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United States Environmental Protection Agency
Office of Water (4305)
EPA 823-B-00-007
November 2000

EPA Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories

Volume 1
Fish Sampling and Analysis
Third Edition

22878

Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories

Volume 1: Fish Sampling and Analysis
Third Edition

Office of Science and Technology
Office of Water
U.S. Environmental Protection Agency
Washington, DC

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Volume 1: Fish Sampling and Analysis

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SECTION 1

INTRODUCTION

1.1 HISTORICAL PERSPECTIVE

Contamination of aquatic resources, including freshwater, estuarine, and marine fish and shellfish, has been documented in the scientific literature for many regions of the United States (NAS, 1991). Environmental concentrations of some pollutants have decreased over the past 25 years as a result of better water quality management practices. However, environmental concentrations of other heavy metals, pesticides, and toxic organic compounds have increased due to intensifying urbanization, industrial development, and use of new agricultural chemicals. Our Nation's waterbodies are among the ultimate repositories of pollutants released from these activities. Pollutants come from permitted point source discharges (e.g., industrial and municipal facilities), accidental spill events, and nonpoint sources (e.g., agricultural practices, resource extraction, urban runoff, in-place sediment contamination, groundwater recharge, vehicular exhaust, and atmospheric deposition from various combustion and incineration processes).

Once these toxic contaminants reach surface waters, they may concentrate through aquatic food chains and bioaccumulate in fish and shellfish tissues. Aquatic organisms may bioaccumulate environmental contaminants to more than 1,000,000 times the concentrations detected in the water column (U.S. EPA, 1992c, 1992d). Thus, fish and shellfish tissue monitoring serves as an important indicator of contaminated sediments and water quality problems, and many states routinely conduct chemical contaminant analyses of fish and shellfish tissues as part of their comprehensive water quality monitoring programs (Cunningham and Whitaker, 1989; Cunningham, 1998; Cunningham and Sullivan,1999). Tissue contaminant monitoring also enables state agencies to detect levels of contamination in fish and shellfish tissue that may be harmful to human consumers. If states conclude that consumption of chemically contaminated fish and shellfish poses an unacceptable human health risk, they may issue local fish consumption advisories or bans for specific waterbodies and specific fish and shellfish species for specific populations.

In 1989, the American Fisheries Society (AFS), at the request of the U.S. Environmental Protection Agency (EPA), conducted a survey of state fish and shellfish consumption advisory practices. Questionnaires were sent to health departments, fisheries agencies, and water quality/environmental management departments in all 50 states and the District of Columbia. Officials in all 50 states and the District responded.

Respondents were asked to provide information on several issues including

- Agency responsibilities
- Sampling strategies
- Sample collection procedures
- Chemical residue analysis procedures
- Risk assessment methodologies
- Data interpretation and advisory development
- State concerns
- Recommendations for federal assistance.

Cunningham et al. (1990) summarized the survey responses and reported that monitoring and risk assessment procedures used by states in their fish and shellfish advisory programs varied widely. States responded to the question concerning assistance from the federal government by requesting that federal agencies

- Provide a consistent approach for state agencies to use in assessing health risks from consumption of chemically contaminated fish and shellfish
- Develop guidance on sample collection procedures
- Develop and/or endorse uniform, cost-effective analytical methods for quantitation of contaminants
- Establish a quality assurance (QA) program that includes use of certified reference materials for chemical analyses.

In March 1991, the National Academy of Sciences (NAS) published a report entitled *Seafood Safety* (NAS, 1991) that reviewed the nature and extent of public health risks associated with seafood consumption and examined the scope and adequacy of current seafood safety programs. After reviewing over 150 reports and publications on seafood contamination, the NAS Institute of Medicine concluded that high concentrations of chemical contaminants exist in various fish species in a number of locations in the country. The report noted that the fish monitoring data available in national and regional studies had two major shortcomings that affected their usefulness in assessing human health risks:

- In some of the more extensive studies, analyses were performed on nonedible portions of finfish (e.g., liver tissue) or on whole fish, which precludes accurate determination of human exposures.

- Studies did not use consistent methods of data reporting (e.g., both geometric and arithmetic means were reported in different studies) or failed to report crucial information on sample size, percent lipid, mean values of contaminant concentrations, or fish size, thus precluding direct comparison of the data from different studies and complicating further statistical analysis and risk assessment.

1.1.1 Establishment of the Fish Contaminant Workgroup

As a result of NAS concerns and state concerns expressed in the AFS survey, EPA's Office of Water established a Fish Contaminant Workgroup. It was composed of representatives from EPA and the following state and federal agencies:

- U.S. Food and Drug Administration (FDA)
- U.S. Fish and Wildlife Service (FWS)
- Ohio River Valley Water Sanitation Commission (ORSANCO)
- National Oceanic and Atmospheric Administration (NOAA)
- Tennessee Valley Authority (TVA)
- United States Geological Survey (USGS)

and representatives from 26 states: Alabama, Arkansas, California, Colorado, Delaware, Florida, Georgia, Illinois, Indiana, Louisiana, Maryland, Massachusetts, Michigan, Minnesota, Missouri, Nebraska, New Hampshire, New Jersey, New York, North Carolina, North Dakota, Ohio, Oregon, Texas, Virginia, and Wisconsin.

The objective of the EPA Fish Contaminant Workgroup was to formulate guidance for states on how to sample and analyze chemical contaminants in fish and shellfish where the primary end uses of the data included development of fish consumption advisories. The Workgroup compiled documents describing protocols currently used by various federal agencies, EPA Regional offices, and states that have extensive experience in fish contaminant monitoring. Using these documents, they selected methods considered most cost-effective and scientifically sound for sampling and analyzing fish and shellfish tissues. These methods were recommended as standard procedures for use by the states and are described in this guidance document.

1.1.2 Development of a National Fish Advisory Database

In addition to initiating work on the national guidance document series in 1993, EPA also initiated work on the development of a national database — The National Listing of Fish and Wildlife Advisories (NLFWA) database — for tracking fish and wildlife advisories issued by the states. The 1998 update of the NLFWA database includes all available information describing state, territorial, tribal, and federal fish consumption advisories issued in the United States (U.S. EPA 1999a, 1999c). The database contains fish consumption advisory information provided to EPA by the states and other jurisdictions from 1993 through December 1998. It also includes information from 1996 through 1997 for 12 Canadian provinces and territories. No updates to information on Canadian advisories were made in 1998. Since the release of the first fish advisory results in 1994, advisory results and trends have been accessible to states, territories, tribal organizations, and the general public by querying the NLFWA database or through summary information reported each year in the *EPA Fact Sheet—Update: National Listing of Fish and*

Wildlife Advisories. Fish advisory results and trends reported in the 1999 Fish Advisory Fact Sheet (U.S. EPA, 1999c) are presented below. The most recent updates of the Fish Advisory Fact Sheet are available on the EPA website at **http://epa.gov/OST/fish**.

1.1.2.1 Background—

The states, U.S. territories, and Native American tribes (hereafter referred to as states) have primary responsibility for protecting residents from the health risks of consuming contaminated noncommercially caught fish and wildlife. They do this by issuing consumption advisories for the general population, including recreational and subsistence fishers, as well as for sensitive subpopulations (such as pregnant women, nursing mothers, and children). These advisories inform the public that high concentrations of chemical contaminants (e.g., mercury and dioxins) have been found in local fish and wildlife. The advisories include recommendations to limit or avoid consumption of certain fish and wildlife species from specified waterbodies or, in some cases, from specific waterbody types (e.g., all inland lakes). Similarly, in Canada, the provinces and territories have primary responsibility for issuing fish consumption advisories for their residents.

States typically issue five major types of advisories and bans to protect both the general population and specific subpopulations.

