recommendations for determining these limits at this time.

[Reviewers' comments or recommendations are requested regarding definition of MLQs and procedures for calculating them.]

The American Chemical Society Committee on Environmental Improvement (Keith et al., 1983) has defined one type of quantitation limit:

- **Limit of Quantitation (LOQ):** The concentration above which quantitative results may be obtained with a specified degree of confidence. The recommended value for the LOQ is 10 times the standard deviation of a method blank in replicate analyses, corresponding to an uncertainty of $\pm$30 percent in the measured value ($10\sigma \pm 3\sigma$) at the 99 percent confidence level.

However, the LOQ does not account for matrix effects or interferences.

The U.S. EPA (1986b) has defined another type of quantitation limit:

- **Practical Quantitation Limit (PQL):** The lowest concentration that can be reliably reported within specified limits of precision and accuracy under routine laboratory operating conditions.

The Puget Sound Estuary Program (1990d) and the National Dioxin Study (U.S. EPA, 1989c) use a PQL based on the lowest concentration of the initial calibration curve, the amount of sample typically analyzed, and the final extract volume of that method. However, the PQL is also applicable only to samples without substantial matrix effects or interferences.

Analysts must use their expertise and professional judgment to determine the best estimate of the MLQ for each target analyte. MLQs, including the estimated degree of confidence in analyte concentrations above the quantitation limit, should be clearly defined in the analytical SOPs and in all data reports.

6.4.3.3.3 Use of Detection and Quantitation Limits--Method detection and quantitation limits should be used to qualify reported data as follows:

- No detected concentrations should be reported below the MLD.
- Concentrations between the MLD and the MLQ should be reported with the qualification that they are below the quantitation limit.
- Concentrations above the MLQ may be reported and used without qualification.

6.4.3.4 Assessment of Analytical Accuracy and Precision--

The accuracy and precision of each analytical method should be assessed and documented for each target analyte of interest prior to the performance of routine analyses and on a regular basis during routine analyses.

6.4.3.4.1 Accuracy--Analytical accuracy may be assessed through analyses of appropriate reference materials (e.g., SRMs or CRMs) (see Section 6.4.3.1), laboratory control samples, matrix spikes, and/or surrogate spikes.

Accuracy is calculated from the results of the analyses of reference materials or laboratory control samples as follows:

$$\% \text{ Accuracy} = [(M - T)/T] \times 100$$

where

M = measured value of the concentration of analyte i

T = "true" value of the concentration of analyte i.

Accuracy is calculated as percent recovery from the analyses of spiked samples as follows:

$$\% \text{ Recovery} = [(M_s - M_u)/T_s] \times 100$$

where

M_s = measured concentration of analyte i in the spiked sample

M_u = measured concentration of analyte i in the unspiked sample

T_s = "true" concentration of analyte i in the spiked sample.

When sample concentrations are less than the MDL, the value of zero should be used as the concentration of the unspiked sample (M_u) in calculating spike recoveries (California, 1990).

The concentrations of target analytes in reference materials should fall within the range of concentrations found in the field samples; however, this is often not possible because of the limited number of certified marine/estuarine tissue reference samples available (see Table 6-9).

Matrix spike samples should be prepared using spike concentrations approximately equal to the concentration found in the unspiked sample. An acceptable range of spike concentrations is 0.5 to 5 times the sample concentrations (U.S. EPA, 1987e).

Method accuracy should be assessed initially by analyses of appropriate reference materials, preferably SRMs or CRMs, in a tissue matrix. The actual number of reference samples required to be analyzed for the initial assessment of method accuracy should be determined by each laboratory for each analytical procedure.

Laboratory control samples and matrix spikes or surrogate spikes should be used for ongoing assessment of accuracy during the routine analyses. **It is recommended that, at a minimum, one laboratory control sample and one matrix spike sample be analyzed with every 20 samples or with each sample batch, whichever is more frequent (Puget Sound Estuary Program, 1990d,e). Ideally, CRMs or SRMs should also be analyzed at this recommended frequency; however, limited availability and cost of these materials often make this impractical. For organic compounds not analyzed by isotope dilution**

techniques (i.e., PCBs and pesticides), surrogate spikes should be added to each sample to assess accuracy.

Spikes should be added to the sample homogenates **prior** to digestion or extraction and dilution steps to provide an assessment of total method (i.e., sample preparation **and** analysis) accuracy. Percent recovery values for spiked samples must fall within control limits specified in the Work/QA Project Plan and in individual analytical SOPs. If the percent recovery falls outside the acceptable recovery range, the analyses should be discontinued, appropriate corrective action taken, and, if possible, the samples associated with the spike reanalyzed. If reanalysis is not possible, all suspect data should be clearly identified.

Poor performance on the analysis of reference materials or poor spike recovery may be caused by inadequate mixing of the sample before aliquotting, inconsistent contamination, inconsistent digestion or extraction procedures, matrix interferences, or instrument problems. If replicate analyses are acceptable (see Section 6.4.3.3.5), matrix interferences or loss of target analytes during sample preparation are indicated.

To check for loss of target analytes during sample preparation, a step-by-step examination of the procedure using spiked blanks should be conducted. For example, to check for loss of metal target analytes during digestion, a postdigestion spike should be prepared and analyzed and the results compared with those from a predigestion spike. If the results are different, the digestion technique should be modified to obtain acceptable recoveries. If there is no difference in the results of pre- and postdigestion spikes, the sample should be diluted by at least a factor of 5 and reanalyzed. If spike recovery is still poor, then the method of standard addition or use of a matrix modifier is indicated (U.S. EPA, 1987e).

6.4.3.4.2 Precision--Precision is defined as the agreement among a set of replicate measurements without assumption of knowledge of the true value. Method precision (i.e., variability due to sample preparation and analysis) is estimated by means of the analyses of duplicate or replicate aliquots of samples containing concentrations of analyte above the MDL. The most commonly used estimates of precision are the relative standard deviation (RSD) or the coefficient of variation (CV),

$$RSD = CV = 100\ S/\bar{x}_1$$

where

$\bar{x}_1$ = arithmetic mean of the x_1 measurements

S = standard deviation of the x_1 measurements

and the relative percent difference (RPD) when only two samples are available,

$$RPD = 100\ [(x_1 - x_2)/\{(x_1 + x_2)/2\}].$$

Method precision may be assessed prior to routine sample analyses by the analysis of replicate samples of reference materials, preferably in tissue matrices, and/or laboratory control samples. Ongoing assessment of method precision during routine analysis should be performed by the analysis of duplicate (or replicate) aliquots of samples (laboratory duplicates) and matrix spike duplicates (or replicates).

For ongoing assessment of method precision, it is recommended that, at a minimum, one laboratory duplicate and one matrix spike duplicate be analyzed with every 20 samples or with each sample batch, whichever is more frequent. In addition, it is recommended that a laboratory control sample be analyzed at the above frequency to allow an ongoing assessment of method performance, including an estimate of method precision over time. Specific procedures for estimating method precision by laboratory and/or matrix spike duplicates and laboratory control samples are given in ASTM (1983). This reference also includes procedures for estimating method precision from spike recoveries and for testing for significant change in method precision.

Precision estimates obtained from the analyses of laboratory duplicates, matrix spike duplicates, and repeated laboratory control sample analyses must fall within control limits specified in the Work/QA Project Plan and in individual analytical SOPs. If these values fall outside the control limits, the analyses must be discontinued, appropriate corrective action taken, and, if possible, the samples associated with the duplicates reanalyzed. If reanalysis is not possible, all suspect data should be clearly identified.

Unacceptable precision estimates derived from the analysis of duplicate or replicate samples may be caused by inadequate mixing of the sample before aliquotting; inconsistent contamination; inconsistent digestion, extraction, or cleanup procedures; or instrumentation problems (U.S. EPA, 1987e).

An alternative approach to assessing laboratory performance using laboratory duplicates, based on testing the null hypothesis that the mean difference in the concentrations of a target contaminant in a number of laboratory duplicates is zero, is given in Section 7.2.1.1.

The analysis of replicate aliquots of final sample extract or digestate solutions **(analytical replicates)** provides only an estimate of analytical precision; it does not provide an

estimate of total method precision. For organic target analytes, such analyses may be conducted at the discretion of the program manager or laboratory supervisor. **For the analysis of target metal analytes by GFAA and CVAA, it is recommended that duplicate injections of each sample be analyzed and the mean concentration be reported. The RPD should be within established control limits or the sample should be reanalyzed (U.S. EPA, 1987e).**

Estimates of the variability of pollutant concentrations in the sample population and of the sampling and analysis procedures can be obtained by the collection and analysis of replicate field samples. Replicate field samples are optional in initial screening studies; however, if resources permit, it is recommended that duplicate samples be collected at 10 percent of the screening sites (see Section 2.1.8). In intensive monitoring studies, five replicate samples should be collected at each sampling location for target contaminant analyses (see Section 2.2.8).

6.4.3.5 Routine Monitoring of Interferences and Contamination--

Because contamination can be a limiting factor in the reliable quantitation of target contaminants in tissue samples, the recommendations for proper materials and handling and cleaning procedures given in Sections 5.2.2. and 6.2 should be followed carefully to avoid serious contamination of samples in the field and laboratory. In addition, the following blank samples should be analyzed **prior** to beginning the sample collection and analyses program and on a routine basis during each monitoring study (U.S. EPA, 1987e):

- **Field blanks** - Rinsates of empty field sample containers (i.e., aluminum foil packets and plastic bags) that are prepared, shipped, and stored as actual field samples should be analyzed to evaluate field sample packaging materials as sources of contamination. Each rinsate should be collected and the volume recorded. The rinsate should be analyzed for target contaminants of interest and the total amount of target contaminant in the rinsate recorded. **It is recommended that one field blank be analyzed with every 20 samples or with each batch of samples, whichever is more frequent.**

- **Processing blanks** - Rinsates of utensils and equipment used for dissecting and homogenizing fish and shellfish should be analyzed, using the procedure described above for field blanks, to evaluate the efficacy of the cleaning procedures used between samples. **It is recommended that processing blanks be analyzed at least once at the beginning of a monitoring study and preferably once with each batch of 20 or fewer samples.**

- **Bottle blanks** - Rinsates of empty bottles used to store and ship sample homogenates should be analyzed, using the procedure described above for field

blanks, to evaluate these sample containers as sources of contamination. **It is recommended, at a minimum, that one bottle blank be analyzed for each lot of sample bottles used and preferably once with each batch of 20 or fewer samples.**

Method blanks - Blank samples, consisting of an analyte-free matrix to which all reagents are added in the same proportion as used in sample preparation, should be analyzed to evaluate contaminants resulting from the total analytical method (e.g., contaminated glassware, reagents, solvents, column packing materials, processing equipment). Note that the method blank is carried through the complete analytical method. **It is recommended that one method blank be analyzed with every 20 samples or with each batch of samples, whichever is more frequent.**

In addition to the routine analysis of the blank samples described above, it is also recommended that each lot of analytical reagents be analyzed for target contaminants of interest prior to use to prevent a potentially serious source of contamination. For organic analyses, each lot of alumina, silica gel, sodium sulfate, or Florasil used in extract drying and cleanup should also be analyzed for target analyte contamination and cleaned as necessary. Surrogate mixtures used in the analysis of organic target analytes have also been found to contain contaminants and interfering impurities and should be verified prior to use (U.S. EPA, 1987e).

In the analysis of organic contaminants by GC/MS or GC/ECD, cross-contamination should be avoided during all steps of analysis. Injection micro-syringes must be cleaned thoroughly between uses. If separate syringes are used for the injection of solutions, possible differences in syringe volumes should be assessed and, if present, corrected for. Particular care should be taken to avoid carryover when high- and low-level samples are analyzed sequentially. Analysis of an appropriate method blank following the analysis of a high-level sample may be required to assess carryover (U.S. EPA, 1987e).

Ideally, there should be no detectable concentration of any target analyte in any blank (i.e., the concentration of target analytes in all blanks should be less than the MDL). However, program managers may set higher acceptance limits (e.g., ≤10-30 percent of sample concentration [U.S. EPA, 1987e]), depending on overall data quality requirements of the monitoring program. If the concentration of any blank is greater than the established acceptance limit, appropriate corrective action should be taken and, if there is sufficient sample material, all samples associated with the blank should be reanalyzed. If reanalysis is not possible, all suspect data should be identified clearly. **Data should not be corrected for**

blank contamination by the reporting laboratory. The blank concentrations should always be reported with each associated sample value.

If the concentration of a target analyte in a blank is greater than the MDL, all steps in the relevant sample handling, processing, and analysis procedures should be reviewed. Many trace metal contamination problems are due to airborne dust. High zinc blanks may result from airborne dust or galvanized iron, while high chromium and nickel blanks often indicate contamination from stainless steel. In the field, the use of mercury thermometers should be avoided, because broken thermometers can be a source of serious mercury contamination. In the laboratory, samples to be analyzed for mercury should be isolated from materials and equipment (e.g., polarograms) that are potential sources of mercury contamination. In organic analyses, phthalates, methylene chloride, and toluene are common laboratory contaminants that are often detected in blanks at concentrations above the MDL. Chromatographic interference by natural substances in the tissue (e.g., fatty acids) may require additional cleanup procedures (U.S. EPA, 1987e).

6.4.3.6 Regular External QA Assessment of Analytical Performance--

Participation in an external QA program by all analytical laboratories in State fish and shellfish consumption advisory programs is recommended for several reasons:

- To enhance the comparability of data between States and Regions.
- To identify potential analytical problems prior to conducting routine analyses and to provide technical assistance to correct these problems.
- To provide an independent ongoing assessment of each laboratory's capability to perform the required analyses.

Two types of external QA programs are recommended to establish most reliably the comparability of data reported from different State and Regional Laboratories: **round-robin** interlaboratory comparisons and **split sample** interlaboratory comparisons.

6.4.3.6.1 Round-Robin Analysis Interlaboratory Comparison Program--At present, the only external round-robin QA program available for analytical laboratories conducting fish/shellfish tissue analyses for environmental pollutants is the QA program administered by NOAA in conjunction with its National Status and Trends Program. This QA program has been designed to ensure proper documentation of sampling and analysis procedures and to reduce intra- and interlaboratory variations among participating laboratories (Cantillo, 1991).

Each laboratory participating in the National Status and Trends QA program is required to conduct yearly analyses of one set each of three organic and three inorganic (i.e., trace metals) environmental and standard reference samples. The organic analytical intercomparison program is coordinated by NIST, and the inorganic analytical intercomparison program is coordinated by the NRCC. The sample types and matrices vary yearly. Sample types include freeze-dried sediments, extracted freeze-dried tissues, and frozen tissues. Sample matrices include mussel, oyster, and fish tissue, and sediments from pristine and contaminated areas. Individual laboratory performance is evaluated against the consensus values (i.e., grand means) of the results reported by all participating laboratories. A second set of samples is provided to a laboratory only after the first set is analyzed successfully. NIST and NRCC also provide technical assistance to participating laboratories that may have problems with the intercomparison analyses. Results of the QA analyses are reviewed by NIST, NRCC, and participants at an annual National Status and Trends QA meeting.

Analytical methods are not specified by NOAA; participants in the QA program may use any analytical method, provided its QA results are within established limits of the consensus values. However, all analytical and sampling protocols used must be documented thoroughly for future reference. Participants in the National Status and Trends QA program are also required to analyze reference materials such as the NIST SRMs and NRCC CRMs (see Table 6-9) as part of routine sample analysis. Results of the routine analysis of reference or control materials must be reported to NOAA. These results and the results of the QA samples are stored electronically in the National Status and Trends database.

Participation in the National Status and Trends QA program is strongly recommended to enhance the credibility and comparability of analytical data among different fish/shellfish monitoring programs. However, because of NOAA's budget constraints, there are only a limited number of openings in the National Status and Trends QA program at present. To address this problem, NOAA is considering expanding the program and charging each participating laboratory a fee to cover administrative costs; however, no estimates of the cost per laboratory are available at this time.

To apply for participation in the National Status and Trends QA program or for additional information, contact Dr. Adriana Cantillo, QA Manager, NOAA/National Status and Trends Program, N/OMA3, Rockville, MD 20852, Tel: 301-443-8655.

6.4.3.6.2 Split Sample Analysis Interlaboratory Comparison Programs--Another useful external QA procedure for assessing interlaboratory comparability of analytical data is a split

sample analysis program in which a percentage (usually 5 to 10 percent) of all field samples analyzed by each State or Region are divided and distributed for analyses among laboratories from other States or Regions. Because actual field samples are used in a split-sample analysis program, the results of the split-sample analyses provide a more direct assessment of the comparability of the reported monitoring results from different States or Regions.

The NOAA National Status and Trends QA program described above does not include an interlaboratory split-sample analysis program. **At a minimum, it is recommended that split-sample analyses be conducted regularly by States and/or Regions that routinely share monitoring results.**

6.4.3.7 Documentation and Reporting of Data--

The results of all chemical analyses (i.e., percent lipid and all target contaminant analyses) must be documented adequately and reported properly to ensure the proper evaluation and interpretation of the data.

Because all analytical data from State fish consumption advisory programs will be stored eventually in the Ocean Data Evaluation System (ODES) database for nationwide use, it will be essential that laboratory documentation procedures be consistent with ODES data reporting requirements.

The documentation of analytical data for each method should include, at a minimum, the following information (U.S. EPA, 1984a):

- Description of the procedure used, including documentation and justification of any deviations from the standard procedure
- Method accuracy and precision for each target analyte
- Method detection limit and limit of quantitation for each target analyte
- Discussion of any analytical problems and corrective action taken
- Sample identification numbers
- Sample weights
- Final dilution volumes
- Date(s) of analysis
- Identification of analyst

- Identification of instrument used (manufacturer, model number, serial number, location)

- Summary calibration data, including identification of calibration materials, dates of calibration and calibration checks, and calibration range(s); for GC/MS analyses, include DFTPP and bromofluorobenzene (BFB) spectra and quantitation report

- Reconstructed ion chromatograms for each sample analyzed by GC/MS

- Mass spectra of detected target compounds for each sample analyzed by GC/MS

- Chromatograms for each sample analyzed by GC/ECD and/or gas chromatography/flame ionization detection (GC/FID)

- Raw data quantitation reports for each sample

- Description of all QA/QC samples associated with each sample (e.g., field blanks, rinsate blanks, method blanks, duplicate or replicate samples, spiked samples, laboratory control samples) and results of all QA/QC analyses. QA/QC reports should include quantitation of all target analytes in each blank, recovery assessments for all spiked samples, and replicate sample summaries. Laboratories should report all surrogate spike recovery data for each sample; the range of recoveries should be included in any reports using these data.

- Analyte concentrations with reporting units identified (as μg/g wet weight to two significant figures unless otherwise justified). **NOTE: Reported data should not be blank-corrected.**

- Percent lipid associated with each sample. **NOTE: Reported data should not be normalized for lipid concentration.**

- Specification of all tentatively identified compounds (if requested) and any quantitation data.

- Data qualifications (including qualification codes and their definitions, if applicable, and a summary of data limitations).

To ensure completeness and consistency, standard forms should be developed and used by each laboratory for recording and reporting data from each analytical method. Standard data forms used in the EPA Contract Laboratory Program (U.S. EPA, 1991a,b) are included in Appendix N as examples of the types of forms that analytical laboratories should use.

All analytical data should be reviewed thoroughly by the analytical laboratory supervisor and, ideally, by a qualified chemist who is independent of the laboratory. In some cases, the analytical laboratory supervisor may conduct the full data review, with a more limited QA

review provided by an independent chemist. The purpose of the data review is to evaluate the data relative to the data quality specifications (e.g., detection and quantitation limits, precision, and accuracy) and other performance criteria established in the Work/QA Project Plan. In many instances, qualifiers may be necessary for reported data values; these qualifiers should always be defined clearly and included in the database.

Summaries of analytical data should be prepared for each target species at a specific sampling location and should include sample size (i.e., number of individuals in each composite sample), measured concentration of each target analyte (for intensive monitoring studies and initial screening studies where replicate QA samples are collected, the **arithmetic mean** and range of measured concentrations of each target analyte), and a measure of variance (standard error or 95 percent confidence limits). Specific data reporting requirements for the initial screening and followup intensive monitoring phases of these programs are given in Sections 2.1.9, 2.2.9, and 7.

SECTION 7
DATA REPORTING, ANALYSIS AND EVALUATION

This section provides guidance on data reporting, data analysis procedures for both initial screening studies and intensive followup studies (Phase I and II) of fish/shellfish contaminant programs, and procedures for evaluating residue data for the issuance of fish or shellfish consumption advisories. A discussion of four types of consumption advisories and bans currently issued by States is provided in Section 7.2.4.

All data reporting, analysis, and evaluation procedures should be documented fully as part of the Work/QA Project Plan for each study, prior to initiating the study. All routine data reporting, analysis, and evaluation procedures should be described in Standard Operating Procedures (SOPs). In particular, the procedures to be used to determine if the concentration of a target contaminant differs significantly from the recommended TV, and the specific decision rules to be used by the State to determine if a consumption advisory should be issued must be clearly documented. EPA has provided guidelines for evaluating fish/shellfish contaminant monitoring data (U.S. EPA, 1989d). Additional recommended data evaluation procedures are included in Sections 4.2 and 7.2 of this guidance document.

7.1 DATA REPORTING

7.1.1 Initial Screening

Data reports should be prepared for each target species sampled at each screening site. These reports should include, at a minimum, the following information:

- Site location (e.g., waterbody name, river mile, latitude/longitude, reach number or State waterbody identification number)
- Scientific name and common name of national target species
- Sampling dates (including rationale for sampling outside of the recommended sampling period [late summer to fall])
- Number of QA/QC replicates (optional; a minimum of one field replicate at 10 percent of the sites is recommended if resources permit)
- Number of individual organisms used in the composite sample (and in the QA/QC replicate, if applicable)

- Characteristics of each individual used in the composite sample (and in the QA/QC replicate, if applicable) (e.g., age, sex, total body length or size, total weight, percent lipid)

- For each target contaminant:

  - Measured concentration (ppm) in the composite sample

  - Measured concentration (ppm) in the QA/QC replicate, if applicable

  - Evaluation of laboratory performance (i.e., description of all QA/QC samples associated with the sample(s) and results of all QA/QC analyses)

  - Comparison of measured concentration with EPA-recommended TV and clear indication of whether TV was exceeded.

In the initial screening study, if a reported contaminant concentration exceeds the TV, a State should initiate an intensive followup study (Phase I, see Section 5.1.2.1) to verify the contamination in species of economic, sport fishing, or subsistence value. If a reported contaminant concentration from the initial screening study is close to the TV but does not exceed the TV (e.g., a reported value of 1.98 as compared to a TV of 2.00), the criteria used to determine if additional Phase I intensive monitoring is warranted should be documented clearly by the State. In this case, a State should reexamine historic data on water, sediment, and fish tissue contamination at the site as well as evaluate data on laboratory performance. If these data indicate that further examination of the site is warranted, the State should initiate a Phase I intensive study to verify the magnitude of the contamination. In Phase I studies, the State may wish to assess the tissue residue concentrations in additional target species or additional age classes of the target species for the contaminant of concern.

States are reminded that several aspects of the EPA-recommended screening study design presented in this guidance document are conservative in nature and are intended to protect the public health because they are based on worst-case exposure assumptions. These include

- Use of target species known to bioaccumulate environmental contaminants

- Use of fish fillets with skin-on and belly flap included

- Use of the oldest individuals in the target species to represent longest exposure times

- Late summer/fall sampling to maximize concentration of bioaccumulants in target species

- Targeting suspected hot spots for sampling
- Use of the 70-yr exposure rate to calculate TVs for carcinogens.

There are, however, several aspects of the screening study design that are of concern - either because they are not based on worst-case exposure assumptions or because they preclude valid statistical analyses of the data. These include the

- Use of fillet tissue samples rather than whole fish which may underestimate contaminant exposures in subpopulations that consume the whole fish.
- Use of composite samples which results in loss of information on the range and variance of the underlying population of individual samples. Such information is critical in bioaccumulation monitoring programs as an early warning sign of potentially harmful levels of contamination (U.S . EPA, 1989d).
- Use of a single sample per site for each target species which precludes estimating the variability of the contamination level at that site and, consequently, of conducting valid statistical comparisons to the target contaminant TVs.
- Use of trigger values calculated for the general U.S. population and not for local populations or subpopulations of recreational or subsistence fishermen.
- Use of a risk level of 10^{-4} for calculating TVs for carcinogens (i.e., as cancer incidence of 1 in 10,000 individuals). Some States are currently using more conservative risk levels of 10^{-5} (9 States) and 10^{-6} (8 States) for calculating TVs for carcinogens (Cunningham et al., 1990).

States should consider the potential effects of these design features on screening study results and should make modifications as appropriate to achieve the specific objectives of their contaminant monitoring programs.

7.1.2 Intensive Monitoring

For each intensive monitoring study (Phase I and Phase II, see Section 5.1.2.1), data reports should be prepared for each target species (by size or age class, if appropriate) at each sampling site within the waterbody under investigation. Note that in Phase II intensive studies, each sampling location is considered to be a separate site. These reports should include, at a minimum, the following information:

- Site location (e.g., waterbody name, river mile, latitude/longitude, reach number or State waterbody identification number)
- Scientific name and common name of regional target species
- Sampling dates (including rationale for sampling periods chosen for target species)

- Sampling design (e.g., two-stage sampling, systematic grid sampling)
- Number of replicates (five minimum)
- Number of individual organisms used in each composite sample (6 to 10 fish; 10 to 50 shellfish)
- Characteristics of individuals used in each composite sample (e.g., age, sex, total length or body size, total weight, percent lipid) and description of fish fillet or edible parts of shellfish used
- For each target contaminant in Phase I or Phase II of the intensive study:
  - Measured contaminant concentrations (ppm) in individual replicate composite samples
  - **Mean** (arithmetic) contaminant concentration for each set of replicate composite samples
  - Range of the contaminant concentrations for each set of replicate composite samples
  - Standard deviation of the contaminant concentrations
  - Comparison of the mean contaminant concentration with the appropriate TV, and clear indication of whether the TV was exceeded.

If the reported mean contaminant concentration is near the TV, the criteria used to determine if the TV was in fact exceeded should be documented clearly. If the study design includes a Phase II intensive study with the specific objectives of making multilocational comparisons to determine the geographic extent of the contamination or performing trend analyses, these results should be presented along with any appropriate statistical results (e.g., analysis of variance [ANOVA], nonparametric multiple comparisons, or trend tests).

NOTE: EPA is currently in the process of modifying the Ocean Data Evaluation System (ODES) database so that it can be used as a national repository for fish and shellfish contaminant monitoring data for both inland and coastal waters. Additional information on data reporting requirements for the ODES database will be available in subsequent drafts of this guidance document.

7.2 DATA ANALYSES AND EVALUATION

7.2.1 Initial Screening

The primary objective of the initial screening study is to assist States in identifying potential hot spots where further investigation of fish/shellfish contamination may be warranted. If the State deems that the measured concentration of a target contaminant in fish or shellfish obtained during the initial screening study warrants further investigation, then the State should initiate a Phase I intensive study at that site. The purpose of the Phase I intensive study is to confirm the findings of the initial screening and to assess the magnitude of the contamination in selected species and age classes of fish and shellfish of commercial, sportfishing, or subsistence importance.

Because duplicate or replicate field composite samples are not required as part of the initial screening study, estimating the variability of the composite contaminant concentration at any site is precluded. States may use duplicate laboratory samples to evaluate a laboratory's performance (see Section 6.4.3).

7.2.1.1 Laboratory Replicates--

States are required to process laboratory duplicate samples as part of the QA/QC protocol for the initial screening study. Consequently, States can conduct quality assurance investigations of their own laboratory or a contract laboratory by pooling duplicate results from multiple sites. Consider, for example, the following concentrations of toxaphene found in duplicate laboratory analyses of field composite samples from n=8 sites:

	Concentration of Toxaphene (ppm)		
Site i	Duplicate #1	Duplicate #2	Difference (d_i)
1	1.00	0.91	0.09
2	0.91	1.12	-0.21
3	0.79	0.93	-0.14
4	1.17	1.19	-0.02
5	0.85	0.67	0.18
6	0.75	0.82	-0.07
7	0.63	0.53	0.10
8	1.02	0.73	0.29

Note that the last column contains the difference (d_i) between the duplicate laboratory concentrations of toxaphene from screening site i. The average difference ($\bar{d}$) in the concentrations of toxaphene over the eight sites is

$$\bar{d} = (\sum_{i=1}^{8} d_i)/8 = 0.03 \ ppm$$

and the standard deviation (s) is

$$s = \sqrt{\sum_{i=1}^{8} (d_i - \bar{d})^2/(8-1)} = 0.17 \ ppm \ .$$

If the participating laboratory (either the State laboratory or a contract laboratory) is performing adequately, the mean difference in toxaphene concentrations is expected to be zero. Data collected at the eight sampled sites are used to test **the null hypothesis that the mean difference in the concentration of toxaphene in laboratory duplicates is zero** versus the two-sided alternative hypothesis that the mean difference is not zero. First, the State should calculate the statistic t^* as

$$\begin{aligned} t^* &= (\bar{d}-0)/(s/\sqrt{n}) \\ &= (0.03)/(0.17/\sqrt{8}) \\ &= 0.50 \ . \end{aligned}$$

Under the assumption of the null hypothesis, t^* has a Student's t-distribution with 7 (=8-1) degrees of freedom. Appendix O contains a table of the percentage points for various Student's t-distributions. If the State sets the Type I error rate (i.e., the probability of rejecting the null hypothesis when, in fact, it is true) at 0.05 (α=0.05) and considers a two-sided alternative hypothesis, then the null hypothesis is rejected if

$$t^* < t(7,0.025) = -2.45 \ or \ t^* > t(7,0.975) = 2.45 \ .$$

In this example, the State would not reject the null hypothesis that the mean difference in toxaphene concentrations found in duplicate laboratory samples is zero. The State could interpret this result as suggesting, with some caution, that the laboratory's analytical performance is acceptable. The cautionary note is deemed necessary because of the small

sample size (i.e., n=8)--there may not be adequate power to detect a difference when a true difference does exist.

Under a different scenario, the State may reject the null hypothesis in favor of the alternative that the mean difference in toxaphene concentrations found in duplicate laboratory samples is not zero. This conclusion makes the laboratory results for toxaphene concentration suspect. In such cases, EPA advises the States to review all field and laboratory procedures for a potential explanation of this finding.

[Reviewers, please provide additional methods for using laboratory replicates data to assess laboratory performance.]

7.2.1.2 Field Replicates--

Field sample replication is optional for the initial screening study. If resources permit and States collect a minimum of five replicate composite samples at a suspected hot spot, then States may pursue the statistical analysis described in the subsequent section for Phase I intensive monitoring studies.

[Reviewers, please provide additional methods for using limited field replicate data to assess total error.]

7.2.2 Intensive Study--Phase I

In the intensive study (Phase I), EPA recommends that the States analyze at least five replicate composite samples for each target species and/or size class of target species at each sampling site. Replicate samples must be as similar to each other as possible. EPA recommends that replicate samples of fish/shellfish be defined as follows:

- All replicate composite samples contain specimens of only a single species.
- Each fish composite sample contains a minimum of 6 fish, with 10 individuals being the optimal number and with each replicate composite containing equal numbers of individuals; each shellfish composite sample contains 10 to 50 individuals, with each replicate composite containing an equal number of individuals
- The smallest individual in any replicate is no less than 75 percent of the total size of the largest individual in the composite sample.
- The relative difference between the overall mean length or size of the replicate samples and the mean length or size of any individual replicate sample is no greater than 10 percent.
- The specimens in all replicates are collected at the same sampling site and within 24 hours of each other (U.S. EPA, 1990b).

States should analyze at least five replicate samples of each species of commercial, sportfishing, or subsistence value in the study area to determine whether fish fillets or edible parts of shellfish (as well as whole fish or shellfish in specific cases) contain tissue residues above the TV for any target contaminant identified in the initial screening study.

The following case studies include appropriate equations and resulting calculations of contaminant tissue residues.

Case Study I

EPA recommends that States collect a minimum of five replicate composite field samples for each secondary target species at each sampling location during the Phase I intensive study. Suppose a State finds the following toxaphene concentrations in 10 replicate composite samples collected at one suspect site during a Phase I intensive study:

Composite sample (j)	Concentration of toxaphene (ppm)(x_j)
1	0.89
2	1.03
3	1.08
4	0.89
5	0.84
6	0.92
7	0.85
8	0.89
9	0.84
10	1.08

Let j index the composite sample and x_j describe the concentration of toxaphene found in the composite sample. Then the mean toxaphene concentration over the 10 samples ($\bar{x}$) is

$$\bar{x} = \sum_{j=1}^{10} x_j/10 = 0.93 \ ppm$$

and the standard deviation (s) is

$$s = \sqrt{\sum_{i=1}^{10} (x_i - \bar{x})^2/(10-1)} = 0.10 \ ppm$$

The State is interested in comparing the average toxaphene concentration at the suspect site to the TV for toxaphene. The TV for toxaphene is 0.98 ppm (see Table 4-6). This TV was calculated using the following equation for carcinogens (see Section 4.2.1.1).

$$TV_c = [(RL/q1^*) \times BW]/CR$$

where

TV_c = trigger value for a carcinogen (m/kg; ppm)

RL = maximum acceptable risk level (10^{-4})

$q1^*$ = carcinogenic potency factor for toxaphene $(1.1 \text{ mg/kg/d})^{-1}$ from IRIS

BW = mean body weight, estimated for the general population (70 kg)

CR = mean daily fish/shellfish consumption rate averaged over a 70-year lifetime for the general population (0.0065 kg/d).

Specifically, the State will test the **null hypothesis that the mean toxaphene concentration at the suspect site is greater than the TV for 70-kg adults in the general population** versus the one-sided alternative hypothesis that the mean site-specific toxaphene concentration is less than the TV for these individuals. To accomplish this, the State first calculates the statistic t^* as

$$t^* = (\bar{x} - 0.98)/(s/\sqrt{n})$$

$$= (0.93 - 0.98)/(0.10/\sqrt{10})$$

$$= -1.58 \ .$$

Under the assumption of the null hypothesis, t^* has a Student's t-distribution with 9 (=10-1) degrees of freedom. Appendix O contains a table of the percentage points for various Student's t-distributions. If the State selects 0.05 (α=0.05) as the Type I error rate, then the null hypothesis is rejected if

$$t^* < t\,(9,0.05) = -1.83 \ .$$

In this example, the State would not reject the null hypothesis that the mean toxaphene concentration in composite samples taken from the suspect site is greater than the toxaphene

TV for the general population (since t* = -1.58 is not less than t(9,0.05) = -1,83). The TV for the general population is within sampling variability of the site-specific mean tissue concentration. Thus, the State might consider issuing a no-consumption advisory for the general population (see Section 7.2.4) because the toxaphene TV based on 0.0065 kg/d consumption by a 70-kg adult has been exceeded. Furthermore, the State should proceed to a Phase II intensive study to determine the geographic extent of the contamination.

Case Study II

States may be confronted with another scenario. Suppose the results of an initial screening study suggest that a State should initiate a Phase I intensive study of toxaphene at a suspect location. During the Phase I study, the State collects nine composite samples at the site:

Composite sample (j)	Concentration of toxaphene (ppm)(x_j)
1	0.58
2	0.83
3	0.85
4	0.71
5	0.90
6	0.57
7	0.79
8	0.96
9	1.01

where the mean toxaphene concentration over the nine samples ($\bar{x}$) is

$$\bar{x} = \sum_{j=1}^{9} x_j/9 = 0.80 \ ppm$$

and the standard deviation (s) is

$$s = \sqrt{\sum_{j=1}^{9} (x_j - \bar{x})^2/(9-1)} = 0.16 \ ppm \ .$$

As in Case Study I, the State will test the **null hypothesis that the mean toxaphene concentration at the suspect site is greater than the TV for adults in the general population** versus the one-sided alternative that the mean site-specific toxaphene

concentration is less than the TV for adults in the general population. The statistic t* is calculated from the data collected by the State:

$$t^* = (\bar{x} - 0.98)/(s/\sqrt{n})$$

$$= (0.80 - 0.98)/(0.16/\sqrt{9})$$

$$= -3.38 \quad .$$

Under the assumption of the null hypothesis, t^* has a Student's t-distribution with 8 (=9-1) degrees of freedom and a Type I error rate of 0.05 (α=0.05). Then the null hypothesis is rejected if

$$t^* = < t(8, 0.05) = -1.86 \quad .$$

In this case, the State would reject the null hypothesis that the mean toxaphene concentration in composite samples taken from the suspect site is greater than the toxaphene TV for adults in the general population in favor of the alternative hypothesis (e.g., the mean toxaphene concentration is less than the TV for toxaphene). Consequently, the State would not have to consider issuing a consumption advisory for the general population.

The corresponding toxaphene TV for adult women is 0.70 ppm based on assumption of a 50-kg rather than a 70-kg body weight. When the State examines an analogous hypothesis using the toxaphene TV for women, the test statistic t^* has the value 1.88. The State cannot reject the null hypothesis that the mean toxaphene concentration in composite samples taken from the suspect site is greater than the toxaphene TV (i.e., 1.88 is not less than -1.86). Consequently, the State should consider issuing a no-consumption advisory for a specific subpopulation (e.g., pregnant women, nursing mothers, and children) (see Section 7.2.4) and should proceed to the Phase II intensive study.

7.2.3 Intensive Study--Phase II

The objective of the Phase II intensive study is to determine both the magnitude of the contamination and the geographic extent of the contamination at various sites dispersed throughout the waterbody under investigation. As described in Section 2 of this guidance document, in order to determine the geographic extent of the contamination, States must collect at least five replicate samples at each of four to six different stations in the waterbody under investigation.

The State's objective for Phase II monitoring is to determine the extent of the geographical area over which the consumption advisory should extend. The location of each sampling station must be determined by State personnel familiar with the specific hydrologic aspects of the waterbody and with the location of additional anthropogenic sources of contamination to the waterbody under investigation. State staff should consult a qualified statistician both in designing Phase II intensive monitoring studies and in interpreting tissue residue data collected at multiple sites throughout a given waterbody.

For some small lakes, States may opt to issue fish consumption advisories after analyzing results of Phase I monitoring rather than conducting further resource-intensive multilocational Phase II monitoring. For large lakes or reservoirs and for riverine or estuarine areas, however, where the economic impact of a commercial or recreational closure may be devastating to the local economy, the State may want to conduct extensive multilocational and multispecies sampling of the targeted hot spot to determine the geographic extent of the advisory and the specific species and age classes affected. The complexity of the monitoring design depends on the complexity of the hydrologic processes operating in the affected waterbody (e.g., estuaries with strong tidal influence or coastal sites influenced by long shore currents).

The following case study illustrates the methodology proposed by EPA for analyzing Phase II intensive study data.

Case Study III

Suppose a State determines in a Phase I study that the mean tissue concentration of toxaphene in a target species exceeds the TV for toxaphene (0.98 ppm) at a riverine site. In this case, EPA recommends that the State proceed to a Phase II intensive study to determine the geographic extent of the contamination at the site.

The State selects four additional riverine sites downstream from the Phase I study site and then collects five replicate composite samples at each of the five sites. For the following fish tissue residue data, Site 1 is the Phase I (and initial screening) site and Sites 2 through 5 are located progressively farther downstream.

Concentration of Toxaphene (ppm) in Replicate Samples

Site 1	Site 2	Site 3	Site 4	Site 5
1.12	1.27	1.30	1.13	0.60
1.40	1.32	1.22	1.28	0.50
1.33	1.35	1.13	1.45	0.55
1.35	1.17	1.29	1.31	0.57
1.29	1.21	1.44	1.29	0.62

To analyze these data, EPA recommends that States use a single-factor ANOVA (Neter and Wasserman, 1974). The appropriate ANOVA table for investigating the **null hypothesis that the mean toxaphene concentration is the same across all sites** versus the alternative hypothesis that at least one site-specific mean toxaphene concentration is different is shown in Table 7-1.

In Table 7-1, j indexes the sites, i indexes the composite samples, r is the number of sites, n is the total number of composite samples, n_j is the number of composite samples at site j, x_{ij} is the concentration of toxaphene in the ith sample at the jth site, $\bar{x}_{..}$ is the mean toxaphene concentration over all sites and samples, and $\bar{x}_{.j}$ is the mean toxaphene concentration at site j.

In Case Study III, r=5; n=25; n_j=5 for j=1,2,3,4 and 5; $\bar{x}_{..}$=1.14 ppm; $\bar{x}_{.1}$=1.30 ppm; $\bar{x}_{.2}$=1.29 ppm; $\bar{x}_{.3}$=1.28 ppm; $\bar{x}_{.4}$=1.26 ppm; and $\bar{x}_{.5}$=0.57 ppm. The ANOVA table for the Case Study III example is shown in Table 7-2.

TABLE 7-1. ANOVA TABLE FOR SINGLE-FACTOR STUDY

Source of variation	Sum of squares (SS)	Degrees of freedom (df)	Mean square (MS)
Between sites	$SSTR = \Sigma n_j(\bar{x}_{.j} - \bar{x}_{..})^2$	$r - 1$	$MSTR = \frac{SSTR}{r-1}$
Error (within sites)	$SSE = \Sigma\Sigma(x_{ij} - \bar{x}_{.j})^2$	$n - r$	$MSE = \frac{SSE}{n-r}$
Total	$SSTO = \Sigma\Sigma(x_{ij} - \bar{x}_{..})^2$	$n - 1$	

TABLE 7-2. ANOVA TABLE FOR THE PHASE II TOXAPHENE STUDY

Source of variation	SS	df	MS
Between sites	2.045	4	0.511
Error	0.181	20	0.009
Total	2.226	24	

To test the null hypothesis described above, the State will first calculate the statistic F^* as the ratio of the mean square for treatments (MSTR) to the mean square error (MSE).

$$F^* = MSTR/MSE = 0.511/0.009 = 56.6 \quad .$$

Under the assumption of the null hypothesis, F^* has an F-distribution with (r-1,n-r) degrees of freedom. Appendix O contains a table of the percentage points for various F-distributions. If the Type I error rate is selected to be 0.05 (α=0.05), then the null hypothesis is rejected if

$$F^* > F(4,20,0.95) = 2.87 \quad .$$

In this case study, the State would reject the null hypothesis that the mean toxaphene concentrations across the five sites are equal and conclude that the mean toxaphene concentration at least one site is different from the other sites. If the State were unable to reject the null hypothesis, the State would conclude that either the null hypothesis was true or that there were not enough data (e.g., replicates) to detect the differences to be tested. At this point, the State might consider revising their Phase II sampling protocol to include additional replicates at each site. In the future, the EPA would like all State fish and shellfish contaminant monitoring data to be entered into the national database, ODES, which contains a statistical power analysis tool for designing contaminant monitoring programs (U.S. EPA, 1987a). Specifically, States may use ODES to determine how best to use their limited monitoring resources (i.e., when to increase the sample size collected in order to achieve adequate power for a statistical test.) States should review the discussion of power analysis provided in U.S. EPA (1989) for the ODES database.

Rejection of the null hypothesis allows the State to pursue pairwise comparisons to determine where the difference in the mean concentration of toxaphene exists (i.e., the geographic extent of the contamination). EPA recommends that States employ Scheffe's

method of multiple comparisons to examine mean concentration differences between sites (Kleinbaum and Kupper, 1978). Scheffe's method, which involves constructing and comparing confidence intervals for all the comparisons, accommodates unequal numbers of samples at each site.

In the illustration, the State may be concerned with the following comparisons: Site 1 vs. Site 5, Site 1 vs. Site 4, Site 1 vs. Site 3, Site 1 vs. Site 2, Site 2 vs. Site 5, Site 2 vs. Site 4, Site 2 vs. Site 3, Site 3 vs. Site 5, Site 3 vs. Site 4, and Site 4 vs. Site 5. For investigating the difference in the mean toxaphene concentrations between Sites 1 and 5, the form of the Scheffe-type confidence interval for this pairwise comparison is

$$(\bar{x}_{.5} - \bar{x}_{.1}) \pm S \times [MSE \times (1/n_5 + 1/n_1)]^{\frac{1}{2}}$$

where

$\bar{x}_{.5}$ = 0.57

$\bar{x}_{.1}$ = 1.30

S = $[(r-1) \times F(r-1, n-r, 1-\alpha)]^{1/2}$

= $(4 \times 2.87)^{1/2}$

= 3.39

MSE = 0.009 (from Table 7-2)

$n_5 = n_1 = 5$

Thus, the confidence interval for comparing Sites 1 and 5 is

$$(0.57 - 1.30) \pm 3.39 \times [0.009 \times (\frac{1}{5} + \frac{1}{5})]^{\frac{1}{2}}$$

$$= -0.73 \pm 3.39 \times 0.06$$

$$= -0.73 \pm 0.20$$

$$= (-0.93, -0.53) \ .$$

This interval does not contain the value zero, which supports rejection of the null hypothesis that the mean toxaphene concentrations at Sites 1 and 5 are equal.

Repeating the above calculation for the comparison between Sites 1 and 4 yields the confidence interval

(-0.19,0.21)

which does contain the value zero. Thus, the State cannot reject the null hypothesis that the mean toxaphene concentrations at Sites 1 and 4 are equal. After investigating for all other site differences similarly, the State would conclude (1) that the mean toxaphene concentration at Sites 1, 2, 3, and 4 are similar and (2) that the mean toxaphene concentration at Site 5 is different (lower) than the mean toxaphene concentration at the other sites. After confirming that the mean toxaphene concentration at Site 5 is less than the toxaphene TV (using the methodology discussed in Section 7.2.2), the State should consider issuing a no-consumption advisory for the general population for Site 1 extending downstream to Site 4 but excluding Site 5 (see Section 7.2.4).

7.2.4 Issuance of Fish/Shellfish Consumption Advisories

After analyzing Phase I and/or Phase II contaminant residue results, a State may find that there is justification for issuing a fish/shellfish consumption advisory. States should review the discussion of estimating TVs for intensive monitoring studies in Section 4.2.3. There are four specific types of advisories that States have issued:

- No consumption advisory that advises against consumption of fish or shellfish species by the general population (NCGP)
- No consumption advisory that advises against consumption of fish or shellfish species by a subpopulation that could be at greater risk (e.g., pregnant women, nursing mothers or children) (NCsp)
- Restricted consumption advisory that advises restricted consumption (e.g., limited number of meals and/or size of meals per unit time) of fish or shellfish species by the general population (RGP)
- Restricted consumption advisory for a subpopulation that advises restricted consumption (e.g., a limited number of meals or size of meals per unit time) of fish or shellfish species by a subpopulation that could be at greater risk (e.g., pregnant women, nursing mothers, and children) (Rsp)

Guidance on issuing each type of advisory is given in Table 7-3.

The four advisory categories were identified from a review of consumption advisories and bans listed by the 50 States, the District of Columbia, and the Virgin Islands and Puerto Rico in their 1990 305(b) reports and were used to develop a database--Current State Fish and Shellfish Consumption Advisories and Bans (RTI, 1991). EPA's Office of Science and

TABLE 7-3. RECOMMENDED GUIDELINES FOR ISSUING VARIOUS TYPES OF FISH/SHELLFISH ADVISORIES[a]

Type of advisory	Conditions under which to issue advisory
NCGP	The contaminant TV is calculated using a 0.0065 kg/d consumption rate for a 70-kg adult and the null hypothesis cannot be rejected--States would conclude that the mean contaminant concentration is greater than the contaminant TV.
NCsp[b]	The contaminant TV is calculated using a consumption rate >0.0065 kg/d for ethnic or subsistence subpopulations or for individuals <70-kg body weight (e.g., women and children) and the null hypothesis cannot be rejected--States would conclude that the mean contaminant concentration is greater than the contaminant TV calculated for the potentially more sensitive subpopulation.
RGP	The contaminant TV is calculated using a 0.0065 kg/d consumption rate for a 70-kg adult and the null hypothesis is rejected--States would conclude that the mean contaminant concentration is less than the TV; however, it is approaching a level of concern based on the best professional judgment of the project team.
Rsp[b]	The contaminant TV is calculated using a consumption rate >0.0065 kg/d for ethnic or subsistence subpopulations or for individuals <70-kg body weight (e.g., women and children) and the null hypothesis is rejected--States would conclude that the mean contaminant concentration is less than the TV; however, it is approaching a level of concern based on the best professional judgment of the project team.

[a] Based on EPA recommended risk assessment procedures discussed in Section 4.2 of this guidance document, assuming a 10^{-4} risk factor for carcinogens. States may employ lower (i.e., 10^{-5} or 10^{-6}) but not higher risk factors.

[b] Subpopulations may include ethnic or subsistence populations that consume more than 0.0065 kg/d of fish or shellfish or individuals with <70 kg body weight such as pregnant women, nursing mothers, and children who may be at potentially greater risk particularly for contaminants that are developmental (fetal) toxicants.

Technology is in the process of developing an electronic bulletin board that will contain this advisory database and will be available to the States in October 1991. For each State advisory listing, the pollutant that triggered the advisory, the type of advisory, the species of fish or shellfish (and for some States, size class of fish affected) for which the advisory was issued, and the waterbody name and extent of the contamination are presented. A sample of the information contained in the consumption advisory database is shown in Figure 7-1. The name, address, and telephone number of a contact person in each State who can discuss the basis of the consumption advisories will also be added to the database.

CURRENT STATE FISH AND SHELLFISH CONSUMPTION ADVISORIES AND BANS

STATE	POLLUTANT	NATURE OF ADVISORY	FISH (common name)	WATERBODY NAME	GEOGRAPHIC EXTENT
VA	PCBs	NCCP	All fish species	N. F. Shenandoah River	Passage Cr to confl. with Shenandoah R
VA	Dioxins •	NCCP	Bottom feeding species	Blackwater River	Union Camp plant to Nottoway R (5 mi)
VA	Dioxins •	NCCP	All fish species	Jackson River	From dam above Dunlap Cr to James River
VA	Dioxins •	NCCP	Bottom feeding species	Nottoway River	Gen. Vaughan Bridge (U.S. 258) to NC border
VA	Dioxins •	NCCP	All fish species	James River	Confluence with Jackson River downstream to Snowden Dam
VI	———	None			
WA	———	None			
WV	Dioxins •	NCCP	Bottom feeders	Kanawha River	(46 mi)
WV	Dioxins •	NCCP	All fish species	Pocatalico River	(2.0 mi)
WV	Dioxins •	NCCP	All fish species	Armour Creek	(2.0 mi)
WV	Dioxins •	NCCP	Bottom feeders	North Br. Potomac	(60.6 mi)
WV	Dioxins •	NCCP	Bottom feeders	Potomac River	(38 mi)
WV	PCBs, chlordane	NCCP	Channel catfish, carp	Ohio River	(277 mi)
WV	PCBs	NCCP	Carp, suckers, channel catfish	Shenandoah River	(19.45 mi)
WI	PCBs, pesticides	NCsp, RCP	Lake trout 20 to 23", coho salmon > 26", chinook salmon 21 to 32", brown trout to 23"	Lake Michigan	
WI	PCBs, pesticides	NCCP	Lake trout > 23", chinook salmon >32", brown trout > 23", carp, catfish	Lake Michigan	
WI	PCBs, pesticides	NCsp, RCP	Splake up to 16"	Green Bay	Menominee, Oconto, & Peshtigo Rivers
WI	PCBs, pesticides	NCCP	Rainbow trout > 22", chinook salmon > 26" brown trout > 12", brook trout > 15", carp, splake > 16", northern pike > 28", walleye > 20", white bass	Green Bay	Menominee, Oconto, & Peshtigo Rivers
WI	PCBs, pesticides	NCsp, RCP	Northern pike, white sucker, walleye 16-18"	Lower Fox River	From mouth at Green Bay up to DePere Dam
WI	PCBs, pesticides	NCCP	White bass, walleye > 18", carp, drum, channel catfish	Lower Fox River	From mouth at Green Bay up to DePere Dam
WI	PCBs, pesticides	NCsp, RCP	Walleye > 15", bullhead	Lower Fox River	From De Pere Dam to Neenah-Menasha Dam
WI	PCBs, pesticides	NCCP	Carp > 17"	Lower Fox River	From De Pere Dam to Neenah-Menasha Dam
WI	PCBs, pesticides	NCsp, RCP	Lake trout 20 to 23", coho salmon > 26" chinook salmon 21 to 32", brown trout to 23"	East and West Twin Rivers	From mouths upstream to first dam
WI	PCBs, pesticides	NCCP	Carp, catfish, lake trout > 23", chinook salmon > 32", brown trout > 23"	East and West Twin Rivers	From mouths upstream to first dam
WI	PCBs, pesticides	NCsp, RCP	Lake trout 20 to 23", coho salmon > 26", chinook salmon > 21", brown trout to 23"	Manitowoc River	Mouth upstream to first dam
WI	PCBs, pesticides	NCCP	Catfish, lake trout > 23", chinook salmon > 32", brown trout > 23",	Manitowoc River	Mouth upstream to first dam
WI	PCBs, pesticides	NCsp, RCP	Rainbow trout, brook trout, coho salmon > 26", chinook salmon 21-32"	Sheboygan River	Fr Sheboygan Falls/Greendale & Weedens Cr
WI	PCBs, pesticides	NCCP	Bluegill, crappie, rock bass, carp, smallmouth bass, walleye, northern pike, brown trout, catfish, chinook salmon > 32"	Sheboygan River	Fr Sheboygan Falls/Greendale & Weedens Cr
WI	PCBs, pesticides	NCsp, RCP	Lake trout 20 to 23", coho salmon > 26", chinook salmon 21-32", brown trout to 23"	Milwaukee River	From mouth to North Avenue Dam
WI	PCBs, pesticides	NCCP	Lake trout > 23", chinook salmon > 32" brown trout > 23", carp, catfish, crappie, northern pike, redhorse, smallmouth bass, white sucker	Milwaukee River	From mouth to North Avenue Dam

Figure 7-1. Sample output from the database–Current State Fish and Shellfish Consumption Advisories and Bans (RTI, 1991).

States will be able to access information from this electronic bulletin board on advisories issued in adjacent States with which they may share waters and may use this information to direct their own contaminant monitoring program. For example, the issuance of an advisory by one State on the upper reaches of a particular river can provide information to an adjacent State on contaminants that might be anticipated in the same fish species at monitoring sites downstream. This is especially important in cases where the geographic extent of the fish consumption advisory for a State ends at the State border because the State's monitoring activities and jurisdiction end at the State line. However, the actual geographic extent of the contamination may continue into the adjacent State's waters for many miles downstream. In addition, information on the bulletin board will allow States to review differences in fish consumption advisories in shared (interstate) waters, where, for example, one State may issue a consumption advisory for one side of a river, while an adjacent State may have no consumption advisory for its jurisdictional waters on the other side of the river. Such inconsistencies in consumption advisories in interstate waters undermine public confidence in State regulations designed to protect public health.

SECTION 8

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APPENDIX G

FORMS

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 TVs *(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 (10 fish optimum)

_____ Shellfish per composite *(specify number to obtain 500 grams of tissue)*

Field Record for Fish Contaminant Monitoring Program — Screening Study

Project Number: _____ Sampling Date and Time: _____

SITE LOCATION

Site Name/Number: _____

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

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

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

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

_____

Predator—Species Name: _____

Composite Sample #: _____ Number of Individuals: _____

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

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

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

_____

Field Record for Shellfish Contaminant Monitoring Program — Screening Study

Project Number: _____ Sampling Date and Time: _____

SITE LOCATION

Site Name/Number: _____

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

State Waterbody 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 (cm)	Bivalve #	Size (cm)	Bivalve #	Size (cm)
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 _____ cm

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

_____

Field Record for Fish Contaminant Monitoring Program — Intensive Study

Project Number: _____ Sampling Date and Time: _____

SITE LOCATION

Site Name/Number: _____

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

State Waterbody 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 (cm)	Sex (M, F, or I)	Fish #	Length (cm)	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 _____ cm

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

_____

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

Composite Sample #: _____ Number of Individuals: _____

Fish #	Length (cm)	Sex (M, F, or I)	Fish #	Length (cm)	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 _____ cm

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

_____

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 (cm)	Sex (M, F, or I)	Fish #	Length (cm)	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 _____ cm

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

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

Composite Sample #: _____ Number of Individuals: _____

Fish #	Length (cm)	Sex (M, F, or I)	Fish #	Length (cm)	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 _____ cm

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

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

Composite Sample #: _____ Number of Individuals: _____

Fish #	Length (cm)	Sex (M, F, or I)	Fish #	Length (cm)	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 _____ cm

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

Field Record for Shellfish Contaminant Monitoring Program — Intensive Study

Project Number: _____ Sampling Date and Time: _____

SITE LOCATION

Site Name/Number: _____

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

State Waterbody 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 (cm)	Sex	Shellfish #	Size (cm)	Sex	Shellfish #	Size (cm)	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					

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

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

_____

Species Name or Code	Sample Type
Total Length or Size (cm)	Sampling Site (name/number)
Specimen Number □□□□□—□□□	Sampling Date/Time

Project Number	Collecting Agency (name, address, phone)			
Sampling Site (name and/or ID number)	Sampler (name and signature)			
Composite Number	Chemical Analyses ☐ All target contaminants ☐ Others (specify) _____		Study Type	
			Screening	Intensive
Sampling Date/Time				
Species Name or Code	Processing		Type of Ice	
	Whole Body	Resection	Wet	Dry
Comments				

Chain-of-Custody Record

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

Composite Number	Sample Nos.	Sampling Time	Study Type: Scr	Study Type: Int	Sampling Site (name/number)	All Target Contaminants	Specific Contaminants Only (Specify)	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

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

Project Number: _____ Sampling Date and Time: _____

STUDY PHASE: Initial Screening ☐; Intensive Monitoring: Phase I ☐ Phase II ☐

SITE LOCATION

Site Name/Number: _____

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

State Waterbody Segment Number: _____ Waterbody Type: _____

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

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

					Left Fillet			Right Fillet		
Fish #	**Weight (g)**	**Scales/Otoliths Removed (✓)**	**Sex (M,F)**	**Resection Performed (✓)**	**Weight (g)**	**Homogenate Prepared (✓)**	**Wt. of Homog. for Composite**	**Weight (g)**	**Homogenate Prepared (✓)**	**Wt. of Homog. for Composite**
001										
002										
003										
004										
005										
006										
007										
008										
009										
010										
Analyst										
Date										

Total Composite Weight (g) **(left)** _____ **(right)** _____

Notes: _____

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

Project Number: _____ Sampling Date and Time: _____

STUDY PHASE: Initial Screening ☐ ; Intensive Monitoring Phase I ☐ Phase II ☐

SITE LOCATION

Site Name/Number: _____

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

State Waterbody Segment Number: _____ Waterbody Type: _____

SHELLFISH COLLECTED

Species Name: _____

Composite Sample #: _____ Number of Individuals: _____

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

Preparation of Composite:

Weight of container + shellfish _____ g

Weight of container _____ g

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

Analyst _____ Date _____

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

Project No. _____ Sampling Date and Time: _____

STUDY PHASE: Initial Screening ☐ ; Intensive Monitoring Phase I ☐ Phase II ☐

SITE LOCATION

Site Name/Number: _____

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

State Waterbody Segment Number: _____ Waterbody Type: _____

Bottom Feeder – Species Name: _____

Composite Sample #: _____ Number of Individuals: _____

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

Total Composite Homogenate Weight _____

Predator – Species Name: _____

Composite Sample #: _____ Number of Individuals: _____

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

Total Composite Homogenate Weight _____

Fish/Shellfish Monitoring Program
Sample Aliquotting Record

Aliquotted by _____ Date _____ Time _____
(name)

Comments _____

Samples from:

Project No. _____ Site # _____ ☐ Screening study ☐ Intensive study

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

Fish/Shellfish Monitoring Program
Sample Transfer Record

Date ____ ____ ____ Time ____ : ____ (24-h clock)
DD MM YY HH MM

Released by: _____
(name)

At: _____
(location)

Shipment Method _____

Shipment Destination _____

Date ____ ____ ____ Time ____ : ____ (24-h clock)
DD MM YY HH MM

Released by: _____
(name)

At: _____
(location)

Shipment Method _____

Shipment Destination _____

Comments _____

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

☐ Intensive—Analyze for *(specify)* _____

Sample IDs:

Laboratory Chain of Custody

Relinquished by	Received by	Purpose	Location

APPENDIX GG

RECOMMENDED PROCEDURES FOR PREPARING WHOLE FISH COMPOSITE SAMPLES

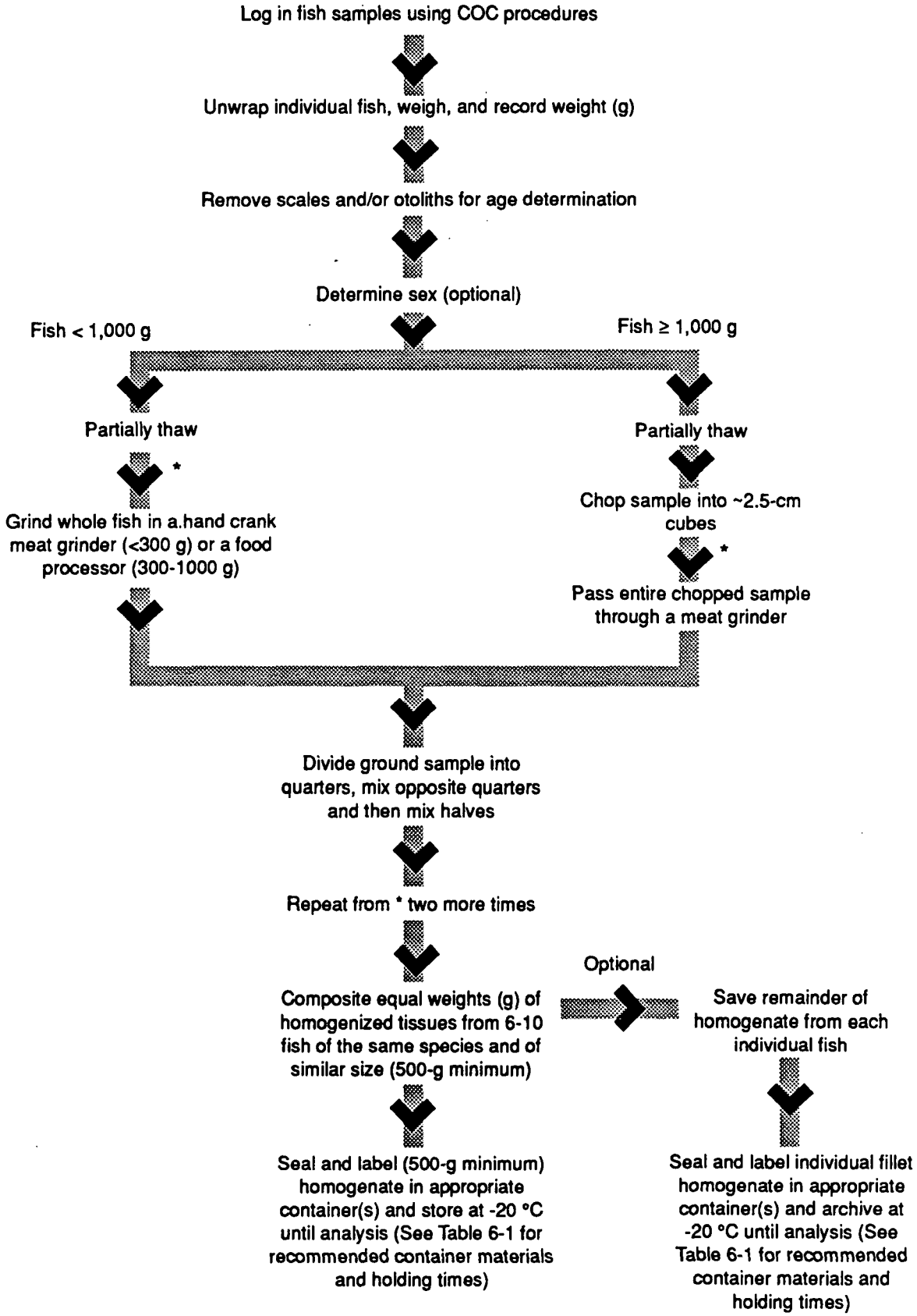
Laboratory processing to prepare whole fish composite samples (diagrammed in Figure GG-1) involves

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

Whole fish samples should be shipped on wet ice or blue ice packets from the field to the sample processing laboratory if next-day delivery is assured (see Section 5.3.2). Fish samples arriving in this manner (chilled but not frozen) should be weighed, scales and/or otoliths removed, and the sex of each fish determined within 24 hours after receipt by the central processing laboratory. The samples should then be frozen (-20 °C) in the laboratory prior to being homogenized. (The grinding/homogenization procedure may be carried out more easily and efficiently if the sample has been frozen previously [Stober, 1991].)

If the fish samples arrive frozen at the sample processing laboratory, precautions should be taken during weighing, removal of scales and/or otoliths, and sex determination to ensure that any liquid formed in thawing remains with the sample. The liquid will contain lipid material that should be included in the analysis scheme.

The thawed or partially thawed whole fish should then be homogenized individually, and equal weight portions of each homogenate should be combined and mixed to form the composite sample. Individual homogenates and/or composite homogenates may be refrozen; however, frozen individual homogenates must be rehomogenized before compositing, and frozen composite homogenates must be rehomogenized before aliquotting for analysis. The maximum holding time from sample collection to analysis for mercury is 28 days at ≤-20 °C; for all other analytes, the holding time is 6 months to 1 year at ≤-20 °C (Stober, 1991). Recommended container materials, preservation methods, and holding times are given in Table GG-1. Fish sample processing procedures are discussed in more detail in the sections below. Each time the samples are transferred from one person to another during processing, the COC form that originated in the field must be signed so that possession and location of the samples can be traced at all times. As each procedure is performed, it should be



COC = Chain of Custody

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

TABLE GG-1. RECOMMENDATIONS FOR CONTAINER MATERIALS, PRESERVATION, AND HOLDING TIMES FOR FISH/SHELLFISH TISSUES FROM DELIVERY AT CENTRAL PROCESSING LABORATORY TO ANALYSIS

Analyte	Matrix	Sample container	Storage	
			Preservation	Holding time
Trace metals (except Hg)	Tissue (whole specimens, edible portions, homogenates)	Plastic, glass	Freeze at $\leq$-20 °C	1 year
Hg	Tissue (whole specimens, edible portions, homogenates)	Plastic, glass	Freeze at $\leq$-20 °C	28 days
Organics	Tissue (whole specimens, edible portions, homogenates)	Glass, teflon	Freeze at $\leq$-20 °C	1 year

documented directly in a bound laboratory notebook or on forms that can be taped or pasted into the notebook. Several existing programs have developed forms similar to the sample processing record for whole fish composite samples shown in Figure GG-2. The use of a form is recommended to ensure consistency and completeness of the record.

Sample Weighing--A wet weight should be determined for each fish collected. If the fish has been shipped on wet ice, it should be unwrapped, placed on a foil-lined balance tray, and the weight recorded to the nearest gram on the sample processing record and/or in the laboratory notebook. To avoid contamination, the foil lining should be replaced between each weighing. Frozen fish should be weighed in tared containers if thawing is expected before the weighing can be completed. Liquid associated with the sample when thawed must be maintained in the container as part of the sample because it will contain lipid material that has separated from the tissue (Stober, 1991).

Removal of Scales and/or Otoliths for Aging--It is recommended that a few scales or otoliths be removed from each fish for age determination by a fisheries biologist. Aging provides a good indication of the length of exposure to pollutants (Versar, 1982). For most warmwater inland gamefish, 5 to 10 scales should be removed from below the lateral line and behind the pectoral fin. On softrayed fish such as trout and salmon, the scale sample should be taken just above the lateral line (Wisconsin, 1988). For catfish and other scaleless fish,

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

Project No. _____ Sampling Date and Time: _____

STUDY PHASE: Initial Screening ; Intensive Monitoring Phase I Phase 2

SITE LOCATION

Site Name/Number: _____

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

State Waterbody Segment Number: _____ Waterbody Type: _____

Bottom Feeder – Species Name: _____

Composite Sample #: _____ Number of Individuals: _____

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

Total Composite Homogenate Weight _____

Predator – Species Name: _____

Composite Sample #: _____ Number of Individuals: _____

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

Total Composite Homogenate Weight _____

Figure GG-2.

the pectoral fin spines should be clipped and saved (Versar, 1982). Otoliths are another indicator of age that may be collected (Jearld, 1983). The scales, spines, or otoliths may be stored by sealing in small envelopes (such as coin envelopes) or plastic bags labeled with, and cross-referenced by, the identification number assigned to the tissue specimen (Versar, 1982). Removal of scales, spines, or otoliths from each fish should be noted on the sample processing record.

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

Preparation of Individual Homogenates--Grinding of biological tissue, especially skin from whole fish samples, is easier when the tissue is partially frozen (Stober, 1991). Chilling the grinder briefly with a few chips of dry ice will reduce the tendency of the tissue to stick to the grinder. However, do not freeze the grinder because it will make it difficult to force frozen tissue through the chopper plate.

Smaller whole fish may be ground in a hand crank meat grinder (fish < 300 g) or a food processor (fish 300-1,000 g). Larger fish may be cut into 2.5-cm cubes with a food service band saw (e.g., Hobart Model 5212) and then ground in either a small (e.g., Hobart, 1/4 hp, Model 4616) or large (e.g., Hobart, 1 hp, Model 4822) meat grinder. To avoid contamination by metals, homogenizers used to grind tissue should have tantalum or titanium parts. The ground sample should be divided into quarters, opposite quarters mixed together by hand, and the two halves mixed back together. The grinding, quartering, and hand mixing should be repeated two more times. If chunks of tissue are present at this point, the grinding/homogenizing should be repeated. No chunks should be discarded. If the sample is to be analyzed for trace metals only, the ground tissue may be mixed by hand in a polyethylene bag (Stober, 1991). Homogenization of each individual fish should be noted on the sample processing record.

Individual whole fish homogenates may be either composited or frozen and stored at ≤-20 °C in cleaned containers that are noncontaminating for the analyses to be performed.

Preparation of Composite Homogenates--If individual whole fish homogenates are frozen, they should be thawed partially and rehomogenized prior to compositing. Any associated liquid should be maintained as a part of the sample. Equal weights should be taken from each individual homogenate and blended to provide a composite sample of sufficient size (500 g minimum) to perform all necessary analyses. Weights of individual homogenates required for a composite sample, based on the total number of fish per composite and the quantity of composite needed, are given in Table GG-2. The actual weight of each individual homogenate that is taken for the composite sample should be recorded on the sample processing record. The remaining individual homogenates should be archived in a freezer at ≤-20 °C, with the designation "Archive" and the expiration date added to each sample label. Location of the archived samples should be indicated on the sample processing record under "Notes." Each composite sample should be divided into quarters, opposite quarters mixed together by hand, and the two halves mixed together. The quartering and mixing should be repeated two more times. If the sample will be analyzed for trace metals only, the composite sample may be mixed by hand in a polyethylene bag. At this point, the composite sample may be frozen and stored at ≤-20 °C or processed for organics and trace metals analyses.

TABLE GG-2. INDIVIDUAL WEIGHTS (g) OF HOMOGENATE REQUIRED FOR A COMPOSITE SAMPLE[a]

Total number of fish per sample	Total homogenate weight		
	500 g (minimum)	1,000 g (average)	2,000 g
6	84	167	334
7	72	143	286
8	63	125	250
9	56	112	223
10	50	100	200

[a] Based on total number of fish per composite and the total homogenate weight required for analysis.

APPENDIX H

EXAMPLE PROCEDURE FOR ANALYSIS OF PERCENT LIPID IN TISSUE SAMPLES

[From: State of California. 1990. Laboratory Quality Assurance Program Plan. Department of Fish and Game, Environmental Services Division. March.]

Method # 2-LIPID

Determination of Percent Lipid in Tissue Samples.

1.0 Scope and Application

1.1 This method determine percent lipid in fish and wildlife tissue samples.

2.0 Summary of Method

2.1 The tissue sample is dried using anhydrous grannular sodium sulfate and the lipid extracted with petroleum ether (PE). The petroleum ether is evaporated and the residue is weighed.

3.0 Interferences

3.1 Each lot of petroleum ether must be tested by evaporating 250 mL of PE to dryness, the residue must be less than 10 mg.

4.0 Apparatus and Materials

4.1 Balance, capable of weighing to the nearest mg.

4.2 Beaker, 250 mL borosilicate glass.

4.3 Buchner Funnel, 8 cm.

4.4 Filter Flask, 500 mL

4.5 Filter Paper, Whatman #42, 8 cm.

4.6 Aluminum Dish, 50 mL.

4.7 Water Bath, heated, with concentric ring cover, capable of temperature control (± 2 ^{o}C), installed in fume hood.

4.8 Desiccator.

4.9 Waring Blender - Glass or stainless steel blender with stainless steel blades and carbon bearings. The motor of the blender must be explosion proof.

5.0 Reagents

5.1 Sodium sulfate, anhydrous, granular meets ACS specifications.

5.2 Petroleum ether, distilled in glass.

6.0 Sample collection, Presevation, and Handling

6.1 All samples must have been collected using a sampling plan that addresses the considerations discussed in this manual.

6.2 All sample containers must be prewashed with detergents, acids, and Type II water. Plastic and glass containers are both suitable.

7.0 Procedure for Sample Preparation

7.1 Weigh 5.0 g of tissue into a 250 mL beaker, and record weight in notebook.

7.2 Add approximately 50 g of anhydrous sodium sulfate and macerate sample with a glass rod to remove moisture. Continue to add sodium sulfate as necessary until sample is free flowing.

7.3 Transfer sample and sodium sulfate to the blender.

7.4 Add 150 mL of petroleum ether (PE) to blender and blend for two minutes at high speed.

7.5 Decant the PE to a Buchner Funnel fitted with the #42 Whateman filter paper. Use vacuum to expedite the filtration process.

7.6 Repeat steps 7.4 and 7.5 using 100 mL PE.

7.7 Preconcentrate the filtrate to 25 mL on a steam bath. Quantitively transfer the 25 mL into a preweighed aluminum dish.

7.8 Evaporate the PE extract on a steam bath, dry in a oven at 103 ^{o}C, store in a desiccator, and reweigh the aluminum dish with the lipid material.

8.0 Analytical Procedure

8.1 Calculation of Per Cent Lipid.

$$\% \text{ Lipid} = \frac{\text{Wt of Al dish with lipid (g)} - \text{wt of Al dish (g)} \times 100}{\text{Weight of sample (g)}}$$

9.0 Quality Control

9.1 All quality control data should be maintained and available for easy reference.

9.2 Analyze at least one blank per batch of samples. See Section 3.0.

9.3 Analyze one duplicate sample for every twenty samples.

9.4 The analytical balance shall be serviced by a qualified service engineer every twelve to twenty-four months.

10.0 Method Performance

10.1 Laboratory duplicates.

# of pairs	Relative Standard Deviation
10	68.3% Confidence limit- 6.4% 95.5% Confidence limit- 9.0% 99.9% Confidence limit-11.6%

11.0 References

11.1 U.S Food and Drug Administration 1970b Method of Analysis. AOAC-Eleventh Edition PAM. Vol. 1, Section 160.

APPENDIX I

EXAMPLE PROCEDURE FOR ANALYSIS OF CADMIUM BY GRAPHITE FURNACE ATOMIC ABSORPTION (GFAA) SPECTROMETRY

[From: State of California. 1990. Laboratory Quality Assurance Program Plan. Department of Fish and Game, Environmental Services Division. March.]

trelesop.dbc

METHOD TRELEDIG

DIGESTION AND ANALYSIS OF TRACE ELEMENTS IN TISSUES BY FLAME AAS AND GRAPHITE FURNACE AAS

1.0 SCOPE AND APPLICATION

1.1 This procedure utilizes an open tube nitric acid digestion for the determination of: aluminum (Al); cadmium (Cd); chromium (Cr); copper (Cu); lead (Pb); manganese (Mn); nickel (Ni); silver (Ag); and zinc (Zn); in whole fish, fish liver, and mussel tissues by flame (FAAS) and graphite furnace (GFAAS) atomic absorption spectrophotometry.

2.0 SUMMARY OF METHOD

2.1 Samples are prepared for analysis by digesting the tissue with concentrated nitric acid in a glass tube inserted in a heating block at elevated temperature. Samples are refluxed for 2-3 hours or until no more nitrogen oxides (reddish brown vapors) are observed in the tubes. The liquid digestate is then evaporated to about 0.5 ml, to remove most of the acid, and then is diluted with 1.0% nitric acid to a final volume of 40.0 ml.

2.2 Whole Fish and Fish Liver

Digestates of whole fish and fish liver are analyzed first by graphite furnace atomic absorption spectrophotometry (GFAAS) on a Perkin-Elmer Model 3030 with Zeeman background correction for Cd, Ag, Pb, Cr, Ni, and Cu. The samples are then analyzed by flame atomic absorption spectrophotometry on a Varian Spectra 300 with deuterium arc background correction for Cu, Zn, and any of the trace elements analyzed by GFAAS present in the samples at a high enough concentration to be detected by flame AAS.

2.3 Mussels

Mussel tissue digestates are analyzed by GFAAS on a Perkin-Elmer Model 3030 Zeeman for Pb, Cr, Ag, and Ni. The samples are then analyzed by flame AAS on a Perkin-Elmer Model 2280 for Cd, Cu, Mn, Zn, and Al.

2.4 The detection limits for this method are as follows:

Whole Fish and Fish Liver	ug/g (ppm) wet*
Cadmium	0.01
Silver	0.01
Lead	0.1
Chromium	0.02
Nickel	0.1
Copper	0.02
Zinc	0.05

* based on 1.0 g sample weight and final volume of 40 ml.

Mussels	ug/g (ppm) dry*
Aluminum	1.0
Cadmium	0.01
Chromium	0.02
Copper	0.02
Lead	0.1
Manganese	0.1
Nickel	0.1
Silver	0.01
Zinc	0.05

* based on 3.0 g sample weight and final volume of 20 mL.

3.0 INTERFERENCES

3.1 Sample Digestion

3.1.1 Tissue samples can cause various problems especially with GFAAS due to the complex matrices involved. A fairly rigorous digestion is needed to remove as much of the sample matrix as possible. The matrix problems can also be addressed by using standard reference materials of similar matrix to the sample and by using the method of standard additions.

3.1.2 Special care must be used in selecting the acid used for digestion. Only redistilled HNO_3 should be used because other reagent grade acids are frequently contaminated with trace levels of metals, especially chromium. Prior to use all acids used in the digestion should be checked for contamination.

3.2 Direct aspiration flame AAS

3.2.1 The most troublesome type of interference in atomic absorption spectrophotometry is usually termed "chemical" and is caused by lack of absorption of atoms bound in molecular combination in the flame.

This phenomenon can occur when the flame is not sufficiently hot to dissociate the molecule, as in the case of phosphate interference with magnesium, or when the dissociated atom is immediately oxidized to a compound that will not dissociate further at the temperature of the flame. The addition of lanthanum will overcome phosphate interference in magnesium, calcium, and barium determinations. Similarly, silica interference in the determination of manganese can be eliminated by the addition of calcium.

3.2.2 Chemical interferences may also be eliminated by separating the metal from the interfering material. Although complexing agents are employed primarily to increase the sensitivity of the analysis, they may also be used to eliminate or reduce interferences.

3.2.3 The presence of high dissolved solids in the sample may result in an interference from nonatomic absorbance such as light scattering. If background correction is not available, a nonabsorbing wavelength should be used. Preferably, samples containing high solids should be extracted.

3.2.4 Ionization interferences occur when the flame temperature is sufficiently high to generate the removal of an electron from a neutral atom, giving a positively charged ion. This type of interference can generally be controlled by the addition, to both standard and sample solutions, of a large excess (1,000 mg/L) of an easily ionized element such as K, Na, Li, or Cs.

3.2.5 Spectral interference can occur when an absorbing wavelength of an element present in the sample but not being determined falls within the width of the absorption line of the element of interest. The results of the determination will then be erroneously high, due to the contribution of the interfering element to the atomic absorption signal. Interference can also occur when resonant energy from another element in a multielement lamp, or from a metal impurity in the lamp cathode, falls within the bandpass of the slit setting when that other metal is present in the sample. This type of interference may sometimes be reduced by narrowing the slit width.

3.2.6 Samples and standards should be monitored for viscosity differences that may alter the aspiration rate.

3.2.7 Some sample solutions may have solids suspended in them from incomplete digestion. These solids can plug the nebulizer tubing and slow or stop the aspiration of sample.

3.2.8 All metals are not equally stable in the digestate, especially if it contains only HNO_3, not HNO_3 and HCl. The digestate should be analyzed as soon as possible, with preference given to Ag, Cd, and Pb.

3.3 Furnace procedure

3.3.1 Although the problem of oxide formation is greatly reduced with furnace procedures because atomization occurs in an inert atmosphere, the technique is still subject to chemical interferences. The composition of the sample matrix can have a major effect on the analysis. It is those effects which must be determined and taken into consideration in the analysis of each different matrix encountered. To help verify the absence of matrix or chemical interference, the serial dilution technique (see Paragraph 9.7) may be used. Those samples which indicate the presence of interference should be treated in one or more of the following ways:

1. Successively dilute and reanalyze the samples to eliminate interferences.

2. Modify the sample matrix either to remove interferences or to stabilize the analyte. Examples are the addition of ammonium nitrate to remove alkali chlorides and the addition of ammonium phosphate to retain cadmium. The mixing of hydrogen with the inert purge gas has also been used to suppress chemical interference. The hydrogen acts as a reducing agent and aids in molecular dissociation.

3. Analyze the sample by method of standard additions while noticing the precautions and limitations of its use (see Paragraph 9.8).

3.3.2 Gases generated in the furnace during atomization ion may have molecular absorption bands encompassing the analytical wavelength. When this occurs, use either background correction or choose an alternate wavelength. Background correction may also compensate for nonspecific broad-band absorption interference.

3.3.3 Continuum background correction cannot correct for all types of background interference. When the background interference cannot be compenstated for, chemically remove the analyte or use an alternate form of background correction, e.g., Zeeman background correction.

3.3.4 Interference from a smoke-producing sample matrix can sometimes be reduced by extending the charring time at a higher temperature or utilizing an ashing cycle in the presence of air. Care must be taken, however, to prevent loss of the analyte.

3.3.5 Samples containing large amounts of organic materials should be oxidized by conventional acid digestion before being placed in the furnace. In this way, broad-band absorption will be minimized.

3.3.6 Anion interference studies in the graphite furnace indicate that, under conditions other than isothermal, the nitrate anion is preferred. Therefore, nitric acid is preferable for any digestion or solubilization step. If another acid in addition to HNO_3 is required, a minimum amount should be used. This applies particularly to hydrochloric and, to a lesser extent, to sulfuric and phosphoric acids.

3.3.7 Carbide formation resulting from the chemical environment of the furnace has been observed. Molybdenum may be cited as an example. When carbides form, the metal is released very slowly from the resulting metal carbide as atomization continues. Molybdenum may require 30 sec or more atomization time before the signal returns to baseline levels. Carbide formation is greatly reduced and the sensitivity increased with the use of pyrolytically coated graphite. Elements that readily form carbides are: Ba, Mo, Ni, and V.

3.3.8 For comments on spectral interference, see Paragraph 3.1.4.

3.3.9 Cross-contamination and contamination of the sample can be major sources of error because of the extreme sensitivities achieved with the furnace. The sample preparation work area should be kept scrupulously clean. All glassware should be cleaned as directed in Paragraphs 4.11 and 7.1. Pipet tips are a frequent source of contamination. If suspected, they should be acid soaked with 1:5 HNO_3 and rinsed thoroughly with tap and deionized (Type II) water. The use of a better grade of pipet tip can greatly reduce this problem. Special attention should be given to reagent blanks in both analysis and in the correction of analytical results. Lastly, pyrolytic graphite, because of the production process and handling, can become contaminated. As many as five to ten high-temperature burns may be required to clean the tube before use.

4.0 APPARATUS AND MATERIALS

4.1 Atomic absorption spectrophotometer

4.1.1 FAAS

Varian Spectra 300 with data system and Mark VI burners for air- and nitrous oxide-acetylene flames or a Perkin-Elmer Model 2280 spectrophotometer with deuterium arc background corrector and digital display.

4.1.2 GFAAS

Perkin-Elmer Model 3030 spectrophotometer with Zeeman effect background correction, HGA-60 furnace controller, AS-60 autosampler, EDL power supply, and PR-100 printer.

4.2 Hollow cathode lamps: Single-element lamps are used and are preferred over multi-element lamps which may be used occasionally. Electrodless discharge lamps may also be used for certain elements.

4.3 Graphite furnace parts:

	Perkin-Elmer P/N
Pyrolytic coated graphite tubes	091504
Pyrolytic coated graphite tubes(grooved)	109322
L'vov platforms	109324

4.4 Pressure-reducing valves: The supplies of fuel and oxidant should be maintained at pressures somewhat higher than the controlled operating pressure of the instrument by suitable valves. (See manufacterer's specifications.)

4.5 Block Thermostat: Liebisch model 2102 with model 2279 programmable controller.

4.6 Digestion Tubes: 25x200 mm glass test tubes with beaded rim.

4.7 Polyethylene caps: Wheaton part No. 227720 caps for BOD bottles, with bottom ring removed.

4.8 Polyethylene (HDPE) bottles: Nalgene part No.2002-002, 2 oz., 60 mL polyethylene (HDPE) bottles.

4.9 Polyethylene cups for AS-60 autosampler: Evergreen part No. 127-0018-020 (case of 1000).

4.10 Pipetors: Preferably all plastic/teflon of various sizes from 100-1000 uL with polyethylene tips. Do not use yellow pipet tips, they are commonly contaminated with cadmium.

4.11 Glassware: All glassware, polypropylene, polyethylene, and Teflon containers, excluding HDPE sample bottles and polyethylene cups for AS-60 autosampler, should be washed in the following sequence: detergent, tap water, 1:1 nitric acid, tap water, 1:1 hydrochloric acid, tap water, and Type II water. (Chromic acid should not be used as a cleaning agent for glassware if chromium is to be included in the analytical scheme.) If it can be documented through an active analytical quality control program using spiked samples and reagent blanks that certain steps in the cleaning procedure are not required for routine samples, those steps may be eliminated from the procedure.

5.0 REAGENTS

5.1 Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

5.2 Type II water (ASTM D1193): Use Type II water for the preparation of all reagents and calibration standards and as dilution water.

5.3 Concentrated nitric acid (HNO_3): Use a spectrograde acid certified for AA use. For graphite furnace work all acids should be checked using reagent blanks for all of the analytes to be reported. Prepare a 1:1 dilution with Type II water by adding the concentrated acid to an equal volume of water.

5.4 Hydrochloric acid (HCl, 1:1): Use a spectrograde acid certified for AA use. Prepare a 1:1 dilution with Type II water by adding the concentrated acid to an equal volume of water.