- When levels of chemical contamination pose a health risk to the general public, states may issue a no consumption advisory for the general population.
- When contaminant levels pose a health risk to sensitive subpopulations, states may issue a no consumption advisory for the sensitive subpopulation.
- In waterbodies where chemical contamination is less severe, states may issue an advisory recommending that either the general population or a sensitive subpopulation restrict their consumption of the specific species for which the advisory is issued.
- The fifth type of state-issued advisory is the commercial fishing ban, which prohibits the commercial harvest and sale of fish, shellfish, and/or wildlife species from a designated waterbody and, by inference, the consumption of all species identified in the fishing ban from that waterbody.

As shown in Table 1-1, advisories of all types increased overall in number from 1993 to 1998.

1.1.2.2 Advisories in Effect—

The database includes information on

- Species and size ranges of fish and/or wildlife sampled
- Chemical contaminants identified in the advisory

Table 1-1. U.S. Advisories Issued from 1993 to 1998 by Type

	1993	1994	1995	1996	1997	1998
No Consumption – General Population	503	462	463	563	545	532
No Consumption – Sensitive Subpopulation	555	720	778	1,022	1,119	1,211
Restricted Consumption – General Population	993	1,182	1,372	1,763	1,843	2,062
Restricted Consumption – Sensitive Subpopulation	689	900	1,042	1,370	1,450	1,595
Commercial Fishing Ban	30	30	55	50	52	50

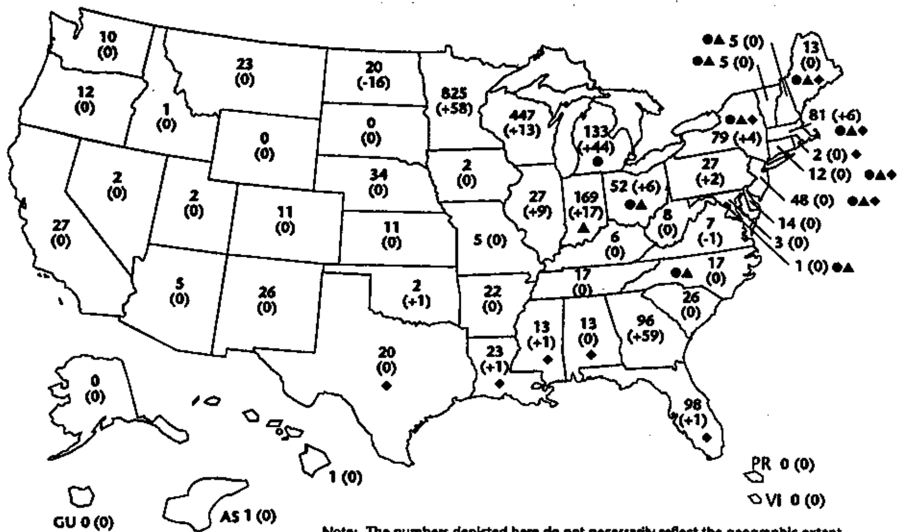
Source: U.S. EPA 1999a, 1999c.

- Geographic location of each advisory (including narrative information on landmarks, river miles, or latitude and longitude coordinates of the affected waterbody and map showing location of waterbody)
- Lake acreage or river miles under advisory
- Population for whom the advisory was issued
- Fish tissue chemical residue data from waterbodies under advisory.

The 1994, 1995, 1996, 1997, and 1998 versions of the NLFWA database can generate national, regional, and state maps that illustrate any combination of these advisory parameters. In addition, the 1996 through 1998 versions of the database can provide information on the percentage of waterbodies in each state currently under an advisory and the percentage of waters assessed. A new feature of the 1998 database provides users access to fish tissue residue data for those waterbodies under advisory in 16 states. The name of each state contact, phone number, FAX number, and e-mail address are also provided so that users can obtain additional information concerning specific advisories. Comparable advisory information (excluding tissue residue data) and contact information for 1996 and 1997 are provided for each Canadian province or territory.

1.1.2.3 Advisory Trends—

The number of waterbodies in the United States under advisory reported in 1998 (2,506) represents a 9% increase from the number reported in 1997 (2,299 advisories) and a 98% increase from the number of advisories issued since 1993 (1,266 advisories). Figure 1-1 shows the number of advisories in effect for each state in 1998 and the number of advisories issued or rescinded since 1997. The increase in advisories issued by the states generally reflects an increase in the number of assessments of the levels of chemical contaminants in fish and wildlife tissues. These additional assessments were conducted as a result of the increased awareness of health risks associated with the consumption of chemically contaminated fish and wildlife. Some of the increase in advisory numbers, however, may be due to the increasing use of EPA risk assessment procedures in setting advisories rather than FDA action levels developed for commercial fisheries.



Note: The numbers depicted here do not necessarily reflect the geographic extent of chemical contamination in each state or the extent of a state's monitoring efforts. The methods used to establish fish advisories vary among the states. Eighteen states have issued statewide advisories for particular pollutants and types of waterbodies. For these states, ● denotes a statewide advisory for lakes, ▲ denotes a statewide advisory for rivers, and ◆ denotes a statewide advisory for coastal waters.

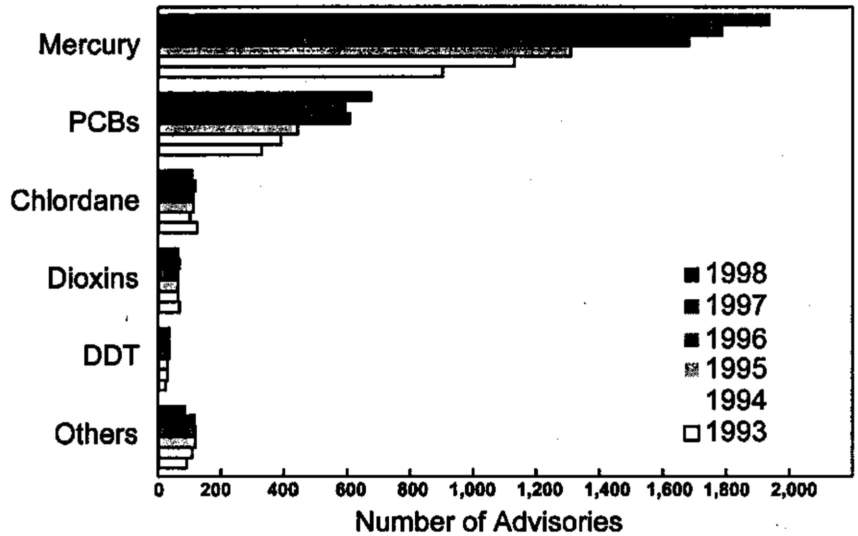
Maine and New York have statewide wildlife advisories for moose liver and kidney and waterfowl, respectively.

Source: U.S. EPA, 1999c.

Figure 1-1. Total number of fish advisories in effect in each state in 1998 (change from 1997).

1.1.2.4 Bioaccumulative Pollutants—

Although U.S. advisories have been issued for a total of 46 chemical contaminants, most advisories issued have involved five primary contaminants. These chemical contaminants are biologically accumulated in the tissues of aquatic organisms at concentrations many times higher than concentrations in the water. In addition, these chemical contaminants persist for relatively long periods in sediments where they can be accumulated by bottom-dwelling organisms and passed up the food chain to fish. Concentrations of these contaminants in the tissues of aquatic organisms may be increased at each successive level of the food chain. As a result, top predators in a food chain, such as largemouth bass, salmon, or walleye, may have concentrations of these chemicals in their tissues that can be a million times higher than the concentrations in the water. Mercury, PCBs, chlordane, dioxins, and DDT (and its degradation products, DDE and DDD) were at least partly responsible for 99 percent of all fish consumption advisories in effect in 1998. (See Figure 1-2.)



Source: U.S. EPA, 1999a, 1999c.

Figure 1-2. Trends in number of advisories issued for various pollutants.