5.5 Fuel and oxidant: Commercial grade acetylene is generally acceptable. Air may be supplied from a compressed air line, a laboratory compressor, or a cylinder of compressed air. Reagent grade nitrous oxide is also required for certain determinations. Standard commercially available argon and nitrogen are required for furnace work.

5.6 Stock standard metal solutions: Stock standard solutions are prepared from high purity metals, oxides, or nonhygroscopic reagent-grade salts using Type II water and redistilled nitric or hydrochloric acids. (See

individual methods for specific instructions.) Sulfuric or phosphoric acids should be avoided as they produce an adverse effect on many elements. The stock solutions are prepared at concentrations of 1,000 mg of the metal per liter. Commercially available standard solutions may also be used if standards from two different vendors are checked against one another and are in agreement. Standards available from the U.S. National Institute of Standards and Technology (NIST) are also acceptable and do not have to be verified. Where the sample viscosity, surface tension, and components cannot be accurately matched with standards, the method of standard additions may be used (see Paragraph 9.8).

5.7 Calibration standards: For those instruments which do not read out directly in concentration, a calibration curve is prepared to cover the appropriate concentration range. Usually, this means the preparation of standards which produce an absorbance of 0.0 to 0.7. Calibration standards are prepared by diluting the stock metal solutions at the time of analysis. For best results, calibration standards should be prepared fresh each time a batch of samples is analyzed or demonstrate that the standards are still good by comparing the standard absorbances with those of SRM 1643b "Trace Elements in Water". The expiration date on the SRM 1643b should be used to validate its use for this purpose. If the standards cannot be validated using the SRM 1643b then the following can be used as a guideline:

less than 0.1 ppm	- prepare daily
0.1 to 1 ppm	- prepare weekly
1.0 to 10 ppm	- prepare monthly
10 to 100 ppm	- prepare quarterly
100+ ppm	- prepare yearly (at a minimum)

Prepare a blank and at least three calibration standards in graduated amounts in the appropriate range of the linear part of the curve. The calibration standards should be prepared using the same type of acid or combination of acids and at the same concentration as will result in the samples following processing, 1% HNO_3 (14 mL concentrated HNO_3/L) for tissues. Beginning with the blank and working toward the highest standard, aspirate the solutions and record the readings. Repeat the operation with both the calibration standards and the samples a sufficient number (minimum of two) of times to secure a reliable average reading for each solution. Calibration standards for furnace procedures should be prepared as described on the individual sheets for that metal.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 All samples must have been collected using a sampling plan that addresses the considerations discussed in this procedure.

6.2 All sample containers must be prewashed with detergents, acids, and Type II water. Plastic, glass, HDPE, and Teflon containers are suitable. Only new HDPE bottles (Nalgene part No. 2002-002) will be used for sample digestates and should not be washed with soap and water. These HDPE bottles should be cleaned and checked according to 7.1.2 and 7.2.

6.3 Samples shall be double-wrapped in aluminum foil or placed in polyethylene bags (do not use polyethylene bags if samples are to be analyzed for organics) and frozen as soon as possible after collection and remain frozen until dissection. After dissection and homogenization the samples should be refrozen until analysis.

7.0 PROCEDURE FOR SAMPLE PREPARATION

7.1 Preparation of glassware

7.1.1 Digestion tubes:

1. Tubes should first be cleaned using the procedure described in section 4.11.

2. Prior to starting digestion add 10 mL 6N nitric acid to tube and fill to the top with with Type II water. Cover with polyethylene cap and leave overnight.

3. The next day discard the acid solution and rinse the tube and the cap three times with Type II water. The tubes are now ready to be checked.

7.1.2 Polyethylene (HDPE) bottles:

1. Fill polyethylene (HDPE) bottles with 6 N nitric acid, cap, and allow to soak for at least 48 hours.

2. Prior to using for digestion tube blanks, empty the bottles, rinse with 0.1 N nitric acid and with Type II water.

7.1.3 Polyethylene digestion tube caps: Store caps in a large polyethylene bottle filled with 1 N nitric acid. Remove from acid bath and rinse with Type II water just prior to use.

7.1.4 Teflon policemen:

1. Policemen should have previously been washed with soap and tap water after the last use.

2. Add fresh solution of 1 N HNO_3 to a milk dilution bottle.

3. Soak Teflon policeman in the acid solution.

4. Rinse the policeman with Type II water.

7.2 Preparation of digestion tube and polyethylene (HDPE) bottle blanks:

1. Each digestion tube should have a corresponding polyethylene (HDPE) bottle with the same number or letter. Each tube/bottle pair should have a unique number or letter to distinguish it from all other tube/bottle pairs.

2. Add 1 mL of redistilled nitric acid to each tube and place in aluminum block with temperature set at 160°C. Heat tubes for about 2 hours. Remove tubes from block and allow to cool. Fill tubes to 20 mL with Type II water and use vortex mixer on slow speed to mix. Transfer solution to pre-cleaned 60 mL Nalgene polyethylene (HDPE) bottle, re-fill tube to 20 mL with Type II water, mix on a vortex mixer on slow speed, and combine with solution in polyethylene (HDPE) bottle.

3. Analyze solution by GFAAS for elements to be analyzed in samples by GFAAS using the procedure described in 8.5.

4. Rinse digestion tubes with 1% redistilled nitric acid and store with caps covering tubes.

5. After analyzing the tube/bottle blanks remove any tube/bottle pairs that are contaminated with any of the elements to be analyzed in the samples. These tube/bottle sets should be taken through the cleaning procedure and rechecked at a later date. It is good policy to clean and check approximately 20% more tube/bottle pairs than will be needed for the current set of samples.

7.3 Weighing Procedures

7.3.1 Prior to weighing samples, prepare lab notebook (i.e. list sample numbers and any special instructions).

7.3.2 Record tube/bottle pair identification number next to the blank or sample identification number as the samples are weighed.

7.3.3 Preparation of method blanks: Prepare two blanks for each set of samples.

1. Add 5 mL of concentrated redistilled HNO_3 to digestion tube, cover with polyethylene cap, and place in heating block.

7.3.4 Standard reference material (SRM): Use reference materials with matrix as close as possible to that of the samples to be analyzed. Weigh at least two SRM's for each set of samples.

1. Weigh 0.25 ± 0.05 g of SRM into tared digestion tube. Be careful to place the sample on the bottom of the tube and not on the sides.

2. Record weight of sample in notebook to at least two places to the right of the decimal point.

3. Add 5 mL of concentrated redistilled HNO_3 to the tube, cover with polyethylene cap, and place in the heater block.

7.3.5 Fish liver samples:

1. Using a clean Teflon policeman, mix liver sample thoroughly.

2. Weigh 1.00 ± 0.10 g of fish liver into a tared digestion tube. Be careful to place the sample on the bottom of the tube and not on the sides.

3. Record weight of sample in notebook to at least two places to the right of the decimal point.

4. Add 5 mL of concentrated redistilled HNO_3 to the tube, cover with polyethylene cap, and place in the heater block.

7.3.6 Whole fish samples:
(Whole fish samples are homogenized using fish:water [1:1]).

1. Using a clean Teflon policeman, mix whole fish thoroughly. Some of the whole fish samples are very watery and should be shaken to thoroughly mix.

2. Weigh 2.00 ± 0.10 g of whole fish homogenate into a tared digestion tube. Be careful to place the sample in the bottom of the tube and not on the sides.

3. Record weight of sample in notebook to at least two places to the right of the decimal point.

4. Add 5 mL of concentrated redistilled HNO_3, cover with polyethylene cap, and place in heater block.

7.3.7 Mussel tissue:

1. Using a clean Teflon policeman, mix mussel sample thoroughly.

2. Weigh 3.00±0.10 g of mussel tissue into a tared digestion tube. Be careful to place the sample into the bottom of the tube and not on the sides.

3. Record weight of sample in notebook to at least two places to the right of the decimal point.

4. Add 5 mL of concentrated redistilled HNO_3, cover with polyethylene cap, and place in heater block.

7.4 Sample digestion procedure

7.4.1 Program temperature of heating block to 70°C at a rate of 600°C/hour. Leave heating block at 70°C for at least one hour.

7.4.2 Program temperature of heating block to 160°C at a rate of 300°C/hour. Leave block at 160°C until no more NO_x (reddish brown fumes) are observed in the tube (at least 2 hours).

7.4.3 After the digestion is completed remove the polyethylene caps from the tubes to allow the acid digestate to evaporate. Evaporate the solution until about 0.5 mL remains in the tube. It may be necessary to elevate the temperature of the block to 170°C.

7.4.4 After the evaporation is completed remove the tubes from the block and allow them to cool. Dilute the remaining digestate with 1% redistilled HNO_3 to the 20 mL mark on the digestion tube. Mix the sample on the vortex mixer and transfer the solution to the 60 mL polyethylene (LPE) bottle. Immediatly add another 20 mL of 1% HNO_3 to the tube, mix on the vortex mixer, and add to the LPE bottle and mix thoroughly. The sample is now ready for analysis.

8.0 ANALYTICAL PROCEDURE

8.1 Sample digestion and dilution steps should result in an extract that is clear and free of undissolved solid materials. If the sample solution is cloudy or has solid materials suspended in solution at the time of analysis, it should be noted in the laboratory note-

book under a "comments" column.

8.2 Samples should be analyzed for silver, cadmium, and lead within 48 hours of digestion. The remaining elements should be analyzed as soon as possible.

8.3 All graphite furnace analyses should be done prior to the flame analyses to prevent cross-contamination between bottles from the aspirator tubing.

8.4 Direct aspiration (flame) procedure

8.4.1 Differences between the various makes and models of atomic absorption spectrophotometers prevent the formulation of detailed instructions applicable to every instrument from being included in this document. Good laboratory practice is to have detailed instructions for the operation of each instrument kept with the instrument for the analyst to use during operation. These instructions should follow the manufacturer's operating instructions for a particular instrument. In general, after choosing the proper lamp for the analysis, allow the lamp to warm up for a minimum of 15 minutes, unless operated in a double-beam mode. During this period, align the instrument, position the monochronometer at the correct wavelength, select the proper monochronometer slit width, and adjust the current according to the manufacturer's recommendation. Some or all of these parameters may be done by the instrument automatically. Subsequently, light the flame and regulate the flow of fuel and oxidant. Adjust the burner and nebulizer flow rate for maximum percent absorption and stability. Balance the photometer. Run a series of standards of the element under analysis. Construct a calibration curve by plotting the concentrations of the standards against absorbances or have the data system construct it. Aspirate the samples and determine the concentrations either directly or from the calibration curve. Standards must be run each time a sample or series of samples is run.

8.5 Furnace procedure

8.5.1 Furnace devices (flameless atomization) are the most useful means of extending detection limits. Because of differences between various makes and models instruments, no detailed operating instructions can be given for each instrument in this document. Detailed operating instructions following the instructions provided by the manufacturer of each instrument are kept with each instrument for the analyst to use during the analysis.

8.5.2 Background correction is important when using flameless atomization, especially below 350 nm. Certain samples, when atomized, may absorb or scatter light from the lamp. This can be caused by the presence of gaseous molecular species, salt particles, or smoke in the sample beam. If no correction is made, sample absorbance will be erroneously high. Zeeman background correction is effective in overcoming composition or structured background interferences. It is particularly useful when analyzing for As in the presence of Al and when analyzing for Se in the presence of Fe.

8.5.3 Memory effects occur when the analyte is not totally volatilized during atomization. This condition depends on several factors: volatility of the element and its chemical form, whether pyrolytic graphite is used, the rate of atomization, and furnace design. This situation is detected through blank burns. The tube should be cleaned by operating the furnace at full power for the required time period, as needed, at regular intervals during the series of determinations.

8.5.4 Inject a measured microliter aliquot of sample into the furnace and atomize. If the concentration found is greater than the highest standard, the sample should be diluted in the same acid matrix and reanalyzed. The use of multiple injections can improve accuracy and help detect furnace pipetting errors.

8.5.5 To verify the absence of interference, follow the serial dilution procedure given in Section 9.7.

8.5.6 A check standard should be run after approximately every 10 sample injections. Standards are run in part to monitor the life and performance of the graphite tube. Lack of reproducibility or significant change in the signal for the standard indicates that the tube should be replaced. Tube life depends on sample matrix and atomization temperature. A conservative estimate would be that a tube will last at least 50 firings. A pyrolytic coating will extend that estimated life by a factor of three.

8.6 Calculation

8.6.1 For determination of metal concentration by direct aspiration and furnace: Read the metal value in mg/L from the calibration curve or directly from the read-out system of the instrument.

8.6.2 Different injection volumes must not be used for samples and standards. Instead, the sample should be diluted and the same size injection volume be used

for both samples and standards. If dilution of sample was required:

$$\text{mg/L metal in sample} = A \left(\frac{C+B}{C}\right)$$

where:

A = mg/L of metal in diluted aliquot from calibration curve.
B = Acid blank matrix used for dilution, mL.
C = Sample aliquot, mL.

8.6.3 For solid samples, report all concentrations as ug/g based on wet. Hence:

$$\text{ug metal g sample} = \frac{AxV}{W}$$

where:

A = mg/L of metal in processed sample from calibration curve.
V = Final volume of the processed sample, mL.
W = Weight of sample, grams.

9.0 QUALITY CONTROL

9.1 All quality control data should be maintained and available for easy reference or inspection.

9.2 A calibration curve must be prepared at least twice each day (one at the beginning and one at the end of each set of samples) for each element analyzed with a minimum of a reagent blank and three standards. The calibration curve should be verified by the use of at least a reagent blank and one quality control check standard at or near the mid-range every 15 samples. Checks throughout the day must be within 20% of the original curve.

9.3 If 20 or more samples per day are analyzed, the working standard curve must be verified by running an additional standard at or near the midrange every 10 samples. Checks must be within $\pm$ 20% of the true value.

9.4 Employ a minimum of one reagent blank per sample batch to determine if contamination or any memory effects are occuring.

9.5 At least one spiked matrix and one replicate sample should be run every 10 samples or per analytical batch, whichever is greater. At least one spiked replicate sample should also be run with each matrix type to verify precision of the method.

9.6 Where the sample matrix is so complex that viscosity, surface tension, and components cannot be accurately matched with standards, the method of standard addition may be used (see Step 9.8 below).

9.7 Serial dilution - Withdraw from the sample two equal aliquots. To one of the aliquots add a known amount of analyte and dilute both aliquots to the same predetermined volume. (The dilution volume should be based on the analysis of the undiluted sample. Preferably, the dilution should be 1:4, while keeping in mind that the diluted value should be at least 5 times the instrument detection limit. Under no circumstances should the dilution be less than 1:1.) The diluted aliquots should then be analyzed, and the unspiked results, multiplied by the dilution factor, should be compared to the original determination. Agreement of the results (within 10%) indicates the absence of interference. Comparison of the actual signal from the spike with the expected response from the analyte in an aqueous standard should help confirm the finding from the dilution analysis.

9.8 Method of standard additions - The standard addition technique involves adding known amounts of standard to one or more aliquots of the processed sample solution. This technique compensates for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interferences which cause a baseline shift.

9.8.1 In the simplest version of this technique is the single addition method, in which two identical aliquots of the sample solution, each of volume Vx, are taken. To the first (labeled A) is added a known volume Vs of a standard analyte solution of concentration Cs. To the second aliquot (labeled B) is added the same volume Vs of the solvent. The analytical signals of A and B are measured and corrected for non-analyte signals. The unknown sample concentration Cx is calculated:

$$Cx = S_B V_S C_S / (S_A - S_B) V_X$$

where S_A and S_B are the analytical signals (corrected for the blank) of solutions A and B, respectively. V_S and C_S should be chosen so that S_A is roughly twice S_B on the average, avoiding excess dilution of the sample. If a separation or concentration step is used, the additions are best made first and carried through the entire procedure.

9.8.2 Improved results can be obtained by employing a series of standard additions. Equal volumes of the

sample are added to a series of standard solutions containing different known quantities of the test analyte, all diluted to the same volume. For example, addition 1 should be prepared so that the resulting concentration is approximately 50 percent of the expected sample absorbance. Additions 2 and 3 should be prepared so that the concentrations are approximately 100 and 150 percent of the expected sample absorbances, respecively. The absorbance of each solution is determined and then plotted on the vertical axis (ordinate) of a graph, with the concentrations of the known standards plotted on the horizontal axis (abscissa). When the resulting line is extrapolated back to zero absorbance, the point of interception of the abscissa is the concentration of the unknown. The abscissa on the left of the ordinate is scaled the same as on the right side, but in the opposite direction from the ordinate. An example of a plot so obtained is shown in Figure 1. Some of the newer instruments (Perkin-Elmer 3030) have standard addition software built into the data system. The AS-60 autosampler on the Perkin-Elmer 3030 will automatically make the standard additions in the graphite tube. All of the calculations for the standard additions technique are done for the operator by the instrument.

9.8.3 For the results of this technique to be valid, the following limitations must be taken into consideration:

1. The absorbance plot of sample and standards must be linear over the concentration range of concern. For best results, the slope of the plot should be nearly the same as the slope of the standard curve. If the slope of the standard addition plot is significantly different (greater than 20%) caution should be exercised.

2. The effect of the interference should not vary as the ratio of analyte concentration to sample matrix changes, and the standard addition should respond in a similar manner as the analyte.

3. The determination must be free of spectral interference and corrected for nonspecific background interference.

9.9 Dilute samples if they are more concentrated than the highest standard or if they fall on the plateau of a calibration curve.

9.10 Duplicates, spiked samples, standard reference materials, and check standards should be routinely analyzed.

9.11 Atomic absorption spectrophotometers (AAS) should be serviced on a regular basis by qualified technicians as part of a regularly scheduled preventive maintenance program.

9.12 A log book should be kept for each AAS that includes: Standard absorbances, photomultiplier voltages, detection limits, maintenance information, and any problems that might occur each time the instrument is used.

10.0 METHOD PERFORMANCE

10.0 See individual methods.

11.0 REFERENCES

1. U.S. Environmental Protection Agency, Test Methods for Evaluating Solid Waste, SW-486 Third Ed., Revision 1, December 1987.

METHOD CDGFAA

CADMIUM (ATOMIC ABSORPTION, FURNACE TECHNIQUE)

1.0 SCOPE AND APPLICATION

1.1 See Section 1.0 of Method TRELEDIG.

2.0 SUMMARY OF METHOD

2.1 See Section 2.0 of Method TRELEDIG.

3.0 INTERFERENCES

3.1 See section 3.0 of Method TRELEDIG if interferences are suspected.

3.2 In addition to the normal interferences experienced during graphite furnace analysis, cadmium analysis can suffer from severe nonspecific absorption and light scattering caused by matrix components during atomization. Simultaneous background correction is required to avoid erroneously high results.

3.3 Cadmium is the most volatile element commonly determined by GFAA. Simple aqueous solutions of Cd produce ashing losses starting at 300°C or 400°C. With the addition of monobasic or dibasic ammonium phosphate, Cd is not lost until about 600°C. If in addition, $Mg(NO_3)_2$ is added to the phosphate, Cd is not lost until 900°C.

3.4 Contamination:

3.4.1 Many plastic tips (yellow) contain cadmium. Use "cadmium free" tips.

3.4.2 The pouring surfaces of glass and plastic ware may be contaminated with cadmium. When pouring solutions to be analyzed by graphite furnace pour a small amount and discard to rinse the pouring surface prior collecting the liquid.

4.0 APPARATUS AND MATERIALS

4.1 For basic apparatus, see Section 4.0 of Method TRELEDIG.

4.2 Instrument parameters (general):

4.2.1 **Drying time and temp:** 60 sec at 120°C.
4.2.2 **Ashing time and temp:** 45 sec at 900°C.
4.2.3 **Atomizing time and temp:** 5 sec at 2500°C.
4.2.4 **Purge gas:** Argon.
4.2.5 **Wavelength:** 228.8 nm.
4.2.6 **Background correction:** Required.
4.2.7 Other operating parameters should be set as specified by the particular instrument manufacturer.
NOTE: The above concentration values and instrument conditions are for a Perkin-Elmer HGA-600, based on a 10-uL injection, stop internal gas flow during atomization, pyrolytic coated graphite tube with L'vov platform.

5.0 REAGENTS

5.1 See section 5.0 of Method TRELEDIG.

5.2 Preparation of standards:

5.2.1 **Stock solution:** Dissolve 1.000 g cadmium metal (analytical reagent grade) in 20 mL of 1:1 HNO_3 and dilute to 1 liter with Type II water. Alternative, procure a standard from a commercial supplier. Analytical standards prepared in the laboratory or purchased from a commercial vendor should be verified by comparison with a second standard. Standards purchased from the U.S. Institute of Standards and Technology (NIST) are certified and do not need to be verified using standards from a second source.

1.0 Standard suppliers and part numbers:

National Institute of Standards and Technology (NIST) Part No. SRM 3108 (Cd-10 mg/mL in 10% HNO_3).

5.2.2 Prepare dilutions of the solution to be used as calibration standards at the time of analysis. The calibration standards should be prepared using the same type of acid and at the same concentration as will result in the sample to be analyzed after processing (1.0% HNO_3).

5.2.3 **Ammonium phosphate-magnesium nitrate solution:** Dissolve 2.42 g of $NH_4H_2PO_4$ and 0.173 g of $Mg(NO_3)_2 \cdot H_2O$ in 100 mL of Type II water. A 10-uL injection of this solution contains 200 ug PO_4 and 10 ug $Mg(NO_3)_2$.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 See Chapter Three, Section 3.1.3, Sample Handling and Preservation.

7.0 PROCEDURE

7.1 Sample preparation: The procedures for preparation of the sample are given in Method TRELEDIG.

7.2 See Method TRELEDIG Paragraph 8.5, Furnace procedure.

8.0 QUALITY CONTROL

8.1 See section 9.0 of Method TRELEDIG.

9.0 METHOD PERFORMANCE

9.1 The performance characteristics for an aqueous sample free of interferences are:

Optimum concentration range: 0.5-10 ug/L (ppb).
Detection limit: 0.1 ug/L (ppb)

9.2 The performance characteristics for a tissue sample free of interferences are:

Optimum concentration range: 0.02 mg/kg (ppm)
Detection limit: 0.01 mg/kg (ppm).

9.3 Precision and accuracy data:

9.3.1 Duplicate data (fish liver-mg/kg):

Duplicates		x	s	%RSD
0.04	0.03	0.035	0.007	20.3%
0.06	0.07	0.065	0.007	10.9%
0.04	0.04	0.04	0.000	0.0%
0.99	1.00	0.995	0.007	0.7%
0.009	0.011	0.010	0.0008	8.0%
0.008	0.001	0.0045	0.0049	109. %
0.052	0.053	0.0525	0.0007	1.3%

9.3.2 Procedural blanks:

n = 11 x = 0.0094 s = 0.0223

9.3.3 Standard Reference Materials:

SRM	cert val(mg/kg)	matrix	n	x	s
DOLT-1	(4.18±0.28)	liver	4	5.81	0.31
DORM-1	(0.086±0.012)	muscle	15	0.098	0.017
NIES#6	(0.82±0.03)	mussel	5	0.96	0.14

9.3.4 Recovery data from spiked samples (fish liver):

Level (ppm)	%Recovery
0.005	100%
0.025	96.0%

10.0 REFERENCES

1. U.S. Environmental Protection Agency, Test Methods for Evaluating Solid Waste, SW-486 Third Ed., Revision 1, December 1987.

2. Slavin, W., G.R. Carnick, D.C. Manning, and E. Pruszkowska, Perkin-Elmer Corp., Recent Experiences with the Stabilized Temperature Platform Furnace and Zeeman Background Correction, Atomic Spectroscopy, Vol. 4, No. 3, 69, 1983.

APPENDIX J

EXAMPLE QA/QC PROCEDURES AND REQUIREMENTS FOR ANALYSIS OF ORGANIC COMPOUNDS

[From: Puget Sound Estuary Program. 1990 (revised). Recommended Guidelines for Measuring Organic Compounds in Puget Sound Sediments and Tissue Samples. Prepared by PTI Environmental Services, Bellevue, WA. In: Recommended Protocols and Guidelines for Measuring Selected Environmental Variables in Puget Sound, U.S. EPA, Region 10, Seattle, WA. (Looseleaf)]

QA/QC PROCEDURES AND REQUIREMENTS

QA/QC requirements are the foundation of this guidance document because they provide information necessary to assess the comparability of data generated by different laboratories or different analytical procedures. The following QA/QC variables are discussed in the order noted:

- Initial and ongoing calibrations (used to establish and verify the quantification technique)
- Surrogate spike compounds (used to evaluate the analytical recovery of each sample)
- Method blanks and field blanks (used to evaluate possible sources of laboratory and field contamination)
- Reference materials (used to evaluate laboratory accuracy)
- Matrix spikes (used to evaluate the effect of sample matrix on the compound of interest)
- Spiked method blanks (used as a procedural check to evaluate method performance prior to and during routine analysis of samples) (also called check standards)
- Analytical replicates (used to evaluate precision of the analytical method and instrumentation)
- Field replicates (used to evaluate field variability).

Data for all QA/QC variables should be submitted by the laboratory as part of the data package. Program managers and project coordinators should verify that requested QA/QC data are included in the data package as supporting information for the summary data, and may wish to review key QA/QC data (e.g., analytical replicate data or surrogate spike recoveries). Acceptable limits for these variables are discussed in the following sections and summarized in Tables 6 and 8. A detailed QA/QC review of the entire data package, especially original quantification reports and standard calibration data, should be conducted by a technical expert. Guidelines on laboratory data validation are available in U.S. EPA (1988).

Screening level analyses (see Table 4) should be conducted according to the QA/QC requirements of the most recent EPA CLP program document. The guidance provided in this section is applicable to low parts-per-billion analyses of both sediment and tissue unless specifically noted. Warning limits are numerical criteria that serve to alert data reviewers and users to possible problems within the analytical system. When a warning limit is exceeded, the laboratory is not obligated to halt analyses, but the reported data may be qualified during subsequent QA/QC review. Action limits are numerical criteria that, when exceeded, require specific action by the laboratory before data may be reported. Action limits are intended to serve as contractual controls on laboratory performance. The warning and action limits are summarized in Table 8.

TABLE 8. SUMMARY OF WARNING AND ACTION LIMITS FOR QUALITY CONTROL SAMPLES

Analysis Type[a]	Recommended Warning Limit	Recommended Action Limit[b]
Ongoing calibration	Project manager decision	25 percent of initial calibration
Surrogate spikes	50 percent recovery[c]	Follow EPA CLP guidelines[c]
Method blanks	Exceeds the limit of detection[d]	Exceeds the practical quantification limit[d]
Reference materials	95 percent confidence interval, if certified	Project manager decision
Matrix spikes	50-150 percent	Project manager decision[e]
Spiked method blanks (check standards)	50-150 relative percent difference	Project manager decision
Analytical replicates	35 percent coefficient of variation	50 percent coefficient of variation (or a factor of 2 for duplicates)
Field replicates	Project manager decision	Project manager decision

[a] The definition of each quality control sample is given in the QA/QC Procedures and Requirements section of this report.

[b] Recommendations for corrective action when action limits are exceeded are given in text.

[c] Except when using the isotope dilution technique; see Appendix C for a summary of acceptance limits and recommended corrective action for EPA Method 1625C.

[d] See Table 5.

[e] Zero percent spike recovery requires rejection of data.

CALIBRATION

The procedure used for calibration of analytical instruments can affect the accuracy of analytical results and therefore can be considered an element of QA/QC. Both external standard calibration and internal standard calibration procedures are used for organic analyses. External standard calibration involves the preparation of standard solutions, independent of the samples, that are used to determine the relationship between instrument response and concentration for the substance being measured. Internal standard calibration is a procedure in which the instrument responses from analytes are determined relative to the responses from one or more internal standards added to every sample prior to extraction and sample processing. An ideal internal standard has chemical and physical properties similar to those of the analyte. This latter calibration technique is discussed in the section entitled *Method Calibration Using the Isotope Dilution Techniques*.

Specific criteria for initial and continuing calibrations using the external standard calibration technique are not supplied in this document because of the diversity of methods that might be used. However, it is critical to adhere to the calibration criteria specified in the analytical method being used.

Initial Calibration Using the External Standard Technique

Initial calibration is performed to determine the response of the instrument across a range of concentrations of each analyte of interest. The relationship between response and concentration is often called linearity. Response factors (RF) for analytes relative to standards at various concentrations are established by calibration.

The procedures and requirements in this section are generally for GC/MS determinations and are consistent with the CLP requirements for external standard calibration of analytical instruments.

Frequency—Equipment should be subject to initial calibration at the beginning of the project before any samples are analyzed, after each major equipment disruption, and when ongoing calibration does not meet criteria.

Number of Calibration Points—RF values must be determined for at least three concentration levels (five concentration levels or a five-point calibration, is preferable). The standard concentrations tested should encompass the range of expected sample concentrations. The lowest standard in this curve is analyzed at an on-column concentration equivalent to the PQL for the sample set.

Reporting of results for an additional standard analyzed near the LOD (e.g., a sample concentration equivalent to approximately 1-5 ng on-column for many compounds on GC/MS) is recommended to provide evidence of the ability to report estimated quantities in the low concentration range between the LOD and PQL. The use of this standard in the calibration curve is not

recommended because random error becomes relatively more significant at concentrations approaching the ultimate detection limit of an instrument, and any random error in the determination of the calibration factor becomes a systematic error when used to calculate concentrations of samples.

Warning Limit—Warning limits are determined at the discretion of the project manager.

Action Limit—For most compounds, action limits are based on the variation among the RRF calculated during the initial calibration. The percent relative standard deviation (percent RSD) obtained from the RRF in the initial calibration should not exceed 30 percent.

Corrective Action—If the percent RSD for the RRF exceeds 30 percent, the initial calibration should be repeated. Failure to meet this calibration before analysis of samples may be cause for omitting the data from regional databases.

Report—Initial calibration results within acceptable limits must be verified prior to the analysis of samples. Summary data documenting initial calibration and any episodes requiring recalibration and the corresponding recalibration data should be included with analytical results.

Ongoing Calibration Using the External Standard Technique

The ongoing calibration (single point) is used to check that the original three-point calibration curve continues to be valid.

Frequency—For GC/MS analyses, compare all area counts of the internal standard to those in the standard for the day.

For GC/MS or GC/FID analyses, calibration should be checked at the beginning of each work shift, at least once every 12 hours (or every 10-12 analyses, whichever is more frequent), and after the last sample of each work shift.

For GC/ECD analyses, calibration should be checked at the beginning of each shift, every 6 hours (or every six samples, whichever is less frequent), and after the last sample of each shift.

Warning Limit—Warning limits are determined at the discretion of the project manager.

Action Limit—The RRF determined for specific compounds should meet the following action limits. The RRF determined for PCB and pesticides analyzed with GC/ECD should be within 25 percent of the initial calibration RRF, as specified in EPA CLP protocols. Those semivolatile and

volatile compounds that must meet the ongoing calibration 25 percent control limits per EPA CLP are shown in Table 9.

Corrective Action—If the action limit is not met, the initial three-point calibration will have to be repeated. The last sample analyzed before the standard analysis that failed criteria should then be reanalyzed. The results from the reanalysis should be within 15 percent of the results from the original analysis. (The expected agreement between replicate injections of a complex extract is 15 percent). If the results exceed a 25 percent difference, the instrument is assumed to have been out of control during the original analysis. Reanalysis of samples should progress in reverse order until it is determined that there is <25 percent difference between initial and reanalysis results. In some cases results from reanalysis may exceed a 25 percent difference because of matrix effects. If the next sample reanalyzed meets the 25 percent requirement, evidence exists for assuming a matrix effect. Requirements for additional reanalysis should be at the discretion of the program manager or project coordinator. For GC/MS, monitor the integrated area for the response of all internal standards. Repeat the initial calibration or reanalyze the sample, if the observed area/amount for any internal standard response varies by more than a factor of 2 when compared to the observed area/amount for the response of the same internal standard of the standard mix analyzed at the beginning of the shift.

Report—Samples requiring reanalysis should be identified. Reanalysis results should be provided with the sample results. A discussion of the values causing exceedance of limits and corrective actions taken should also be provided.

Method Calibration Using the Isotope Dilution Technique

The following introduction to calibration using the isotope dilution technique is excerpted from Kirchmer et al. (1986). Isotope dilution mass spectrometry is a type of internal standard calibration and analysis, in which the internal standard is an isotopically labeled analog of the analyte. When added initially to the sample, the internal standard serves to correct for losses during the processing of samples, and to compensate for errors owing to differences in injected volume and unnoticed variations in instrument sensitivity. A stable isotope-labeled analog of the analyte is an ideal internal standard, because its chemical and physical properties can be expected to be almost identical to the analyte, thus assuring negligible differences in extraction, cleanup, and chromatographic properties during sample processing (Watson 1976).

Internal standard calibration requires both a calibration solution for instrument calibration and a spiking solution. The calibration standard is used to determine the relative responses of an analyte and an internal standard, while the spiking solution is used to add a known amount of internal standard to each sample prior to extraction, processing, and analysis. EPA Methods 1624C and 1625C contain isotope dilution calibration and analysis procedures. In these procedures, the isotopically labeled internal standards are added to the sample prior to extraction, and the results are corrected for losses that occur during sample processing but do not occur in the instrument calibration standards because they are not processed before analysis.

TABLE 9. MINIMUM COMPOUNDS REQUIRED TO MEET ONGOING CALIBRATION CONTROL LIMITS

Semivolatiles	Volatiles
phenol	vinyl chloride
1,4-dichlorobenzene	1,1-dichloroethane
2-nitrophenol	chloroform
2,4-dichlorophenol	1,2-dichloropropane
hexachlorobutadiene	toluene
4-chloro-3-methylphenol	ethylbenzene
2,4,6-trichlorophenol	
acenaphthene	
N-nitrosodiphenylamine	
pentachlorophenol	
fluoranthene	
di-n-octyl phthalate	
benzo(a)pyrene	

It is important to note that the isotope dilution technique (or any other spiking technique) can not be used to correct for the efficiency of extraction because some analytes may be more tightly bound to particles in the sample than are the isotopically labeled internal standards spiked into the sample. Hence, tested or proven extraction procedures are considered essential to ensure complete extraction of all analytes from the sample matrix.

In all methods used for the analysis of volatile organic compounds, including the isotope dilution technique, the procedures used for calibration are identical to those used for analysis. Because calibration bias can only occur when the procedures used for instrument calibration standards differ from those used for complete analysis of samples, an isotope dilution technique such as EPA Method 1624C offers no substantial reduction in calibration bias when compared to a non-isotope dilution technique such as EPA Method 624. However, because random errors in calibration can be converted to a bias for quantification of sample responses, it is important that a sufficient number of calibrations standards be run to reduce bias.

In EPA Method 1625C, an instrument internal standard (2,2'-difluorobiphenyl) is added to the final extract prior to instrument analysis to determine the physical percent recoveries of the isotopically labeled internal standards that were added to the sample prior to extraction. The physical percent recoveries of the isotopically labeled internal standards should meet QA/QC criteria for the isotope dilution technique to be valid. Acceptance limits and recommendations for corrective action are given in EPA Method 1625C and are reproduced in Appendix B.

Use of an instrument internal standard is only to obtain QA/QC data and not to measure the analytes in the sample. The instrument internal standard is used to quantify selected analytes under the following conditions:

- An isotopically labeled analog of an analyte is not available, and there is no closely eluting and structurally similar surrogate that can be substituted for an isotopically labeled analog of the analyte (e.g., d_8-naphthalene could be used to quantify 2-methylnaphthalene)
- Certain QA/QC criteria specified in the method are not met for an analyte.

SURROGATE SPIKE COMPOUNDS

A surrogate is a type of check standard that is added to each sample in a known amount prior to extraction or purging. The surrogate is not one of the target compounds for the analyses, but should have analytical properties similar to those compounds. Because surrogate spikes are the only means of checking method performance on a sample-by-sample basis, they are required for all methods except isotope dilution methods.

Frequency

Surrogate spikes should be added to each sample unless the isotope dilution technique is used.

Compound Type

A minimum of five surrogate spikes should be added to each sample (three neutral and two acid compounds) when analyzing for semivolatile organic compounds. These surrogate spikes should cover a wide elution range and include one of the more volatile compounds (e.g., d_5-phenol) as well as a degradable PAH [e.g., d_{12}-perylene or d_{12}-benzo(a)pyrene]. Three surrogate spikes are required for the analysis of volatile compounds.

Surrogates need not be isotopically labeled. They need only be compounds that are physically and chemically similar to the analytes. Surrogates should be compounds that are not expected to be present in the samples.

At least one surrogate spike is required as a check on recovery of pesticides and PCB mixtures. This compound must be well-resolved, must not co-elute with any PCB or pesticide analyte, and should behave similarly to the analytes. This surrogate will likely not be a perfect PCB/pesticide analog. Possible standards are dibutylchlorendate (used in the EPA CLP), hexabromobenzene (used at EPA/Ecology Manchester laboratory), dibromooctofluorobiphenyl (used by Northwest NMFS and by EPA/Ecology Manchester laboratory), and isodrin (the endo-endo isomer of aldrin).

Warning and Action Limits

The warning and action limits in the most recent EPA CLP methods are recommended for use in evaluating surrogate recoveries. These limits are only valid if surrogates are added at the concentrations specified in the CLP methods.

Corrective Action

The corrective actions specified in the most recent EPA CLP protocols should be followed when action limits for surrogate recoveries are exceeded.

Report

Percent recovery values in sample and method blanks for all surrogate compounds analyzed should accompany the data. Data are not to be recovery corrected.

METHOD BLANKS AND FIELD BLANKS

Method blanks are analyzed to assess possible laboratory contamination of samples associated with all stages of preparation and analysis of sample extracts. Contamination is of concern because it can result in a false positive result (i.e., erroneous reports of the compound as present in the sample) or overestimates of sample concentrations. Alternatively, it is possible that

method blanks could incorrectly indicate contamination to be present in a sample. If analyte data are incorrectly rejected on the basis of method blank results, then a false negative result would occur. Protection against false positive results is given greatest weight in programs that generate data for possible use in litigation. Guidelines consistent with EPA CLP functional guidelines for QA review (EPA 1988) are recommended in this section for qualifying data associated with significant blank contamination.

Frequency

At a minimum, one method blank should be run for every extraction batch (or for volatile compound analyses, every 12-hour shift, whichever is more frequent).

Warning Limit

The warning limit is reached for a contaminant in a blank when its concentration exceeds the LOD.

Action Limit

The action limit for a contaminant is reached when its concentration in a blank exceeds the PQL.

Corrective Action

If any warning limit is exceeded, likely sources of contamination should be discussed in the cover letter of the data report. If action limits are exceeded, analyses should be halted until the contaminant source is eliminated or greatly reduced, or the data recipient has been notified and an acceptable plan of action has been determined.

The following compounds are some of the common laboratory contaminants that often appear in method blanks: methylene chloride, acetone, toluene, 2-butanone (all volatile compounds), and selected phthalate esters (semivolatile compounds including bis-ethylhexyl phthalate, butyl benzyl phthalate, and di-n-octyl phthalate). Sample data should be qualified as undetected at either the higher of the sample results or at the PQL when the sample concentration is less than 10 times the blank concentration for these compounds (i.e., the blank response is $\geq$10 percent of the sample response). The appropriate qualifiers for such data are *ZU*, indicating a detection limit established because of significant blank contamination.

Sample data for other contaminants should be qualified as undetected (*ZU* qualifiers) at the higher of the sample result or the PQL when the sample concentration is less than 5 times the blank concentration (i.e., the blank response is $\geq$20 percent of the sample response). If gross contamination exists (i.e., saturated peaks by GC/MS in the method blank), concentration data for

all compounds affected should be rejected (*R* qualifier) and not incorporated into regional databases.

Report

Laboratories should report original sample data without blank correction and should report data for all method blanks such that the contribution to associated samples can be determined. If contamination exists but does not exceed the guidelines in this section, then corrections may be applied to the data during independent QA review at the discretion of the project manager to minimize the effects of laboratory contamination on what may otherwise be unqualified analyte concentrations. For such corrections, the blank analyses are assumed to be representative of the potential contamination in sample extracts. However, blank correction is not acceptable under the EPA CLP (U.S. EPA 1988).

Any reported concentrations that have been blank-corrected must be qualified with a *Z* qualifier. Data sets that have not been blank-corrected must be explicitly identified as such. In all cases, results for method blanks and a cross-reference to identify associated samples for each method blank analysis must be summarized in data reports.

Blank analyses may not involve the same weight, volume, or dilution factors as the associated samples. These factors must be taken into consideration when blank-correcting data or applying the following guidelines for data qualification, such that a comparison of the total amount of contamination is actually made.

REFERENCE MATERIALS

The following definitions of reference materials will be adhered to throughout these guidelines:

- Reference Material—A material or substance, one or more properties of which are sufficiently well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials. In Puget Sound, a regional reference material (RRM) has been developed for marine sediments by NOAA/NMFS for EPA, NOAA, and other agencies and laboratories. The RRM is a fresh-frozen sediment homogenate from Sequim Bay, spiked with selected organic acid and neutral compounds at low concentrations. Available samples of the RRM can be requested from the EPA Region 10 Office of Puget Sound. This RRM has been analyzed in interlaboratory studies using NOAA methods, the results of which have ben compared with analyses by various investigators using different methods. Although not certified, this RRM is useful for intercomparing Puget Sound studies and is strongly recommended in every project.

- Certified Reference Material (CRM)—A reference material, one or more of whose property values are certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation that is issued by a certifying body (e.g., National Research Council of Canada, National Institute of Standards and Technology). A standard reference material is a CRM issued by the National Institute for Standards and Technology. There is no marine sediment CRM available for organic compounds of concern in Puget Sound, except for a marine sediment certified by the National Research Council (Canada) for organotin compounds (i.e., PACS-1). Tissue homogenates are sometimes available as reference materials (e.g., mega mussel sample, EPA, Environmental Research Laboratory, Narragansett, Rhode Island). An oyster CRM may be available by special request for selected organic contaminants.

RM and CRM provide information on the accuracy (i.e., how near the measurement is to its true value) as opposed to precision (i.e., how near replicate measurements are to each other). When analyzed in replicate, RM and CRM provide information on both accuracy and precision for a particular matrix type. Routine analysis of the RRM for Puget Sound sediment is recommended to provide data for interlaboratory comparisons.

Frequency

If five or fewer samples are submitted for analysis, one RM (or CRM, if available) is recommended, at the discretion of the project coordinator. If analysis of an available reference material is not included, the data may be qualified before entry in regional databases. If 6-50 samples are submitted, at least one RM should be analyzed. For submittals of more than 50 samples, one RM should be analyzed for each 50 samples.

Warning Limits

For analyses of CRM, the reported values should be within the 95 percent confidence interval certified by the agency dispensing the CRM. If more than two analytes fall outside of the 95 percent confidence interval, corrective action should be taken. If CRM are unavailable, control limits may not be appropriate, but analyses of RM can still be used to assess overall accuracy or method bias (in conjunction with matrix spikes and surrogate compounds).

Action Limits

Action limits are only appropriate for analysis of CRMs (i.e., action limits are not recommended for RM analyses). Action limits may be determined at the discretion of the project manager.

Corrective Action

It is recommended that the RM, if available, be analyzed prior to analysis of any samples. If values are outside the action limits, the RM should be reanalyzed to confirm the results. If the values are still outside action limits in the repeat analysis, the samples may be analyzed and reported with statements that describe the possible bias of the results in the cover letter accompanying the data. Alternatively, the laboratory may be required to repeat the analyses until action limits are met before continuing with sample analyses. Determination of the appropriate corrective action is the responsibility of the program manager or project coordinator and should be specified in the statement of work for the laboratory.