1.1.2.5 Wildlife Advisories—

In addition to advisories for fish and shellfish, the database also contains several wildlife advisories. Four states have issued consumption advisories for turtles: Arizona (3), Massachusetts (1), Minnesota (8), and New York (statewide advisory). One state (Massachusetts) has an advisory for frogs, New York has a statewide advisory for waterfowl (including mergansers), Arkansas has an advisory for woodducks, and Utah has an advisory for American coot and ducks. Maine issued a statewide advisory for moose liver and kidneys due to cadmium levels. No new wildlife advisories were issued in 1998.

1.1.2.6 1998 United States Advisories—

The 1998 database lists 2,506 advisories in 47 states, the District of Columbia, and the U.S. Territory of American Samoa. Some of these advisories represent statewide advisories for certain types of waterbodies (e.g., lakes, rivers, and/or coastal waters). An advisory may represent one waterbody or one type of waterbody within a state's jurisdiction. Statewide advisories are counted as one advisory. The database counts one advisory for each waterbody name or type of waterbody regardless of the number of fish or wildlife species that are affected or the number of chemical contaminants detected at concentrations of human health concern. Eighteen states (Alabama, Connecticut, District of Columbia, Florida, Indiana, Louisiana, Maine, Massachusetts, Michigan, Mississippi, New Hampshire, New Jersey, New York, North Carolina, Ohio, Rhode Island, Texas, and Vermont) currently have statewide advisories in effect (see Table 1-2). Missouri rescinded its statewide advisories for lakes and rivers in 1998, and Mississippi added a statewide coastal advisory for mercury. A statewide advisory is issued to warn the public of the potential for widespread contamination of certain species of fish in certain types of waterbodies (e.g., lakes, rivers and streams, or coastal waters) or certain species of wildlife (e.g., moose or waterfowl). In such a case, the state may have found a level of contamination of a specific pollutant in a particular fish or wildlife species over a relatively wide geographic area that warrants advising the public of the situation.

The statewide advisories and 2,506 specifically named waterbodies represent approximately 15.8 percent of the Nation's total lake acreage and 6.8% of the Nation's total river miles. In addition, 100 percent of the Great Lakes waters and their connecting waters are also under advisory due to one or more contaminants (e.g., PCBs, dioxins, mercury, and/or chlordane). The Great Lakes waters are considered separately from other lakes, and their connecting waters are considered separately from other river miles.

Several states also have issued fish advisories for all of their coastal waters. Using coastal mileages calculated by the National Oceanic and Atmospheric Administration (NOAA), an estimated 58.9 percent of the coastline of the contiguous 48 states currently is under advisory. This includes 61.5 percent of the Atlantic Coast and 100 percent of the Gulf Coast. No Pacific Coast state has issued a statewide advisory for any of its coastal waters although several localized areas along the Pacific Coast are under advisory. The Atlantic coastal advisories have been issued for a wide variety of chemical contaminants including mercury, PCBs, dioxins, and cadmium, while all of the Gulf Coast advisories have been issued for mercury.

1.1.2.7 Database Use and Access—

The NLFWA database was developed by EPA to help federal, state, and local government agencies and Native American tribes assess the potential for human health risks associated with consumption of chemical contaminants in noncommercially caught fish and wildlife. The data contained in this database may also be used by the general public to make informed decisions about the waterbodies in which they choose to fish or harvest wildlife; the frequency with which they fish these waterbodies; the species, size, and number of fish they collect; and the frequency with which they consume fish from specific waterbodies. Note: State fish advisory contact information and hyperlinks to state fish advisory websites are also provided.

EPA provides this 1998 update of the NLFWA database available on the Internet at

http://www.epa.gov/OST/fish

Table 1-2. Summary of Statewide Advisories in Effect in 1998

State	Lakes	Rivers	Coastal Waters
Alabama	—	—	Mercury
Connecticut	Mercury	Mercury	PCBs
District of Columbia	PCBs	PCBs	—
Florida	—	—	Mercury
Indiana	—	Mercury PCBs	—
Louisiana	—	—	Mercury
Maine	Mercury	Mercury	Dioxins
Massachusetts	Mercury	Mercury	PCBs Organics
Michigan	Mercury	—	—
Mississippi	—	—	Mercury
New Hampshire	Mercury	Mercury	PCBs
New Jersey	Mercury	Mercury	PCBs Cadmium Dioxins
New York	PCBs Chlordane Mirex DDT	PCBs Chlordane Mirex DDT	PCBs Cadmium Dioxins
North Carolina	Mercury	Mercury	—
Ohio	Mercury	Mercury	—
Rhode Island	—	—	PCBs
Texas	—	—	Mercury
Vermont	Mercury	Mercury	—

Source: U.S. EPA, 1999a, 1999c.

Further information on specific advisories within a particular state is available from the appropriate state agency contact listed in the database. This is particularly important for advisories recommending that consumers restrict their consumption of fish from certain waterbodies. State health departments provide more specific information for restricted consumption advisories (RGP and RSP) on the appropriate meal size and meal frequency (number of meals per week or month) that is considered safe to consume for a specific consumer group (e.g., the general public versus pregnant women, nursing mothers, and young children). For further information on Canadian advisories, contact the appropriate Province contact given in the database.

For more information concerning the National Fish and Wildlife Contamination Program, contact:

U.S. Environmental Protection Agency
Office of Science and Technology
National Fish and Wildlife Contamination Program—4305
1200 Pennsylvania Avenue, NW
Washington, DC 20460
Phone 202 260-7301 FAX 202 260-9830
e-mail: Bigler.Jeff@epa.gov

1.2 PURPOSE

The purpose of this manual is to provide overall guidance to states on methods for sampling and analyzing contaminants in fish and shellfish tissue that will promote consistency in the data they use to determine the need for fish consumption advisories. This manual provides guidance only and does not constitute a regulatory requirement for the states. It is intended to describe what EPA believes to be scientifically sound methods for sample collection, chemical analyses, and statistical analyses of fish and shellfish tissue contaminant data for use in fish contaminant monitoring programs that have as their objective the protection of public health. This nonregulatory, technical guidance manual is intended for use as a handbook by state and local agencies that are responsible for sampling and analyzing fish and shellfish tissue. Adherence to this guidance will enhance the comparability of fish and shellfish contaminant data, especially in interstate waters and thus provide more standardized information on fish contamination problems.

It should be noted that the EPA methodology described in Volumes 1 and 2 of this guidance series offers great flexibility to state users. These documents are designed to meet the objectives of state monitoring and risk assessment programs by providing options to meet specific state or study needs within state budgetary constraints. The users of this fish advisory guidance document should recognize that it is the consistent application of the EPA methodology and processes rather than individual elements of the program sampling design that are of major importance in improving consistency among state fish advisory programs. For example, whether a state elects to collect three composite samples of five individual fish or four composite samples of eight individual fish as the basis of its state program is of less importance than a state designing and executing its monitoring program with attention to all elements of the EPA methodology having been considered and addressed during the planning and implementation phases.

One major factor currently affecting the comparability of fish advisory information nationwide, is the fact that the states employ different methodologies to determine the necessity for issuing an advisory. For example, some states currently do not use the EPA methodology at all or use it only in their assessment of health risks for certain chemical contaminants. Often these states rely instead on exceedances of FDA action levels or tolerances to determine the need to issue an advisory. FDA's mission is to protect the public health with respect to levels of chemical contaminants in all foods, including fish and shellfish sold in interstate commerce. FDA has developed both action levels and tolerances to address levels of contamination in foods. FDA may establish an action level when food contains a chemical from sources of contamination that cannot be avoided even by adherence to good agricultural or manufacturing practices, such as contamination by a pesticide that persists in the environment. An action level is an administrative guideline or instruction to the agency field unit that defines the extent of contamination at which FDA may regard food as adulterated. An action level represents the limit at or above which FDA may take legal action to remove products from the marketplace. Under the Food, Drug, and Cosmetic Act, FDA also may set tolerances for unavoidably added poisonous or deleterious substances, that is, substances that are either required in the production of food or are otherwise unavoidable by good manufacturing practices. A tolerance is a regulation that is established following formal rulemaking procedures; an action level is a guideline or "instruction" and is not a formal regulation (Boyer et al., 1991).

FDA's jurisdiction in setting action levels or tolerances is limited to contaminants in food shipped and marketed in interstate commerce. Thus, the methodology used by FDA in establishing action levels or tolerances is directed at determining the health risks of chemical contaminants in fish and shellfish that are bought and sold in interstate commerce rather than in locally harvested fish and shellfish (Bolger et al., 1990). FDA action levels and tolerances are indicators of chemical residue levels in fish and shellfish that should not be exceeded for the general population who consume fish and shellfish typically purchased in supermarkets or fish markets that sell products that are harvested from a wide geographic area, including imported fish and shellfish products. However, the underlying assumptions used in the FDA methodology were never intended to be protective of recreational, tribal, ethnic, and subsistence fishers who typically consume larger quantities of fish than the general population and often harvest the fish and shellfish they consume from the same local waterbodies repeatedly over many years. If these local fishing and harvesting areas contain fish and shellfish with elevated tissue levels of chemical contaminants, these individuals potentially could have increased health risks associated with their consumption of the contaminated fish and shellfish.

The following chemical contaminants discussed in this volume have FDA action levels for their concentration in the edible portion of fish and shellfish: chlordane, DDT, DDE, DDD, heptachlor epoxide, mercury, and mirex. FDA has not set an action level for PCBs in fish but has established a tolerance in fish for this chemical. Table 1-3 compares the FDA action levels and tolerance for these six chemical contaminants with EPA's recommended screening values (SVs) for recreational and subsistence fishers calculated for these target analytes using the EPA methodology.

The EPA SV for each chemical contaminant is defined as the concentration of the chemical in fish tissue that is of potential public health concern and that is used as a threshold value against which tissue residue levels of the contaminant in fish and shellfish can be compared. The SV is calculated based on both the

Table 1-3. Comparison of FDA Action Levels and Tolerances with EPA Screening Values

Chemical contaminant	FDA Action Level[a] (ppm)	EPA SV for Recreational Fishers (ppm)	EPA SV for Subsistence Fishers (ppm)
Chlordane	0.3	0.114	0.014
Total DDT	5	0.117	0.014
Dieldrin	0.3	2.50×10^{-3}	3.07×10^{-4}
Heptachlor epoxide	0.3	4.39×10^{-3}	5.40×10^{-4}
Mercury	1 .0	0.40	0.049
Mirex	0.1	0.80	0.098
	FDA Tolerance Level (ppm)		
PCBs	2	0.02	2.45×10^{-3}

[a]U.S. FDA 1998.

noncarcinogenic and carcinogenic effects of the chemical contaminant, which are discussed in detail in Section 5 of this volume. EPA recommends that the more conservative of the calculated values derived from the noncarcinogenic rather than the carcinogenic effects be used because it is more protective of the consumer population (either recreational or subsistence fishers). As can be seen in Table 1-3 for the recreational fisher SV, the EPA-recommended values typically range from 2 to 120 times lower and are thus more protective than the corresponding FDA action or tolerance level. This difference is even more striking for subsistence fishers for whom the SVs are 20 to 997 times lower than the FDA values.

EPA and FDA have agreed that the use of FDA Action Levels for the purpose of making local advisory determinations is inappropriate. In letters to all states, guidance documents, and annual conferences, this practice has been discouraged by EPA and FDA in favor of EPA's risk-based approach to derive local fish consumption advisories.

EPA has provided this guidance to be especially protective of recreational fishers and subsistence fishers within the general U.S. population. EPA recognizes, however, that Native American subsistence fishers are a unique subsistence fisher population that needs to be considered separately. For Native American subsistence fishers, eating fish is not simply a dietary choice that can be completely eliminated if chemical contamination reaches unacceptable levels; rather, eating fish is an integral part of their lifestyle and culture. This traditional lifestyle is a living religion that includes values about environmental responsibility and community health as taught by elders and tribal religious leaders (Harris and Harper, 1977). Therefore, methods for balancing benefits and risks from eating contaminated fish must be evaluated differently than for the general fisher population (see Section 5.1.3.2).

To enhance the use of this guidance as a working document, EPA will issue additional information and updates to users as appropriate. It is anticipated that updates will include minor revisions such as the addition or deletion of chemicals from the recommended list of target analytes, new screening values as new toxicologic data become available, and new chemical analysis procedures for some target analytes as they are developed. A new edition of this document will be issued to include the addition of major new areas of guidance or when major changes are made to the Agency's risk assessment procedures.

EPA's Office of Water realizes that adoption of these recommended methods requires adequate funding. In practice, funding varies among states and resource limitations will cause states to tailor their fish and shellfish contaminant monitoring programs to meet their own needs. States must consider tradeoffs among the various parameters when developing their fish contaminant monitoring programs. These parameters include

- Total number of stations sampled
- Intensity of sampling at each site
- Number of chemical analyses and their cost
- Resources expended on data storage and analysis, QA and quality control (QC), and sample archiving.

Consideration of these tradeoffs will determine the number of sites sampled, number of target analytes analyzed at each site, number of target species collected, and number of replicate samples of each target species collected at each site (Crawford and Luoma, 1993).

1.3 OBJECTIVES

The specific objectives of this manual are to

1. Recommend a tiered monitoring strategy designed to

    - Screen waterbodies (**Tier 1**) to identify those harvested sites where chemical contaminant concentrations in the edible portions of fish and shellfish exceed human consumption levels of potential concern (screening values [**SVs**]). SVs for contaminants with carcinogenic effects are calculated based on selection of an acceptable cancer risk level. SVs for contaminants with noncarcinogenic effects are concentrations determined to be without appreciable noncancer health risk. For a contaminant with both carcinogenic and noncarcinogenic effects, EPA recommends that the lower (more conservative) of these two calculated SVs be used.

- Conduct intensive followup sampling (**Tier 2, Phase I**) to determine the magnitude of the contamination in edible portions of fish and shellfish species commonly consumed by humans in waterbodies identified in the screening process.
- Conduct intensive sampling at additional sites (**Tier 2, Phase II**) in a waterbody where screening values were exceeded to determine the geographic extent of contamination in various size classes of fish and shellfish.
- Conduct intensive followup sampling in waterbodies where none of the 25 SVs are exceeded in order to establish areas of unrestricted fish consumption or "green areas."

2. Recommend target species and criteria for selecting additional species if the recommended target species are not present at a site.

3. Recommend target analytes to be analyzed in fish and shellfish tissue and criteria for selecting additional analytes.

4. Recommend risk-based procedures for calculating target analyte screening values.

5. Recommend standard field procedures including

   - Site selection
   - Sampling time
   - Sample type and number of replicates
   - Sample collection procedures including sampling equipment
   - Field recordkeeping and chain of custody
   - Sample processing, preservation, and shipping.

6. Recommend cost-effective, technically sound analytical methods and associated QA and QC procedures, including identification of

   - Analytical methods for target analytes with detection limits capable of measuring tissue concentrations at or below SVs
   - Sources of recommended certified reference materials
   - Federal agencies currently conducting QA interlaboratory comparison programs.

7. Recommend procedures for data analysis and reporting of fish and shellfish contaminant data.

8. Recommend QA and QC procedures for all phases of the monitoring program and provide guidance for documenting QA and QC requirements in a QA plan or in a combined work/QA project plan.