Report

The laboratory should keep a running record of results obtained for each analysis of a RM. Observed results should be compared to the mean provided by the originator of the RM, the observed mean obtained from repeated analyses by the laboratory, and acceptable range limits. Minimum reporting of RM results with laboratory data should include observed and expected values and the acceptable range limits. The steps for corrective action and observed bias relative to existing RM values should be reported and discussed in the cover letter.

MATRIX SPIKES

Matrix spike results are a common form of recovery data provided by laboratories, and are required by the EPA CLP protocol for screening level analyses. Matrix spike results are of less value than RM results, because the efficiency of the extraction of the compounds of interest from the sample matrix is not accounted for in matrix spike results. Matrix spikes are preferred as QC samples only in the absence of a suitable RM. Matrix spikes should include a wide range of representative analyte types (preferably all analytes). Compounds should be spiked at ca. 5 times the concentration of compounds in the sample or 5 times the PQL.

It was agreed in a 1989 work group that matrix spike samples will be recommended to provide data for cross-comparing the isotope dilution technique and matrix spike results, which are usually obtained at different concentration levels and serve different purposes. Matrix spike results are used to provide an indication of interferences during sample processing and analysis using native compounds typically at moderate concentrations. The isotope dilution technique is used to correct for losses during the processing of samples and to compensate for sample-specific instrument analysis errors using isotopically-labeled analogs at moderate to low concentrations.

Spiking concentrations that are low relative to sample concentrations increase random error in the matrix spike analysis. Spiking concentrations that are too high reduce the value of matrix spike analyses for interpreting sample interferences at representative concentrations of pollutants.

For comparison, EPA CLP spiking levels for sediments (U.S. EPA 1988) result in approximately 100 ng on-column for organic base/neutral compounds and 200 ng on-column for organic acids assuming a 1-mL final dilution volume, 100 percent recovery, and undetected concentrations

in the unspiked sample. These levels represent approximately 6,700-13,000 µg/kg dry weight assuming a 30-gram sediment sample with 50 percent moisture, or approximately 10-20 times the lowest contract required limit of 330 µg/kg wet weight (660 µg/kg dry weight in this example).

The same spiked amount in a 100-gram sample with 50 percent moisture would result in approximately 2,000-4,000 µg/kg dry weight concentrations under the same assumptions for other variables. This spiking level would be approximately 40-80 times an LOD of 50 µg/kg dry weight for modified CLP procedures (i.e., assuming lowest calibration at 10 ng on-column and 0.5-mL final dilution volume). Matrix spikes for marine samples should be similar to the levels that are expected in the environment, assuming that the analytical technique is sufficient to produce reproducible results at these concentrations. In many areas of Puget Sound, environmental concentrations of organic contaminants are closer to the PQL than to the spiking levels used for hazardous waste samples in the CLP. The EPA CLP spiked amount is most appropriate for highly contaminated samples that occur in small areas of Puget Sound.

The range of LOD in Table 5 will bracket or exceed the concentrations of many organic compounds in Puget Sound reference area sediments. Concentrations of compounds in contaminated urban bay samples may exceed 10-100 times reference area concentrations, and will often exceed the PQL in Table 5. Ideally, matrix spike results would be obtained for a range of sample types. Given limited resources, it is probably of greater value to assess possible interferences in moderately contaminated samples than in reference area samples.

Frequency

If fewer than 20 samples are submitted, at least one matrix spike and one matrix spike duplicate should be run. If 20 or more samples are submitted, one matrix spike and one matrix spike duplicate should be run for each 20 samples.

Warning and Action Limits

Recovery of ≥50 percent of matrix spike compounds accompanied by good precision is considered to be acceptable. Low matrix spike recoveries may result from matrix interferences in the sample. Therefore, poor results alone should not be cause for data qualification. Rigorous control limits for qualifying data are not recommended because of the potential difficulty in determining when matrix spike results indicate bias due to sample interferences rather than the expected random error of the difference between sample results before and after spiking. However, sample data should be rejected whenever zero percent recovery of an associated matrix spike compound has occurred.

Corrective Action

In the event of poor matrix spike performance, alternative QA measures should be considered before any associated sample data are qualified as estimates (*E*) or underestimates (*G*), or in very extreme cases, rejected (*R*). These measures include results of reference material analyses,

surrogate recoveries, and the physical percent recoveries of isotopically labeled internal standards, if using the isotope dilution technique. Professional judgment must be used to determine which samples should be associated with each matrix spike analysis.

Report

An explanation of low percent recovery values for matrix spike results should be discussed in the cover letter accompanying the data package.

SPIKED METHOD BLANKS

Spiked method blanks, sometimes called check standards, are method blanks spiked with surrogate compounds and analytes. Such samples are useful in verifying acceptable method performance prior to and during routine analysis of samples. Spiked method blanks do not take into account sample matrix effects, but can be used to identify basic problems in procedural steps. Spiked method blanks can also provide minimum recovery data when no suitable RM is available or when insufficient sample size exists for matrix spikes. Target analyte compounds and surrogate compounds should be added to a method blank prior to extraction.

Frequency

A spiked method blank should be analyzed before analysis of samples when a method is used for the first time in a project and after each method modification.

Warning and Action Limits

The warning and action limits in the most recent EPA CLP methods are recommended for use in evaluating spiked method blank recoveries. These limits are only valid if spiked compounds are added at the concentrations specified in the CLP methods.

Corrective Action

Analysis of actual samples should not begin until results are within action limits.

Report

Detailed notes should be kept in a laboratory notebook. The notes should discuss method spike results exceeding recommended limits, corrective action, and verification of instrument response within acceptable criteria. This information need not be included with data package results because analysis cannot continue until all results are within action limits.

ANALYTICAL REPLICATES

Analytical replicates (usually duplicates are sufficient when using a protocol that is well proven in the laboratory) provide precision information on the actual samples. Replicate analyses are useful in assessing potential sample heterogeneity and matrix effects.

Frequency

If five or fewer samples are submitted for analysis, a minimum of one replicate is recommended, at the discretion of the program manager or project coordinator. If 6-19 samples are submitted, at least one analytical replicate should be analyzed. If at least 20 samples are submitted, one blind replicate (i.e., unknown to the laboratory) analysis should be required, for a minimum replication of 5 percent overall.

Pooling of variances in duplicate analyses from different sample batches is recommended for estimating the standard deviation of replicate analyses. This technique is preferred to the analysis of a blind triplicate sample. Blind replicates also provide information on potential laboratory bias in analyzing known QA samples. Because there are limited numbers of blind replicates analyzed in a sample case, there is some value in analyzing a triplicate measurement (i.e., there may be no other blind replicates that can be pooled). However, the use of a triplicate analysis is at the discretion of the project manager.

Warning and Action Limits

Based on data of Horwitz et al. (1980), who charted interlaboratory precision as a function of concentration, a 30 percent coefficient of variation (a statistical measure of precision) is expected for concentrations ranging between 1 and 50 μg/kg dry weight. Compound-specific advisory limits are provided in the EPA CLP protocols.

These advisory limits are recommended as warning limits. Extensive discussion of precision requirements occurred at a Puget Sound organics workshop in 1985 and in subsequent work sessions. Based on professional judgment of analysts and regional program managers in attendance, it was decided that a difference of no more than a factor of 2 among replicates would be the basis for the laboratory action limit (i.e., approximately 50 percent coefficient of variation). Exceedance of the action limit would require automatic reanalysis to confirm the results. Many compound analyses are more precise. There was discussion about easing the action limit if the results were well beyond some regulatory guideline for acceptable contamination, and tightening the action limit if the results were close to some regulatory guideline. However, most data will have multiple uses and adjustable limits will be difficult to apply as a laboratory control.

Corrective Action

If results fall outside the action limit for more than two compounds, a repeat analysis is required to determine the origin of the problem before any data can be reported. If results continue to exceed action limits, subsequent corrective action is at the discretion of the program manager or project coordinator.

Report

A discussion of the results of duplicate sample analysis should include probable sources of laboratory error and an assessment of natural sample variability. If data are to be qualified on the basis of duplicate results, justification for assigning the data qualifier should be provided.

FIELD REPLICATES

Field replicates are separate samples collected at the identical station in the field and submitted for analysis. These QA samples are useful in determining total sample variability (i.e., analytical variability plus field variability).

Frequency

The program manager or project coordinator determines the frequency with which field replicates are collected. Laboratory replicates must be coordinated with field replicates so that sampling and analytical variability will be measured for the same station.

Warning and Action Limits

Warning and action limits are not appropriate when measuring field sampling variability since the analytical laboratory does not have control over the variability due to field sampling.

Corrective Action

No corrective action is recommended for field replicate analyses.

Report

If it is determined that variability observed in field duplicate results can be partially explained by analytical or sampling variability, it should be noted and discussed in a QA/QC evaluation of the data.

DATA REPORTING REQUIREMENTS

The following items are recommended to be provided by the analytical laboratory. The items listed below include most, but not all, of the documentation required by the EPA CLP. This documentation is necessary for independent QA/QC review of the data, and its delivery (or availability for inspection at the laboratory) should be required in the original statement of work if an independent QA/QC review is to be conducted:

- A cover letter discussing analytical problems (if any) and referencing or describing the procedure used
- Reconstructed ion chromatograms for GC/MS analyses for each sample
- Mass spectra of detected target compounds (GC/MS) for each sample
- GC/ECD or GC/FID chromatograms for each sample
- Raw data quantification reports for each sample
- A calibration data summary reporting the calibration range used [and for GC/MS, spectra and quantification reports for decafluorotriphenylphosphine (for semivolatile analyses), bromofluorobenzene (for volatile analyses), or an appropriate substitute standard]
- Final dilution volumes, sample size, wet-to-dry ratios, and instrument detection limit
- Analyte concentrations with reporting units identified to two significant figures unless otherwise justified
- Quantification of all analytes in method blanks (ng/sample rather than using a hypothetical sediment weight to calculate ng/g)
- Method blanks associated with each sample
- Tentatively identified compounds (if requested) and methods of quantification (include spectra)
- Recovery assessments and a replicate sample summary (laboratories should report all surrogate spike recovery data for each sample; a statement of the range of recoveries should be included in reports using these data)
- Data qualification codes and their definitions (qualifier codes used by PSEP are shown in Table 10).

TABLE 10. QUALIFIER CODES USED BY PSEP

Qualifier Code	Description
C	Combined with unresolved substances
E	Estimate
G	Estimate is greater than value shown
K	Detected at less than detection limit shown
L	Value is less than the maximum shown
M	Value is a mean
Q	Questionable value
T	Detected below quantification limit shown
U	Undetected at the detection limit shown
X	Recovery less than 10 percent
Z	Blank-corrected

RECOVERY AND BLANK CORRECTIONS

Recovery corrections based on a limited number of internal standards should not be applied. However, correction for bias due to compound losses in sample processing is inherent to isotope dilution techniques such as EPA Method 1625C, and is acceptable.

Blank corrections should not be applied by the laboratory. Concentrations of analytes in method blanks should be reported by the laboratory as part of the data report; corrections may then be made by program or project data managers. All such corrections must be indicated by assigning the *Z* data qualifier to the data value (or to the detection limit if the contamination is significant as described in the *QA/QC Procedures and Requirements* section). Whether data are corrected or not, the concentration of analytes in method blanks should always be given in reports. Results for several analytes are often suspect because they are commonly reported in method blanks. For example, reported concentrations of phthalates, methylene chloride, acetone, chloroform, benzene, 2-butanone, and toluene in samples should be carefully compared to those in the method blank before the compounds are assessed as environmental contaminants of concern.

DETECTION AND QUANTIFICATION LIMITS

Concentrations, LOD, and PQL are reported in terms of µg/kg dry weight sediment and µg/kg wet weight tissue. No detected concentrations should be reported below the LOD. Concentrations reported between the LOD and PQL are usable after qualification as estimates using the *T* qualifier (Table 10). Concentrations reported above the PQL are usable without qualification unless qualification is deemed appropriate during QA review. Laboratory statements of work that reference PSEP protocols for low-level analyses must, at a minimum, specify the PQL as the maximum acceptable limit to be reported for samples without significant interferences.

COST IMPLICATIONS

Higher analytical costs may be required to achieve lower LOD and to increase the precision of results (Table 11). Lowering LOD to achieve project goals can increase costs, particularly if additional sample cleanup is required. Additional sample cleanup may also improve precision because interferences are removed. However, the range of precision expected at a given detection limit in Table 11 reflects primarily differences in the analytical variability of a set of diverse compound types. For example, hydrocarbons can typically be recovered at the lower end of each range of precision estimates shown, while phthalates and some acid compounds are often analyzed much less precisely (i.e., higher coefficient of variation). Hence, a wide range of precision may be found at constant cost when analyses cover a wide range of compounds.

The major determinants of the range of analytical costs at a given detection limit are individual laboratory efficiencies and the specific analytical technique used (i.e., methods having large differences in cost can yield similar detection limits and precision of results). Nevertheless, lowering the required detection limits tends to raise the minimum cost expected for the analysis; a range in costs can still be expected above this minimum for different laboratories.

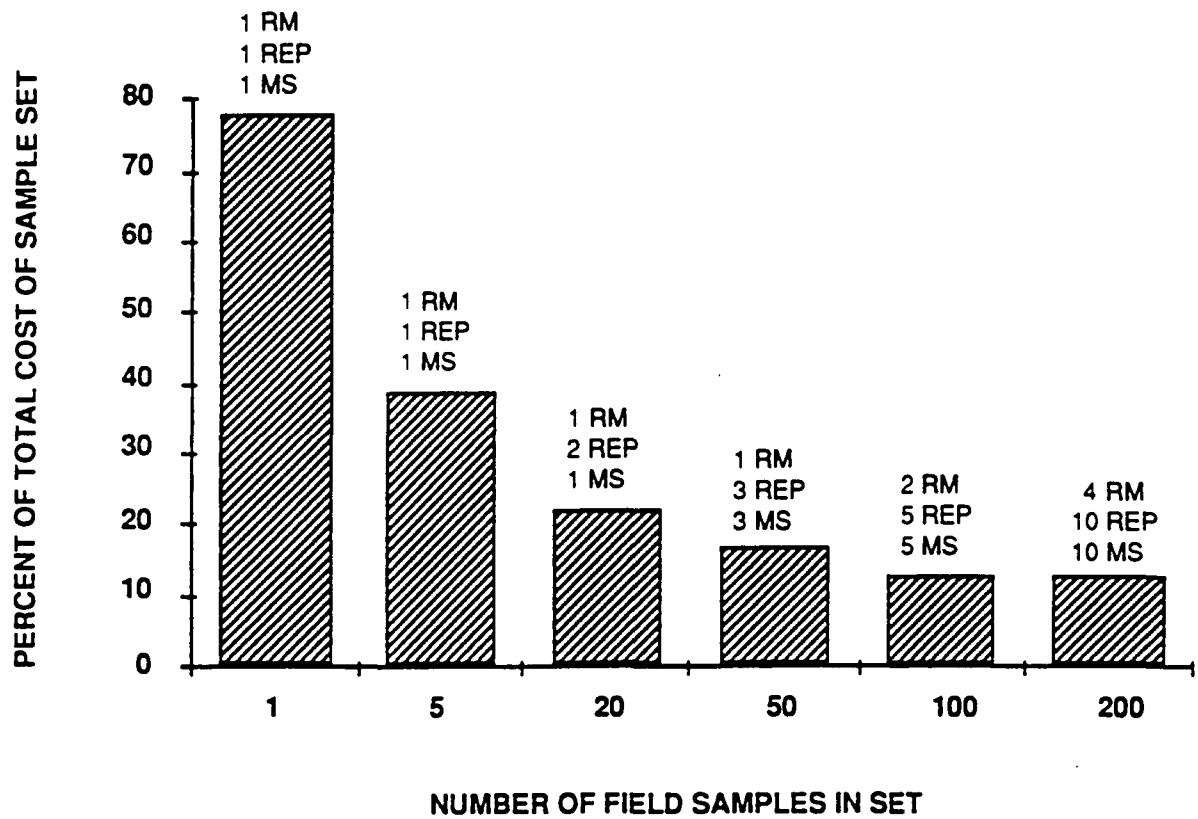
The major goal of QA/QC activities is to improve and control the accuracy of results. A successful QA/QC program will minimize the quantity of data that are rejected (a waste of sampling and analysis resources), improve the legal defensibility of the data set, and enable an assessment of comparability among data sets. Additional analytical costs are incurred to achieve these goals because QC samples must be analyzed with each sample set. The percent of the total analytical cost attributed to QC samples as a function of the number of samples submitted for analysis is shown in Figure 1. The number of QC samples for each sample set is based on the minimum frequency of analysis recommended in the *QA Procedures and Requirements* section. The percent of total costs attributed to QC samples rapidly declines as the number of samples submitted for analysis increases from 1 to 20. The percent QC cost is constant at 10 to 15 percent of total costs (depending on whether matrix spike analyses are conducted) in sets of greater than 50 samples.

TABLE 11. APPROXIMATE COST RANGE OF ANALYSES AS A FUNCTION OF MATRIX, DETECTION LIMITS, AND PRECISION[a]

Matrix	Practical Quantitation Limit (µg/kg dry weight)	Typical Precision (µg/kg dry weight)	Approximate Cost Range[b] (Per Analysis)
Sediments			
Extractable acid/base/neutrals	>200	±20% - >±100%	$404 - >$600
	<200	±20% - >±100%	$404 - >$700
PCB/pesticides	0.01 - 15	<±5% - >±50%	$153 - $555
Volatiles	2 - 10	<±5% - >±50%	$213 - $300
Tissues			
Extractable acid/base/neutrals	>330	<±5% - >±100%	$454 - $900
	20 - 100	<±5% - >±100%	$454 - $700
PCB/pesticides	0.1 - 20	<±5% - >±100%	$203 - >$555
Volatiles	5 - 20	<±10% - >±100%	$263 - >$400

[a] Cost range is based on multiple quotes compiled in 1989 for specific applications and greater than five samples. The actual costs may vary from the range shown. The table provides a general perspective of the relative difference in costs.

[b] NOTE: Each cost range is mainly the result of laboratory differences in technique pricing and number of analytes, not the range in precision or detection limits shown.



Assumes calibration runs and method blanks included in per sample cost, and method has been checked with spiked blanks.

RM - Certified Reference Material
REP - Replicate Analysis
MS - Matrix Spike Sample

Figure 1. Percent of the total analytical cost attributed to QC samples for analysis of organic compounds in Puget Sound as a function of the number of samples submitted for analysis

APPENDIX K

EXAMPLE QA/QC PROCEDURES AND REQUIREMENTS FOR ANALYSIS OF METALS

[From: Puget Sound Estuary Program. 1990 (revised). Recommended Protocols for Measuring Metals in Puget Sound Water, Sediments, and Tissue Samples. Prepared by PTI Environmental Services, Bellevue, WA. In: Recommended Protocols and Guidelines for Measuring Selected Environmental Variables in Puget Sound, U.S. EPA, Region 10, Seattle, WA. (Looseleaf)]

QUALITY ASSURANCE/QUALITY CONTROL

QUALITY ASSURANCE/QUALITY CONTROL MEASURES INITIATED IN THE ANALYTICAL LABORATORY

Standard laboratory practices for cleanliness of laboratory ware, reagents, solvents, gases, and instruments must be followed. For additional guidelines not covered in this report, see Sections 4 and 5 of *Handbook for Analytical Quality Control in Water and Wastewater Laboratories* (U.S. EPA 1979b) and U.S. EPA (1988).

Instrument Quality Assurance/Quality Control Checks

Instrument QA/QC checks necessary for all the EPA-approved methods discussed in the previous section include:

- Calibration blank
- Initial calibration and initial calibration verification
- Continuing calibration verification
- CRMs
- ICP interference check sample analysis (for ICP only).

Guidelines for instrument calibration are given in *Methods for Chemical Analysis of Water and Wastes* (U.S. EPA 1979a). In general, calibrations must be conducted each time the instrument is set up, and or on a daily basis when analyses are in progress. Calibration procedures should follow the procedures specified for each analysis in the EPA protocols. In addition, as specified for the CLP (U.S. EPA 1987), after an instrument system has been calibrated, the accuracy of the initial calibration should be verified and documented for every analyte by the analysis of EPA quality control solutions. Where a certified solution of an analyte is not available from EPA or any source (e.g., tin), analyses should be conducted on an independent standard at a concentration other than that used for calibration, but within the calibration range. When measurements for the certified components exceed the action limits, the analysis must be terminated, the problem corrected, the instrument recalibrated, and the recalibration verified.

For ICP and AA analyses, all work should be performed using continuing calibration as outlined in the EPA CLP Statement of Work. Frequency of continuing calibration analysis is 10 percent of the samples or every 2 hours during an analysis run, whichever is more frequent.

Method Quality Assurance/Quality Control Checks

Laboratories should perform the quality control checks listed below:

- Procedural or method blank
- Spiked sample analysis
- Replicate or duplicate sample analysis
- GFAA method of standard addition (if necessary)
- Laboratory control sample or CRM analysis.

Details on the use and application of these checks, along with action limits, reporting requirements, and corrective actions, are discussed in U.S. EPA (1987). The frequencies of application of these checks are 5 percent or one per batch, whichever is more frequent. The action limits are ±20 relative percent difference for duplicates, 75-125 percent recovery for spikes, and 80-120 percent recovery for the analysis of CRMs. Other recovery limits may be accepted if they are specified for a particular CRM. For the purpose of QA/QC, the required LODs are listed in Table A-6 of Appendix A. For batches of five or fewer samples, the minimum QC checks should be two blanks and the analysis of a CRM. Since spiked metals do not necessarily equilibrate with metals in the original matrix, a CRM from an ambient sample is preferred over a CRM with spiked metals. For the analysis of total or dissolved metals in ambient estuarine or coastal seawater, the National Research Council of Canada's CASS-1 nearshore seawater reference material has become the accepted standard of trace metal chemists (NRCC Marine Chemistry Standards, Ottawa, Canada K1A OR6). If an analyte is not in the CRM, a matrix spike must be analyzed for that particular analyte.

In general, for small batches of fewer than five samples, the priority of QC checks should be: CRMs > check standards > analytical duplicates > matrix spikes. If several small batches of the same matrix are analyzed sequentially (e.g., for several small projects), a CRM can be analyzed at a frequency of 5 percent overall, with at least one sample duplicate analyzed per individual batch. If any QA/QC check does not meet the established criteria, the laboratory QA officer should notify the project QA coordinator and the methods should be adjusted or, if necessary, data qualifications and reasons for noncompliance with QA/QC criteria should be submitted with the analytical data report.

QUALITY ASSURANCE/QUALITY CONTROL MEASURES INITIATED IN THE FIELD

In addition to the QA/QC checks listed above, the following five checks may be initiated at the time of sample collection (Plumb 1981):

- Transfer (preservation) blanks
- Cross-contamination blanks
- Blind replicate samples

- Field replicate samples
- Blind CRMs.

These checks may not replace any of the QA/QC measures outlined previously, but may be included as part of the overall QA/QC program.

Transfer (Preservation) Blanks

Reagents used for sample preservation can become contaminated after a period of use in the field. Analysis of the transfer blank will enable detection of contaminants in reagents and contaminants introduced during shipping.

To obtain a transfer blank, a sample container is filled in the field with deionized or distilled water to the same volume as that of samples. The transfer blank is preserved as if it were a normal water sample and sent to the laboratory for analysis.

Cross-Contamination Blanks

Carry-over from one sample to the next can occur if field equipment is not thoroughly cleaned between samples. The cross-contamination blank is designed to verify the absence of carry-over.

To obtain a cross-contamination blank, decontaminated sample-handling equipment (e.g., spatulas, augers, core barrels) is rinsed with deionized or distilled water, and the rinse water is collected. This sample is preserved as if it were a normal water sample and sent to the laboratory for analysis.

Blind Replicate Samples

To obtain blind replicates (i.e., replicates that are not known to be replicates by the laboratory) a collected sample is homogenized and split in the field into at least two identical aliquots, and each aliquot is treated and identified as a separate sample. Caution must be exercised to prevent field contamination. The replicates are sent blind to the laboratory. Homogenization and splitting may also be done in the laboratory. However, the identity of such samples must be unknown to the analyst. The mean, standard deviation, and relative percent standard deviation are calculated by the project QA coordinator.

In addition, a collected sample may be split in the field into two aliquots, and one aliquot sent to a different laboratory for analysis. The relative percent difference is calculated by the project QA coordinator. If project constraints require the use of more than one laboratory, comparability of the laboratories must be established using CRMs.

Field Replicate Samples

Field replicates are separate samples collected at the identical station and submitted for analysis. These samples are useful in determining total sample variability (i.e., analytical variability plus field variability).

Blind Certified Reference Materials

Blind analysis of CRMs can be conducted to determine the accuracy of laboratory analyses. To conduct analysis of a blind CRM sample, a subsample of a CRM is placed in a sample container and sent blind to the laboratory. The percent recovery is calculated by the project QA coordinator.

It is recommended that the same action limits be established for blind analysis of CRMs as for the laboratory QA/QC checks. The project QA coordinator must inform the laboratory if the action limits are exceeded and corrective actions must be taken (see below).

CORRECTIVE ACTIONS

If the concentration of the field or laboratory blank is greater than the detection limit required in the contract, all steps in the sample handling should be reviewed. For blank contamination, see U.S. EPA (1988) for appropriate corrective action. Many trace metal contamination problems are due to airborne dust. Contamination from airborne dust can be minimized by keeping containers closed and by rinsing all handling equipment immediately before use. See the *Contaminant Sources* section of this report (above) for further information.

Poor replication may be caused by inadequate mixing of the sample before taking aliquots, inconsistent contamination, inconsistent digestion procedures, or instrumentation problems. Instrumentation problems may be corrected by recalibration and calibration blank analysis. Inconsistent digestion may be caused by analyte loss during digestion; see below for corrective action. Also, hotplates may not hold a constant temperature across their surfaces. This problem can be alleviated by changing the position of digestion vessels at regular intervals during heating.

Poor performance on the analysis of CRMs or poor spike recovery may be caused by the same factors that were discussed above for poor replication. However, if replicate results are acceptable, poor CRM performance or spike recovery may be caused by loss of analyte during digestion. To check for analyte loss during digestion and for low recovery due to interferences during analysis, spike the sample after digestion and compare results to those of the predigestion spike. If the results are different, the digestion technique should be adjusted. If the results are not significantly different, dilute the sample by at least a factor of 5 and reanalyze. If spike recovery is still poor, standard additions, matrix modifiers, or the use of another method is required.

DATA REPORTING

Concentrations of elements in sediment, tissue, and water samples should be corrected for method (or procedural) blanks. However, some EPA programs do not allow for blank correction of results (e.g., CLP). Therefore, to distinguish these data, any result that has been blank corrected should be qualified with the *Z* qualifier (blank correction). Results for sediments should be reported on a dry-weight basis. Results for tissues should be reported on a wet-weight basis along with the percent moisture content (wet/dry ratio) of the tissue. If the tissue sample is too small to do both a metals analysis and a moisture determination, omit the latter analysis.

DATA REPORT PACKAGE

The data report package for analyses of each sample should include the following items:

- A summary of the digestion procedure.
- Tabulated results in units of mg/kg (dry weight) for sediment, mg/kg (wet weight) for tissue, and μg/L for water (validated and signed in original by the laboratory manager or designee).
- Method blanks for each batch of samples.
- Results from analysis of CRMs and matrix spikes.
- All data qualifications and explanations for all departures from the analytical protocols.
- Results for all the QA/QC checks initiated by the laboratory.
- Tabulation of instrument detection limits and LODs achieved for the samples. The LOD value reported by the laboratory for the analyses should be calculated as three times the standard deviation of the method blanks. A minimum of three method blanks need to be analyzed to calculate the LOD. When the concentration of the metal in a sample is less than the LOD after the method blank is subtracted, the *ZU* qualifiers (blank corrected to the detection limit) should be entered together with the LOD in the data report. Data reviewers may qualify data for which the method blank concentration exceeds 20 percent of the original metal concentration.

BACKUP DOCUMENTATION

All laboratories are required to submit results that are supported by sufficient backup data and quality assurance results to enable independent QA reviewers to conclusively determine the quality of the data.

Legible photocopies of original data sheets should be available from the laboratory with sufficient information to identify unequivocally the following items:

- Calibration results
- Calibration and method blanks
- Samples and dilutions
- Duplicates and spikes
- Control samples or CRMs
- Any anomalies in instrument performance or unusual instrument adjustments.

APPENDIX L

SOURCES OF EPA-CERTIFIED REFERENCE MATERIALS AND STANDARDS

EPA-certified analytical reference materials for priority pollutants and related compounds are currently produced under Cooperative Research and Development Agreements (CRADAs) by the following organizations:

- EPA-certified organic quality control samples, including standards for pesticides in fish tissue, are produced by:

  Supelco, Inc.
  Supelco Park
  Bellefonte, PA 16823-0048
  TEL: 1-800-247-6628 or 1-814-359-3441
  FAX: 1-814-359-3044
  Contact: Linda Alexander

- EPA-certified organic solution standards for toxic and hazardous materials (formerly the EPA Toxic and Hazardous Materials Repository) are produced by:

  NSI Environmental Solutions, Inc.
  P. O. Box 12313
  2 Triangle Drive
  Research Triangle Park, NC 27709
  TEL: 1-800-234-7837 or 1-919-549-8980
  FAX: 1-919-544-0334

- EPA-certified neat organic standards, including neat pesticide standards (formerly the EPA Pesticide Repository), are produced by:

  Ultra Scientific
  250 Smith Street
  North Kingston, RI 02852
  TEL: 1-401-294-9400
  FAX: 1-401-295-2330
  Contact: Dr. Bill Russo

- EPA-certified inorganic quality control samples, including trace metals, minerals, and nutrients, are produced by:

  SPEX Industries, Inc.
  3880 Park Avenue
  Edison, NJ 08820
  TEL: 1-201-549-7144 or 1-800-GET-SPEX
  FAX: 1-201-549-5125

The most recent information on EPA-certified materials is available on the EPA Electronic Bulletin Board (Modum No. 513-569-7610). Names and addresses of retailers of EPA-certified CRADA QA/QC samples or standards as of February 20, 1991, are given below. When ordering these materials, specify "EPA Certified Materials."

Retailers of EPA-Certified Organic Quality Control Samples

Accurate Chemical and Scientific
300 Shamee Drive
Westbury, NY 11590
TEL: 516-443-4900
FAX: 516-997-4938
Contact: Rudy Rosenberg

Accustandard
25 Science Park Road
New Haven, CT 06511
TEL: 203-786-5290
FAX: 203-786-5287
Contact: Mike Bolgar

Aldrich Chemical Company, Inc.
940 West Saint Paul Avenue
Milwaukee, WI 53233
TEL: 414-273-3850
FAX: 800-962-9591
Contact: Roy Pickering

Alltech Associates/Applied Science/Wescan Instruments
2051 Waukegan Road
Deerfield, IL 60015
TEL: 708-948-8600
FAX: 708-948-1078
Contact: Tom Rendl

Analytical Products Group
2730 Washington Boulevard
Belpre, OH 45714
TEL: 614-423-4200
FAX: 614-423-5588
Contact: Tom Coyner

Bodman Chemicals
P. O. Box 2221
Aston, PA 19014
TEL: 215-459-5600
FAX: 215-459-8036
Contact: Kirk Lind

Chemical Research Supply
P. O. Box 888
Addison, IL 60101
TEL: 708-543-0290
FAX: 708-543-0294
Contact: Nelson Armstrong

Crescent Chemical Corporation
1324 Motor Parkway
Hauppauge, NY 11788
TEL: 516-348-0333
FAX: 516-348-0913
Contact: Eric Rudnick

Curtis Matheson Scientific
P. O. Box 1546
9999 Veterans Memorial Drive
Houston, TX 77251-1546
TEL: 713-820-9898
FAX: 713-878-2221
Contact: Mitchel Martin

Environmental Research Associates
5540 Marshall Street
Arvada, CO 80002
TEL: 303-431-8454
FAX: 303-421-0159
Contact: Mark Carter

Restek Corporation
110 Benner Circle
Bellefonte, PA 16823
TEL: 814-353-1300
FAX: 814-353-1309
Contact: Eric Steindle

Supelco
Supelco Park
Bellefonte, PA 16823-0048
TEL: 800-247-6628 or 814-359-3441
FAX: 814-359-3044
Contact: Linda Alexander

Ultra Scientific
250 Smith Street
North Kingston, RI 02852
TEL: 401-294-9400
FAX: 401-295-2330
Contact: Dr. Bill Russo

Retailers of EPA-Certified Organic Solution Standards
(Formerly the EPA Toxic and Hazardous Materials Repository)

Absolute Standards
498 Russel Street
New Haven, CT 06513
TEL: 800-368-1131
FAX: 203-468-7407
Contact: Jack Ciscio

Accustandard
25 Science Park Road
New Haven, CT 06511
TEL: 203-786-5290
FAX: 203-786-5287
Contact: Mike Bolgar

Alltech Associates
2051 Waukegan Road
Deerfield, IL 60015
TEL: 708-948-8600
FAX: 708-948-1078
Contact: Tom Rendl

Alameda Chemical and Scientific
922 East Southern Pacific Drive
Phoenix, AZ 85034
TEL: 602-256-7044
FAX: 602-256-6566

Bodman Chemicals
P.O. Box 2221
Aston, PA 19014
TEL: 215-459-5600
FAX: 215-459-8036
Contact: Kirk Lind

Cambridge Isotope Laboratories
20 Commerce Way
Woburn, MA 01801-9894
TEL: 800-322-1174 or 617-938-0067
FAX: 617-932-9721

NSI Environmental Solutions, Inc.
P.O. Box 12313
2 Triangle Drive
Research Triangle Park, NC 27709
TEL: 800-234-7837 or 919-549-8980
FAX: 919-544-0334
Contact: Zora Bunn

Promochem
Postfach 1246
D 4230 Wesel
West Germany
TEL: 0281/530081
FAX: 0281/89991-93

Ultra Scientific
250 Smith Street
North Kingston, RI 02852
TEL: 401-294-9400
FAX: 401-295-2330
Contact: Dr. Bill Russo

Retailers of EPA-Certified Neat Organic Standards
(Including the Former EPA Pesticide Repository Standards)

Absolute Standards
498 Russel Street
New Haven, CT 06513
TEL: 800-368-1131
FAX: 203-468-7407
Contact: Jack Ciscio

Accustandard
25 Science Park Road
New Haven, CT 06511
TEL: 203-786-5290
FAX: 203-786-5287
Contact: Mike Bolgar

Alltech Associates
2051 Waukegan Road
Deerfield, IL 60015
TEL: 708-948-8600
FAX: 708-948-1078
Contact: Tom Rendl

Ultra Scientific
250 Smith Street
North Kingston, RI 02852
TEL: 401-294-9400
FAX: 401-295-2330
Contact: Dr. Bill Russo

Retailers of EPA-Certified Inorganic Quality Control Samples

SPEX Industries, Inc.
3880 Park Avenue
Edison, NJ 08820
TEL: 1-201-549-7144 or 1-800-GET-SPEX
FAX: 1-201-549-5125

APPENDIX M

DEFINITION AND PROCEDURE FOR THE DETERMINATION OF THE METHOD DETECTION LIMIT

[From: U.S. Environmental Protection Agency. 1982. Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater. James E. Longbottom and James J. Lichtenberg (eds.). EPA-600/4-82-057. Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.]

Definition and Procedure for the Determination of the Method Detection Limit

The method detection limit (MDL) is defined as the minimum concentration of a substance that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero and determined from analysis of a sample in a given matrix containing analyte.

Scope and Application

This procedure is designed for applicability to a wide variety of sample types ranging from reagent (blank) water containing analyte to wastewater containing analyte. The MDL for an analytical procedure may vary as a function of sample type. The procedure requires a complete, specific and well defined analytical method. It is essential that all sample processing steps of the analytical method be included in the determination of the method detection limit.

The MDL obtained by this procedure is used to judge the significance of a single measurement of a future sample.

The MDL procedure was designed for applicability to a broad variety of physical and chemical methods. To accomplish this, the procedure was made device- or instrument-independent.

Procedure

1. Make an estimate of the detection limit using one of the following:

   (a) The concentration value that corresponds to an instrument signal/noise ratio in the range of 2.5 to 5. If the criteria for qualitative identification of the analyte is based upon pattern recognition techniques, the least abundant signal necessary to achieve identification must be considered in making the estimate.
   (b) The concentration value that corresponds to three times the standard deviation of replicate instrumental measurements for the analyte in reagent water.
   (c) The concentration value that corresponds to the region of the standard curve where there is a significant change in sensitivity at low analyte concentrations, i.e., a break in the slope of the standard curve.
   (d) The concentration value that corresponds to known instrumental limitations.

   It is recognized that the experience of the analyst is important to this process. However, the analyst must include the above considerations in the estimate of the detection limit.

2. Prepare reagent (blank) water that is as free of analyte as possible. Reagent or interference free water is defined as a water sample in which analyte and interferent concentrations are not detected at the method detection limit of each analyte of interest. Interferences are defined as systematic errors in the measured analytical signal of an established procedure caused by the presence of interfering species (interferent). The interferent concentration is presupposed to be normally distributed in representative samples of a given matrix.

3. (a) If the MDL is to be determined in reagent water (blank), prepare a laboratory standard (analyte in reagent water) at a concentration which is at least equal to or in the same concentration range as the estimated MDL. (Recommend between 1 and 5 times the estimated MDL.) Proceed to Step 4.

(b) If the MDL is to be determined in another sample matrix, analyze the sample. If the measured level of the analyte is in the recommended range of one to five times the estimated MDL, proceed to Step 4.

If the measured concentration of analyte is less than the estimated MDL, add a known amount of analyte to bring the concentration of analyte to between one and five times the MDL. In the case where an interference is coanalyzed with the analyte.

If the measured level of analyte is greater than five times the estimated MDL, there are two options:

(1) Obtain another sample of lower level of analyte in same matrix if possible.
(2) The sample may be used as is for determining the MDL if the analyte level does not exceed 10 times the MDL of the analyte in reagent water. The variance of the analytical method changes as the analyte concentration increases from the MDL, hence the MDL determined under these circumstances may not truly reflect method variance at lower analyte concentrations.

4. (a) Take a minimum of seven aliquots of the sample to be used to calculate the MDL and process each through the entire analytical method. Make all computations according to the defined method with final results in the method reporting units. If blank measurements are required to calculate the measured level of analyte, obtain separate blank measurements for each sample aliquot analyzed. The average blank measurement is subtracted from the respective sample measurements.

(b) It may be economically and technically deirable to evaluate the estimated MDL before proceeding with 4a. This will: (1) prevent repeating this entire procedure when the costs of analyses are high and (2) insure that the procedure is being conducted at the correct concentration. It is quite possible that an incorrect MDL can be calculated from data obtained at many times the real MDL even though the background concentration of analyte is less than five times the calculated MDL. To insure that the estimate of the MDL is a good estimate, it is necessary to determine that a lower concentration of analyte will not result in a significantly lower MDL. Take two aliquots of the sample to be used to calculate the MDL and process each through the entire method, including blank measurements as described above in 4a. Evaluate these data:

(1) If these measurements indicate the sample is in the desirable range for determining the MDL, take five additional aliquots and proceed. Use all seven measurements to calculate the MDL.
(2) If these measurements indicate the sample is not in the correct range, reestimate the MDL, obtain new sample as in 3 and repeat either 4a or 4b.

5. Calculate the variance (S^2) and standard deviation (S) of the replicate measurements, as follows:

$$S^2 = \frac{1}{n-1}\left[\sum_{i=1}^{n} X_i^2 - \left(\sum_{i=1}^{n} X_i\right)^2 \Big/ n\right]$$

$$S = (S^2)^{1/2}$$

where: the x_i, i = 1 to n are the analytical results in the final method reporting units obtained from the n sample aliquots and $\sum_{i=1}^{n} X_i^2$ refers to the sum of the X values from i = 1 to n.

6. (a) Compute the MDL as follows:

$$MDL = t_{(n-1,\ 1-\alpha\ =\ .99)}(S)$$

where

MDL = the method detection

$t_{(n-1,\ 1-\alpha\ =\ .99)}$ = the students' t value appropriate for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom See Table.

S = standard deviation of the replicate analyses.

(b) The 95% confidence limits for the MDL derived in 6a are computed according to the following equations derived from percentiles of the chi square over degrees of freedom distribution (X^2/df) and calculated as follows:

$MDL_{LCL} = 0.69\ MDL$
$MDL_{UCL} = 1.92\ MDL$

where MDL_{LCL} and MDL_{UCL} are the lower and upper 95% confidence limits respectively based on seven aliquots.

7. Optional iterative procedure to verify the reasonableness of the estimated MDL and calculated MDL of subsequent MDL determinations.

(a) If this is the initial attempt to compute MDL based on the estimated MDL in Step 1, take the MDL as calculated in Step 6, spike in the matrix at the calculated MDL and proceed through the procedure starting with Step 4.

(b) If the current MDL determination is an iteration of the MDL procedure for which the spiking level does not permit qualitative identification, report the MDL as that concentration between the current spike level and the previous spike level which allows qualitative identification.

(c) If the current MDL determination is an iteration of the MDL procedure and the spiking level allows qualitative identification, use S^2 from the current MDL calculation and S^2 from the previous MDL calculation to compute the F ratio.

$$\text{if } \frac{S_A^2}{S_B^2} < 3.05$$

then compute the pooled standard deviation by the following equation:

$$S_{pooled} = \left[\frac{6S_A^2 + 6S_B^2}{12}\right]^{1/2}$$

if $\frac{S_A^2}{S_B^2} > 3.05$, respike at the last calculated MDL and process the samples through the procedure starting with Step 4.

(c) Use the S_{pooled} as calculated in 7b to compute the final MDL according to the following equation:

$$MDL = 2.681\ (S_{pooled})$$

where 2.681 is equal to $t_{(12,\ 1-\alpha\ =\ .99)}$.

(d) The 95% confidence limits for MDL derived in 7c are computed according to the following equations derived from percentiles of the chi squared over degrees of freedom distribution.

$MDL_{LCL} = 0.72\ MDL$
$MDL_{UCL} = 1.65\ MDL$

where LCL and UCL are the lower and upper 95% confidence limits respectively based on 14 aliquots.

Reporting

The analytical method used must be specifically identified by number or title and the MDL for each analyte expressed in the appropriate method reporting units. If the analytical method permits options which affect the method detection limit, these conditions must be specified with the MDL value. The sample matrix used to

determine the MDL must also be identified with the MDL value Report the mean analyte level with the MDL If a laboratory standard or a sample that contained a known amount analyte was used for this determination, report the mean recovery and indicate if the MDL determination was iterated

If the level of the analyte in the sample matrix exceeds 10 times the MDL of the analyte in reagent water, do not report a value for the MDL.

Reference

Glaser, J. A., Foerst, D. L., McKee, G. D., Quave, S. A., and Budde, W. L., "Trace Analysis for Wastewaters," *Environmental Science and Technology, 15,* 1426 (1981).

Table of Students' t Values at the 99 Percent Confidence Level

Number of Replicates	*Degrees of Freedom (n-1)*	$t_{(n-1,\ 1-\alpha\ =\ 99)}$
7	*6*	*3.143*
8	*7*	*2.998*
9	*8*	*2.896*
10	*9*	*2.821*
11	*10*	*2.764*
16	*15*	*2.602*
21	*20*	*2.528*
26	*25*	*2.485*
31	*30*	*2.457*
61	*60*	*2.390*
∞	∞	*2.326*

• U.S. GOVERNMENT PRINTING OFFICE 1982 - 559-092/0443

APPENDIX N

EXAMPLE DATA FORMS FOR ANALYSIS OF METALS AND ORGANIC TARGET CONTAMINANTS

[From: U.S. Environmental Protection Agency. 1991. Contract Laboratory Program Statement of Work for Inorganic Analysis, Multi-Media, Multi-Concentration, SOW #788, July, and Contract Laboratory Program Statement of Work for Organic Analysis, February. Washington, DC.]