1.4 RELATIONSHIP OF MANUAL TO OTHER GUIDANCE DOCUMENTS

This manual is the first in a series of four documents to be prepared by EPA's Office of Water as part of a Federal Assistance Plan to help states standardize fish consumption advisories. This series of four documents—*Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories* includes

- Volume 1: *Fish Sampling and Analysis* (EPA 823-R-93-002), published August 1993; a second edition, published September 1995; and the current third edition (EPA-823-B-00-007) to be published in November 2000.
- Volume 2: *Risk Assessment and Fish Consumption Limits* (EPA 823-B-94-004), published June 1994; a second edition (EPA 823-B-97-009), published in July 1997; and a third edition (EPA-823-B-00-008) to be published in November 2000.
- Volume 3: *Overview of Risk Management* (EPA 823-B-96-006), published in June 1996.
- Volume 4: *Risk Communication* (EPA 823-R-95-001), published March 1995.

This sampling and analysis manual is not intended to be an exhaustive guide to all aspects of sampling, statistical design, development of risk-based screening values, laboratory analyses, QA and QC considerations, data analysis, and reporting for fish and shellfish contaminant monitoring programs. Key references are provided in Section 10, Literature Cited, that detail various aspects of these topics.

1.5 CONTENTS OF VOLUME 1

Figure 1-3 shows how Volume 1 fits into the overall guidance series and lists the major categories of information provided. The first five sections discuss the history of the EPA Fish and Wildlife Contamination Program, monitoring strategy, including selection of target fish and shellfish species, selection of target analytes, and calculation of screening values for all target analytes. Section 6 provides guidance on field sampling and preservation procedures. Sections 7 and 8 provide guidance on laboratory procedures including sample handling and analysis, and Section 9 discusses data analysis and reporting procedures.

Appropriate QA and QC considerations are integral parts of each of the recommended procedures. Section 10 is a compilation of all literature cited in Sections 1 through 9 of this document. New information or revisions to existing information contained in previous editions of this guidance document are briefly described in Section 1.6.

Section 1 of this document reviews the historical development of this guidance document series, describes the purpose and objectives of the Volume 1 manual,

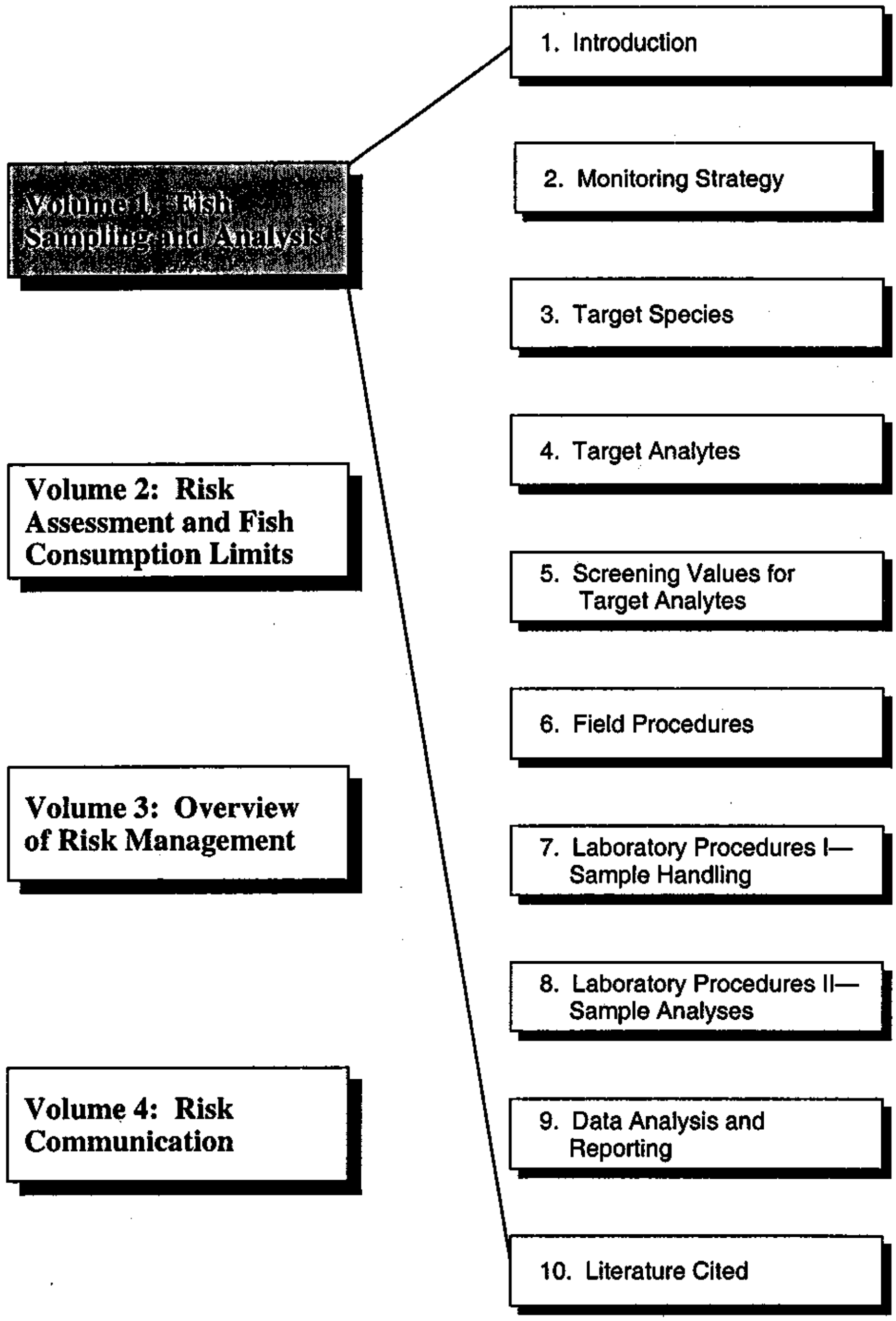


Figure 1-3. Series summary: Guidance for assessing chemical contamination data for use in fish advisories.

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outlines the relationship of the manual to the other three documents in the series, describes the contents of the manual, and identifies new revisions made to the guidance of this third edition.

Section 2 outlines the recommended strategy for state fish and shellfish contaminant monitoring programs. This strategy is designed to (1) routinely screen waterbodies to identify those locations where chemical contaminants in edible portions of fish and shellfish exceed human health screening values, (2) sample more intensively those waterbodies where exceedances of these SVs have been found in order to assess the magnitude and the geographic extent of the contamination, and (3) identify those areas where chemical contaminant concentrations are low and would allow states to designate areas where unrestricted fish consumption may be permitted.

Section 3 discusses the purpose of using target species and criteria for selection of target species for both screening and intensive studies. Lists of recommended target species are provided for inland fresh waters, Great Lakes waters, and seven distinct estuarine and coastal marine regions of the United States.

Section 4 presents a list of recommended target analytes to be considered for inclusion in screening and intensive studies, briefly discusses the original criteria used in selecting these analytes, provides a summary of the toxicological information available for each analyte as well as pertinent information on the analyte's detection in national and regional fish monitoring studies.

Section 5 describes the new EPA risk-based procedure for calculating screening values for target analytes using (1) an adult body weight of 70 kg, (2) a lifetime exposure of 70 years, and (3) new consumption rate default values for both the general population and recreational fishers (17.5 g/d) and subsistence fishers (142.4 g/d). The last part of this section describes how to compare these new SVs against results obtained in fish tissue residue analysis.

Section 6 recommends field procedures to be followed from the time fish or shellfish samples are collected until they are delivered to the laboratory for processing and analysis. Guidance is provided on site selection and sample collection procedures; the guidance addresses material and equipment requirements, time of sampling, size of animals to be collected, sample type, and number of samples. Sample identification, handling, preservation, shipping, and storage procedures are also described.

Section 7 describes recommended laboratory procedures for sample handling including: sample measurements, sample processing procedures, and sample preservation and storage procedures.