EXAMPLE DATA FORMS FOR METALS ANALYSIS

U.S. EPA - CLP

1 INORGANIC ANALYSIS DATA SHEET

EPA SAMPLE NO.

Lab Name: _____ Contract: _____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Matrix (soil/water): _____ Lab Sample ID: _____

Level (low/med): _____ Date Received: _____

% Solids: _____

Concentration Units (ug/L or mg/kg dry weight): _____

CAS No.	Analyte	Concentration	C	Q	M
7429-90-5	Aluminum				
7440-36-0	Antimony				
7440-38-2	Arsenic				
7440-39-3	Barium				
7440-41-7	Beryllium				
7440-43-9	Cadmium				
7440-70-2	Calcium				
7440-47-3	Chromium				
7440-48-4	Cobalt				
7440-50-8	Copper				
7439-89-6	Iron				
7439-92-1	Lead				
7439-95-4	Magnesium				
7439-96-5	Manganese				
7439-97-6	Mercury				
7440-02-0	Nickel				
7440-09-7	Potassium				
7782-49-2	Selenium				
7440-22-4	Silver				
7440-23-5	Sodium				
7440-28-0	Thallium				
7440-62-2	Vanadium				
7440-66-6	Zinc				
	Cyanide				

Color Before: _____ Clarity Before: _____ Texture: _____

Color After: _____ Clarity After: _____ Artifacts: _____

Comments:

_____

_____

_____

_____

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COVER PAGE - INORGANIC ANALYSES DATA PACKAGE

Lab Name: _____ Contract: _____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

SOW No. _____

EPA Sample No.	Lab Sample ID

Were ICP interelement corrections applied? Yes/No ___

Were ICP background corrections applied? Yes/No ___

If yes, were raw data generated before application of background corrections? Yes/No ___

Comments: _____

I certify that this data package is in compliance with the terms and conditions of the contract, both technically and for completeness, for other than the conditions detailed above. Release of the data contained in this hardcopy data package and in the computer-readable data submitted on floppy diskette has been authorized by the Laboratory Manager or the Manager's designee, as verified by the following signature.

Signature: _____ Name: _____

Date: _____ Title: _____

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2A

INITIAL AND CONTINUING CALIBRATION VERIFICATION

Lab Name: _____ Contract: _____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Initial Calibration Source: _____

Continuing Calibration Source: _____

Concentration Units: ug/L

	Initial Calibration			Continuing Calibration					
Analyte	True	Found	%R(1)	True	Found	%R(1)	Found	%R(1)	M
Aluminum									
Antimony									
Arsenic									
Barium									
Beryllium									
Cadmium									
Calcium									
Chromium									
Cobalt									
Copper									
Iron									
Lead									
Magnesium									
Manganese									
Mercury									
Nickel									
Potassium									
Selenium									
Silver									
Sodium									
Thallium									
Vanadium									
Zinc									
Cyanide									

(1) Control Limits: Mercury 80-120; Other Metals 90-110; Cyanide 85-115

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2B
CRDL STANDARD FOR AA AND ICP

Lab Name: _____ Contract: _____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

AA CRDL Standard Source: _____

ICP CRDL Standard Source: _____

Concentration Units: ug/L

	CRDL Standard for AA			CRDL Standard for ICP				
				Initial			Final	
Analyte	True	Found	%R	True	Found	%R	Found	%R
Aluminum								
Antimony								
Arsenic								
Barium								
Beryllium								
dmium								
alcium								
Chromium								
Cobalt								
Copper								
Iron								
Lead								
Magnesium								
Manganese								
Mercury								
Nickel								
Potassium								
Selenium								
Silver								
Sodium								
Thallium								
Vanadium								
Zinc								

3
BLANKS

Lab Name: _____ Contract: _____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Preparation Blank Matrix (soil/water): _____

Preparation Blank Concentration Units (ug/L or mg/kg): _____

Analyte	Initial Calib. Blank (ug/L)	C	Continuing Calibration Blank (ug/L) 1	C	2	C	3	C	Prepa-ration Blank	C	M
Aluminum											
Antimony											
Arsenic											
Barium											
Beryllium											
Cadmium											
Calcium											
Chromium											
Cobalt											
Copper											
Iron											
Lead											
Magnesium											
Manganese											
Mercury											
Nickel											
Potassium											
Selenium											
Silver											
Sodium											
Thallium											
Vanadium											
Zinc											
Cyanide											

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4

ICP INTERFERENCE CHECK SAMPLE

Lab Name: _____ Contract: _____

Lab Code: _____ Case No: _____ SAS No.: _____ SDG No.: _____

ICP ID Number: _____ ICS Source: _____

Concentration Units: ug/L

Analyte	True Sol. A	True Sol. AB	Initial Found Sol. A	Initial Found Sol. AB	Initial Found %R	Final Found Sol. A	Final Found Sol. AB	Final Found %R
Aluminum								
Antimony								
Arsenic								
Barium								
Beryllium								
Cadmium								
Calcium								
Chromium								
Cobalt								
Copper								
Iron								
Lead								
Magnesium								
Manganese								
Mercury								
Nickel								
Potassium								
Selenium								
Silver								
Sodium								
Thallium								
Vanadium								
Zinc								

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5A
SPIKE SAMPLE RECOVERY

EPA SAMPLE NO.

Lab Name: _____ Contract: _____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Matrix: _____ Level (low/med): _____

% Solids for Sample: _____

Concentration Units (ug/L or mg/kg dry weight): _____

Analyte	Control Limit %R	Spiked Sample Result (SSR)	C	Sample Result (SR)	C	Spike Added (SA)	%R	Q	M
Aluminum									
Antimony									
Arsenic									
Barium									
Beryllium									
Cadmium									
Calcium									
Chromium									
Cobalt									
Copper									
Iron									
Lead									
Magnesium									
Manganese									
Mercury									
Nickel									
Potassium									
Selenium									
Silver									
Sodium									
Thallium									
Vanadium									
Zinc									
Cyanide									

Comments:

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5B
POST DIGEST SPIKE SAMPLE RECOVERY

EPA SAMPLE NO.

Lab Name: _____ Contract: _____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Matrix: _____ Level (low/med): _____

Concentration Units: ug/L

Analyte	Control Limit %R	Spiked Sample Result (SSR)	C	Sample Result (SR)	C	Added (SA)	%R	Q	M
Aluminum									
Antimony									
Arsenic									
Barium									
Beryllium									
Cadmium									
Calcium									
Chromium									
Cobalt									
Copper									
Iron									
Lead									
Magnesium									
Manganese									
Mercury									
Nickel									
Potassium									
Selenium									
Silver									
Sodium									
Thallium									
Vanadium									
Zinc									

Comments:

_____

_____

_____

_____

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6
DUPLICATES

EPA SAMPLE NO.

Lab Name: _____ Contract: _____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Matrix (soil/water): _____ Level (low/med): _____

% Solids for Sample: _____ % Solids for Duplicate: _____

Concentration Units (ug/L or mg/kg dry weight): _____

Analyte	Control Limit	Sample (S)	C	Duplicate (D)	C	RPD	Q	M
Aluminum								
Antimony								
Arsenic								
Barium								
Beryllium								
Cadmium								
Calcium								
Chromium								
Cobalt								
Copper								
Iron								
Lead								
Magnesium								
Manganese								
Mercury								
Nickel								
Potassium								
Selenium								
Silver								
Sodium								
Thallium								
Vanadium								
Zinc								
Cyanide								

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7

LABORATORY CONTROL SAMPLE

Lab Name: _____ Contract: _____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Solid LCS Source: _____

Aqueous LCS Source: _____

Analyte	Aqueous (ug/L) True	Found	%R	Solid (mg/kg) True	Found	C	Limits		%R
Aluminum									
Antimony									
Arsenic									
Barium									
Beryllium									
Cadmium									
Calcium									
Chromium									
Cobalt									
Copper									
Iron									
Lead									
Magnesium									
Manganese									
Mercury									
Nickel									
Potassium									
Selenium									
Silver									
Sodium									
Thallium									
Vanadium									
Zinc									
Cyanide									

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8
STANDARD ADDITION RESULTS

Lab Name: ______________________ Contract: __________

Lab Code: ______ Case No.: ______ SAS No.: ______ SDG No.: ______

Concentration Units: ug/L

EPA Sample No.	An	0 ADD ABS	1 ADD CON	1 ADD ABS	2 ADD CON	2 ADD ABS	3 ADD CON	3 ADD ABS	Final Conc.	r	Q

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9

ICP SERIAL DILUTIONS

EPA SAMPLE NO.

Lab Name: _____ Contract: _____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Matrix (soil/water): _____ Level (low/med): _____

Concentration Units: ug/L

Analyte	Initial Sample Result (I)	C	Serial Dilution Result (S)	C	% Difference	Q	M
Aluminum							
Antimony							
Arsenic							
Barium							
Beryllium							
Cadmium							
Calcium							
Chromium							
Cobalt							
Copper							
Iron							
Lead							
Magnesium							
Manganese							
Mercury							
Nickel							
Potassium							
Selenium							
Silver							
Sodium							
Thallium							
Vanadium							
Zinc							

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10

Instrument Detection Limits (Quarterly)

Lab Name: _____ Contract: _____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

ICP ID Number: _____ Date: _____

Flame AA ID Number: _____

Furnace AA ID Number: _____

Analyte	Wave-length (nm)	Back-ground	CRDL (ug/L)	IDL (ug/L)	M
Aluminum			200		
Antimony			60		
Arsenic			10		
Barium			200		
Beryllium			5		
Cadmium			5		
Calcium			5000		
Chromium			10		
Cobalt			50		
Copper			25		
Iron			100		
Lead			3		
Magnesium			5000		
Manganese			15		
Mercury			0.2		
Nickel			40		
Potassium			5000		
Selenium			5		
Silver			10		
Sodium			5000		
Thallium			10		
Vanadium			50		
Zinc			20		

Comments:

_____

_____

_____

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11A

ICP Interelement Correction Factors (Annually)

Lab Name: _____ Contract: _____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

ICP ID Number: _____ Date: _____

Analyte	Wavelength (nm)	Interelement Correction Factors for: Al	Ca	Fe	Mg	---
Aluminum						
Antimony						
Arsenic						
Barium						
Beryllium						
Cadmium						
Calcium						
Chromium						
Cobalt						
Copper						
Iron						
Lead						
Magnesium						
Manganese						
Mercury						
Nickel						
Potassium						
Selenium						
Silver						
Sodium						
Thallium						
Vanadium						
Zinc						

Comments:

_____

_____

_____

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11B

ICP Interelement Correction Factors (Annually)

Lab Name: _____ Contract: _____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

ICP ID Number: _____ Date: _____

Analyte	Wavelength (nm)	Interelement Correction Factors for: ---	---	---	---	---
Aluminum						
Antimony						
Arsenic						
Barium						
Beryllium						
Cadmium						
Calcium						
Chromium						
Cobalt						
Copper						
Iron						
Lead						
Magnesium						
Manganese						
Mercury						
Nickel						
Potassium						
Selenium						
Silver						
Sodium						
Thallium						
Vanadium						
Zinc						

Comments:

EXAMPLE DATA FORMS FOR ORGANICS ANALYSIS

SEMIVOLATILE ORGANICS ANALYSIS DATA SHEET

EPA SAMPLE NO.

b Name: _____ Contract: _____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Matrix: (soil/water) _____ Lab Sample ID: _____

Sample wt/vol: _____(g/mL)____ Lab File ID: _____

Level: (low/med) _____ Date Received: _____

% Moisture: not dec._____ dec._____ Date Extracted:_____

Extraction: (SepF/Cont/Sonc) ____ Date Analyzed: _____

GPC Cleanup: (Y/N)___ pH:____ Dilution Factor: _____

CAS NO.	COMPOUND	CONCENTRATION UNITS: (ug/L or ug/Kg)_____	Q
108-95-2	Phenol		
111-44-4	bis(2-Chloroethyl)ether		
95-57-8	2-Chlorophenol		
541-73-1	1,3-Dichlorobenzene		
106-46-7	1,4-Dichlorobenzene		
100-51-6	Benzyl alcohol		
95-50-1	1,2-Dichlorobenzene		
95-48-7	2-Methylphenol		
108-60-1	bis(2-Chloroisopropyl)ether		
106-44-5	4-Methylphenol		
621-64-7	N-Nitroso-di-n-propylamine		
67-72-1	Hexachloroethane		
98-95-3	Nitrobenzene		
78-59-1	Isophorone		
88-75-5	2-Nitrophenol		
105-67-9	2,4-Dimethylphenol		
65-85-0	Benzoic acid		
111-91-1	bis(2-Chloroethoxy)methane		
120-83-2	2,4-Dichlorophenol		
120-82-1	1,2,4-Trichlorobenzene		
91-20-3	Naphthalene		
106-47-8	4-Chloroaniline		
87-68-3	Hexachlorobutadiene		
59-50-7	4-Chloro-3-methylphenol		
91-57-6	2-Methylnaphthalene		
77-47-4	Hexachlorocyclopentadiene		
88-06-2	2,4,6-Trichlorophenol		
95-95-4	2,4,5-Trichlorophenol		
91-58-7	2-Chloronaphthalene		
88-74-4	2-Nitroaniline		
131-11-3	Dimethylphthalate		
208-96-8	Acenaphthylene		
606-20-2	2,6-Dinitrotoluene		

SEMIVOLATILE ORGANICS ANALYSIS DATA SHEET

EPA SAMPLE NO.

Lab Name:_____ Contract:_____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Matrix: (soil/water)_____ Lab Sample ID: _____

Sample wt/vol: _____(g/mL)____ Lab File ID: _____

Level: (low/med) _____ Date Received: _____

% Moisture: not dec._____ dec._____ Date Extracted:_____

Extraction: (SepF/Cont/Sonc) _____ Date Analyzed: _____

GPC Cleanup: (Y/N)___ pH:____ Dilution Factor: _____

CAS NO.	COMPOUND	CONCENTRATION UNITS: (ug/L or ug/Kg)_____	Q
99-09-2	3-Nitroaniline		
83-32-9	Acenaphthene		
51-28-5	2,4-Dinitrophenol		
100-02-7	4-Nitrophenol		
132-64-9	Dibenzofuran		
121-14-2	2,4-Dinitrotoluene		
84-66-2	Diethylphthalate		
7005-72-3	4-Chlorophenyl-phenylether		
86-73-7	Fluorene		
100-01-6	4-Nitroaniline		
534-52-1	4,6-Dinitro-2-methylphenol		
86-30-6	N-Nitrosodiphenylamine (1)		
101-55-3	4-Bromophenyl-phenylether		
118-74-1	Hexachlorobenzene		
87-86-5	Pentachlorophenol		
85-01-8	Phenanthrene		
120-12-7	Anthracene		
84-74-2	Di-n-butylphthalate		
206-44-0	Fluoranthene		
129-00-0	Pyrene		
85-68-7	Butylbenzylphthalate		
91-94-1	3,3'-Dichlorobenzidine		
56-55-3	Benzo(a)anthracene		
218-01-9	Chrysene		
117-81-7	bis(2-Ethylhexyl)phthalate		
117-84-0	Di-n-octylphthalate		
205-99-2	Benzo(b)fluoranthene		
207-08-9	Benzo(k)fluoranthene		
50-32-8	Benzo(a)pyrene		
193-39-5	Indeno(1,2,3-cd)pyrene		
53-70-3	Dibenz(a,h)anthracene		
191-24-2	Benzo(g,h,i)perylene		

(1) - Cannot be separated from Diphenylamine

EPA SAMPLE NO.

PESTICIDE ORGANICS ANALYSIS DATA SHEET

ab Name:_____ Contract:_____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Matrix: (soil/water)_____ Lab Sample ID: _____

Sample wt/vol: _____(g/mL)____ Lab File ID: _____

Level: (low/med) _____ Date Received: _____

% Moisture: not dec._____ dec._____ Date Extracted:_____

Extraction: (SepF/Cont/Sonc) _____ Date Analyzed: _____

GPC Cleanup: (Y/N)___ pH:____ Dilution Factor: _____

CAS NO.	COMPOUND	CONCENTRATION UNITS: (ug/L or ug/Kg)_____	Q
319-84-6	alpha-BHC		
319-85-7	beta-BHC		
319-86-8	delta-BHC		
58-89-9	gamma-BHC (Lindane)		
76-44-8	Heptachlor		
309-00-2	Aldrin		
1024-57-3	Heptachlor epoxide		
959-98-8	Endosulfan I		
60-57-1	Dieldrin		
72-55-9	4,4'-DDE		
72-20-8	Endrin		
33213-65-9	Endosulfan II		
72-54-8	4,4'-DDD		
1031-07-8	Endosulfan sulfate		
50-29-3	4,4'-DDT		
72-43-5	Methoxychlor		
53494-70-5	Endrin ketone		
5103-71-9	alpha-Chlordane		
5103-74-2	gamma-Chlordane		
8001-35-2	Toxaphene		
12674-11-2	Aroclor-1016		
11104-28-2	Aroclor-1221		
11141-16-5	Aroclor-1232		
53469-21-9	Aroclor-1242		
12672-29-6	Aroclor-1248		
11097-69-1	Aroclor-1254		
11096-82-5	Aroclor-1260		

EPA SAMPLE NO.

SEMIVOLATILE ORGANICS ANALYSIS DATA SHEET
TENTATIVELY IDENTIFIED COMPOUNDS

Name:_____ Contract:_____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Matrix: (soil/water)_____ Lab Sample ID: _____

Sample wt/vol: _____(g/mL)____ Lab File ID: _____

Level: (low/med) _____ Date Received: _____

% Moisture: not dec._____ dec._____ Date Extracted:_____

Extraction: (SepF/Cont/Sonc) _____ Date Analyzed: _____

GPC Cleanup: (Y/N)___ pH:____ Dilution Factor: _____

Number TICs found: _____ CONCENTRATION UNITS: (ug/L or ug/Kg)_____

	CAS NUMBER	COMPOUND NAME	RT	EST. CONC.	Q
1.					
2.					
3.					
4.					
5.					
6.					
7.					
8.					
9.					
10.					
11.					
12.					
13.					
14.					
15.					
16.					
17.					
18.					
19.					
20.					
21.					
22.					
23.					
24.					
25.					
26.					
27.					
28.					
29.					
30.					

SEMIVOLATILE SURROGATE RECOVERY

Name:_____ Contract:_____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Level: (low/med)_____

	EPA SAMPLE NO.	S1 (NBZ) #	S2 (FBP) #	S3 (TPH) #	S4 (PHL) #	S5 (2FP) #	S6 (TBP) #	OTHER	TOT OUT
01									
02									
03									
04									
05									
06									
07									
08									
09									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									

	QC LIMITS
S1 (NBZ) = Nitrobenzene-d5	(23-120)
S2 (FBP) = 2-Fluorobiphenyl	(30-115)
S3 (TPH) = Terphenyl-d14	(18-137)
S4 (PHL) = Phenol-d6	(24-113)
S5 (2FP) = 2-Fluorophenol	(25-121)
S6 (TBP) = 2,4,6-Tribromophenol	(19-122)

Column to be used to flag recovery values
* Values outside of contract required QC limits
D Surrogates diluted out

PESTICIDE SURROGATE RECOVERY

Lab Name:_____ Contract:_____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.:_____

	EPA SAMPLE NO.	S1 (DBC) #	OTHER
01			
02			
03			
04			
05			
06			
07			
08			
09			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			
28			
29			
30			

ADVISORY QC LIMITS (24-154)

S1 (DBC) = Dibutylchlorendate

Column to be used to flag recovery values

* Values outside of QC limits

D Surrogates diluted out

SEMIVOLATILE MATRIX SPIKE/MATRIX SPIKE DUPLICATE RECOVERY

Lab Name:_____ Contract:_____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Matrix Spike - EPA Sample No.: _____

COMPOUND	SPIKE ADDED (ug/L)	SAMPLE CONCENTRATION (ug/L)	MS CONCENTRATION (ug/L)	MS % REC #	QC LIMITS REC.
Phenol					12- 89
2-Chlorophenol					27-123
1,4-Dichlorobenzene					36- 97
N-Nitroso-di-n-prop.(1)					41-116
1,2,4-Trichlorobenzene					39- 98
4-Chloro-3-methylphenol					23- 97
Acenaphthene					46-118
4-Nitrophenol					10- 80
2,4-Dinitrotoluene					24- 96
Pentachlorophenol					9-103
Pyrene					26-127

COMPOUND	SPIKE ADDED (ug/L)	MSD CONCENTRATION (ug/L)	MSD % REC #	% RPD #	QC LIMITS RPD	QC LIMITS REC.
Phenol					42	12- 89
2-Chlorophenol					40	27-123
1,4-Dichlorobenzene					28	36- 97
N-Nitroso-di-n-prop.(1)					38	41-116
1,2,4-Trichlorobenzene					28	39- 98
4-Chloro-3-methylphenol					42	23- 97
Acenaphthene					31	46-118
4-Nitrophenol					50	10- 80
2,4-Dinitrotoluene					38	24- 96
Pentachlorophenol					50	9-103
Pyrene					31	26-127

(1) N-Nitroso-di-n-propylamine

Column to be used to flag recovery and RPD values with an asterisk
* Values outside of QC limits

RPD:_____ out of _____ outside limits
Spike Recovery:_____ out of _____ outside limits

COMMENTS: _____

_____

PESTICIDE MATRIX SPIKE/MATRIX SPIKE DUPLICATE RECOVERY

Lab Name:_____ Contract:_____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Matrix Spike - EPA Sample No.: _____

COMPOUND	SPIKE ADDED (ug/L)	SAMPLE CONCENTRATION (ug/L)	MS CONCENTRATION (ug/L)	MS % REC #	QC. LIMITS REC.
gamma-BHC (Lindane)					56-123
Heptachlor					40-131
Aldrin					40-120
Dieldrin					52-126
Endrin					56-121
4,4'-DDT					38-127

COMPOUND	SPIKE ADDED (ug/L)	MSD CONCENTRATION (ug/L)	MSD % REC #	% RPD #	QC LIMITS RPD	QC LIMITS REC.
gamma-BHC (Lindane)					15	56-123
Heptachlor					20	40-131
Aldrin					22	40-120
Dieldrin					18	52-126
Endrin					21	56-121
4,4'-DDT					27	38-127

Column to be used to flag recovery and RPD values with an asterisk

* Values outside of QC limits

RPD:_____ out of _____ outside limits
Spike Recovery:_____ out of _____ outside limits

COMMENTS: _____

_____

SEMIVOLATILE METHOD BLANK SUMMARY

Lab Name:_____ Contract:_____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Lab File ID: _____ Lab Sample ID: _____

Date Extracted: _____ Extraction:(SepF/Cont/Sonc) _____

Date Analyzed: _____ Time Analyzed: _____

Matrix: (soil/water) _____ Level:(low/med) _____

Instrument ID: _____

THIS METHOD BLANK APPLIES TO THE FOLLOWING SAMPLES, MS AND MSD:

	EPA SAMPLE NO.	LAB SAMPLE ID	LAB FILE ID	DATE ANALYZED
01				
02				
03				
04				
05				
06				
07				
08				
09				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				
29				
30				

COMMENTS: _____

_____

PESTICIDE METHOD BLANK SUMMARY

Lab Name:_____ Contract:_____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Lab Sample ID: _____ Lab File ID: _____

Matrix:(soil/water) _____ Level:(low/med) _____

Date Extracted: _____ Extraction: (SepF/Cont/Sonc)_____

Date Analyzed (1): _____ Date Analyzed (2): _____

Time Analyzed (1): _____ Time Analyzed (2): _____

Instrument ID (2): _____ Instrument ID (2): _____

GC Column ID (1): _____ GC Column ID (1): _____

THIS METHOD BLANK APPLIES TO THE FOLLOWING SAMPLES, MS AND MSD:

	EPA SAMPLE NO.	LAB SAMPLE ID	DATE ANALYZED 1	DATE ANALYZED 2
01				
02				
03				
04				
05				
06				
07				
08				
09				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				

COMMENTS: _____

_____

U. S. EPA - CLP

12

ICP Linear Ranges (Quarterly)

Lab Name: _____ Contract: _____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

ICP ID Number: _____ Date: _____

Analyte	Integ. Time (sec.)	Concentration (ug/L)	M
Aluminum			
Antimony			
Arsenic			
Barium			
Beryllium			
Cadmium			
Calcium			
Chromium			
Cobalt			
Copper			
Iron			
Lead			
Magnesium			
Manganese			
Mercury			
Nickel			
Potassium			
Selenium			
Silver			
Sodium			
Thallium			
Vanadium			
Zinc			

Comments:

_____

_____

_____

_____

U.S. EPA - CLP

13
PREPARATION LOG

Lab Name: ______________________ Contract: __________

Lab Code: ______ Case No.: ______ SAS No.: ______ SDG No.: ______

Method: __

EPA Sample No.	Preparation Date	Weight (gram)	Volume (mL)

U.S. EPA - CLP

14
ANALYSIS RUN LOG

ab Name: ______________________ Contract: __________

ab Code: ______ Case No.: ______ SAS No.: ______ SDG No.: ______

nstrument ID Number: ____________ Method: __

tart Date: ________ End Date: ________

EPA Sample No.	D/F	Time	% R	Analytes																							
				AL	SB	AS	BA	BE	CD	CA	CR	CO	CU	FE	PB	MG	MN	HG	NI	K	SE	AG	NA	TL	V	ZN	CN

FORM XIV - IN 7/88

EXAMPLE DATA FORMS FOR METALS ANALYSIS

U.S. EPA - CLP

1

INORGANIC ANALYSIS DATA SHEET

EPA SAMPLE NO.

Lab Name: _____ Contract: _____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Matrix (soil/water): _____ Lab Sample ID: _____

Level (low/med): _____ Date Received: _____

% Solids: _____

Concentration Units (ug/L or mg/kg dry weight): _____

CAS No.	Analyte	Concentration	C	Q	M
7429-90-5	Aluminum				
7440-36-0	Antimony				
7440-38-2	Arsenic				
7440-39-3	Barium				
7440-41-7	Beryllium				
7440-43-9	Cadmium				
7440-70-2	Calcium				
7440-47-3	Chromium				
7440-48-4	Cobalt				
7440-50-8	Copper				
7439-89-6	Iron				
7439-92-1	Lead				
7439-95-4	Magnesium				
7439-96-5	Manganese				
7439-97-6	Mercury				
7440-02-0	Nickel				
7440-09-7	Potassium				
7782-49-2	Selenium				
7440-22-4	Silver				
7440-23-5	Sodium				
7440-28-0	Thallium				
7440-62-2	Vanadium				
7440-66-6	Zinc				
	Cyanide				

Color Before: _____ Clarity Before: _____ Texture: _____

Color After: _____ Clarity After: _____ Artifacts: _____

Comments:

U.S. EPA - CLP

2A

INITIAL AND CONTINUING CALIBRATION VERIFICATION

Lab Name: _____ Contract: _____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Initial Calibration Source: _____

Continuing Calibration Source: _____

Concentration Units: ug/L

Analyte	Initial Calibration True	Found	%R(1)	Continuing Calibration True	Found	%R(1)	Found	%R(1)	M
Aluminum									
Antimony									
Arsenic									
Barium									
Beryllium									
Cadmium									
Calcium									
Chromium									
Cobalt									
Copper									
Iron									
Lead									
Magnesium									
Manganese									
Mercury									
Nickel									
Potassium									
Selenium									
Silver									
Sodium									
Thallium									
Vanadium									
Zinc									
Cyanide									

(1) Control Limits: Mercury 80-120; Other Metals 90-110; Cyanide 85-115

U.S. EPA - CLP

2B
CRDL STANDARD FOR AA AND ICP

Lab Name: _____ Contract: _____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

AA CRDL Standard Source: _____

ICP CRDL Standard Source: _____

Concentration Units: ug/L

	CRDL Standard for AA			CRDL Standard for ICP				
				Initial			Final	
Analyte	True	Found	%R	True	Found	%R	Found	%R
Aluminum								
Antimony								
Arsenic								
Barium								
Beryllium								
Cadmium								
Calcium								
Chromium								
Cobalt								
Copper								
Iron								
Lead								
Magnesium								
Manganese								
Mercury								
Nickel								
Potassium								
Selenium								
Silver								
Sodium								
Thallium								
Vanadium								
Zinc								

CRDL= Contract required detection limit.

3
BLANKS

Lab Name: _____ Contract: _____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Preparation Blank Matrix (soil/water): _____

Preparation Blank Concentration Units (ug/L or mg/kg): _____

Analyte	Initial Calib. Blank (ug/L)	C	Continuing Calibration Blank (ug/L) 1	C	2	C	3	C	Prepa-ration Blank	C	M
Aluminum											
Antimony											
Arsenic											
Barium											
Beryllium											
Cadmium											
Calcium											
Chromium											
Cobalt											
Copper											
Iron											
Lead											
Magnesium											
Manganese											
Mercury											
Nickel											
Potassium											
Selenium											
Silver											
Sodium											
Thallium											
Vanadium											
Zinc											
Cyanide											

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5A
SPIKE SAMPLE RECOVERY

EPA SAMPLE NO.

Lab Name: _____ Contract: _____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Matrix (soil/water): _____ Level (low/med): _____

% Solids for Sample: _____

Concentration Units (ug/L or mg/kg dry weight): _____

Analyte	Control Limit %R	Spiked Sample Result (SSR)	C	Sample Result (SR)	C	Spike Added (SA)	%R	Q	M
Aluminum									
Antimony									
Arsenic									
Barium									
Beryllium									
Cadmium									
Calcium									
Chromium									
Cobalt									
Copper									
Iron									
Lead									
Magnesium									
Manganese									
Mercury									
Nickel									
Potassium									
Selenium									
Silver									
Sodium									
Thallium									
Vanadium									
Zinc									
Cyanide									

Comments:

_____

_____

_____

_____

U.S. EPA - CLP

5B

POST DIGEST SPIKE SAMPLE RECOVERY

EPA SAMPLE NO.

Lab Name: _____ Contract: _____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Matrix (soil/water): _____ Level (low/med): _____

Concentration Units: ug/L

Analyte	Control Limit %R	Spiked Sample Result (SSR)	C	Sample Result (SR)	C	Spike Added (SA)	%R	Q	M
Aluminum									
Antimony									
Arsenic									
Barium									
Beryllium									
Cadmium									
Calcium									
Chromium									
Cobalt									
Copper									
Iron									
Lead									
Magnesium									
Manganese									
Mercury									
Nickel									
Potassium									
Selenium									
Silver									
Sodium									
Thallium									
Vanadium									
Zinc									
Cyanide									

Comments:

_____

_____

_____

_____

U.S. EPA - CLP

6
DUPLICATES

EPA SAMPLE NO.

Lab Name: _____ Contract: _____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Matrix (soil/water): _____ Level (low/med): _____

% Solids for Sample: _____ % Solids for Duplicate: _____

Concentration Units (ug/L or mg/kg dry weight): _____

Analyte	Control Limit	Sample (S)	C	Duplicate (D)	C	RPD	Q	M
Aluminum								
Antimony								
Arsenic								
Barium								
Beryllium								
Cadmium								
Calcium								
Chromium								
Cobalt								
Copper								
Iron								
Lead								
Magnesium								
Manganese								
Mercury								
Nickel								
Potassium								
Selenium								
Silver								
Sodium								
Thallium								
Vanadium								
Zinc								
Cyanide								

7

LABORATORY CONTROL SAMPLE

Lab Name: _____ Contract: _____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Solid LCS Source: _____

Aqueous LCS Source: _____

Analyte	Aqueous (ug/L) True	Found	%R	Solid (mg/kg) True	Found	C	Limits		%R
Aluminum									
Antimony									
Arsenic									
Barium									
Beryllium									
Cadmium									
Calcium									
Chromium									
Cobalt									
Copper									
Iron									
Lead									
Magnesium									
Manganese									
Mercury									
Nickel									
Potassium									
Selenium									
Silver									
Sodium									
Thallium									
Vanadium									
Zinc									
Cyanide									

8

STANDARD ADDITION RESULTS

Lab Name: ______________________ Contract: __________

Lab Code: ______ Case No.: ______ SAS No.: ______ SDG No.: ______

Concentration Units: ug/L

EPA Sample No.	An	0 ADD ABS	1 ADD CON	1 ADD ABS	2 ADD CON	2 ADD ABS	3 ADD CON	3 ADD ABS	Final Conc.	r	Q

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10

INSTRUMENT DETECTION LIMITS (QUARTERLY)

Lab Name: _____ Contract: _____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

ICP ID Number: _____ Date: _____

Flame AA ID Number: _____

Furnace AA ID Number: _____

Analyte	Wave-length (nm)	Back-ground	CRDL (ug/L)	IDL (ug/L)	M
Aluminum			200		
Antimony			60		
Arsenic			10		
Barium			200		
Beryllium			5		
Cadmium			5		
Calcium			5000		
Chromium			10		
Cobalt			50		
Copper			25		
Iron			100		
Lead			3		
Magnesium			5000		
Manganese			15		
Mercury			0.2		
Nickel			40		
Potassium			5000		
Selenium			5		
Silver			10		
Sodium			5000		
Thallium			10		
Vanadium			50		
Zinc			20		

Comments:

_____

_____

_____

_____

13

PREPARATION LOG

Lab Name: ____________________ Contract: __________

Lab Code: ________ Case No.: ______ SAS No.: ______ SDG No.: _______

Method: __

EPA Sample No.	Preparation Date	Weight (gram)	Volume (mL)

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14

ANALYSIS RUN LOG

Lab Name: ______________________________ Contract: __________

Lab Code: _______ Case No.: _______ SAS No.: _______ SDG No.: _______

Instrument ID Number: _______________ Method: __

Start Date: __________ End Date: __________

EPA Sample No.	D/F	Time	% R	Analytes																							
				AL	SB	AS	BA	BE	CD	CA	CR	CO	CU	FE	PB	MG	MN	HG	NI	K	SE	AG	NA	TL	V	ZN	CN

EXAMPLE DATA FORMS FOR ORGANICS ANALYSIS

1B

SEMIVOLATILE ORGANICS ANALYSIS DATA SHEET

EPA SAMPLE NO.

Lab Name:_____ Contract:_____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Matrix: (soil/water)_____ Lab Sample ID: _____

Sample wt/vol: _____(g/mL)____ Lab File ID: _____

Level: (low/med) _____ Date Received: _____

% Moisture: _____ decanted: (Y/N)___ Date Extracted:_____

Concentrated Extract Volume: _____(uL) Date Analyzed: _____

Injection Volume: _____(uL) Dilution Factor: _____

GPC Cleanup: (Y/N)___ pH:____

CAS NO.	COMPOUND	CONCENTRATION UNITS: (ug/L or ug/Kg)_____	Q
108-95-2	Phenol		
111-44-4	bis(2-Chloroethyl)ether		
95-57-8	2-Chlorophenol		
541-73-1	1,3-Dichlorobenzene		
106-46-7	1,4-Dichlorobenzene		
95-50-1	1,2-Dichlorobenzene		
95-48-7	2-Methylphenol		
108-60-1	2,2'-oxybis(1-Chloropropane)		
106-44-5	4-Methylphenol		
621-64-7	N-Nitroso-di-n-propylamine		
67-72-1	Hexachloroethane		
98-95-3	Nitrobenzene		
78-59-1	Isophorone		
88-75-5	2-Nitrophenol		
105-67-9	2,4-Dimethylphenol		
111-91-1	bis(2-Chloroethoxy)methane		
120-83-2	2,4-Dichlorophenol		
120-82-1	1,2,4-Trichlorobenzene		
91-20-3	Naphthalene		
106-47-8	4-Chloroaniline		
87-68-3	Hexachlorobutadiene		
59-50-7	4-Chloro-3-methylphenol		
91-57-6	2-Methylnaphthalene		
77-47-4	Hexachlorocyclopentadiene		
88-06-2	2,4,6-Trichlorophenol		
95-95-4	2,4,5-Trichlorophenol		
91-58-7	2-Chloronaphthalene		
88-74-4	2-Nitroaniline		
131-11-3	Dimethylphthalate		
208-96-8	Acenaphthylene		
606-20-2	2,6-Dinitrotoluene		
99-09-2	3-Nitroaniline		
83-32-9	Acenaphthene		

1C

SEMIVOLATILE ORGANICS ANALYSIS DATA SHEET

EPA SAMPLE NO.

Lab Name:_____ Contract:_____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Matrix: (soil/water)_____ Lab Sample ID: _____

Sample wt/vol: _____(g/mL)____ Lab File ID: _____

Level: (low/med) _____ Date Received: _____

% Moisture: _____ decanted: (Y/N)___ Date Extracted:_____

Concentrated Extract Volume: _____(uL) Date Analyzed: _____

Injection Volume: _____(uL) Dilution Factor: _____

GPC Cleanup: (Y/N)___ pH:____

CAS NO.	COMPOUND	CONCENTRATION UNITS: (ug/L or ug/Kg)_____	Q
51-28-5	2,4-Dinitrophenol		
100-02-7	4-Nitrophenol		
132-64-9	Dibenzofuran		
121-14-2	2,4-Dinitrotoluene		
84-66-2	Diethylphthalate		
7005-72-3	4-Chlorophenyl-phenylether		
86-73-7	Fluorene		
100-01-6	4-Nitroaniline		
534-52-1	4,6-Dinitro-2-methylphenol		
86-30-6	N-Nitrosodiphenylamine (1)		
101-55-3	4-Bromophenyl-phenylether		
118-74-1	Hexachlorobenzene		
87-86-5	Pentachlorophenol		
85-01-8	Phenanthrene		
120-12-7	Anthracene		
86-74-8	Carbazole		
84-74-2	Di-n-butylphthalate		
206-44-0	Fluoranthene		
129-00-0	Pyrene		
85-68-7	Butylbenzylphthalate		
91-94-1	3,3'-Dichlorobenzidine		
56-55-3	Benzo(a)anthracene		
218-01-9	Chrysene		
117-81-7	bis(2-Ethylhexyl)phthalate		
117-84-0	Di-n-octylphthalate		
205-99-2	Benzo(b)fluoranthene		
207-08-9	Benzo(k)fluoranthene		
50-32-8	Benzo(a)pyrene		
193-39-5	Indeno(1,2,3-cd)pyrene		
53-70-3	Dibenz(a,h)anthracene		
191-24-2	Benzo(g,h,i)perylene		

(1) - Cannot be separated from Diphenylamine

1D

PESTICIDE ORGANICS ANALYSIS DATA SHEET

EPA SAMPLE NO.

Lab Name:_____ Contract:_____

Lab Code: ____.___ Case No.: _____ SAS No.: _____ SDG No.: _____

Matrix: (soil/water)_____ Lab Sample ID: _____

Sample wt/vol: _____(g/mL)____ Lab File ID: _____

% Moisture: _____ decanted: (Y/N)___ Date Received: _____

Extraction: (SepF/Cont/Sonc) _____ Date Extracted:_____

Concentrated Extract Volume: _____(uL) Date Analyzed: _____

Injection Volume: _____(uL) Dilution Factor: _____

GPC Cleanup: (Y/N)___ pH:____ Sulfur Cleanup: (Y/N) ___

CAS NO.	COMPOUND	CONCENTRATION UNITS: (ug/L or ug/Kg)_____	Q
319-84-6	alpha-BHC		
319-85-7	beta-BHC		
319-86-8	delta-BHC		
58-89-9	gamma-BHC (Lindane)		
76-44-8	Heptachlor		
309-00-2	Aldrin		
1024-57-3	Heptachlor epoxide		
959-98-8	Endosulfan I		
60-57-1	Dieldrin		
72-55-9	4,4'-DDE		
72-20-8	Endrin		
33213-65-9	Endosulfan II		
72-54-8	4,4'-DDD		
1031-07-8	Endosulfan sulfate		
50-29-3	4,4'-DDT		
72-43-5	Methoxychlor		
53494-70-5	Endrin ketone		
7421-36-3	Endrin aldehyde		
5103-71-9	alpha-Chlordane		
5103-74-2	gamma-Chlordane		
8001-35-2	Toxaphene		
12674-11-2	Aroclor-1016		
11104-28-2	Aroclor-1221		
11141-16-5	Aroclor-1232		
53469-21-9	Aroclor-1242		
12672-29-6	Aroclor-1248		
11097-69-1	Aroclor-1254		
11096-82-5	Aroclor-1260		

1F

EPA SAMPLE NO.

SEMIVOLATILE ORGANICS ANALYSIS DATA SHEET
TENTATIVELY IDENTIFIED COMPOUNDS

Lab Name:_____ Contract:_____

Lab Code: ____ Case No.: _____ SAS No.: _____ SDG No.: _____

Matrix: (soil/water)_____ Lab Sample ID: _____

Sample wt/vol: _____(g/mL)____ Lab File ID: _____

Level: (low/med) _____ Date Received: _____

% Moisture: _____ decanted: (Y/N)___ Date Extracted:_____

Concentrated Extract Volume: _____(uL) Date Analyzed: _____

Injection Volume: _____(uL) Dilution Factor: _____

GPC Cleanup: (Y/N)___ pH:____

Number TICs found: _____ CONCENTRATION UNITS: (ug/L or ug/Kg)_____

CAS NUMBER	COMPOUND NAME	RT	EST. CONC.	Q
1.				
2.				
3.				
4.				
5.				
6.				
7.				
8.				
9.				
10.				
11.				
12.				
13.				
14.				
15.				
16.				
17.				
18.				
19.				
20.				
21.				
22.				
23.				
24.				
25.				
26.				
27.				
28.				
29.				
30.				