Section 8 presents recommended laboratory procedures for sample analyses, including cost-effective analytical methods and associated QC procedures; and information on sources of certified reference materials; recommended analytical techniques for target analytes, including revised detection and quantitation limits; information on the per-sample cost of chemical analysis for each target analyte; and information on federal agencies currently conducting interlaboratory comparison programs.

Section 9 includes procedures for data analysis to determine the need for additional monitoring and risk assessment and for data reporting.

Supporting documentation for this guidance is provided in Section 10, Literature Cited and in Appendixes A through N.

1.6 NEW INFORMATION AND REVISIONS TO VOLUME 1

This 3rd edition of Volume 1 contains newly prepared material as well as major updates and revisions to existing information. A brief summary of major additions and revisions is provided below.

Section 1

- New information is presented on the NLFWA database, including the 5-year trend in the total number of advisories issued nationwide, the number of advisories issued for five major pollutants of concern, and the issuance of increasing numbers of statewide advisories for freshwater lakes and/or rivers and coastal marine areas.

- Additional information describes the flexibility that is built into the EPA methodology, which allows the method to be used to meet a wide variety of state or tribal study needs within budgetary constraints.

- *Clarification of the FDA methodology is provided emphasizing the* inappropriateness of the method and reasons states should adopt and use the EPA methodology when issuing fish consumption advisories to protect their recreational and subsistence fishers.

Section 2

- Updated information is presented in Table 2-1 to be consistent with monitoring design and risk assumptions used in this 3rd edition.

- New discussion of the criteria states may use to identify green areas where chemical contaminant concentrations are at or below the screening values for recreational or subsistence fishers is introduced with more detailed information provided in Appendix B.

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Section 3

- Several tables, including Tables 3-7 and 3-19, were updated to include new information from the 1998 NLFWA database on the number of states that have issued fish advisories for freshwater and marine species.

- Table 3-9 was updated and associated narrative text was revised to include information on studies using turtles as biomonitors of environmental contaminants.

Section 4

- Information on the environmental sources, toxicology, and the number of fish advisories issued in 1998 for each of the 25 target analytes was updated.

- New information is included on the range in concentrations of each contaminant detected in the FWS National Contaminant Biomonitoring Program and the EPA National Study of Chemical Residues in Fish as well as information on more recent regional studies.

- A procedure is described for the selection and prioritization of target analytes for analysis predicated on a watershed-based approach that takes into consideration land use categories, as well as geological characteristics, regional differences, national fish advisory trends, and monitoring and analysis costs.

- Additional guidance is presented on organophosphate pesticides and when and under what situations to monitor fish tissues for these compounds.

- A clarification is provided of the recommendation for selection of target species, especially bivalve molluscs and/or crustaceans when PAH contamination is suspected.

- A new discussion is provided to reflect the Agency's position on using Aroclor and congener analysis for calculating total PCB concentration.

- A new discussion is provided for determining the TEQ value for dioxins, which are now defined as including the 17 2,3,7,8 congeners of dioxin and 2,3,7,8 congeners of dibenzofuran, and the 12 coplanar PCBs with dioxin-like properties based on recent guidance from the World Health Organization (Van den Berg et al., 1998).

- Several tables, including Tables 4-1, 4-2, 4-7, and 4-9 were revised with new information. Tables 4-3, 4-4, 4-5, 4-6, and 4-8 are new to the document.

- All of the toxicological information was revised in light of the most current information concerning each target analyte.

Section 5

- Revisions were made describing major changes in the assumptions used in the risk assessment equations to calculate screening values including use of default consumption rates of 17.5 g/d for the general population and recreational fishers and 142.4 g/d for subsistence fishers based on more recent information from the 1994 to 1996 Continuing Survey of Food Intake by Individuals study conducted by the U.S. Department of Agriculture.

- Additional guidance is provided on how states should handle the interpretation and risk assessment of chemicals that have detection limits higher than the risk-based screening values.

- Tables 5-1, 5-3, 5-4, and 5-5 were revised to reflect changes in consumption rates. Screening values shown in Tables 5-3 and 5-4 were developed using the new consumption rates as well as the most recent RfD and cancer slope factors available.

- Additional information is provided on Native American subsistence fishers, and Table 5-2 was added to summarize several recent studies on Native American fish consumption rates.

- Additional guidance is provided on how states should deal with interpreting analytical results in cases where the screening value is lower than the detection limit for a particular analyte.

- New guidance is provided on determining total PCBs by summary Aroclor equivalents or PCB congeners.

- New information from the World Health Organization (Van den Berg et al., 1998) is included in Table 5-6 showing the most recent Toxic Equivalency Factors (TEF) for the 2,3,7,8-substituted dioxins, dibenzofurans, and the 12 coplanar PCBs.

Section 6

- Additional information is provided on the statistical implications associated with deviations from the recommended sampling design, including the use of unequal numbers of fish per composite, sizes of fish exceeding the size range recommendations for composites, and the use of unequal numbers of replicate samples across sampling sites.

- Clarification is provided on the recommended number of fish that should make up a composite sample.

- More explicit information is provided regarding exceedances of screening values and the statistical basis for issuing a new advisory or rescinding an existing advisory.

- Discussion is provided on the number of samples necessary to characterize different waterbody types and sizes of waterbodies with consideration given to the home range and mobility of the target species.

- How regional data should be used in the risk assessment process to address statewide advisories is discussed.

- Additional guidance is provided on how sample type selection should be based on the study objectives as well as on the sample type consumed by the target population.

- Clarification is provided as to EPA's position on the use of dead, lacerated, or mutilated fish for human health risk assessments.

- New information is provided on U.S. Fish and Wildlife Service and National Marine Fisheries permit requirements in situations where concerns exist about the impact of sampling for the target species in areas inhabited by threatened or endangered species.

- Revisions were made in recordkeeping for field sampling associated with use of the Year 2000 compliant format (YYYYMMDD) for sampling date information.

Section 7

- Revisions were made in recordkeeping forms to initiate use of the Year 2000 compliant format for the date of sampling and analysis procedures.

Section 8

- Updated information is included in Tables 8-1 through 8-5.

- Updated information is provided on the EPA Environmental Monitoring Methods Index System (EMMI).

- Revised information is provided in Section 8.3.3.8.1 concerning round-robin analysis interlaboratory comparison programs.

Section 9

- New information is included on the National Tissue Residue Data Repository, now housed within the NLFWA database.

- Recommended data reporting requirements were updated (Figure 9-1) to include Year 2000 compliant format.

- Detailed information is provided on the Internet-based data entry facility contained within the NLFWA database that can accept fish contaminant residue data to support state fish advisories.

- An example of the new data tables (Figure 9-2) currently used in the fish tissue residue data repository is provided.

Section 10

- Literature citations were revised to include all new references cited in Sections 1 through 9.

Appendixes:

- The following appendixes were revised or added:

  A - EPA 1993 Fish Contamination Workgroup Members
  B - Screening Values for Defining Green Areas
  D - Fish and Shellfish Species for Which State Consumption Advisories Have Been Issued
  F - Pesticide and Herbicides Recommended as Target Analytes
  G - Target Analyte Dose-Response Variables and Associated Information
  I - Quality Assurance and Quality Control Guidance
  M - Sources of Reference Materials

SECTION 2

MONITORING STRATEGY

The objective of this section is to describe the strategy recommended by the EPA Office of Water for use by states in their fish and shellfish contaminant monitoring programs. A two-tiered strategy is recommended as the most cost-effective approach for State contaminant monitoring programs to obtain data necessary to evaluate the need to issue fish or shellfish consumption advisories. This monitoring strategy is shown schematically in Figure 2-1 and consists of

- **Tier 1—Screening studies** of a large number of sites for chemical contamination where sport, subsistence, and/or commercial fishing is conducted. This screening will help states identify those sites where concentrations of chemical contaminants in edible portions of commonly consumed fish and shellfish indicate the potential for significant health risks to human consumers.