2C
SEMIVOLATILE SURROGATE RECOVERY

Lab Name:_____ Contract:_____

Lab Code: ____ Case No.: _____ SAS No.: _____ SDG No.: _____

	EPA SAMPLE NO.	S1 (NBZ) #	S2 (FBP) #	S3 (TPH) #	S4 (PHL) #	S5 (2FP) #	S6 (TBP) #	S7 (2CP) #	S8 (DCB) #	TOT OUT
01										
02										
03										
04										
05										
06										
07										
08										
09										
10										
11										
12										
13										
14										
15										
16										
17										
18										
19										
20										
21										
22										
23										
24										
25										
26										
27										
28										
29										
30										

		QC LIMITS	
S1 (NBZ)	= Nitrobenzene-d5	(35-114)	
S2 (FBP)	= 2-Fluorobiphenyl	(43-116)	
S3 (TPH)	= Terphenyl-d14	(33-141)	
S4 (PHL)	= Phenol-d5	(10-110)	
S5 (2FP)	= 2-Fluorophenol	(21-110)	
S6 (TBP)	= 2,4,6-Tribromophenol	(10-123)	
S7 (2CP)	= 2-Chlorophenol-d4	(33-110)	(advisory)
S8 (DCB)	= 1,2-Dichlorobenzene-d4	(16-110)	(advisory)

Column to be used to flag recovery values
* Values outside of contract required QC limits
D Surrogate diluted out

2E
PESTICIDE SURROGATE RECOVERY

Lab Name:_____ Contract:_____

Lab Code: ____. Case No.: _____ SAS No.: _____ SDG No.:_____

GC Column(1): _____ ID: _____(mm) GC Column(2): _____ ID: _____(mm)

	EPA SAMPLE NO.	TCX 1 %REC #	TCX 2 %REC #	DCB 1 %REC #	DCB 2 %REC #	OTHER (1)	OTHER (2)	TOT OUT
01								
02								
03								
04								
05								
06								
07								
08								
09								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								
23								
24								
25								
26								
27								
28								
29								
30								

	ADVISORY QC LIMITS
TCX = Tetrachloro-m-xylene	(60-150)
DCB = Decachlorobiphenyl	(60-150)

Column to be used to flag recovery values
* Values outside of QC limits
D Surrogate diluted out

3C

SEMIVOLATILE MATRIX SPIKE/MATRIX SPIKE DUPLICATE RECOVERY

Lab Name:_____ Contract:_____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Matrix Spike - EPA Sample No.: _____

COMPOUND	SPIKE ADDED (ug/L)	SAMPLE CONCENTRATION (ug/L)	MS CONCENTRATION (ug/L)	MS % REC #	QC. LIMITS REC.
Phenol					12-110
2-Chlorophenol					27-123
1,4-Dichlorobenzene					36- 97
N-Nitroso-di-n-prop.(1)					41-116
1,2,4-Trichlorobenzene					39- 98
4-Chloro-3-methylphenol					23- 97
Acenaphthene					46-118
4-Nitrophenol					10- 80
2,4-Dinitrotoluene					24- 96
Pentachlorophenol					9-103
Pyrene					26-127

COMPOUND	SPIKE ADDED (ug/L)	MSD CONCENTRATION (ug/L)	MSD % REC #	% RPD #	QC LIMITS RPD	QC LIMITS REC.
Phenol					42	12-110
2-Chlorophenol					40	27-123
1,4-Dichlorobenzene					28	36- 97
N-Nitroso-di-n-prop.(1)					38	41-116
1,2,4-Trichlorobenzene					28	39- 98
4-Chloro-3-methylphenol					42	23- 97
Acenaphthene					31	46-118
4-Nitrophenol					50	10- 80
2,4-Dinitrotoluene					38	24- 96
Pentachlorophenol					50	9-103
Pyrene					31	26-127

(1) N-Nitroso-di-n-propylamine

Column to be used to flag recovery and RPD values with an asterisk
* Values outside of QC limits

RPD:_____ out of _____ outside limits
Spike Recovery:_____ out of _____ outside limits

COMMENTS: _____

_____

3E

PESTICIDE MATRIX SPIKE/MATRIX SPIKE DUPLICATE RECOVERY

Lab Name:_____ Contract:_____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Matrix Spike - EPA Sample No.: _____

COMPOUND	SPIKE ADDED (ug/L)	SAMPLE CONCENTRATION (ug/L)	MS CONCENTRATION (ug/L)	MS % REC #	QC. LIMITS REC.
gamma-BHC (Lindane)					56-123
Heptachlor					40-131
Aldrin					40-120
Dieldrin					52-126
Endrin					56-121
4,4'-DDT					38-127

COMPOUND	SPIKE ADDED (ug/L)	MSD CONCENTRATION (ug/L)	MSD % REC #	% RPD #	QC LIMITS RPD	QC LIMITS REC.
gamma-BHC (Lindane)					15	56-123
Heptachlor					20	40-131
Aldrin					22	40-120
Dieldrin					18	52-126
Endrin					21	56-121
4,4'-DDT					27	38-127

Column to be used to flag recovery and RPD values with an asterisk

* Values outside of QC limits

RPD:_____ out of _____ outside limits
Spike Recovery:_____ out of _____ outside limits

COMMENTS: _____

_____

4B

SEMIVOLATILE METHOD BLANK SUMMARY

EPA SAMPLE NO.

Lab Name: _____ Contract: _____

Lab Code: ____ Case No.: _____ SAS No.: _____ SDG No.: _____

Lab File ID: _____ Lab Sample ID: _____

Instrument ID: _____ Date Extracted: _____

Matrix: (soil/water) _____ Date Analyzed: _____

Level: (low/med) _____ Time Analyzed: _____

THIS METHOD BLANK APPLIES TO THE FOLLOWING SAMPLES, MS AND MSD:

	EPA SAMPLE NO.	LAB SAMPLE ID	LAB FILE ID	DATE ANALYZED
01				
02				
03				
04				
05				
06				
07				
08				
09				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				
29				
30				

COMMENTS:

_____

_____

4C

PESTICIDE METHOD BLANK SUMMARY

EPA SAMPLE NO.

Lab Name: _____ Contract: _____

Lab Code: ____ Case No.: _____ SAS No.: _____ SDG No.: _____

Lab Sample ID: _____ Lab File ID: _____

Matrix: (soil/water) _____ Extraction: (SepF/Cont/Sonc) _____

Sulfur Cleanup: (Y/N) __ Date Extracted: _____

Date Analyzed (1): _____ Date Analyzed (2): _____

Time Analyzed (1): _____ Time Analyzed (2): _____

Instrument ID (1): _____ Instrument ID (2): _____

GC Column (1): _____ ID: _____ (mm) GC Column (2): _____ ID: _____ (mm)

THIS METHOD BLANK APPLIES TO THE FOLLOWING SAMPLES, MS AND MSD:

	EPA SAMPLE NO.	LAB SAMPLE ID	DATE ANALYZED 1	DATE ANALYZED 2
01				
02				
03				
04				
05				
06				
07				
08				
09				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				

COMMENTS: _____

_____

5B

SEMIVOLATILE ORGANIC INSTRUMENT PERFORMANCE CHECK
DECAFLUOROTRIPHENYLPHOSPHINE (DFTPP)

Lab Name:_____ Contract:_____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Lab File ID: _____ DFTPP Injection Date:_____

Instrument ID: _____ DFTPP Injection Time:_____

m/e	ION ABUNDANCE CRITERIA	% RELATIVE ABUNDANCE
51	30.0 - 80.0% of mass 198	
68	Less than 2.0% of mass 69	( )1
69	Mass 69 relative abundance	
70	Less than 2.0% of mass 69	( )1
127	25.0 - 75.0% of mass 198	
197	Less than 1.0% of mass 198	
198	Base Peak, 100% relative abundance	
199	5.0 to 9.0% of mass 198	
275	10.0 - 30.0% of mass 198	
365	Greater than 0.75% of mass 198	
441	Present, but less than mass 443	
442	40.0 - 110.0% of mass 198	
443	15.0 - 24.0% of mass 442	( )2

1-Value is % mass 69 2-Value is % mass 442

THIS CHECK APPLIES TO THE FOLLOWING SAMPLES, MS, MSD, BLANKS, AND STANDARDS:

	EPA SAMPLE NO.	LAB SAMPLE ID	LAB FILE ID	DATE ANALYZED	TIME ANALYZED
01					
02					
03					
04					
05					
06					
07					
08					
09					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					

SEMIVOLATILE ORGANICS INITIAL CALIBRATION DATA

Lab Name:_____ Contract:_____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Instrument ID: _____ Calibration Date(s):_____ _____

Calibration Times: _____ _____

LAB FILE ID: RRF20 =_____ RRF50 =_____

RRF80 =_____ RRF120=_____ RRF160=_____

COMPOUND	RRF20	RRF50	RRF80	RRF120	RRF160	$\overline{RRF}$	% RSD
Phenol	*						*
bis(2-Chloroethyl)ether	*						*
2-Chlorophenol	*						*
1,3-Dichlorobenzene	*						*
1,4-Dichlorobenzene	*						*
1,2-Dichlorobenzene	*						*
2-Methylphenol	*						*
2,2'-oxybis(1-Chloropropane)							
4-Methylphenol	*						*
N-Nitroso-di-n-propylamine	*						*
Hexachloroethane	*						*
Nitrobenzene	*						*
Isophorone	*						*
2-Nitrophenol	*						*
2,4-Dimethylphenol	*						*
bis(2-Chloroethoxy)methane	*						*
2,4-Dichlorophenol	*						*
1,2,4-Trichlorobenzene	*						*
Naphthalene	*						*
4-Chloroaniline							
Hexachlorobutadiene							
4-Chloro-3-methylphenol	*						*
2-Methylnaphthalene	*						*
Hexachlorocyclopentadiene							
2,4,6-Trichlorophenol	*						*
2,4,5-Trichlorophenol	*						*
2-Chloronaphthalene	*						*
2-Nitroaniline							
Dimethylphthalate							
Acenaphthylene	*						*
2,6-Dinitrotoluene	*						*
3-Nitroaniline							
Acenaphthene	*						*
2,4-Dinitrophenol							
4-Nitrophenol							
Dibenzofuran	*						*
2,4-Dinitrotoluene	*						*

* Compounds with required minimum RRF and maximum %RSD values. All other compounds must meet a minimum RRF of 0.010.

6C

SEMIVOLATILE ORGANICS INITIAL CALIBRATION DATA

Lab Name:_____ Contract:_____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Instrument ID: _____ Calibration Date(s):_____ _____

Calibration Times: _____ _____

LAB FILE ID: RRF20 =_____ RRF50 =_____

RRF80 =_____ RRF120=_____ RRF160=_____

COMPOUND	RRF20	RRF50	RRF80	RRF120	RRF160	$\overline{RRF}$	% RSD
Diethylphthalate							
4-Chlorophenyl-phenylether	*						*
Fluorene	*						*
4-Nitroaniline							
4,6-Dinitro-2-methylphenol							
N-Nitrosodiphenylamine (1)							
4-Bromophenyl-phenylether	*						*
Hexachlorobenzene	*						*
Pentachlorophenol	*						*
Phenanthrene	*						*
Anthracene	*						*
Carbazole							
Di-n-butylphthalate							
Fluoranthene	*						*
Pyrene	*						*
Butylbenzylphthalate							
3,3'-Dichlorobenzidine							
Benzo(a)anthracene	*						*
Chrysene	*						*
bis(2-Ethylhexyl)phthalate							
Di-n-octylphthalate							
Benzo(b)fluoranthene	*						*
Benzo(k)fluoranthene	*						*
Benzo(a)pyrene	*						*
Indeno(1,2,3-cd)pyrene	*						*
Dibenz(a,h)anthracene	*						*
Benzo(g,h,i)perylene	*						*
Nitrobenzene-d5							*
2-Fluorobiphenyl	*						*
Terphenyl-d14	*						*
Phenol-d5	*						*
2-Fluorophenol	*						*
2,4,6-Tribromophenol							
2-Chlorophenol-d4	*						*
1,2-Dichlorobenzene-d4	*						*

(1) Cannot be separated from Diphenylamine

* Compounds with required minimum RRF and maximum %RSD values. All other compounds must meet a minimum RRF of 0.010.

6D

PESTICIDE INITIAL CALIBRATION OF SINGLE COMPONENT ANALYTES

ab Name:_____ Contract:_____

ab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

nstrument ID: _____ Level (x low): low _____ mid _____ high _____

C Column: _____ ID: _____(mm) Date(s) Analyzed: _____ _____

COMPOUND	RT OF STANDARDS LOW	MID	HIGH	MEAN RT	RT WINDOW FROM	TO
alpha-BHC						
beta-BHC						
delta-BHC						
gamma-BHC (Lindane)						
Heptachlor						
Aldrin						
Heptachlor epoxide						
Endosulfan I						
Dieldrin						
4,4'-DDE						
Endrin						
Endosulfan II						
4,4'-DDD						
Endosulfan sulfate						
4,4'-DDT						
Methoxychlor						
Endrin ketone						
Endrin aldehyde						
alpha-Chlordane						
gamma-Chlordane						
Tetrachloro-m-xylene						
Decachlorobiphenyl						

* Surrogate retention times are measured from Standard Mix A analyses.

Retention time windows are ± 0.05 minutes for all compounds that elute before Heptachlor epoxide, ±0.07 minutes for all other compounds, except ±0.10 minutes for Decachlorobiphenyl.

6E

PESTICIDE INITIAL CALIBRATION OF SINGLE COMPONENT ANALYTES

Lab Name:_____ Contract:_____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Instrument ID: _____ Level (x low): low _____ mid _____ high _____

GC Column: _____ ID: _____(mm) Date(s) Analyzed: _____ _____

COMPOUND	CALIBRATION FACTORS LOW	MID	HIGH	MEAN	%RSD
alpha-BHC					
beta-BHC					
delta-BHC					
gamma-BHC (Lindane)					
Heptachlor					
Aldrin					
Heptachlor epoxide					
Endosulfan I					
Dieldrin					
4,4'-DDE					
Endrin					
Endosulfan II					
4,4'-DDD					
Endosulfan sulfate					
4,4'-DDT					
Methoxychlor					
Endrin ketone					
Endrin aldehyde					
alpha-Chlordane					
gamma-Chlordane					
Tetrachloro-m-xylene					
Decachlorobiphenyl					

* Surrogate calibration factors are measured from Standard Mix A analyses.

%RSD must be less than or equal 20.0 % for all compounds except the surrogates, where %RSD must be less than or equal to 30.0%. Up to two target compounds, but not surrogates, may have %RSD greater than 20.0% but less than or equal to 30.0%.

6F

PESTICIDE INITIAL CALIBRATION OF MULTICOMPONENT ANALYTES

Lab Name:_____ Contract:_____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Instrument ID: _____ Date(s) Analyzed: _____ _____

GC Column: _____ ID: _____(mm)

COMPOUND	AMOUNT (ng)	PEAK	RT	RT WINDOW FROM	RT WINDOW TO	CALIBRATION FACTOR
Toxaphene		*1				
		*2				
		*3				
		4				
		5				
Aroclor 1016		*1				
		*2				
		*3				
		4				
		5				
Aroclor 1221		*1				
		*2				
		*3				
		4				
		5				
Aroclor 1232		*1				
		*2				
		*3				
		4				
		5				
Aroclor 1242		*1				
		*2				
		*3				
		4				
		5				
Aroclor 1248		*1				
		*2				
		*3				
		4				
		5				
Aroclor 1254		*1				
		*2				
		*3				
		4				
		5				
Aroclor 1260		*1				
		*2				
		*3				
		4				
		5				

* Denotes required peaks

GG

PESTICIDE ANALYTE RESOLUTION SUMMARY

Lab Name:_____ Contract:_____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

GC Column (1): _____ ID:_____(mm) Instrument ID (1): _____

EPA Sample No. (Standard 1): _____ Lab Sample ID (1): _____

Date Analyzed (1): _____ Time Analyzed (1): _____

	ANALYTE	RT	RESOLUTION (%)
01			
02			
03			
04			
05			
06			
07			
08			
09			

GC Column (2): _____ ID:_____(mm) Instrument ID (2): _____

EPA Sample No. (Standard 2): _____ Lab Sample ID (2): _____

Date Analyzed (2): _____ Time Analyzed (2): _____

	ANALYTE	RT	RESOLUTION (%)
01			
02			
03			
04			
05			
06			
07			
08			
09			

Resolution of two adjacent peaks must be calculated as a percentage of the height of the smaller peak, and must be greater than or equal to 60.0%.

SEMIVOLATILE CONTINUING CALIBRATION CHECK

Lab Name: _____ Contract: _____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Instrument ID: _____ Calibration Date: _____ Time: _____

Lab File ID: _____ Init. Calib. Date(s): _____ _____

Init. Calib. Times: _____ _____

COMPOUND	$\overline{RRF}$	RRF50	MIN RRF	%D	MAX %D
Phenol			0.800		25.0
bis(2-Chloroethyl)ether			0.700		25.0
2-Chlorophenol			0.800		25.0
1,3-Dichlorobenzene			0.600		25.0
1,4-Dichlorobenzene			0.500		25.0
1,2-Dichlorobenzene			0.400		25.0
2-Methylphenol			0.700		25.0
2,2'-oxybis(1-Chloropropane)					
4-Methylphenol			0.600		25.0
N-Nitroso-di-n-propylamine			0.500		25.0
Hexachloroethane			0.300		25.0
Nitrobenzene			0.200		25.0
Isophorone			0.400		25.0
2-Nitrophenol			0.100		25.0
2,4-Dimethylphenol			0.200		25.0
bis(2-Chloroethoxy)methane			0.300		25.0
2,4-Dichlorophenol			0.200		25.0
1,2,4-Trichlorobenzene			0.200		25.0
Naphthalene			0.700		25.0
4-Chloroaniline					
Hexachlorobutadiene					
4-Chloro-3-methylphenol			0.200		25.0
2-Methylnaphthalene			0.400		25.0
Hexachlorocyclopentadiene					
2,4,6-Trichlorophenol			0.200		25.0
2,4,5-Trichlorophenol			0.200		25.0
2-Chloronaphthalene			0.800		25.0
2-Nitroaniline					
Dimethylphthalate					
Acenaphthylene			1.300		25.0
2,6-Dinitrotoluene			0.200		25.0
3-Nitroaniline					
Acenaphthene			0.800		25.0
2,4-Dinitrophenol					
4-Nitrophenol					
Dibenzofuran			0.800		25.0
2,4-Dinitrotoluene			0.200		25.0

All other compounds must meet a minimum RRF of 0.010.

7C

SEMIVOLATILE CONTINUING CALIBRATION CHECK

ıb Name:_____ Contract:_____

ıb Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

ıstrument ID: _____ Calibration Date:_____ Time:_____

ıb File ID: _____ Init. Calib. Date(s):_____ _____

Init. Calib. Times: _____ _____

COMPOUND	RRF	RRF50	MIN RRF	%D	MAX %D
Diethylphthalate					
4-Chlorophenyl-phenylether			0.400		25.0
Fluorene			0.900		25.0
4-Nitroaniline					
4,6-Dinitro-2-methylphenol					
N-Nitrosodiphenylamine (1)					
4-Bromophenyl-phenylether			0.100		25.0
Hexachlorobenzene			0.100		25.0
Pentachlorophenol			0.050		25.0
Phenanthrene			0.700		25.0
Anthracene			0.700		25.0
Carbazole					
Di-n-butylphthalate					
Fluoranthene			0.600		25.0
Pyrene			0.600		25.0
Butylbenzylphthalate					
3,3'-Dichlorobenzidine					
Benzo(a)anthracene			0.800		25.0
Chrysene			0.700		25.0
bis(2-Ethylhexyl)phthalate					
Di-n-octylphthalate					
Benzo(b)fluoranthene			0.700		25.0
Benzo(k)fluoranthene			0.700		25.0
Benzo(a)pyrene			0.700		25.0
Indeno(1,2,3-cd)pyrene			0.500		25.0
Dibenz(a,h)anthracene			0.400		25.0
Benzo(g,h,i)perylene			0.500		25.0
Nitrobenzene-d5			0.200		25.0
2-Fluorobiphenyl			0.700		25.0
Terphenyl-d14			0.500		25.0
Phenol-d5			0.800		25.0
2-Fluorophenol			0.600		25.0
2,4,6-Tribromophenol					
2-Chlorophenol-d4			0.800		25.0
1,2-Dichlorobenzene-d4			0.400		25.0

(1) Cannot be separated from Diphenylamine
All other compounds must meet a minimum RRF of 0.010.

7D

PESTICIDE CALIBRATION VERIFICATION SUMMARY

Lab Name:_____ Contract:_____

Lab Code: ____.____ Case No.: _____ SAS No.: _____ SDG No.: _____

GC Column: _____ ID: _____(mm) Init. Calib. Date(s):_____ _____

EPA Sample No.(PIBLK): _____ Date Analyzed :_____

Lab Sample ID (PIBLK):_____ Time Analyzed :_____

EPA Sample No.(PEM): _____ Date Analyzed :_____

Lab Sample ID (PEM):_____ Time Analyzed :_____

PEM COMPOUND	RT	RT WINDOW FROM	RT WINDOW TO	CALC AMOUNT (ng)	NOM AMOUNT (ng)	RPD
alpha-BHC						
beta-BHC						
gamma-BHC (Lindane)						
Endrin						
4,4'-DDT						
Methoxychlor						

4,4'-DDT % breakdown (1): _____ Endrin % breakdown (1): _____

Combined % breakdown (1): _____

QC LIMITS:

RPD of amounts in PEM must be less than or equal to 25.0%

4,4'-DDT breakdown must be less than or equal to 20.0%

Endrin breakdown must be less than or equal to 20.0%

Combined breakdown must be less than or equal to 30.0%

7E

PESTICIDE CALIBRATION VERIFICATION SUMMARY

Lab Name:_____ Contract:_____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

GC Column: _____ ID: _____(mm) Init. Calib. Date(s):_____ _____

EPA Sample No.(PIBLK): _____ Date Analyzed :_____

Lab Sample ID (PIBLK): _____ Time Analyzed :_____

EPA Sample No.(INDA): _____ Date Analyzed :_____

Lab Sample ID (INDA): _____ Time Analyzed :_____

INDIVIDUAL MIX A COMPOUND	RT	RT WINDOW FROM	RT WINDOW TO	CALC AMOUNT (ng)	NOM AMOUNT (ng)	RPD
alpha-BHC						
gamma-BHC (Lindane)						
Heptachlor						
Endosulfan I						
Dieldrin						
Endrin						
4,4'-DDD						
4,4'-DDT						
Methoxychlor						
Tetrachloro-m-xylene						
Decachlorobiphenyl						

EPA Sample No.(INDB): _____ Date Analyzed :_____

Lab Sample ID (INDB): _____ Time Analyzed :_____

INDIVIDUAL MIX B COMPOUND	RT	RT WINDOW FROM	RT WINDOW TO	CALC AMOUNT (ng)	NOM AMOUNT (ng)	RPD
beta-BHC						
delta-BHC						
Aldrin						
Heptachlor epoxide						
4,4'-DDE						
Endosulfan II						
Endosulfan sulfate						
Endrin ketone						
Endrin aldehyde						
alpha-Chlordane						
gamma-Chlordane						
Tetrachloro-m-xylene						
Decachlorobiphenyl						

QC LIMITS: RPD of amounts in the Individual Mixes must be less than or equal to 25.0%.

8B

SEMIVOLATILE INTERNAL STANDARD AREA AND RT SUMMARY

Lab Name:_____ Contract:_____

Lab Code: ____ Case No.: _____ SAS No.: _____ SDG No.: _____

Lab File ID (Standard): _____ Date Analyzed:_____

Instrument ID: _____ Time Analyzed:_____

	EPA SAMPLE NO.	IS1(DCB) AREA #	RT #	IS2(NPT) AREA #	RT #	IS3(ANT) AREA #	RT #
	12 HOUR STD						
	UPPER LIMIT						
	LOWER LIMIT						
01							
02							
03							
04							
05							
06							
07							
08							
09							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							
21							
22							

IS1 (DCB) = 1,4-Dichlorobenzene-d4
IS2 (NPT) = Naphthalene-d8
IS3 (ANT) = Acenaphthene-d10

AREA UPPER LIMIT = +100% of internal standard area
AREA LOWER LIMIT = - 50% of internal standard area
RT UPPER LIMIT = +0.50 minutes of internal standard RT
RT LOWER LIMIT = -0.50 minutes of internal standard RT

Column used to flag internal standard area values with an asterisk.
* Values outside of QC limits.

page __ of __

8C

SEMIVOLATILE INTERNAL STANDARD AREA AND RT SUMMARY

Lab Name:_____ Contract:_____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Lab File ID (Standard): _____ Date Analyzed:_____

Instrument ID: _____ Time Analyzed:_____

	IS4(PHN) AREA	#	RT	#	IS5(CRY) AREA	#	RT	#	IS6(PRY) AREA	#	RT	#
12 HOUR STD												
UPPER LIMIT												
LOWER LIMIT												
EPA SAMPLE NO.												
01												
02												
03												
04												
05												
06												
07												
08												
09												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												

IS4 (PHN) = Phenanthrene-d10
IS5 (CRY) = Chrysene-d12
IS6 (PRY) = Perylene-d12

AREA UPPER LIMIT = +100% of internal standard area
AREA LOWER LIMIT = - 50% of internal standard area
RT UPPER LIMIT = +0.50 minutes of internal standard RT
RT LOWER LIMIT = -0.50 minutes of internal standard RT

Column used to flag internal standard area values with an asterisk.
* Values outside of QC limits.

8D

PESTICIDE ANALYTICAL SEQUENCE

Lab Name: _____ Contract: _____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

GC Column: _____ ID: _____(mm) Init. Calib. Date(s): _____ _____

Instrument ID: _____

THE ANALYTICAL SEQUENCE OF PERFORMANCE EVALUATION MIXTURES, BLANKS, SAMPLES, AND STANDARDS IS GIVEN BELOW:

MEAN SURROGATE RT FROM INITIAL CALIBRATION
TCX: _____ DCB: _____

	EPA SAMPLE NO.	LAB SAMPLE ID	DATE ANALYZED	TIME ANALYZED	TCX RT #	DCB RT #
01						
02						
03						
04						
05						
06						
07						
08						
09						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						
21						
22						
23						
24						
25						
26						
27						
28						
29						
30						
31						
32						

QC LIMITS

TCX = Tetrachloro-m-xylene (± 0.05 MINUTES)
DCB = Decachlorobiphenyl (± 0.10 MINUTES)

Column used to flag retention time values with an asterisk.
* Values outside of QC limits.

9A

PESTICIDE FLORISIL CARTRIDGE CHECK

Lab Name:_____ Contract:_____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Florisil Cartridge Lot Number: _____ Date of Analysis: _____

GC Column(1): _____ ID: _____(mm) GC Column(2): _____ ID: _____(mm)

COMPOUND	SPIKE ADDED (ng)	SPIKE RECOVERED (ng)	% REC	#	QC LIMITS
alpha-BHC					80-120
gamma-BHC (Lindane)					80-120
Heptachlor					80-120
Endosulfan I					80-120
Dieldrin					80-120
Endrin					80-120
4,4'-DDD					80-120
4,4'-DDT					80-120
Methoxychlor					80-120
Tetrachloro-m-xylene					80-120
Decachlorobiphenyl					80-120

Column to be used to flag recovery with an asterisk.
* Values outside of QC limits.

THIS CARTRIDGE LOT APPLIES TO THE FOLLOWING SAMPLES, BLANKS, MS, AND MSD:

	EPA SAMPLE NO.	LAB SAMPLE ID	DATE ANALYZED 1	DATE ANALYZED 2
01				
02				
03				
04				
05				
06				
07				
08				
09				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				

PESTICIDE GPC CALIBRATION

Lab Name:_____ Contract: _____

Lab Code: ____ Case No.: _____ SAS No.: _____ SDG No.: _____

GPC Column: _____ Calibration Date: _____

GC Column(1): _____ ID: _____(mm) GC Column(2): _____ ID: _____(mm)

COMPOUND	SPIKE ADDED (ng)	SPIKE RECOVERED (ng)	% REC #	QC. LIMITS REC.
gamma-BHC (Lindane)				80-110
Heptachlor				80-110
Aldrin				80-110
Dieldrin				80-110
Endrin				80-110
4,4'-DDT				80-110

\# Column to be used to flag recovery values with an asterisk
* Values outside of QC limits

THIS GPC CALIBRATION APPLIES TO THE FOLLOWING SAMPLES, BLANKS, MS AND MSD:

	EPA SAMPLE NO.	LAB SAMPLE ID	DATE ANALYZED 1	DATE ANALYZED 2
01				
02				
03				
04				
05				
06				
07				
08				
09				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				

10A

PESTICIDE IDENTIFICATION SUMMARY
FOR SINGLE COMPONENT ANALYTES

EPA SAMPLE NO.

Lab Name: _____ Contract: _____

Lab Code: ____ Case No.: _____ SAS No.: _____ SDG No.: _____

Lab Sample ID : _____ Date(s) Analyzed: _____ _____

Instrument ID (1): _____ Instrument ID (2): _____

GC Column(1): _____ ID: _____(mm) GC Column(2): _____ ID: _____(mm

ANALYTE	COL	RT	RT WINDOW FROM	RT WINDOW TO	CONCENTRATION	%D
	1					
	2					
	1					
	2					
	1					
	2					
	1					
	2					
	1					
	2					
	1					
	2					
	1					
	2					
	1					
	2					

10B
PESTICIDE IDENTIFICATION SUMMARY
FOR MULTICOMPONENT ANALYTES

EPA SAMPLE NO.

Lab Name: ______________ Contract: ________

Lab Code: ______ Case No.: ______ SAS No.: ______ SDG No.: ______

Lab Sample ID : __________ Date(s) Analyzed: ______ ______

Instrument ID (1): __________ Instrument ID (2): __________

GC Column(1): __________ ID: _____(mm) GC Column(2): __________ ID: _____(mm)

ANALYTE	PEAK	RT	RT WINDOW FROM	TO	CONCENTRATION	MEAN CONCENTRATION	%D
	1						
	2						
	3						
COLUMN 1	4						
	5						
	1						
	2						
	3						
COLUMN 2	4						
	5						
	1						
	2						
	3						
COLUMN 1	4						
	5						
	1						
	2						
	3						
COLUMN 2	4						
	5						
	1						
	2						
	3						
COLUMN 1	4						
	5						
	1						
	2						
	3						
COLUMN 2	4						
	5						

At least 3 peaks are required for identification of multicomponent analytes

page __ of __

SAMPLE LOG-IN SHEET

Lab Name: ______________________ Page ____ of ____

Received By (Print Name): ______________________ Log-in Date: ____________

Received By (Signature): ______________________

Case Number: ____________

Sample Delivery Group No.: ____________

SAS Number: ____________

REMARKS:

1. Custody Seal(s) — Present/Absent* Intact/Broken
2. Custody Seal Nos.: ________ ________
3. Chain-of-Custody Records — Present/Absent*
4. Traffic Reports or Packing List — Present/Absent*
5. Airbill — Airbill/Sticker Present/Absent*
6. Airbill No.: ________ ________
7. Sample Tags — Present/Absent*

   Sample Tag Numbers — Listed/Not Listed on Chain-of-Custody
8. Sample Condition: Intact/Broken*/ Leaking
9. Does information on custody records, traffic reports, and sample tags agree? Yes/No*
10. Date Received at Lab: ________
11. Time Received: ________

Sample Transfer

Fraction: ________ ________

Area #: ________ ________

By: ________ ________

On: ________ ________

	CORRESPONDING		
EPA SAMPLE #	SAMPLE TAG #	ASSIGNED LAB #	REMARKS: CONDITION OF SAMPLE SHIPMENT, ETC.

* Contact SMO and attach record of resolution

Reviewed By: ______________________

Date: ______________________

Logbook No.: ______________________

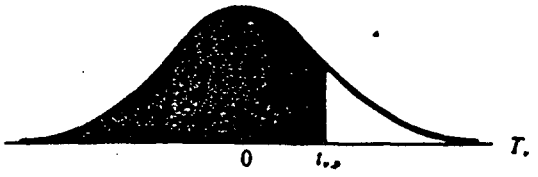
Logbook Page No: ______________________

FORM DC-1

APPENDIX O

STATISTICAL TABLES

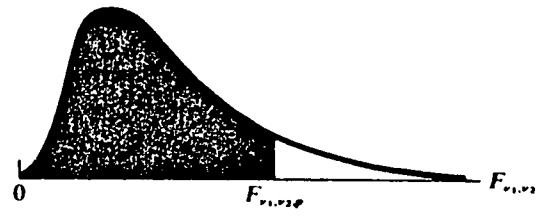
TABLE A-2 *Percentiles of the t distribution*



(a) *Student's t distribution*

df \ %	55	65	75	85	90	95	97.5	99	99.5	99.95
1	0.158	0.510	1.000	1.963	3.078	6.314	12.706	31.821	63.657	636.619
2	0.142	0.445	0.816	1.386	1.886	2.920	4.303	6.965	9.925	31.599
3	0.137	0.424	0.765	1.250	1.638	2.353	3.182	4.541	5.841	12.924
4	0.134	0.414	0.741	1.190	1.533	2.132	2.776	3.747	4.604	8.610
5	0.132	0.408	0.727	1.156	1.476	2.015	2.571	3.365	4.032	6.869
6	0.131	0.404	0.718	1.134	1.440	1.943	2.447	3.143	3.707	5.959
7	0.130	0.402	0.711	1.119	1.415	1.895	2.365	2.998	3.499	5.408
8	0.130	0.399	0.706	1.108	1.397	1.860	2.306	2.896	3.355	5.041
9	0.129	0.398	0.703	1.100	1.383	1.833	2.262	2.821	3.250	4.781
10	0.129	0.397	0.700	1.093	1.372	1.812	2.228	2.764	3.169	4.587
11	0.129	0.396	0.697	1.088	1.363	1.796	2.201	2.718	3.106	4.437
12	0.128	0.395	0.695	1.083	1.356	1.782	2.179	2.681	3.055	4.318
13	0.128	0.394	0.694	1.079	1.350	1.771	2.160	2.650	3.012	4.221
14	0.128	0.393	0.692	1.076	1.345	1.761	2.145	2.624	2.977	4.140
15	0.128	0.393	0.691	1.074	1.341	1.753	2.131	2.602	2.947	4.073
16	0.128	0.392	0.690	1.071	1.337	1.746	2.120	2.583	2.921	4.015
17	0.128	0.392	0.689	1.069	1.333	1.740	2.110	2.567	2.898	3.965
18	0.127	0.392	0.688	1.067	1.330	1.734	2.101	2.552	2.878	3.922
19	0.127	0.391	0.688	1.066	1.328	1.729	2.093	2.539	2.861	3.883
20	0.127	0.391	0.687	1.064	1.325	1.725	2.086	2.528	2.845	3.850
21	0.127	0.391	0.686	1.063	1.323	1.721	2.080	2.518	2.831	3.819
22	0.127	0.390	0.686	1.061	1.321	1.717	2.074	2.508	2.819	3.792
23	0.127	0.390	0.685	1.060	1.319	1.714	2.069	2.500	2.807	3.768
24	0.127	0.390	0.685	1.059	1.318	1.711	2.064	2.492	2.797	3.745
25	0.127	0.390	0.684	1.058	1.316	1.708	2.060	2.485	2.787	3.725
26	0.127	0.390	0.684	1.058	1.315	1.706	2.056	2.479	2.779	3.707
27	0.127	0.389	0.684	1.057	1.314	1.703	2.052	2.473	2.771	3.690
28	0.127	0.389	0.683	1.056	1.313	1.701	2.048	2.467	2.763	3.674
29	0.127	0.389	0.683	1.055	1.311	1.699	2.045	2.462	2.756	3.659
30	0.127	0.389	0.683	1.055	1.310	1.697	2.042	2.457	2.750	3.646
35	0.127	0.388	0.682	1.052	1.306	1.690	2.030	2.438	2.724	3.591
40	0.126	0.388	0.681	1.050	1.303	1.684	2.021	2.423	2.704	3.551
45	0.126	0.388	0.680	1.049	1.301	1.679	2.014	2.412	2.690	3.520
50	0.126	0.388	0.679	1.047	1.299	1.676	2.009	2.403	2.678	3.496
60	0.126	0.387	0.679	1.045	1.296	1.671	2.000	2.390	2.660	3.460
70	0.126	0.387	0.678	1.044	1.294	1.667	1.994	2.381	2.648	3.435
80	0.126	0.387	0.678	1.043	1.292	1.664	1.990	2.374	2.639	3.416
90	0.126	0.387	0.677	1.042	1.291	1.662	1.987	2.368	2.632	3.402
100	0.126	0.386	0.677	1.042	1.290	1.660	1.984	2.364	2.626	3.390
120	0.126	0.386	0.677	1.041	1.289	1.658	1.980	2.358	2.617	3.373
140	0.126	0.386	0.676	1.040	1.288	1.656	1.977	2.353	2.611	3.361
160	0.126	0.386	0.676	1.040	1.287	1.654	1.975	2.350	2.607	3.352
180	0.126	0.386	0.676	1.039	1.286	1.653	1.973	2.547	2.603	3.345
200	0.126	0.386	0.676	1.039	1.286	1.653	1.972	2.345	2.601	3.340
∞	0.126	0.385	0.674	1.036	1.282	1.645	1.960	2.326	2.576	3.291

TABLE A-4 *Percentiles of the F distribution*



(c) *F distribution*

Upper 25% point of the F distribution

DEGREES OF FREEDOM FOR DENOMINATOR	DEGREES OF FREEDOM FOR NUMERATOR																										
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	25	30	40	50	100	150	200
1	5.83	7.50	8.20	8.58	8.82	8.98	9.10	9.19	9.26	9.32	9.37	9.41	9.44	9.47	9.49	9.52	9.53	9.55	9.57	9.58	9.63	9.67	9.71	9.74	9.80	9.81	9.82
2	2.57	3.00	3.15	3.23	3.28	3.31	3.34	3.35	3.37	3.38	3.39	3.39	3.40	3.41	3.41	3.41	3.42	3.42	3.42	3.43	3.44	3.44	3.45	3.46	3.47	3.47	3.47
3	2.02	2.28	2.36	2.39	2.41	2.42	2.43	2.44	2.44	2.44	2.45	2.45	2.45	2.45	2.46	2.46	2.46	2.46	2.46	2.46	2.46	2.47	2.47	2.47	2.47	2.47	2.47
4	1.81	2.00	2.05	2.06	2.07	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08
5	1.69	1.85	1.88	1.89	1.89	1.89	1.89	1.89	1.89	1.89	1.89	1.89	1.89	1.89	1.89	1.88	1.88	1.88	1.88	1.88	1.88	1.88	1.88	1.88	1.87	1.87	1.87
6	1.62	1.76	1.78	1.79	1.79	1.78	1.78	1.78	1.77	1.77	1.77	1.77	1.77	1.76	1.76	1.76	1.76	1.76	1.76	1.76	1.75	1.75	1.75	1.75	1.74	1.74	1.74
7	1.57	1.70	1.72	1.72	1.71	1.71	1.70	1.70	1.69	1.69	1.69	1.68	1.68	1.68	1.68	1.68	1.67	1.67	1.67	1.67	1.67	1.66	1.66	1.66	1.65	1.65	1.65
8	1.54	1.66	1.67	1.66	1.66	1.65	1.64	1.64	1.63	1.63	1.63	1.62	1.62	1.62	1.62	1.62	1.61	1.61	1.61	1.61	1.60	1.60	1.59	1.59	1.58	1.58	1.58
9	1.51	1.62	1.63	1.63	1.62	1.61	1.60	1.60	1.59	1.59	1.58	1.58	1.58	1.57	1.57	1.57	1.57	1.56	1.56	1.56	1.55	1.55	1.54	1.54	1.53	1.53	1.53
10	1.49	1.60	1.60	1.59	1.59	1.58	1.57	1.56	1.56	1.55	1.55	1.54	1.54	1.54	1.53	1.53	1.53	1.53	1.53	1.52	1.52	1.51	1.51	1.50	1.49	1.49	1.49
11	1.47	1.58	1.58	1.57	1.56	1.55	1.54	1.53	1.53	1.52	1.52	1.51	1.51	1.51	1.50	1.50	1.50	1.50	1.49	1.49	1.49	1.48	1.47	1.47	1.46	1.46	1.46
12	1.46	1.56	1.56	1.55	1.54	1.53	1.52	1.51	1.51	1.50	1.49	1.49	1.49	1.48	1.48	1.48	1.47	1.47	1.47	1.47	1.46	1.45	1.45	1.44	1.43	1.43	1.43
13	1.45	1.55	1.55	1.53	1.52	1.51	1.50	1.49	1.49	1.48	1.47	1.47	1.47	1.46	1.46	1.46	1.45	1.45	1.45	1.45	1.44	1.43	1.42	1.42	1.41	1.41	1.40
14	1.44	1.53	1.53	1.52	1.51	1.50	1.49	1.48	1.47	1.46	1.46	1.45	1.45	1.44	1.44	1.44	1.44	1.43	1.43	1.43	1.42	1.41	1.41	1.40	1.39	1.39	1.38
15	1.43	1.52	1.52	1.51	1.49	1.48	1.47	1.46	1.46	1.45	1.44	1.44	1.43	1.43	1.43	1.42	1.42	1.42	1.41	1.41	1.40	1.40	1.39	1.38	1.37	1.37	1.37
16	1.42	1.51	1.51	1.50	1.48	1.47	1.46	1.45	1.44	1.44	1.43	1.43	1.42	1.42	1.41	1.41	1.41	1.40	1.40	1.40	1.39	1.38	1.37	1.37	1.36	1.35	1.35
17	1.42	1.51	1.50	1.49	1.47	1.46	1.45	1.44	1.43	1.43	1.42	1.41	1.41	1.41	1.40	1.40	1.39	1.39	1.39	1.39	1.38	1.37	1.36	1.36	1.34	1.34	1.34
18	1.41	1.50	1.49	1.48	1.46	1.45	1.44	1.43	1.42	1.42	1.41	1.40	1.40	1.40	1.39	1.39	1.38	1.38	1.38	1.38	1.37	1.36	1.35	1.34	1.33	1.33	1.32
19	1.41	1.49	1.49	1.47	1.46	1.44	1.43	1.42	1.41	1.41	1.40	1.40	1.39	1.39	1.38	1.38	1.37	1.37	1.37	1.37	1.36	1.35	1.34	1.33	1.32	1.31	1.31
20	1.40	1.49	1.48	1.47	1.45	1.44	1.43	1.42	1.41	1.40	1.39	1.39	1.38	1.38	1.37	1.37	1.37	1.36	1.36	1.36	1.35	1.34	1.33	1.32	1.31	1.30	1.30
21	1.40	1.48	1.48	1.46	1.44	1.43	1.42	1.41	1.40	1.39	1.39	1.38	1.37	1.37	1.37	1.36	1.36	1.36	1.35	1.35	1.34	1.33	1.32	1.32	1.30	1.30	1.29
22	1.40	1.48	1.47	1.45	1.44	1.42	1.41	1.40	1.39	1.39	1.38	1.37	1.37	1.36	1.36	1.36	1.35	1.35	1.35	1.34	1.33	1.32	1.31	1.31	1.29	1.29	1.28
23	1.39	1.47	1.47	1.45	1.43	1.42	1.41	1.40	1.39	1.38	1.37	1.37	1.36	1.36	1.35	1.35	1.35	1.34	1.34	1.34	1.33	1.32	1.31	1.30	1.29	1.28	1.28
24	1.39	1.47	1.46	1.44	1.43	1.41	1.40	1.39	1.38	1.38	1.37	1.36	1.36	1.35	1.35	1.34	1.34	1.34	1.33	1.33	1.32	1.31	1.30	1.29	1.28	1.27	1.27
25	1.39	1.47	1.46	1.44	1.42	1.41	1.40	1.39	1.38	1.37	1.36	1.36	1.35	1.35	1.34	1.34	1.33	1.33	1.33	1.33	1.31	1.31	1.29	1.29	1.27	1.27	1.26
26	1.38	1.46	1.45	1.44	1.42	1.41	1.39	1.38	1.37	1.37	1.36	1.35	1.35	1.34	1.34	1.33	1.33	1.33	1.32	1.32	1.31	1.30	1.29	1.28	1.27	1.26	1.26
27	1.38	1.46	1.45	1.43	1.42	1.40	1.39	1.38	1.37	1.36	1.35	1.35	1.34	1.34	1.33	1.33	1.33	1.32	1.32	1.32	1.30	1.30	1.28	1.28	1.26	1.25	1.25
28	1.38	1.46	1.45	1.43	1.41	1.40	1.39	1.38	1.37	1.36	1.35	1.34	1.34	1.33	1.33	1.32	1.32	1.32	1.31	1.31	1.30	1.29	1.28	1.27	1.25	1.25	1.25
29	1.38	1.46	1.45	1.43	1.41	1.40	1.38	1.37	1.36	1.35	1.35	1.34	1.33	1.33	1.32	1.32	1.32	1.31	1.31	1.31	1.30	1.29	1.27	1.27	1.25	1.24	1.24
30	1.38	1.45	1.44	1.42	1.41	1.39	1.38	1.37	1.36	1.35	1.34	1.34	1.33	1.33	1.32	1.32	1.31	1.31	1.31	1.30	1.29	1.28	1.27	1.26	1.25	1.24	1.24
32	1.37	1.45	1.44	1.42	1.40	1.39	1.37	1.36	1.35	1.34	1.34	1.33	1.32	1.32	1.31	1.31	1.31	1.30	1.30	1.30	1.28	1.28	1.26	1.25	1.24	1.23	1.23
34	1.37	1.44	1.43	1.42	1.40	1.38	1.37	1.36	1.35	1.34	1.33	1.33	1.32	1.31	1.31	1.30	1.30	1.30	1.29	1.29	1.28	1.27	1.26	1.25	1.23	1.22	1.22
36	1.37	1.44	1.43	1.41	1.39	1.38	1.36	1.35	1.34	1.33	1.33	1.32	1.31	1.31	1.30	1.30	1.30	1.29	1.29	1.29	1.27	1.26	1.25	1.24	1.22	1.22	1.21
38	1.36	1.44	1.43	1.41	1.39	1.37	1.36	1.35	1.34	1.33	1.32	1.32	1.31	1.30	1.30	1.29	1.29	1.29	1.28	1.28	1.27	1.26	1.24	1.24	1.22	1.21	1.21
40	1.36	1.44	1.42	1.40	1.39	1.37	1.36	1.35	1.34	1.33	1.32	1.31	1.31	1.30	1.30	1.29	1.29	1.28	1.28	1.28	1.26	1.25	1.24	1.23	1.21	1.20	1.20
42	1.36	1.43	1.42	1.40	1.38	1.37	1.35	1.34	1.33	1.32	1.32	1.31	1.30	1.30	1.29	1.29	1.28	1.28	1.28	1.27	1.26	1.25	1.24	1.23	1.21	1.20	1.20
44	1.36	1.43	1.42	1.40	1.38	1.36	1.35	1.34	1.33	1.32	1.31	1.31	1.30	1.29	1.29	1.28	1.28	1.28	1.27	1.27	1.25	1.24	1.23	1.22	1.20	1.19	1.19
46	1.36	1.43	1.42	1.40	1.38	1.36	1.35	1.34	1.33	1.32	1.31	1.30	1.30	1.29	1.28	1.28	1.28	1.27	1.27	1.26	1.25	1.24	1.23	1.22	1.20	1.19	1.19
48	1.36	1.43	1.41	1.39	1.38	1.36	1.35	1.33	1.32	1.31	1.31	1.30	1.29	1.29	1.28	1.28	1.27	1.27	1.27	1.26	1.25	1.24	1.22	1.21	1.19	1.19	1.18
50	1.35	1.43	1.41	1.39	1.37	1.36	1.34	1.33	1.32	1.31	1.30	1.30	1.29	1.28	1.28	1.27	1.27	1.27	1.26	1.26	1.25	1.23	1.22	1.21	1.19	1.18	1.18
60	1.35	1.42	1.41	1.38	1.37	1.35	1.33	1.32	1.31	1.30	1.29	1.29	1.28	1.27	1.27	1.26	1.26	1.26	1.25	1.25	1.23	1.22	1.21	1.20	1.18	1.17	1.16
70	1.35	1.41	1.40	1.38	1.36	1.34	1.33	1.32	1.31	1.30	1.29	1.28	1.27	1.27	1.26	1.26	1.25	1.25	1.24	1.24	1.23	1.21	1.20	1.19	1.16	1.16	1.15
80	1.34	1.41	1.40	1.38	1.36	1.34	1.32	1.31	1.30	1.29	1.28	1.27	1.27	1.26	1.26	1.25	1.25	1.24	1.24	1.23	1.22	1.21	1.19	1.18	1.16	1.15	1.14
90	1.34	1.41	1.39	1.37	1.35	1.33	1.32	1.31	1.30	1.29	1.28	1.27	1.26	1.26	1.25	1.25	1.24	1.24	1.23	1.23	1.21	1.20	1.19	1.18	1.15	1.14	1.13
100	1.34	1.41	1.39	1.37	1.35	1.33	1.32	1.30	1.29	1.28	1.27	1.27	1.26	1.25	1.25	1.24	1.24	1.23	1.23	1.23	1.21	1.20	1.18	1.17	1.14	1.13	1.13
125	1.34	1.40	1.39	1.36	1.34	1.33	1.31	1.30	1.29	1.28	1.27	1.26	1.25	1.25	1.24	1.24	1.23	1.23	1.22	1.22	1.20	1.19	1.17	1.16	1.14	1.12	1.12
150	1.33	1.40	1.38	1.36	1.34	1.32	1.31	1.30	1.28	1.27	1.27	1.26	1.25	1.24	1.24	1.23	1.23	1.22	1.22	1.21	1.20	1.19	1.17	1.16	1.13	1.12	1.11
200	1.33	1.40	1.38	1.36	1.34	1.32	1.30	1.29	1.28	1.27	1.26	1.25	1.24	1.24	1.23	1.23	1.22	1.22	1.21	1.21	1.19	1.18	1.16	1.15	1.12	1.11	1.10
300	1.33	1.39	1.38	1.35	1.33	1.32	1.30	1.29	1.27	1.26	1.26	1.25	1.24	1.23	1.23	1.22	1.22	1.21	1.21	1.20	1.19	1.17	1.15	1.14	1.11	1.10	1.09
500	1.33	1.39	1.37	1.35	1.33	1.31	1.30	1.28	1.27	1.26	1.25	1.24	1.24	1.23	1.22	1.22	1.21	1.21	1.20	1.20	1.18	1.17	1.15	1.14	1.10	1.09	1.08
1000	1.32	1.39	1.37	1.35	1.33	1.31	1.29	1.28	1.27	1.26	1.25	1.24	1.23	1.23	1.22	1.21	1.21	1.20	1.20	1.19	1.18	1.16	1.14	1.13	1.10	1.08	1.07