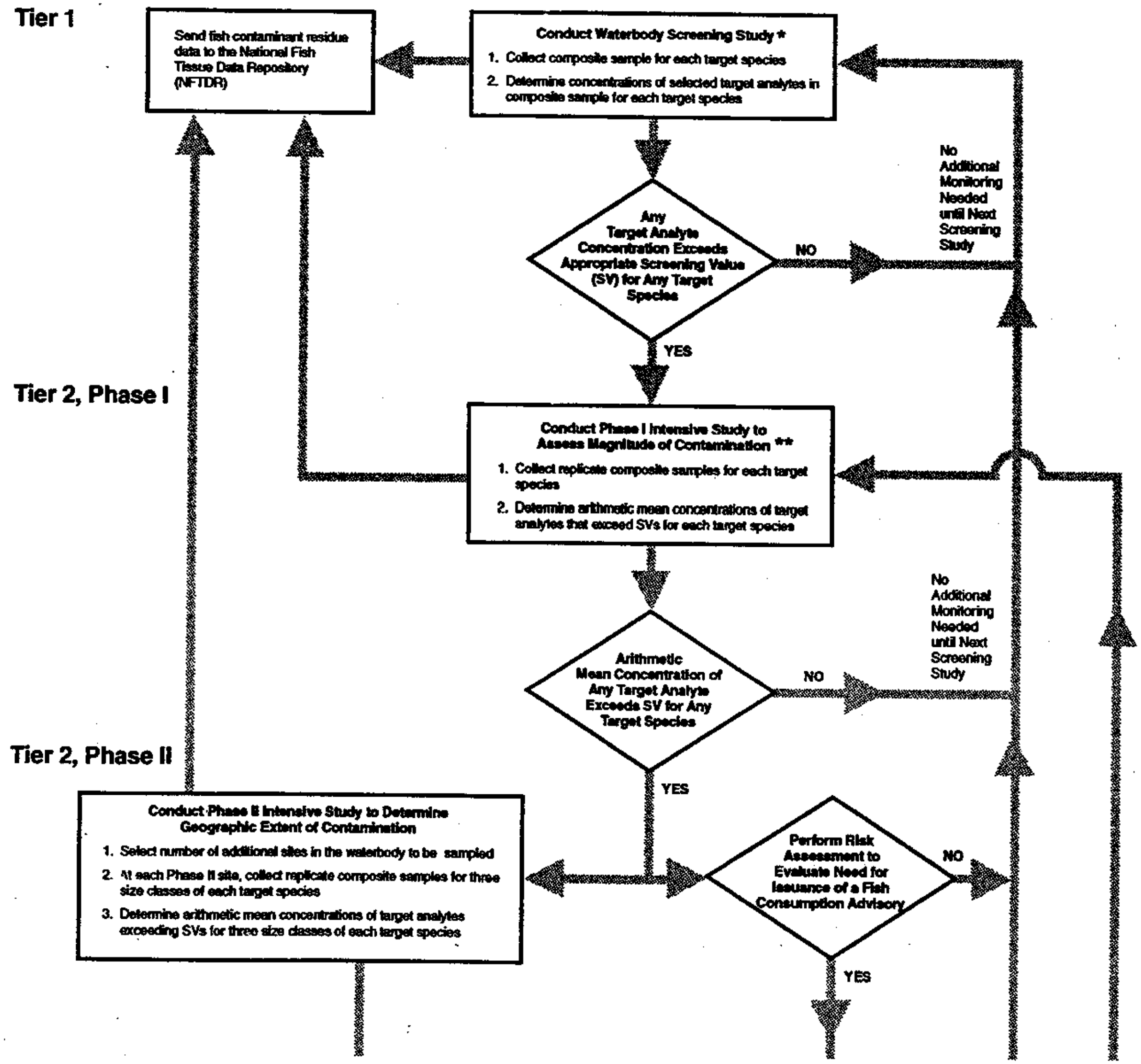
- **Tier 2—Two-phase intensive studies** of problem areas identified in screening studies to determine the magnitude of contamination in edible portions of commonly consumed fish and shellfish species (**Phase I**), to determine size-specific levels of contamination, and to assess the geographic extent of the contamination (**Phase II**).

One key objective in the recommendation of this approach is to improve the data used by states for issuing fish and shellfish consumption advisories. Other specific aims of the recommended strategy are

- To ensure that resources for fish contaminant monitoring programs are allocated in the most cost-effective way. By limiting the number of sites targeted for intensive studies, as well as the number of target analytes at each intensive sampling site, screening studies help to reduce overall program costs while still allowing public health protection objectives to be met.

- To ensure that sampling data are appropriate for developing risk-based consumption advisories.

- To ensure that sampling data are appropriate for determining contaminant concentrations in various size (age) classes of each target species so that states can give size-specific advice on contaminant concentrations (as appropriate).


Tier 1
Send fish contaminant residue data to the National Fish Tissue Data Repository (NFTDR)
Conduct Waterbody Screening Study *
1. Collect composite sample for each target species
2. Determine concentrations of selected target analytes in composite sample for each target species
Any Target Analyte Concentration Exceeds Appropriate Screening Value (SV) for Any Target Species
NO
No Additional Monitoring Needed until Next Screening Study
YES
Tier 2, Phase I
Conduct Phase I Intensive Study to Assess Magnitude of Contamination **
1. Collect replicate composite samples for each target species
2. Determine arithmetic mean concentrations of target analytes that exceed SVs for each target species
Arithmetic Mean Concentration of Any Target Analyte Exceeds SV for Any Target Species
NO
No Additional Monitoring Needed until Next Screening Study
YES
Tier 2, Phase II
Conduct Phase II Intensive Study to Determine Geographic Extent of Contamination
1. Select number of additional sites in the waterbody to be sampled
2. At each Phase II site, collect replicate composite samples for three size classes of each target species
3. Determine arithmetic mean concentrations of target analytes exceeding SVs for three size classes of each target species
Perform Risk Assessment to Evaluate Need for Issuance of a Fish Consumption Advisory
NO
YES