TABLE A-4 *Percentiles of the F distribution (continued)*

Upper 10% point of the F distribution

DEGREES OF FREEDOM FOR DENOMINATOR	DEGREES OF FREEDOM FOR NUMERATOR																										
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	25	30	40	50	100	150	200
1	39.9	49.5	53.6	55.8	57.2	58.2	58.9	59.4	59.9	60.2	60.5	60.7	60.9	61.1	61.2	61.3	61.5	61.6	61.7	61.7	62.1	62.3	62.5	62.7	63.0	63.1	63.2
2	8.53	9.00	9.16	9.24	9.29	9.33	9.35	9.37	9.38	9.39	9.40	9.41	9.41	9.42	9.42	9.43	9.43	9.44	9.44	9.44	9.45	9.46	9.47	9.47	9.48	9.48	9.49
3	5.54	5.46	5.39	5.34	5.31	5.28	5.27	5.25	5.24	5.23	5.22	5.22	5.21	5.20	5.20	5.20	5.19	5.19	5.19	5.18	5.17	5.17	5.16	5.15	5.14	5.14	5.14
4	4.54	4.32	4.19	4.11	4.05	4.01	3.98	3.95	3.94	3.92	3.91	3.90	3.89	3.88	3.87	3.86	3.86	3.85	3.85	3.84	3.83	3.82	3.80	3.80	3.78	3.77	3.77
5	4.06	3.78	3.62	3.52	3.45	3.40	3.37	3.34	3.32	3.30	3.28	3.27	3.26	3.25	3.24	3.23	3.22	3.22	3.21	3.21	3.19	3.17	3.16	3.15	3.13	3.12	3.12
6	3.78	3.46	3.29	3.18	3.11	3.05	3.01	2.98	2.96	2.94	2.92	2.90	2.89	2.88	2.87	2.86	2.85	2.85	2.84	2.84	2.81	2.80	2.78	2.77	2.75	2.74	2.73
7	3.59	3.26	3.07	2.96	2.88	2.83	2.78	2.75	2.72	2.70	2.68	2.67	2.65	2.64	2.63	2.62	2.61	2.61	2.60	2.59	2.57	2.56	2.54	2.52	2.50	2.49	2.48
8	3.46	3.11	2.92	2.81	2.73	2.67	2.62	2.59	2.56	2.54	2.52	2.50	2.49	2.48	2.46	2.45	2.45	2.44	2.43	2.42	2.40	2.38	2.36	2.35	2.32	2.31	2.31
9	3.36	3.01	2.81	2.69	2.61	2.55	2.51	2.47	2.44	2.42	2.40	2.38	2.36	2.35	2.34	2.33	2.32	2.31	2.30	2.30	2.27	2.25	2.23	2.22	2.19	2.18	2.17
10	3.29	2.92	2.73	2.61	2.52	2.46	2.41	2.38	2.35	2.32	2.30	2.28	2.27	2.26	2.24	2.23	2.22	2.22	2.21	2.20	2.17	2.16	2.13	2.12	2.09	2.08	2.07
11	3.23	2.86	2.66	2.54	2.45	2.39	2.34	2.30	2.27	2.25	2.23	2.21	2.19	2.18	2.17	2.16	2.15	2.14	2.13	2.12	2.10	2.08	2.05	2.04	2.01	1.99	1.99
12	3.18	2.81	2.61	2.48	2.39	2.33	2.28	2.24	2.21	2.19	2.17	2.15	2.13	2.12	2.10	2.09	2.08	2.08	2.07	2.06	2.03	2.01	1.99	1.97	1.94	1.93	1.92
13	3.14	2.76	2.56	2.43	2.35	2.28	2.23	2.20	2.16	2.14	2.12	2.10	2.08	2.07	2.05	2.04	2.03	2.02	2.01	2.01	1.98	1.96	1.93	1.92	1.88	1.87	1.86
14	3.10	2.73	2.52	2.39	2.31	2.24	2.19	2.15	2.12	2.10	2.07	2.05	2.04	2.02	2.01	2.00	1.99	1.98	1.97	1.96	1.93	1.91	1.89	1.87	1.83	1.82	1.82
15	3.07	2.70	2.49	2.36	2.27	2.21	2.16	2.12	2.09	2.06	2.04	2.02	2.00	1.99	1.97	1.96	1.95	1.94	1.93	1.92	1.89	1.87	1.85	1.83	1.79	1.78	1.77
16	3.05	2.67	2.46	2.33	2.24	2.18	2.13	2.09	2.06	2.03	2.01	1.99	1.97	1.95	1.94	1.93	1.92	1.91	1.90	1.89	1.86	1.84	1.81	1.79	1.76	1.74	1.74
17	3.03	2.64	2.44	2.31	2.22	2.15	2.10	2.06	2.03	2.00	1.98	1.96	1.94	1.93	1.91	1.90	1.89	1.88	1.87	1.86	1.83	1.81	1.78	1.76	1.73	1.71	1.71
18	3.01	2.62	2.42	2.29	2.20	2.13	2.08	2.04	2.00	1.98	1.95	1.93	1.92	1.90	1.89	1.87	1.86	1.85	1.84	1.84	1.80	1.78	1.75	1.74	1.70	1.68	1.68
19	2.99	2.61	2.40	2.27	2.18	2.11	2.06	2.02	1.98	1.96	1.93	1.91	1.89	1.88	1.86	1.85	1.84	1.83	1.82	1.81	1.78	1.76	1.73	1.71	1.67	1.66	1.65
20	2.97	2.59	2.38	2.25	2.16	2.09	2.04	2.00	1.96	1.94	1.91	1.89	1.87	1.86	1.84	1.83	1.82	1.81	1.80	1.79	1.76	1.74	1.71	1.69	1.65	1.64	1.63
21	2.96	2.57	2.36	2.23	2.14	2.08	2.02	1.98	1.95	1.92	1.90	1.87	1.86	1.84	1.83	1.81	1.80	1.79	1.78	1.78	1.74	1.72	1.69	1.67	1.63	1.62	1.61
22	2.95	2.56	2.35	2.22	2.13	2.06	2.01	1.97	1.93	1.90	1.88	1.86	1.84	1.83	1.81	1.80	1.79	1.78	1.77	1.76	1.73	1.70	1.67	1.65	1.61	1.60	1.59
23	2.94	2.55	2.34	2.21	2.11	2.05	1.99	1.95	1.92	1.89	1.87	1.84	1.83	1.81	1.80	1.78	1.77	1.76	1.75	1.74	1.71	1.69	1.66	1.64	1.59	1.58	1.57
24	2.93	2.54	2.33	2.19	2.10	2.04	1.98	1.94	1.91	1.88	1.85	1.83	1.81	1.80	1.78	1.77	1.76	1.75	1.74	1.73	1.70	1.67	1.64	1.62	1.58	1.56	1.56
25	2.92	2.53	2.32	2.18	2.09	2.02	1.97	1.93	1.89	1.87	1.84	1.82	1.80	1.79	1.77	1.76	1.75	1.74	1.73	1.72	1.68	1.66	1.63	1.61	1.56	1.55	1.54
26	2.91	2.52	2.31	2.17	2.08	2.01	1.96	1.92	1.88	1.86	1.83	1.81	1.79	1.77	1.76	1.75	1.73	1.72	1.71	1.71	1.67	1.65	1.61	1.59	1.55	1.54	1.53
27	2.90	2.51	2.30	2.17	2.07	2.00	1.95	1.91	1.87	1.85	1.82	1.80	1.78	1.76	1.75	1.74	1.72	1.71	1.70	1.70	1.66	1.64	1.60	1.58	1.54	1.52	1.52
28	2.89	2.50	2.29	2.16	2.06	2.00	1.94	1.90	1.87	1.84	1.81	1.79	1.77	1.75	1.74	1.73	1.71	1.70	1.69	1.69	1.65	1.63	1.59	1.57	1.53	1.51	1.50
29	2.89	2.50	2.28	2.15	2.06	1.99	1.93	1.89	1.86	1.83	1.80	1.78	1.76	1.75	1.73	1.72	1.71	1.69	1.68	1.68	1.64	1.62	1.58	1.56	1.52	1.50	1.49
30	2.88	2.49	2.28	2.14	2.05	1.98	1.93	1.88	1.85	1.82	1.79	1.77	1.75	1.74	1.72	1.71	1.70	1.69	1.68	1.67	1.63	1.61	1.57	1.55	1.51	1.49	1.48
32	2.87	2.48	2.26	2.13	2.04	1.97	1.91	1.87	1.83	1.81	1.78	1.76	1.74	1.72	1.71	1.69	1.68	1.67	1.66	1.65	1.62	1.59	1.56	1.53	1.49	1.47	1.46
34	2.86	2.47	2.25	2.12	2.02	1.96	1.90	1.86	1.82	1.79	1.77	1.75	1.73	1.71	1.69	1.68	1.67	1.66	1.65	1.64	1.60	1.58	1.54	1.52	1.47	1.46	1.45
36	2.85	2.46	2.24	2.11	2.01	1.94	1.89	1.85	1.81	1.78	1.76	1.73	1.71	1.70	1.68	1.67	1.66	1.65	1.64	1.63	1.59	1.56	1.53	1.51	1.46	1.44	1.43
38	2.84	2.45	2.23	2.10	2.01	1.94	1.88	1.84	1.80	1.77	1.75	1.72	1.70	1.69	1.67	1.66	1.65	1.63	1.62	1.61	1.58	1.55	1.52	1.49	1.45	1.43	1.42
40	2.84	2.44	2.23	2.09	2.00	1.93	1.87	1.83	1.79	1.76	1.74	1.71	1.70	1.68	1.66	1.65	1.64	1.63	1.62	1.61	1.57	1.54	1.51	1.48	1.43	1.42	1.41
42	2.83	2.43	2.22	2.08	1.99	1.92	1.86	1.82	1.78	1.75	1.73	1.71	1.69	1.67	1.65	1.64	1.63	1.62	1.61	1.60	1.56	1.53	1.50	1.47	1.42	1.40	1.40
44	2.82	2.43	2.21	2.08	1.98	1.91	1.86	1.81	1.78	1.75	1.72	1.70	1.68	1.66	1.65	1.63	1.62	1.61	1.60	1.59	1.55	1.52	1.49	1.46	1.41	1.39	1.39
46	2.82	2.42	2.21	2.07	1.98	1.91	1.85	1.81	1.77	1.74	1.71	1.69	1.67	1.65	1.64	1.63	1.61	1.60	1.59	1.58	1.54	1.52	1.48	1.46	1.40	1.39	1.38
48	2.81	2.42	2.20	2.07	1.97	1.90	1.85	1.80	1.77	1.73	1.71	1.69	1.67	1.65	1.63	1.62	1.61	1.59	1.58	1.57	1.54	1.51	1.47	1.45	1.40	1.38	1.37
50	2.81	2.41	2.20	2.06	1.97	1.90	1.84	1.80	1.76	1.73	1.70	1.68	1.66	1.64	1.63	1.61	1.60	1.59	1.58	1.57	1.53	1.50	1.46	1.44	1.39	1.37	1.36
60	2.79	2.39	2.18	2.04	1.95	1.87	1.82	1.77	1.74	1.71	1.68	1.66	1.64	1.62	1.60	1.59	1.58	1.56	1.55	1.54	1.50	1.48	1.44	1.41	1.36	1.34	1.33
70	2.78	2.38	2.16	2.03	1.93	1.86	1.80	1.76	1.72	1.69	1.66	1.64	1.62	1.60	1.59	1.57	1.56	1.55	1.54	1.53	1.49	1.46	1.42	1.39	1.34	1.31	1.30
80	2.77	2.37	2.15	2.02	1.92	1.85	1.79	1.75	1.71	1.68	1.65	1.63	1.61	1.59	1.57	1.56	1.55	1.53	1.52	1.51	1.47	1.44	1.40	1.38	1.32	1.30	1.28
90	2.76	2.36	2.15	2.01	1.91	1.84	1.78	1.74	1.70	1.67	1.64	1.62	1.60	1.58	1.56	1.55	1.54	1.52	1.51	1.50	1.46	1.43	1.39	1.36	1.30	1.28	1.27
100	2.76	2.36	2.14	2.00	1.91	1.83	1.78	1.73	1.69	1.66	1.64	1.61	1.59	1.57	1.56	1.54	1.53	1.52	1.50	1.49	1.45	1.42	1.38	1.35	1.29	1.27	1.26
125	2.75	2.35	2.13	1.99	1.89	1.82	1.77	1.72	1.68	1.65	1.62	1.60	1.58	1.56	1.54	1.53	1.51	1.50	1.49	1.48	1.44	1.41	1.36	1.34	1.27	1.25	1.23
150	2.74	2.34	2.12	1.98	1.89	1.81	1.76	1.71	1.67	1.64	1.61	1.59	1.57	1.55	1.53	1.52	1.50	1.49	1.48	1.47	1.43	1.40	1.35	1.33	1.26	1.23	1.22
200	2.73	2.33	2.11	1.97	1.88	1.80	1.75	1.70	1.66	1.63	1.60	1.58	1.56	1.54	1.52	1.51	1.49	1.48	1.47	1.46	1.41	1.38	1.34	1.31	1.24	1.21	1.20
300	2.72	2.32	2.10	1.96	1.87	1.79	1.74	1.69	1.65	1.62	1.59	1.57	1.55	1.53	1.51	1.49	1.48	1.47	1.46	1.45	1.40	1.37	1.32	1.29	1.22	1.19	1.18
500	2.72	2.31	2.09	1.96	1.86	1.79	1.73	1.68	1.64	1.61	1.58	1.56	1.54	1.52	1.50	1.49	1.47	1.46	1.45	1.44	1.39	1.36	1.31	1.28	1.21	1.18	1.16
1000	2.71	2.31	2.09	1.95	1.85	1.78	1.72	1.68	1.64	1.61	1.58	1.55	1.53	1.51	1.49	1.48	1.46	1.45	1.44	1.43	1.38	1.35	1.30	1.27	1.20	1.16	1.15

TABLE A-4 *Percentiles of the F distribution (continued)*

Upper 5% point of the F distribution

DEGREES OF FREEDOM FOR DENOMINATOR	DEGREES OF FREEDOM FOR NUMERATOR																										
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	25	30	40	60	100	150	200
1	161	200	216	225	230	234	237	239	241	242	243	244	245	245	246	246	247	247	248	248	249	250	251	252	253	253	254
2	18.5	19.0	19.2	19.2	19.3	19.3	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.5	19.5	19.5	19.5	19.5	19.5	19.5
3	10.1	9.55	9.28	9.12	9.01	8.94	8.89	8.85	8.81	8.79	8.76	8.74	8.73	8.71	8.70	8.69	8.68	8.67	8.67	8.66	8.63	8.62	8.59	8.58	8.55	8.54	8.54
4	7.71	6.94	6.59	6.39	6.26	6.16	6.09	6.04	6.00	5.96	5.94	5.91	5.89	5.87	5.86	5.84	5.83	5.82	5.81	5.80	5.77	5.75	5.72	5.70	5.66	5.65	5.65
5	6.61	5.79	5.41	5.19	5.05	4.95	4.88	4.82	4.77	4.74	4.70	4.68	4.66	4.64	4.62	4.60	4.59	4.58	4.57	4.56	4.52	4.50	4.46	4.44	4.41	4.39	4.39
6	5.99	5.14	4.76	4.53	4.39	4.28	4.21	4.15	4.10	4.06	4.03	4.00	3.98	3.96	3.94	3.92	3.91	3.90	3.88	3.87	3.83	3.81	3.77	3.75	3.71	3.70	3.69
7	5.59	4.74	4.35	4.12	3.97	3.87	3.79	3.73	3.68	3.64	3.60	3.57	3.55	3.53	3.51	3.49	3.48	3.47	3.46	3.44	3.40	3.38	3.34	3.32	3.27	3.26	3.25
8	5.32	4.46	4.07	3.84	3.69	3.58	3.50	3.44	3.39	3.35	3.31	3.28	3.26	3.24	3.22	3.20	3.19	3.17	3.16	3.15	3.11	3.08	3.04	3.02	2.97	2.96	2.95
9	5.12	4.26	3.86	3.63	3.48	3.37	3.29	3.23	3.18	3.14	3.10	3.07	3.05	3.03	3.01	2.99	2.97	2.96	2.95	2.94	2.89	2.86	2.83	2.80	2.76	2.74	2.73
10	4.96	4.10	3.71	3.48	3.33	3.22	3.14	3.07	3.02	2.98	2.94	2.91	2.89	2.86	2.85	2.83	2.81	2.80	2.79	2.77	2.73	2.70	2.66	2.64	2.59	2.57	2.56
11	4.84	3.98	3.59	3.36	3.20	3.09	3.01	2.95	2.90	2.85	2.82	2.79	2.76	2.74	2.72	2.70	2.69	2.67	2.66	2.65	2.60	2.57	2.53	2.51	2.46	2.44	2.43
12	4.75	3.89	3.49	3.26	3.11	3.00	2.91	2.85	2.80	2.75	2.72	2.69	2.66	2.64	2.62	2.60	2.58	2.57	2.56	2.54	2.50	2.47	2.43	2.40	2.35	2.33	2.32
13	4.67	3.81	3.41	3.18	3.03	2.92	2.83	2.77	2.71	2.67	2.63	2.60	2.58	2.55	2.53	2.51	2.50	2.48	2.47	2.46	2.41	2.38	2.34	2.31	2.26	2.24	2.23
14	4.60	3.74	3.34	3.11	2.96	2.85	2.76	2.70	2.65	2.60	2.57	2.53	2.51	2.48	2.46	2.44	2.43	2.41	2.40	2.39	2.34	2.31	2.27	2.24	2.19	2.17	2.16
15	4.54	3.68	3.29	3.06	2.90	2.79	2.71	2.64	2.59	2.54	2.51	2.48	2.45	2.42	2.40	2.38	2.37	2.35	2.34	2.33	2.28	2.25	2.20	2.18	2.12	2.10	2.10
16	4.49	3.63	3.24	3.01	2.85	2.74	2.66	2.59	2.54	2.49	2.46	2.42	2.40	2.37	2.35	2.33	2.32	2.30	2.29	2.28	2.23	2.19	2.15	2.12	2.07	2.05	2.04
17	4.45	3.59	3.20	2.96	2.81	2.70	2.61	2.55	2.49	2.45	2.41	2.38	2.35	2.33	2.31	2.29	2.27	2.26	2.24	2.23	2.18	2.15	2.10	2.08	2.02	2.00	1.99
18	4.41	3.55	3.16	2.93	2.77	2.66	2.58	2.51	2.46	2.41	2.37	2.34	2.31	2.29	2.27	2.25	2.23	2.22	2.20	2.19	2.14	2.11	2.06	2.04	1.98	1.96	1.95
19	4.38	3.52	3.13	2.90	2.74	2.63	2.54	2.48	2.42	2.38	2.34	2.31	2.28	2.26	2.23	2.21	2.20	2.18	2.17	2.16	2.11	2.07	2.03	2.00	1.94	1.92	1.91
20	4.35	3.49	3.10	2.87	2.71	2.60	2.51	2.45	2.39	2.35	2.31	2.28	2.25	2.22	2.20	2.18	2.17	2.15	2.14	2.12	2.07	2.04	1.99	1.97	1.91	1.89	1.88
21	4.32	3.47	3.07	2.84	2.68	2.57	2.49	2.42	2.37	2.32	2.28	2.25	2.22	2.20	2.18	2.16	2.14	2.12	2.11	2.10	2.05	2.01	1.96	1.94	1.88	1.86	1.84
22	4.30	3.44	3.05	2.82	2.66	2.55	2.46	2.40	2.34	2.30	2.26	2.23	2.20	2.17	2.15	2.13	2.11	2.10	2.08	2.07	2.02	1.98	1.94	1.91	1.85	1.83	1.82
23	4.28	3.42	3.03	2.80	2.64	2.53	2.44	2.37	2.32	2.27	2.24	2.20	2.18	2.15	2.13	2.11	2.09	2.08	2.06	2.05	2.00	1.96	1.91	1.88	1.82	1.80	1.79
24	4.26	3.40	3.01	2.78	2.62	2.51	2.42	2.36	2.30	2.25	2.22	2.18	2.15	2.13	2.11	2.09	2.07	2.05	2.04	2.03	1.97	1.94	1.89	1.86	1.80	1.78	1.77
25	4.24	3.39	2.99	2.76	2.60	2.49	2.40	2.34	2.28	2.24	2.20	2.16	2.14	2.11	2.09	2.07	2.05	2.04	2.02	2.01	1.96	1.92	1.87	1.84	1.78	1.76	1.75
26	4.23	3.37	2.98	2.74	2.59	2.47	2.39	2.32	2.27	2.22	2.18	2.15	2.12	2.09	2.07	2.05	2.03	2.02	2.00	1.99	1.94	1.90	1.85	1.82	1.76	1.74	1.73
27	4.21	3.35	2.96	2.73	2.57	2.46	2.37	2.31	2.25	2.20	2.17	2.13	2.10	2.08	2.06	2.04	2.02	2.00	1.99	1.97	1.92	1.88	1.84	1.81	1.74	1.72	1.71
28	4.20	3.34	2.95	2.71	2.56	2.45	2.36	2.29	2.24	2.19	2.15	2.12	2.09	2.06	2.04	2.02	2.00	1.99	1.97	1.96	1.91	1.87	1.82	1.79	1.73	1.70	1.69
29	4.18	3.33	2.93	2.70	2.55	2.43	2.35	2.28	2.22	2.18	2.14	2.10	2.08	2.05	2.03	2.01	1.99	1.97	1.96	1.94	1.89	1.85	1.81	1.77	1.71	1.69	1.67
30	4.17	3.32	2.92	2.69	2.53	2.42	2.33	2.27	2.21	2.16	2.13	2.09	2.06	2.04	2.01	1.99	1.98	1.96	1.95	1.93	1.88	1.84	1.79	1.76	1.70	1.67	1.66
32	4.15	3.29	2.90	2.67	2.51	2.40	2.31	2.24	2.19	2.14	2.10	2.07	2.04	2.01	1.99	1.97	1.95	1.94	1.92	1.91	1.85	1.82	1.77	1.74	1.67	1.64	1.63
34	4.13	3.28	2.88	2.65	2.49	2.38	2.29	2.23	2.17	2.12	2.08	2.05	2.02	1.99	1.97	1.95	1.93	1.92	1.90	1.89	1.83	1.80	1.75	1.71	1.65	1.62	1.61
36	4.11	3.26	2.87	2.63	2.48	2.36	2.28	2.21	2.15	2.11	2.07	2.03	2.00	1.98	1.95	1.93	1.92	1.90	1.88	1.87	1.81	1.78	1.73	1.69	1.62	1.60	1.59
38	4.10	3.24	2.85	2.62	2.46	2.35	2.26	2.19	2.14	2.09	2.05	2.02	1.99	1.96	1.94	1.92	1.90	1.88	1.87	1.85	1.80	1.76	1.71	1.68	1.61	1.58	1.57
40	4.08	3.23	2.84	2.61	2.45	2.34	2.25	2.18	2.12	2.08	2.04	2.00	1.97	1.95	1.92	1.90	1.89	1.87	1.85	1.84	1.78	1.74	1.69	1.66	1.59	1.56	1.55
42	4.07	3.22	2.83	2.59	2.44	2.32	2.24	2.17	2.11	2.06	2.03	1.99	1.96	1.94	1.91	1.89	1.87	1.86	1.84	1.83	1.77	1.73	1.68	1.65	1.57	1.55	1.53
44	4.06	3.21	2.82	2.58	2.43	2.31	2.23	2.16	2.10	2.05	2.01	1.98	1.95	1.92	1.90	1.88	1.86	1.84	1.83	1.81	1.76	1.72	1.67	1.63	1.56	1.53	1.52
46	4.05	3.20	2.81	2.57	2.42	2.30	2.22	2.15	2.09	2.04	2.00	1.97	1.94	1.91	1.89	1.87	1.85	1.83	1.82	1.80	1.75	1.71	1.65	1.62	1.55	1.52	1.51
48	4.04	3.19	2.80	2.57	2.41	2.29	2.21	2.14	2.08	2.03	1.99	1.96	1.93	1.90	1.88	1.86	1.84	1.82	1.81	1.79	1.74	1.70	1.64	1.61	1.54	1.51	1.49
50	4.03	3.18	2.79	2.56	2.40	2.29	2.20	2.13	2.07	2.03	1.99	1.95	1.92	1.89	1.87	1.85	1.83	1.81	1.80	1.78	1.73	1.69	1.63	1.60	1.52	1.50	1.48
60	4.00	3.15	2.76	2.53	2.37	2.25	2.17	2.10	2.04	1.99	1.95	1.92	1.89	1.86	1.84	1.82	1.80	1.78	1.76	1.75	1.69	1.65	1.59	1.56	1.48	1.45	1.44
70	3.98	3.13	2.74	2.50	2.35	2.23	2.14	2.07	2.02	1.97	1.93	1.89	1.86	1.84	1.81	1.79	1.77	1.75	1.74	1.72	1.66	1.62	1.57	1.53	1.45	1.42	1.40
80	3.96	3.11	2.72	2.49	2.33	2.21	2.13	2.06	2.00	1.95	1.91	1.88	1.84	1.82	1.79	1.77	1.75	1.73	1.72	1.70	1.64	1.60	1.54	1.51	1.43	1.39	1.38
90	3.95	3.10	2.71	2.47	2.32	2.20	2.11	2.04	1.99	1.94	1.90	1.86	1.83	1.80	1.78	1.76	1.74	1.72	1.70	1.69	1.63	1.59	1.53	1.49	1.41	1.38	1.36
100	3.94	3.09	2.70	2.46	2.31	2.19	2.10	2.03	1.97	1.93	1.89	1.85	1.82	1.79	1.77	1.75	1.73	1.71	1.69	1.68	1.62	1.57	1.52	1.48	1.39	1.36	1.34
125	3.92	3.07	2.68	2.44	2.29	2.17	2.08	2.01	1.96	1.91	1.87	1.83	1.80	1.77	1.75	1.73	1.71	1.69	1.67	1.66	1.59	1.55	1.49	1.45	1.36	1.33	1.31
150	3.90	3.06	2.66	2.43	2.27	2.16	2.07	2.00	1.94	1.89	1.85	1.82	1.79	1.76	1.73	1.71	1.69	1.67	1.66	1.64	1.58	1.54	1.48	1.44	1.34	1.31	1.29
200	3.89	3.04	2.65	2.42	2.26	2.14	2.06	1.98	1.93	1.88	1.84	1.80	1.77	1.74	1.72	1.69	1.67	1.66	1.64	1.62	1.56	1.52	1.46	1.41	1.32	1.28	1.26
300	3.87	3.03	2.63	2.40	2.24	2.13	2.04	1.97	1.91	1.86	1.82	1.78	1.75	1.72	1.70	1.68	1.66	1.64	1.62	1.61	1.54	1.50	1.43	1.39	1.30	1.26	1.23
500	3.86	3.01	2.62	2.39	2.23	2.12	2.03	1.96	1.90	1.85	1.81	1.77	1.74	1.71	1.69	1.66	1.64	1.62	1.61	1.59	1.53	1.48	1.42	1.38	1.28	1.23	1.21
1000	3.85	3.00	2.61	2.38	2.22	2.11	2.02	1.95	1.89	1.84	1.80	1.76	1.73	1.70	1.68	1.65	1.63	1.61	1.60	1.58	1.52	1.47	1.41	1.36	1.26	1.22	1.19

TABLE A-4 *Percentiles of the F distribution (continued)*

Upper 2.5% point of the F distribution

DEGREES OF FREEDOM FOR DENOMINATOR	DEGREES OF FREEDOM FOR NUMERATOR																										
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	25	30	40	50	100	150	200
1	548	800	864	900	922	937	948	957	963	969	973	977	980	983	985	987	989	990	992	993	998	1001	1006	1008	1013	1015	1016
2	38 5	39 0	39.2	39.2	39.3	39.3	39.4	39.4	39.4	39.4	39.4	39.4	39.4	39.4	39.4	39.4	39 4	39.4	39.4	39 4	39.5	39 5	39 5	39 5	39 5	39 5	39.5
3	17 4	16 0	15 4	15 1	14 9	14.7	14.6	14.5	14.5	14.4	14 4	14.3	14.3	14.3	14 3	14 2	14.2	14 2	14 2	14 2	14 1	14 1	14 0	14.0	14 0	13 9	13 9
4	12 2	10 6	9 98	9 60	9 36	9 20	9 07	8 98	8.90	8 84	8.79	8.75	8.71	8 68	8 66	8.63	8 61	8.59	8.58	8.56	8.50	8 46	8 41	8.38	8.32	8 30	8 29
5	10.0	8.43	7.76	7 39	7.15	6.98	6.85	6.76	6.68	6.62	6.57	6.52	6.49	6.46	6.43	6.40	6.38	6.36	6 34	6.33	6.27	6 23	6.18	6 14	6 08	6 06	6 05
6	8 81	7.26	6.60	6.23	5.99	5.82	5 70	5.60	5.52	5.46	5.41	5.37	5.33	5 30	5.27	5 24	5.22	5 20	5.18	5 17	5.11	5 07	5 01	4 98	4.92	4.89	4 88
7	8 07	6.54	5 89	5 52	5 29	5 12	4.99	4.90	4.82	4.76	4.71	4.67	4.63	4.60	4 57	4 54	4.52	4 50	4.48	4 47	4 40	4 36	4 31	4 28	4 21	4 19	4 18
8	7 57	6 06	5.42	5.05	4 82	4 65	4.53	4 43	4 36	4 30	4 24	4 20	4 16	4 13	4 10	4.08	4.05	4 03	4 02	4 00	3 94	3 89	3 84	3 81	3 74	3 72	3 70
9	7.21	5 71	5.08	4 72	4.48	4 32	4 20	4.10	4 03	3 96	3.91	3 87	3 83	3.80	3 77	3.74	3 72	3 70	3 68	3 67	3 60	3.56	3 51	3 47	3 40	3 38	3 37
10	6.94	5 46	4.83	4 47	4 24	4.07	3 95	3 85	3.78	3.72	3 66	3.62	3 58	3 55	3.52	3 50	3.47	3 45	3.44	3.42	3.35	3.31	3 26	3.22	3 15	3 13	3 12
11	6.72	5.26	4 63	4 28	4 04	3 88	3.76	3 66	3.59	3 53	3.47	3.43	3 39	3 36	3 33	3.30	3 28	3 26	3.24	3.23	3 16	3 12	3 06	3 03	2.96	2 93	2 92
12	6.55	5 10	4 47	4 12	3.89	3.73	3 61	3 51	3 44	3 37	3 32	3.28	3.24	3.21	3 18	3 15	3 13	3 11	3 09	3 07	3 01	2 96	2 91	2 87	2 80	2 78	2 76
13	6.41	4 97	4 35	4 00	3 77	3 60	3 48	3.39	3.31	3.25	3 20	3.15	3.12	3 08	3.05	3 03	3 00	2.98	2 96	2 95	2.88	2 84	2 78	2 74	2.67	2 65	2 63
14	6.30	4 86	4 24	3 89	3 66	3 50	3 38	3 29	3.21	3 15	3.09	3.05	3 01	2.98	2.95	2 92	2 90	2 88	2.86	2 84	2.78	2.73	2 67	2.64	2 56	2 54	2 53
15	6.20	4 77	4 15	3 80	3.58	3.41	3.29	3 20	3.12	3 06	3 01	2 96	2.92	2.89	2 86	2.84	2.81	2 79	2.77	2 76	2 69	2 64	2.59	2.55	2 47	2 45	2 44
16	6.12	4 69	4 08	3 73	3 50	3 34	3 22	3.12	3 05	2 99	2 93	2 89	2 85	2 82	2.79	2.76	2.74	2 72	2 70	2 68	2 61	2 57	2 51	2 47	2 40	2.37	2 36
17	6 04	4 62	4 01	3 66	3 44	3.28	3 16	3 06	2 98	2 92	2.87	2.82	2 79	2.75	2.72	2.70	2 67	2 65	2.63	2 62	2.55	2 50	2 44	2 41	2 33	2 30	2 29
18	5 98	4.56	3 95	3 61	3 38	3 22	3.10	3 01	2.93	2.87	2 81	2 77	2 73	2.70	2.67	2.64	2 62	2 60	2.58	2.56	2 49	2 44	2.38	2 35	2 27	2 24	2 23
19	5.92	4.51	3 90	3 56	3 33	3 17	3.05	2 96	2 88	2.82	2 76	2.72	2.68	2 65	2.62	2.59	2.57	2 55	2 53	2 51	2.44	2 39	2 33	2.30	2.22	2 19	2 18
20	5 87	4.46	3.86	3 51	3.29	3 13	3 01	2.91	2.84	2 77	2.72	2 68	2 64	2 60	2 57	2.55	2 52	2 50	2 48	2 46	2 40	2 35	2 29	2 25	2 17	2.14	2 13
21	5 83	4 42	3 82	3 48	3 25	3 09	2.97	2 87	2 80	2 73	2 68	2 64	2 60	2 56	2 53	2 51	2 48	2 46	2 44	2 42	2 36	2 31	2 25	2 21	2 13	2 10	2 09
22	5 79	4 38	3 78	3 44	3 22	3 05	2 93	2 84	2 76	2 70	2 65	2 60	2 56	2 53	2 50	2 47	2 45	2 43	2 41	2 39	2 32	2 27	2 21	2 17	2 09	2 06	2 05
23	5 75	4 35	3 75	3 41	3 18	3 02	2.90	2 81	2 73	2 67	2 62	2 57	2.53	2 50	2 47	2 44	2 42	2 39	2 37	2 36	2 29	2 24	2 18	2 14	2 06	2 03	2 01
24	5 72	4 32	3 72	3 38	3 15	2 99	2 87	2 78	2 70	2 64	2 59	2 54	2 50	2 47	2 44	2 41	2 39	2 36	2 35	2 33	2 26	2 21	2 15	2 11	2 02	2 00	1 98
25	5 69	4 29	3 69	3 35	3 13	2 97	2.85	2 75	2 68	2 61	2 56	2 51	2 48	2 44	2 41	2 38	2 36	2 34	2 32	2 30	2 23	2 18	2 12	2 08	2 00	1 97	1 95
26	5 66	4 27	3 67	3 33	3 10	2 94	2 82	2 73	2 65	2 59	2.54	2 49	2 45	2 42	2 39	2 36	2 34	2 31	2 29	2 28	2 21	2 16	2 09	2 05	1.97	1 94	1 92
27	5 63	4 24	3 65	3 31	3 08	2 92	2 80	2 71	2 63	2 57	2 51	2 47	2 43	2 39	2 36	2 34	2 31	2 29	2 27	2 25	2 18	2 13	2 07	2 03	1 94	1 91	1 90
28	5 61	4 22	3 63	3 29	3 06	2 90	2 78	2 69	2 61	2 55	2 49	2 45	2 41	2 37	2 34	2 32	2 29	2 27	2 25	2 23	2 16	2 11	2 05	2 01	1 92	1 89	1 88
29	5 59	4 20	3 61	3 27	3 04	2 88	2 76	2.67	2 59	2 53	2 48	2 43	2 39	2 36	2 32	2 30	2 27	2 25	2 23	2 21	2 14	2 09	2 03	1 99	1 90	1 87	1 86
30	5 57	4 18	3 59	3 25	3 03	2 87	2 75	2 65	2 57	2 51	2 46	2 41	2 37	2 34	2 31	2 28	2.26	2 23	2 21	2 20	2 12	2 07	2 01	1.97	1 88	1 85	1 84
32	5 53	4 15	3 56	3 22	3 00	2 84	2 71	2 62	2 54	2 48	2 43	2 38	2 34	2.31	2 28	2 25	2 22	2 20	2 18	2 16	2 09	2 04	1 98	1 93	1 85	1 82	1 80
34	5 50	4 12	3 53	3 19	2 97	2 81	2 69	2 59	2 52	2 45	2.40	2 35	2 31	2 28	2 25	2.22	2 20	2 17	2 15	2 13	2 06	2 01	1 95	1 90	1 82	1 78	1 77
36	5 47	4 09	3 50	3 17	2 94	2 78	2 66	2 57	2 49	2 43	2 37	2 33	2 29	2 25	2 22	2 20	2 17	2 15	2 13	2 11	2 04	1 99	1 92	1 88	1 79	1 76	1 74
38	5 45	4 07	3 48	3 15	2 92	2 76	2 64	2 55	2 47	2 41	2 35	2 31	2 27	2 23	2 20	2 17	2 15	2 13	2 11	2 09	2 01	1 96	1 90	1 85	1 76	1 73	1 71
40	5 42	4 05	3 46	3 13	2 90	2 74	2 62	2 53	2 45	2 39	2 33	2 29	2 25	2 21	2 18	2 15	2 13	2 11	2 09	2 07	1 99	1 94	1 88	1 83	1 74	1 71	1 69
42	5 40	4 03	3 45	3 11	2 89	2 73	2 61	2 51	2 43	2 37	2 32	2 27	2 23	2 20	2 16	2 14	2 11	2 09	2 07	2 05	1 98	1 92	1 86	1 81	1 72	1 69	1 67
44	5 39	4 02	3 43	3 09	2 87	2 71	2 59	2 50	2 42	2 36	2 30	2 26	2 22	2 18	2 15	2 12	2 10	2 07	2 05	2 03	1 96	1 91	1 84	1 80	1 70	1 67	1 65
46	5 37	4 00	3 42	3 08	2 86	2 70	2 58	2 48	2 41	2 34	2 29	2 24	2 20	2 17	2 13	2 11	2 08	2 06	2 04	2 02	1 94	1 89	1 82	1 78	1.69	1 65	1 63
48	5 35	3 99	3 40	3 07	2 84	2 69	2 56	2 47	2 39	2 33	2 27	2 23	2 19	2 15	2 12	2 09	2 07	2 05	2 02	2 01	1 93	1 88	1 81	1 77	1 67	1 64	1 62
50	5 34	3 97	3 39	3 05	2 83	2 67	2 55	2 46	2 38	2 32	2 26	2 22	2 18	2 14	2 11	2 08	2.06	2.03	2 01	1 99	1 92	1 87	1 80	1 75	1 66	1 62	1 60
60	5 29	3 93	3 34	3 01	2 79	2 63	2 51	2 41	2 33	2 27	2 22	2 17	2 13	2 09	2 06	2 03	2 01	1 98	1 96	1 94	1 87	1 82	1 74	1 70	1 60	1 56	1 54
70	5 25	3 89	3 31	2 97	2 75	2 59	2 47	2 38	2 30	2 24	2 18	2 14	2 10	2 06	2 03	2 00	1 97	1 95	1 93	1 91	1 83	1 78	1 71	1 66	1 56	1 52	1 50
80	5 22	3 86	3 28	2 95	2 73	2 57	2 45	2 35	2 28	2 21	2 16	2 11	2 07	2 03	2 00	1 97	1 95	1 92	1 90	1 88	1 81	1 75	1 68	1 63	1 53	1 49	1 47
90	5 20	3 84	3 26	2 93	2 71	2 55	2 43	2 34	2 26	2 19	2 14	2 09	2 05	2 02	1 98	1 95	1 93	1 91	1 88	1 86	1 79	1 73	1 66	1 61	1 50	1 46	1 44
100	5 18	3 83	3 25	2 92	2 70	2 54	2 42	2 32	2 24	2 18	2 12	2 08	2 04	2 00	1 97	1 94	1 91	1 89	1 87	1 85	1 77	1 71	1 64	1 59	1 48	1 44	1 42
125	5 15	3 80	3 22	2 89	2 67	2 51	2 39	2 30	2.22	2 15	2 10	2 05	2 01	1 97	1 94	1 91	1 89	1 86	1 84	1 82	1 74	1 68	1 61	1 56	1 45	1 40	1 38
150	5 13	3 78	3 20	2 87	2 65	2 49	2 37	2 28	2 20	2 13	2 08	2 03	1 99	1 95	1 92	1 89	1 87	1 84	1 82	1 80	1 72	1 67	1 59	1 54	1 42	1 38	1 35
200	5 10	3 76	3 18	2 85	2 63	2 47	2 35	2 26	2 18	2 11	2 06	2 01	1 97	1 93	1 90	1 87	1 84	1 82	1 80	1 78	1 70	1 64	1 56	1 51	1 39	1 35	1 32
300	5 07	3 73	3 16	2 83	2 61	2 45	2 33	2 23	2 16	2 09	2 04	1 99	1 95	1 91	1 88	1 85	1 82	1 80	1 77	1 75	1 67	1 62	1 54	1 48	1 36	1 31	1 28
500	5 05	3 72	3 14	2 81	2 59	2 43	2 31	2 22	2 14	2 07	2 02	1 97	1 93	1 89	1 86	1 83	1 80	1 78	1 76	1 74	1 65	1 60	1 52	1 46	1 34	1 28	1 25
1000	5 04	3 70	3 13	2 80	2 58	2 42	2 30	2 20	2 13	2 06	2 01	1 96	1 92	1 88	1 85	1 82	1 79	1 77	1 74	1 72	1 64	1 58	1 50	1 45	1 32	1 26	1 23

TABLE A-4 *Percentiles of the F distribution (continued)*

Upper 1% point of the F distribution

DEGREES OF FREEDOM FOR DENOMINATOR	DEGREES OF FREEDOM FOR NUMERATOR																										
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	25	30	40	50	100	150	200
1	4052	5000	5403	5625	5764	5859	5928	5981	6022	6056	6083	6106	6126	6143	6157	6170	6181	6192	6201	6209	6240	6261	6287	6303	6334	6345	6350
2	98 5	99 0	99 2	99 2	99.3	99 3	99 4	99 4	99 4	99 4	99 4	99 4	99 4	99 4	99 4	99 4	99 4	99 4	99.4	99 4	99 5	99 5	99 5	99 5	99 5	99 5	99 5
3	34 1	30 8	29 5	28 7	28 2	27 9	27 7	27 5	27 3	27 2	27 1	27 1	27 0	26.9	26.9	26.8	26 8	26 8	26.7	26 7	26 6	26 5	26 4	26 4	26 2	26 2	26 2
4	21.2	18 0	16 7	16 0	15 5	15 2	15 0	14 8	14 7	14 5	14 5	14 4	14 3	14 2	14 2	14 2	14 1	14.1	14 0	14 0	13 9	13.8	13 7	13 7	13 6	13 5	13 5
5	16 3	13.3	12.1	11 4	11 0	10.7	10 5	10.3	10.2	10 1	9 96	9 89	9 82	9.77	9 72	9 68	9.64	9 61	9.58	9 55	9 45	9.38	9 29	9 24	9 13	9 09	9 08
6	13 7	10 9	9.78	9 15	8.75	8 47	8 26	8.10	7 98	7.87	7 79	7.72	7 66	7.60	7 56	7 52	7.48	7 45	7 42	7 40	7 30	7.23	7 14	7.09	6 99	6 95	6 93
7	12 2	9 55	8 45	7 85	7.46	7 19	6 99	6 84	6 72	6 62	6 54	6 47	6 41	6 36	6 31	6 28	6.24	6 21	6 18	6 16	6 06	5 99	5.91	5 86	5.75	5 72	5 70
8	11 3	8 65	7 59	7 01	6 63	6 37	6 18	6 03	5 91	5 81	5 73	5 67	5 61	5 56	5 52	5 48	5 44	5.41	5 38	5 36	5.26	5 20	5 12	5 07	4 96	4 93	4 91
9	10 6	8 02	6 99	6 42	6 06	5 80	5 61	5 47	5 35	5 26	5 18	5 11	5 05	5 01	4 96	4 92	4 89	4 86	4 83	4 81	4 71	4 65	4 57	4 52	4 41	4 38	4 36
10	10 0	7 56	6 55	5 99	5.64	5 39	5 20	5 06	4 94	4 85	4 77	4 71	4 65	4 60	4 56	4 52	4 49	4 46	4 43	4 41	4 31	4 25	4 17	4 12	4 01	3 98	3 96
11	9 65	7 21	6 22	5 67	5.32	5 07	4 89	4 74	4 63	4 54	4 46	4 40	4.34	4.29	4 25	4.21	4 18	4.15	4 12	4 10	4 01	3 94	3 86	3.81	3.71	3 67	3.66
12	9 33	6 93	5 95	5 41	5 06	4 82	4 64	4 50	4 39	4 30	4.22	4 16	4 10	4 05	4 01	3 97	3 94	3 91	3 88	3 86	3 76	3 70	3 62	3.57	3 47	3 43	3 41
13	9 07	6 70	5 74	5 21	4 86	4 62	4 44	4 30	4.19	4 10	4 02	3 96	3 91	3 86	3 82	3 78	3 75	3 72	3 69	3 66	3 57	3 51	3 43	3 38	3 27	3 24	3 22
14	8 88	6 51	5 56	5 04	4 69	4 46	4.28	4 14	4 03	3 94	3 86	3 80	3 75	3 70	3 66	3 62	3 59	3 56	3 53	3 51	3 41	3 35	3 27	3 22	3 11	3 08	3 06
15	8 68	6 36	5 42	4 89	4.56	4 32	4 14	4 00	3 89	3.80	3 73	3 67	3 61	3 56	3 52	3 49	3.45	3 42	3 40	3 37	3 28	3 21	3 13	3 08	2 98	2 94	2 92
16	8 53	6 23	5 29	4 77	4 44	4 20	4 03	3 89	3 78	3.69	3 62	3 55	3 50	3 45	3 41	3 37	3 34	3 31	3 28	3 26	3 16	3 10	3 02	2 97	2 86	2 83	2 81
17	8 40	6 11	5 19	4 67	4 34	4 10	3 93	3 79	3 68	3 59	3 52	3 46	3 40	3 35	3 31	3 27	3 24	3 21	3 19	3 16	3 07	3 00	2 92	2 87	2 76	2 73	2 71
18	8 29	6 01	5 09	4 58	4 25	4 01	3 84	3 71	3 60	3 51	3 43	3 37	3 32	3 27	3 23	3 19	3.16	3 13	3 10	3 08	2 98	2 92	2 84	2 78	2 68	2 64	2 62
19	8 18	5 93	5 01	4 50	4.17	3 94	3 77	3 63	3 52	3 43	3 36	3 30	3 24	3.19	3 15	3.12	3 08	3 05	3 03	3 00	2 91	2 84	2 76	2 71	2 60	2 57	2 55
20	8 10	5 85	4 94	4 43	4 10	3 87	3 70	3 56	3 46	3 37	3 29	3 23	3 18	3.13	3.09	3 05	3 02	2 99	2 96	2 94	2.84	2.78	2.69	2 64	2 54	2 50	2 48
21	8 02	5 78	4 87	4 37	4 04	3 81	3 64	3 51	3 40	3 31	3 24	3 17	3.12	3.07	3 03	2 99	2 96	2 93	2 90	2 88	2 79	2 72	2 64	2 58	2 48	2 44	2 42
22	7 95	5 72	4 82	4 31	3.99	3.76	3 59	3 45	3 35	3 26	3 18	3 12	3 07	3.02	2 98	2 94	2 91	2 88	2 85	2 83	2 73	2 67	2 58	2 53	2 42	2 38	2 36
23	7 88	5 66	4 76	4 26	3 94	3 71	3 54	3 41	3 30	3.21	3 14	3 07	3 02	2 97	2 93	2 89	2 86	2 83	2 80	2 78	2 69	2 62	2.54	2 48	2 37	2 34	2 32
24	7 82	5 61	4 72	4 22	3 90	3 67	3 50	3 36	3 26	3.17	3 09	3 03	2 98	2 93	2 89	2 85	2 82	2 79	2 76	2 74	2 64	2 58	2 49	2 44	2.33	2 29	2 27
25	7 77	5 57	4 68	4 18	3 85	3 63	3 46	3 32	3 22	3 13	3 06	2 99	2 94	2 89	2 85	2 81	2 78	2 75	2.72	2 70	2 60	2 54	2 45	2 40	2 29	2 25	2 23
26	7 72	5 53	4 64	4 14	3.82	3 59	3 42	3 29	3 18	3 09	3 02	2 96	2 90	2 86	2 81	2 78	2 75	2 72	2 69	2 66	2 57	2 50	2 42	2 36	2 25	2 21	2 19
27	7 68	5 49	4 60	4 11	3 78	3 56	3 39	3 26	3 15	3 06	2 99	2 93	2 87	2 82	2 78	2 75	2 71	2 68	2 66	2 63	2 54	2 47	2 38	2 33	2.22	2 18	2 16
28	7 64	5 45	4 57	4 07	3 75	3 53	3 36	3 23	3 12	3 03	2 96	2 90	2 84	2 79	2 75	2 72	2 68	2 65	2 63	2 60	2 51	2 44	2 35	2 30	2 19	2 15	2 13
29	7 60	5 42	4 54	4 04	3 73	3 50	3 33	3 20	3 09	3 00	2 93	2 87	2 81	2 77	2 73	2 69	2 66	2 63	2 60	2 57	2 48	2 41	2 33	2 27	2 16	2 12	2 10
30	7 56	5 39	4 51	4 02	3 70	3 47	3 30	3 17	3 07	2 98	2 91	2 84	2 79	2 74	2 70	2 66	2 63	2 60	2 57	2 55	2 45	2 39	2.30	2 25	2 13	2 09	2.07
32	7 50	5 34	4 46	3 97	3 65	3 43	3 26	3 13	3 02	2 93	2 86	2 80	2 74	2.70	2 65	2 62	2 58	2 55	2.53	2 50	2 41	2 34	2 25	2 20	2 08	2 04	2 02
34	7 44	5 29	4 42	3 93	3 61	3 39	3 22	3 09	2 98	2 89	2 82	2 76	2.70	2 66	2 61	2 58	2 54	2 51	2.49	2.46	2 37	2 30	2 21	2 16	2 04	2 00	1 98
36	7 40	5 25	4 38	3 89	3 57	3 35	3 18	3 05	2 95	2 86	2 79	2 72	2 67	2 62	2 58	2 54	2 51	2 48	2 45	2 43	2 33	2 26	2 18	2 12	2 00	1 96	1 94
38	7 35	5 21	4 34	3 86	3 54	3 32	3 15	3 02	2 92	2 83	2 75	2 69	2 64	2 59	2 55	2 51	2 48	2 45	2 42	2 40	2 30	2 23	2 14	2 09	1 97	1 93	1 90
40	7 31	5 18	4 31	3 83	3 51	3 29	3 12	2 99	2 89	2 80	2 73	2 66	2 61	2 56	2 52	2 48	2 45	2 42	2 39	2 37	2 27	2.20	2 11	2 06	1 94	1 90	1 87
42	7 28	5 15	4 29	3 80	3 49	3 27	3 10	2 97	2 86	2 78	2 70	2 64	2 59	2 54	2 50	2.46	2.43	2 40	2 37	2 34	2 25	2 18	2 09	2 03	1 91	1 87	1 85
44	7 25	5 12	4 26	3 78	3 47	3 24	3 08	2 95	2 84	2 75	2 68	2 62	2 56	2 52	2 47	2 44	2 40	2 37	2 35	2 32	2 22	2 15	2 07	2 01	1 89	1 84	1 82
46	7 22	5 10	4 24	3 76	3 44	3 22	3 06	2 93	2 82	2 73	2 66	2 60	2 54	2 50	2 45	2 42	2 38	2 35	2 33	2 30	2 20	2 13	2 04	1 99	1 86	1 82	1 80
48	7 19	5 08	4 22	3 74	3 43	3 20	3 04	2 91	2 80	2 71	2 64	2 58	2 53	2 48	2 44	2 40	2 37	2 33	2 31	2 28	2 18	2 12	2 02	1 97	1 84	1 80	1 78
50	7 17	5 06	4 20	3 72	3 41	3 19	3 02	2 89	2 78	2 70	2 63	2 56	2 51	2 46	2 42	2 38	2 35	2 32	2 29	2 27	2 17	2 10	2 01	1 95	1 82	1 78	1 76
60	7 08	4 98	4 13	3 65	3 34	3 12	2 95	2 82	2 72	2 63	2 56	2 50	2 44	2 39	2 35	2 31	2 28	2 25	2 22	2 20	2 10	2 03	1 94	1 88	1 75	1 70	1 68
70	7 01	4 92	4 07	3 60	3 29	3 07	2 91	2 78	2 67	2 59	2 51	2 45	2 40	2 35	2 31	2 27	2 23	2 20	2 18	2 15	2 05	1 98	1 89	1 83	1 70	1 65	1 62
80	6 96	4 88	4 04	3 56	3 26	3 04	2 87	2 74	2 64	2 55	2 48	2 42	2 36	2 31	2 27	2 23	2 20	2 17	2 14	2 12	2 01	1 94	1 85	1 79	1 65	1 61	1 58
90	6 93	4 85	4 01	3 53	3 23	3 01	2 84	2 72	2 61	2 52	2 45	2 39	2 33	2 29	2 24	2 21	2 17	2 14	2 11	2 09	1 99	1 92	1 82	1 76	1 62	1 57	1 55
100	6 90	4 82	3 98	3 51	3 21	2 99	2 82	2 69	2 59	2 50	2 43	2 37	2 31	2 27	2 22	2 19	2 15	2 12	2 09	2 07	1 97	1 89	1 80	1 74	1 60	1 55	1 52
125	6 84	4 78	3 94	3 47	3 17	2 95	2 79	2 66	2 55	2 47	2 39	2 33	2 28	2 23	2 19	2 15	2 11	2 08	2 05	2 03	1 93	1 85	1 76	1 69	1 55	1 50	1 47
150	6 81	4 75	3 91	3 45	3 14	2 92	2 76	2 63	2 53	2 44	2 37	2 31	2 25	2 20	2 16	2 12	2 09	2 06	2 03	2 00	1 90	1 83	1 73	1 66	1 52	1 46	1 43
200	6 76	4 71	3 88	3 41	3 11	2 89	2 73	2 60	2 50	2 41	2 34	2 27	2 22	2 17	2 13	2 09	2 06	2 03	2 00	1 97	1 87	1 79	1 69	1 63	1 48	1 42	1 39
300	6 72	4 68	3 85	3 38	3 08	2 86	2 70	2 57	2 47	2 38	2 31	2 24	2 19	2 14	2 10	2 06	2 03	1 99	1 97	1 94	1 84	1 76	1 66	1 59	1 44	1 38	1 35
500	6 69	4 65	3 82	3 36	3 05	2 84	2 68	2 55	2 44	2 36	2 28	2 22	2 17	2 12	2 07	2 04	2 00	1 97	1 94	1 92	1 81	1 74	1 63	1 57	1 41	1 34	1 31
1000	6 66	4 63	3 80	3 34	3 04	2 82	2 66	2 53	2 43	2 34	2 27	2 20	2 15	2 10	2 06	2 02	1 98	1 95	1 92	1 90	1 79	1 72	1 61	1 54	1 38	1 32	1 28

TABLE A-4 *Percentiles of the F distribution (continued)*

Upper 0.5% point of the F distribution

DEGREES OF FREEDOM FOR DENOMINATOR	DEGREES OF FREEDOM FOR NUMERATOR																										
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	25	30	40	50	100	150	200
1	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
2	199	199	199	199	199	199	199	199	199	199	199	199	199	199	199	199	199	199	199	199	199	199	199	199	199	199	199
3	55.6	49.8	47.5	46.2	45.4	44.8	44.4	44.1	43.9	43.7	43.5	43.4	43.3	43.2	43.1	43.0	42.9	42.9	42.8	42.8	42.6	42.5	42.3	42.2	42.0	42.0	41.9
4	31.3	26.3	24.3	23.2	22.5	22.0	21.6	21.4	21.1	21.0	20.8	20.7	20.6	20.5	20.4	20.4	20.3	20.3	20.2	20.2	20.0	19.9	19.8	19.7	19.5	19.4	19.4
5	22.8	18.3	16.5	15.6	14.9	14.5	14.2	14.0	13.8	13.6	13.5	13.4	13.3	13.2	13.1	13.1	13.0	13.0	12.9	12.9	12.8	12.7	12.5	12.5	12.3	12.2	12.2
6	18.6	14.5	12.9	12.0	11.5	11.1	10.8	10.6	10.4	10.3	10.1	10.0	9.95	9.88	9.81	9.76	9.71	9.66	9.62	9.59	9.45	9.36	9.24	9.17	9.03	8.98	8.95
7	16.2	12.4	10.9	10.1	9.52	9.16	8.89	8.68	8.51	8.38	8.27	8.18	8.10	8.03	7.97	7.91	7.87	7.83	7.79	7.75	7.62	7.53	7.42	7.35	7.22	7.17	7.15
8	14.7	11.0	9.60	8.81	8.30	7.95	7.69	7.50	7.34	7.21	7.10	7.01	6.94	6.87	6.81	6.76	6.72	6.68	6.64	6.61	6.48	6.40	6.29	6.22	6.09	6.04	6.02
9	13.6	10.1	8.72	7.96	7.47	7.13	6.88	6.69	6.54	6.42	6.31	6.23	6.15	6.09	6.03	5.98	5.94	5.90	5.86	5.83	5.71	5.62	5.52	5.45	5.32	5.28	5.26
10	12.8	9.43	8.08	7.34	6.87	6.54	6.30	6.12	5.97	5.85	5.75	5.66	5.59	5.53	5.47	5.42	5.38	5.34	5.31	5.27	5.15	5.07	4.97	4.90	4.77	4.73	4.71
11	12.2	8.91	7.60	6.88	6.42	6.10	5.86	5.68	5.54	5.42	5.32	5.24	5.16	5.10	5.05	5.00	4.96	4.92	4.89	4.86	4.74	4.65	4.55	4.49	4.36	4.31	4.29
12	11.8	8.51	7.23	6.52	6.07	5.76	5.52	5.35	5.20	5.09	4.99	4.91	4.84	4.77	4.72	4.67	4.63	4.59	4.56	4.53	4.41	4.33	4.23	4.17	4.04	3.99	3.97
13	11.4	8.19	6.93	6.23	5.79	5.48	5.25	5.08	4.94	4.82	4.72	4.64	4.57	4.51	4.46	4.41	4.37	4.33	4.30	4.27	4.15	4.07	3.97	3.91	3.78	3.74	3.71
14	11.1	7.92	6.68	6.00	5.56	5.26	5.03	4.86	4.72	4.60	4.51	4.43	4.36	4.30	4.25	4.20	4.16	4.12	4.09	4.06	3.94	3.86	3.76	3.70	3.57	3.53	3.50
15	10.8	7.70	6.48	5.80	5.37	5.07	4.85	4.67	4.54	4.42	4.33	4.25	4.18	4.12	4.07	4.02	3.98	3.95	3.91	3.88	3.77	3.69	3.58	3.52	3.39	3.35	3.33
16	10.6	7.51	6.30	5.64	5.21	4.91	4.69	4.52	4.38	4.27	4.18	4.10	4.03	3.97	3.92	3.87	3.83	3.80	3.76	3.73	3.62	3.54	3.44	3.37	3.25	3.20	3.18
17	10.4	7.35	6.16	5.50	5.07	4.78	4.56	4.39	4.25	4.14	4.05	3.97	3.90	3.84	3.79	3.75	3.71	3.67	3.64	3.61	3.49	3.41	3.31	3.25	3.12	3.07	3.05
18	10.2	7.21	6.03	5.37	4.96	4.66	4.44	4.28	4.14	4.03	3.94	3.86	3.79	3.73	3.68	3.64	3.60	3.56	3.53	3.50	3.38	3.30	3.20	3.14	3.01	2.96	2.94
19	10.1	7.09	5.92	5.27	4.85	4.56	4.34	4.18	4.04	3.93	3.84	3.76	3.70	3.64	3.59	3.54	3.50	3.46	3.43	3.40	3.29	3.21	3.11	3.04	2.91	2.87	2.85
20	9.94	6.99	5.82	5.17	4.76	4.47	4.26	4.09	3.96	3.85	3.76	3.68	3.61	3.55	3.50	3.46	3.42	3.38	3.35	3.32	3.20	3.12	3.02	2.96	2.83	2.78	2.76
21	9.83	6.89	5.73	5.09	4.68	4.39	4.18	4.01	3.88	3.77	3.68	3.60	3.54	3.48	3.43	3.38	3.34	3.31	3.27	3.24	3.13	3.05	2.95	2.88	2.75	2.71	2.68
22	9.73	6.81	5.65	5.02	4.61	4.32	4.11	3.94	3.81	3.70	3.61	3.54	3.47	3.41	3.36	3.31	3.27	3.24	3.21	3.18	3.06	2.98	2.88	2.82	2.69	2.64	2.62
23	9.63	6.73	5.58	4.95	4.54	4.26	4.05	3.88	3.75	3.64	3.55	3.47	3.41	3.35	3.30	3.25	3.21	3.18	3.15	3.12	3.00	2.92	2.82	2.76	2.62	2.58	2.56
24	9.55	6.66	5.52	4.89	4.49	4.20	3.99	3.83	3.69	3.59	3.50	3.42	3.35	3.30	3.25	3.20	3.16	3.12	3.09	3.06	2.95	2.87	2.77	2.70	2.57	2.52	2.50
25	9.48	6.60	5.46	4.84	4.43	4.15	3.94	3.78	3.64	3.54	3.45	3.37	3.30	3.25	3.20	3.15	3.11	3.08	3.04	3.01	2.90	2.82	2.72	2.65	2.52	2.47	2.45
26	9.41	6.54	5.41	4.79	4.38	4.10	3.89	3.73	3.60	3.49	3.40	3.33	3.26	3.20	3.15	3.11	3.07	3.03	3.00	2.97	2.85	2.77	2.67	2.61	2.47	2.43	2.40
27	9.34	6.49	5.36	4.74	4.34	4.06	3.85	3.69	3.56	3.45	3.36	3.28	3.22	3.16	3.11	3.07	3.03	2.99	2.96	2.93	2.81	2.73	2.63	2.57	2.43	2.38	2.36
28	9.28	6.44	5.32	4.70	4.30	4.02	3.81	3.65	3.52	3.41	3.32	3.25	3.18	3.12	3.07	3.03	2.99	2.95	2.92	2.89	2.77	2.69	2.59	2.53	2.39	2.35	2.32
29	9.23	6.40	5.28	4.66	4.26	3.98	3.77	3.61	3.48	3.38	3.29	3.21	3.15	3.09	3.04	2.99	2.95	2.92	2.88	2.86	2.74	2.66	2.56	2.49	2.36	2.31	2.29
30	9.18	6.35	5.24	4.62	4.23	3.95	3.74	3.58	3.45	3.34	3.25	3.18	3.11	3.06	3.01	2.96	2.92	2.89	2.85	2.82	2.71	2.63	2.52	2.46	2.32	2.28	2.25
32	9.09	6.28	5.17	4.56	4.17	3.89	3.68	3.52	3.39	3.29	3.20	3.12	3.06	3.00	2.95	2.90	2.86	2.83	2.80	2.77	2.65	2.57	2.47	2.40	2.26	2.22	2.19
34	9.01	6.22	5.11	4.50	4.11	3.84	3.63	3.47	3.34	3.24	3.15	3.07	3.01	2.95	2.90	2.85	2.81	2.78	2.75	2.72	2.60	2.52	2.42	2.35	2.21	2.16	2.14
36	8.94	6.16	5.06	4.46	4.06	3.79	3.58	3.42	3.30	3.19	3.10	3.03	2.96	2.90	2.85	2.81	2.77	2.73	2.70	2.67	2.56	2.48	2.37	2.30	2.17	2.12	2.09
38	8.88	6.11	5.02	4.41	4.02	3.75	3.54	3.39	3.26	3.15	3.06	2.99	2.92	2.87	2.82	2.77	2.73	2.70	2.66	2.63	2.52	2.44	2.33	2.27	2.12	2.08	2.05
40	8.83	6.07	4.98	4.37	3.99	3.71	3.51	3.35	3.22	3.12	3.03	2.95	2.89	2.83	2.78	2.74	2.70	2.66	2.63	2.60	2.48	2.40	2.30	2.23	2.09	2.04	2.01
42	8.78	6.03	4.94	4.34	3.95	3.68	3.48	3.32	3.19	3.09	3.00	2.92	2.86	2.80	2.75	2.71	2.67	2.63	2.60	2.57	2.45	2.37	2.26	2.20	2.06	2.00	1.98
44	8.74	5.99	4.91	4.31	3.92	3.65	3.45	3.29	3.16	3.06	2.97	2.89	2.83	2.77	2.72	2.68	2.64	2.60	2.57	2.54	2.42	2.34	2.24	2.17	2.03	1.97	1.95
46	8.70	5.96	4.88	4.28	3.90	3.62	3.42	3.26	3.14	3.03	2.94	2.87	2.80	2.75	2.70	2.65	2.61	2.58	2.54	2.51	2.40	2.32	2.21	2.14	2.00	1.95	1.92
48	8.66	5.93	4.85	4.25	3.87	3.60	3.40	3.24	3.11	3.01	2.92	2.85	2.78	2.72	2.67	2.63	2.59	2.55	2.52	2.49	2.37	2.29	2.19	2.12	1.97	1.92	1.90
50	8.63	5.90	4.83	4.23	3.85	3.58	3.38	3.22	3.09	2.99	2.90	2.82	2.76	2.70	2.65	2.61	2.57	2.53	2.50	2.47	2.35	2.27	2.16	2.10	1.95	1.90	1.87
60	8.49	5.79	4.73	4.14	3.76	3.49	3.29	3.13	3.01	2.90	2.82	2.74	2.68	2.62	2.57	2.53	2.49	2.45	2.42	2.39	2.27	2.19	2.08	2.01	1.86	1.81	1.78
70	8.40	5.72	4.66	4.08	3.70	3.43	3.23	3.08	2.95	2.85	2.76	2.68	2.62	2.56	2.51	2.47	2.43	2.39	2.36	2.33	2.21	2.13	2.02	1.95	1.80	1.74	1.71
80	8.33	5.67	4.61	4.03	3.65	3.39	3.19	3.03	2.91	2.80	2.72	2.64	2.58	2.52	2.47	2.43	2.39	2.35	2.32	2.29	2.17	2.08	1.97	1.90	1.75	1.69	1.66
90	8.28	5.62	4.57	3.99	3.62	3.35	3.15	3.00	2.87	2.77	2.68	2.61	2.54	2.49	2.44	2.39	2.35	2.32	2.28	2.25	2.13	2.05	1.94	1.87	1.71	1.65	1.62
100	8.24	5.59	4.54	3.96	3.59	3.33	3.13	2.97	2.85	2.74	2.66	2.58	2.52	2.46	2.41	2.37	2.33	2.29	2.26	2.23	2.11	2.02	1.91	1.84	1.68	1.62	1.59
125	8.17	5.53	4.49	3.91	3.54	3.28	3.08	2.93	2.80	2.70	2.61	2.54	2.47	2.42	2.37	2.32	2.28	2.24	2.21	2.18	2.06	1.98	1.86	1.79	1.63	1.56	1.53
150	8.12	5.49	4.45	3.88	3.51	3.25	3.05	2.89	2.77	2.67	2.58	2.51	2.44	2.38	2.33	2.29	2.25	2.21	2.18	2.15	2.03	1.94	1.83	1.76	1.59	1.53	1.49
200	8.06	5.44	4.41	3.84	3.47	3.21	3.01	2.86	2.73	2.63	2.54	2.47	2.40	2.35	2.30	2.25	2.21	2.18	2.14	2.11	1.99	1.91	1.79	1.71	1.54	1.48	1.44
300	8.00	5.39	4.36	3.80	3.43	3.17	2.97	2.82	2.69	2.59	2.51	2.43	2.37	2.31	2.26	2.21	2.17	2.14	2.10	2.07	1.95	1.87	1.75	1.67	1.50	1.43	1.39
500	7.95	5.35	4.33	3.76	3.40	3.14	2.94	2.79	2.66	2.56	2.48	2.40	2.34	2.28	2.23	2.19	2.14	2.11	2.07	2.04	1.92	1.84	1.72	1.64	1.46	1.39	1.35
1000	7.91	5.33	4.30	3.74	3.37	3.11	2.92	2.77	2.64	2.54	2.45	2.38	2.32	2.26	2.21	2.16	2.12	2.09	2.05	2.02	1.90	1.81	1.69	1.61	1.43	1.36	1.31

TABLE A-4 *Percentiles of the F distribution (continued)*

Upper 0.1% point of the F distribution

DEGREES OF FREEDOM FOR DENOMINATOR	DEGREES OF FREEDOM FOR NUMERATOR																										
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	25	30	40	50	100	150	200
1	****	****	****	****	****	****	****	****	****	****	****	****	****	****	****	****	****	****	****	****	****	****	****	****	****	****	****
2	999	999	999	999	999	999	999	999	999	999	999	999	999	999	999	999	999	999	999	999	999	999	999	999	999	999	999
3	167	148	141	137	135	133	132	131	130	129	129	128	128	128	127	127	127	127	127	126	126	125	125	125	124	124	124
4	74.1	61.2	56.2	53.4	51.7	50.5	49.7	49.0	48.5	48.1	47.7	47.4	47.2	46.9	46.8	46.6	46.5	46.3	46.2	46.1	45.7	45.4	45.1	44.9	44.5	44.3	44.3
5	47.2	37.1	33.2	31.1	29.8	28.8	28.2	27.6	27.2	26.9	26.6	26.4	26.2	26.1	25.9	25.8	25.7	25.6	25.5	25.4	25.1	24.9	24.6	24.4	24.1	24.0	24.0
6	35.5	27.0	23.7	21.9	20.8	20.0	19.5	19.0	18.7	18.4	18.2	18.0	17.8	17.7	17.6	17.4	17.4	17.3	17.2	17.1	16.9	16.7	16.4	16.3	16.0	15.9	15.9
7	29.2	21.7	18.8	17.2	16.2	15.5	15.0	14.6	14.3	14.1	13.9	13.7	13.6	13.4	13.3	13.2	13.1	13.1	13.0	12.9	12.7	12.5	12.3	12.2	12.0	11.9	11.8
8	25.4	18.5	15.8	14.4	13.5	12.9	12.4	12.0	11.8	11.5	11.4	11.2	11.1	10.9	10.8	10.8	10.7	10.6	10.5	10.5	10.3	10.1	9.92	9.80	9.57	9.49	9.45
9	22.9	16.4	13.9	12.6	11.7	11.1	10.7	10.4	10.1	9.89	9.72	9.57	9.44	9.33	9.24	9.15	9.08	9.01	8.95	8.90	8.69	8.55	8.37	8.26	8.04	7.96	7.93
10	21.0	14.9	12.6	11.3	10.5	9.93	9.52	9.20	8.96	8.75	8.59	8.45	8.32	8.22	8.13	8.05	7.98	7.91	7.86	7.80	7.60	7.47	7.30	7.19	6.98	6.91	6.87
11	19.7	13.8	11.6	10.3	9.58	9.05	8.66	8.35	8.12	7.92	7.76	7.63	7.51	7.41	7.32	7.24	7.17	7.11	7.06	7.01	6.81	6.68	6.52	6.42	6.21	6.14	6.10
12	18.6	13.0	10.8	9.63	8.89	8.38	8.00	7.71	7.48	7.29	7.14	7.00	6.89	6.79	6.71	6.63	6.57	6.51	6.45	6.40	6.22	6.09	5.93	5.83	5.63	5.56	5.52
13	17.8	12.3	10.2	9.07	8.35	7.86	7.49	7.21	6.98	6.80	6.65	6.52	6.41	6.31	6.23	6.16	6.09	6.03	5.98	5.93	5.75	5.63	5.47	5.37	5.17	5.10	5.07
14	17.1	11.8	9.73	8.62	7.92	7.44	7.08	6.80	6.58	6.40	6.26	6.13	6.02	5.93	5.85	5.78	5.71	5.66	5.60	5.56	5.38	5.25	5.10	5.00	4.81	4.74	4.71
15	16.6	11.3	9.34	8.25	7.57	7.09	6.74	6.47	6.26	6.08	5.94	5.81	5.71	5.62	5.54	5.46	5.40	5.35	5.29	5.25	5.07	4.95	4.80	4.70	4.51	4.44	4.41
16	16.1	11.0	9.01	7.94	7.27	6.80	6.46	6.19	5.98	5.81	5.67	5.55	5.44	5.35	5.27	5.20	5.14	5.09	5.04	4.99	4.82	4.70	4.54	4.45	4.26	4.19	4.16
17	15.7	10.7	8.73	7.68	7.02	6.56	6.22	5.96	5.75	5.58	5.44	5.32	5.22	5.13	5.05	4.99	4.92	4.87	4.82	4.78	4.60	4.48	4.33	4.24	4.05	3.98	3.95
18	15.4	10.4	8.49	7.46	6.81	6.35	6.02	5.76	5.56	5.39	5.25	5.13	5.03	4.94	4.87	4.80	4.74	4.68	4.63	4.59	4.42	4.30	4.15	4.06	3.87	3.80	3.77
19	15.1	10.2	8.28	7.27	6.62	6.18	5.85	5.59	5.39	5.22	5.08	4.97	4.87	4.78	4.70	4.64	4.58	4.52	4.47	4.43	4.26	4.14	3.99	3.90	3.71	3.65	3.61
20	14.8	9.95	8.10	7.10	6.46	6.02	5.69	5.44	5.24	5.08	4.94	4.82	4.72	4.64	4.56	4.49	4.44	4.38	4.33	4.29	4.12	4.00	3.86	3.77	3.58	3.51	3.48
21	14.6	9.77	7.94	6.95	6.32	5.88	5.56	5.31	5.11	4.95	4.81	4.70	4.60	4.51	4.44	4.37	4.31	4.26	4.21	4.17	4.00	3.88	3.74	3.64	3.46	3.39	3.36
22	14.4	9.61	7.80	6.81	6.19	5.76	5.44	5.19	4.99	4.83	4.70	4.58	4.49	4.40	4.33	4.26	4.20	4.15	4.10	4.06	3.89	3.78	3.63	3.54	3.35	3.28	3.25
23	14.2	9.47	7.67	6.70	6.08	5.65	5.33	5.09	4.89	4.73	4.60	4.48	4.39	4.30	4.23	4.16	4.10	4.05	4.00	3.96	3.79	3.68	3.53	3.44	3.25	3.19	3.16
24	14.0	9.34	7.55	6.59	5.98	5.55	5.23	4.99	4.80	4.64	4.51	4.39	4.30	4.21	4.14	4.07	4.02	3.96	3.92	3.87	3.71	3.59	3.45	3.36	3.17	3.10	3.07
25	13.9	9.22	7.45	6.49	5.89	5.46	5.15	4.91	4.71	4.56	4.42	4.31	4.22	4.13	4.06	3.99	3.94	3.88	3.84	3.79	3.63	3.52	3.37	3.28	3.09	3.03	2.99
26	13.7	9.12	7.36	6.41	5.80	5.38	5.07	4.83	4.64	4.48	4.35	4.24	4.14	4.06	3.99	3.92	3.86	3.81	3.77	3.72	3.56	3.44	3.30	3.21	3.02	2.95	2.92
27	13.6	9.02	7.27	6.33	5.73	5.31	5.00	4.76	4.57	4.41	4.28	4.17	4.08	3.99	3.92	3.86	3.80	3.75	3.70	3.66	3.49	3.38	3.23	3.14	2.96	2.89	2.86
28	13.5	8.93	7.19	6.25	5.66	5.24	4.93	4.69	4.50	4.35	4.22	4.11	4.01	3.93	3.86	3.80	3.74	3.69	3.64	3.60	3.43	3.32	3.18	3.09	2.90	2.83	2.80
29	13.4	8.85	7.12	6.19	5.59	5.18	4.87	4.64	4.45	4.29	4.16	4.05	3.96	3.88	3.80	3.74	3.68	3.63	3.59	3.54	3.38	3.27	3.12	3.03	2.84	2.78	2.74
30	13.3	8.77	7.05	6.12	5.53	5.12	4.82	4.58	4.39	4.24	4.11	4.00	3.91	3.82	3.75	3.69	3.63	3.58	3.53	3.49	3.33	3.22	3.07	2.98	2.79	2.73	2.69
32	13.1	8.64	6.94	6.01	5.43	5.02	4.72	4.48	4.30	4.14	4.02	3.91	3.81	3.73	3.66	3.60	3.54	3.49	3.44	3.40	3.24	3.13	2.98	2.89	2.70	2.64	2.60
34	13.0	8.52	6.83	5.92	5.34	4.93	4.63	4.40	4.22	4.06	3.94	3.83	3.74	3.65	3.56	3.52	3.46	3.41	3.37	3.33	3.16	3.05	2.91	2.82	2.63	2.56	2.52
36	12.8	8.42	6.74	5.84	5.26	4.86	4.56	4.33	4.14	3.99	3.87	3.76	3.67	3.59	3.51	3.45	3.40	3.34	3.30	3.26	3.10	2.98	2.84	2.75	2.56	2.49	2.46
38	12.7	8.33	6.66	5.76	5.19	4.79	4.49	4.26	4.08	3.93	3.80	3.70	3.60	3.52	3.45	3.39	3.34	3.28	3.24	3.20	3.04	2.92	2.78	2.69	2.50	2.43	2.40
40	12.6	8.25	6.59	5.70	5.13	4.73	4.44	4.21	4.02	3.87	3.75	3.64	3.55	3.47	3.40	3.34	3.28	3.23	3.19	3.14	2.98	2.87	2.73	2.64	2.44	2.38	2.34
42	12.5	8.18	6.53	5.64	5.07	4.68	4.38	4.16	3.97	3.83	3.70	3.59	3.50	3.42	3.35	3.29	3.23	3.18	3.14	3.10	2.94	2.83	2.68	2.59	2.40	2.33	2.29
44	12.4	8.12	6.48	5.59	5.02	4.63	4.34	4.11	3.93	3.78	3.66	3.55	3.46	3.38	3.31	3.25	3.19	3.14	3.10	3.06	2.89	2.78	2.64	2.55	2.35	2.28	2.25
46	12.4	8.06	6.42	5.54	4.98	4.59	4.30	4.07	3.89	3.74	3.62	3.51	3.42	3.34	3.27	3.21	3.15	3.10	3.06	3.02	2.86	2.74	2.60	2.51	2.31	2.24	2.21
48	12.3	8.00	6.38	5.50	4.94	4.55	4.26	4.03	3.85	3.70	3.58	3.48	3.38	3.31	3.24	3.17	3.12	3.07	3.02	2.98	2.82	2.71	2.56	2.47	2.28	2.21	2.17
50	12.2	7.96	6.34	5.46	4.90	4.51	4.22	4.00	3.82	3.67	3.55	3.44	3.35	3.27	3.20	3.14	3.09	3.04	2.99	2.95	2.79	2.68	2.53	2.44	2.25	2.18	2.14
60	12.0	7.77	6.17	5.31	4.76	4.37	4.09	3.86	3.69	3.54	3.42	3.32	3.23	3.15	3.08	3.02	2.96	2.91	2.87	2.83	2.67	2.55	2.41	2.32	2.12	2.05	2.01
70	11.8	7.64	6.06	5.20	4.66	4.28	3.99	3.77	3.60	3.45	3.33	3.23	3.14	3.06	2.99	2.93	2.88	2.83	2.78	2.74	2.58	2.47	2.32	2.23	2.03	1.95	1.92
80	11.7	7.54	5.97	5.12	4.58	4.20	3.92	3.70	3.53	3.39	3.27	3.16	3.07	3.00	2.93	2.87	2.81	2.76	2.72	2.68	2.52	2.41	2.26	2.16	1.96	1.89	1.85
90	11.6	7.47	5.91	5.06	4.53	4.15	3.87	3.65	3.48	3.34	3.22	3.11	3.02	2.95	2.88	2.82	2.76	2.71	2.67	2.63	2.47	2.36	2.21	2.11	1.91	1.83	1.79
100	11.5	7.41	5.86	5.02	4.48	4.11	3.83	3.61	3.44	3.30	3.18	3.07	2.99	2.91	2.84	2.78	2.73	2.68	2.63	2.59	2.43	2.32	2.17	2.08	1.87	1.79	1.75
125	11.4	7.30	5.77	4.93	4.40	4.03	3.75	3.54	3.37	3.23	3.11	3.00	2.92	2.84	2.77	2.71	2.66	2.61	2.56	2.52	2.36	2.25	2.10	2.01	1.79	1.71	1.67
150	11.3	7.24	5.71	4.88	4.35	3.98	3.71	3.49	3.32	3.18	3.06	2.96	2.87	2.80	2.73	2.67	2.61	2.56	2.52	2.48	2.32	2.21	2.06	1.96	1.74	1.66	1.62
200	11.2	7.15	5.63	4.81	4.29	3.92	3.65	3.43	3.26	3.12	3.00	2.90	2.82	2.74	2.67	2.61	2.56	2.51	2.46	2.42	2.26	2.15	2.00	1.90	1.68	1.60	1.55
300	11.0	7.07	5.56	4.75	4.22	3.86	3.59	3.38	3.21	3.07	2.95	2.85	2.76	2.69	2.62	2.56	2.50	2.46	2.41	2.37	2.21	2.10	1.94	1.85	1.62	1.53	1.48
500	11.0	7.00	5.51	4.69	4.18	3.81	3.54	3.33	3.16	3.02	2.91	2.81	2.72	2.64	2.58	2.52	2.46	2.41	2.37	2.33	2.17	2.05	1.90	1.80	1.57	1.48	1.43
1000	10.9	6.96	5.46	4.65	4.14	3.78	3.51	3.30	3.13	2.99	2.87	2.77	2.69	2.61	2.54	2.48	2.43	2.38	2.34	2.30	2.14	2.02	1.87	1.77	1.53	1.44	1.38