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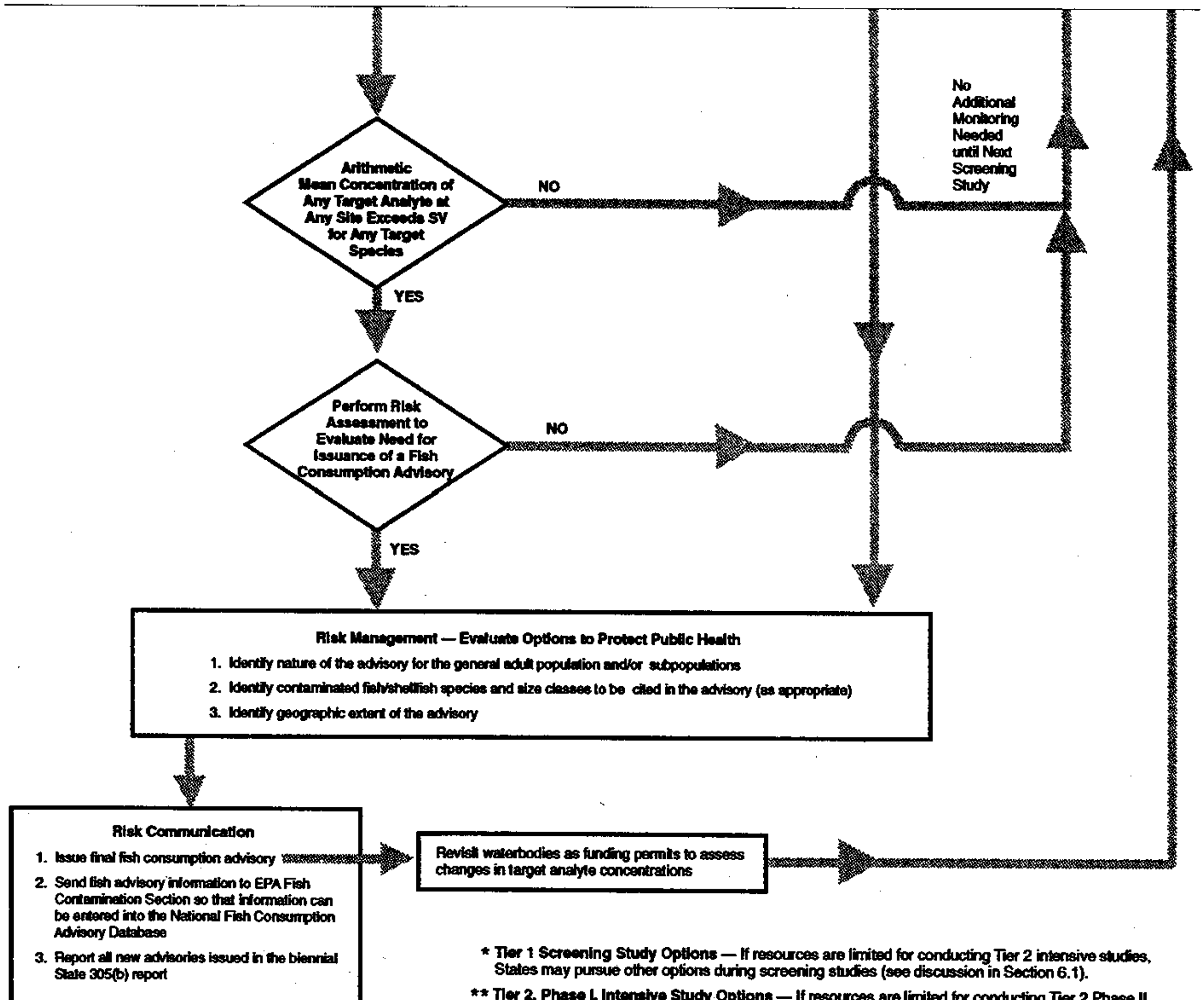

* **Tier 1 Screening Study Options** — If resources are limited for conducting Tier 2 intensive studies, States may pursue other options during screening studies (see discussion in Section 6.1).

** **Tier 2, Phase I, Intensive Study Options** — If resources are limited for conducting Tier 2 Phase II studies, States may pursue other options during Phase I (see discussion in Section 6.2).

Figure 2-1. Recommended strategy for state fish and shellfish contaminant monitoring programs.

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- To ensure that sampling designs are appropriate to allow statistical hypothesis testing. Such sampling designs permit the use of statistical tests to detect a difference between the average tissue contaminant concentration at a site and the human health screening value for any analyte.

The following elements must be considered when planning either screening studies or more intensive followup sampling studies:

- Study objective
- Target species (and size classes)
- Target analytes
- Target analyte screening values
- Sampling locations
- Sampling times
- Sample type
- Sample replicates
- Sample analysis
- Data analysis and reporting.

Detailed guidance for each of these elements, for screening studies (**Tier 1**) and for both Phase I and Phase II of intensive studies (**Tier 2**), is provided in this document. The key elements of the monitoring strategy are summarized in Table 2-1, with reference to the section number of this document where each element is discussed.

2.1 SCREENING STUDIES (TIER 1)

The primary aim of screening studies is to identify frequently fished sites where concentrations of chemical contaminants in edible fish and shellfish composite samples exceed specified human health screening values and thus require more intensive followup sampling. Ideally, screening studies should include all waterbodies where commercial, recreational, or subsistence fishing is practiced; specific sampling sites should include areas where various types of fishing are conducted routinely (e.g., from a pier, from shore, or from private and commercial boats), thereby exposing a significant number of individuals to potentially adverse health effects. Composites of skin-on fillets (except for catfish and other scaleless species, which are usually prepared as skin-off fillets) and edible portions of shellfish are recommended for contaminant analyses in screening studies to provide conservative estimates of typical exposures for the general population. If consumers remove the skin and fatty areas from a fish before preparing it for eating, exposures to some contaminants can be reduced (see U.S. EPA, 2000a, Appendix C of Volume 2 of this guidance document series).

Note: If the target population of consumers includes primarily ethnic or subsistence fishers who consume the whole fish or tissues of the fish not typically consumed by the general population, state monitoring programs should include the fish sample type associated with the target consumers' dietary and/or culinary preference (see Section 6.1.1.6, Sample Type, for additional information.)

Table 2-1. Recommended Strategy for State Fish and Shellfish Contaminant Monitoring Programs

Program element	Tier 1 Screening study	Tier 2 Intensive study (Phase I)	Tier 2 Intensive study (Phase II)
Objective (see Section 2)	Identify frequently fished sites where commonly consumed fish and shellfish target species are contaminated and may pose potential human health risk.	Assess and verify magnitude of tissue contamination at screening site for commonly consumed target species.	Assess geographic extent of contamination in selected size classes of commonly consumed target species.
Target species and size classes (see Sections 3 and 6)	Select target species from commonly consumed species using the following additional criteria: known to bioaccumulate high concentrations of contaminants and distributed over a wide geographic area. Recommended types of target species: Inland fresh waters: 1 bottom-feeder, 1 predator Great Lakes: 1 bottom-feeder, 1 predator Estuarine/marine: 1 shellfish and 1 fish species or 2 fish species (one species should be bottom-feeder).	Resample target species at sites where they were found to be contaminated in screening study.	Resample at additional sites in the waterbody 3 size classes of the target species found to be contaminated in Phase I study.
	OPTIONAL: If resources are limited and a state cannot conduct Tier 2 intensive studies, the state may find it more cost-effective to collect additional samples during the Tier 1 screening study. States **may** collect (1) one composite sample of each of three size classes for each target species, (2) replicate composite samples for each target species, or (3) replicate composite samples of each of three size classes for each target species.	OPTIONAL: If resources are limited and a state cannot conduct Tier 2, Phase II, intensive studies, the state may find it more cost-effective to collect additional samples during the Tier 2, Phase I, intensive study. States **may** collect replicate composite samples of three size classes of the target species found to be contaminated to assess size-specific contaminant concentrations. Other commonly consumed target species may also be sampled if resources allow.	OPTIONAL: If resources allow, select additional commonly consumed target species using same criteria as in Phase I study.

See notes at end of table.

(continued)

Table 2-1. (continued)

Program element	Tier 1 Screening study	Tier 2 Intensive study (Phase I)	Tier 2 Intensive study (Phase II)
Target analytes (see Section 4)	Consider all target analytes listed in Table 4-1 for analysis but prioritize the 25 target analytes based on water and sediment sampling results, land use within the watershed, geographic characteristics, regional and national advisory trends and analytical costs. Include additional site-specific target analytes as appropriate based on current or historic data.	Analyze only for those target analytes from Tier 1 screening study that exceeded SVs.	Analyze only for those target analytes from Tier 2, Phase I, study that exceeded SVs.
Screening values (see Section 5)	Calculate SVs using oral RfDs for noncarcinogens and using oral slope factors and an appropriate risk level (10^{-4} to 10^{-7}) for carcinogens, for adults consuming 17.5 g/d and 142.4 g/d of fish and shellfish (default values) or based on site-specific dietary data. **Note:** In this guidance document, EPA's Office of Water used 17.5 g/d (for recreational fishers) and 142.4 g/d (for subsistence fishers) consumption rates, 70-kg adult body weight, and, for carcinogens, used a 10^{-5} risk level, 70-year exposure, and assumed no loss of contaminants during preparation or cooking. States may use other SVs for site-specific exposure scenarios by adjusting values for consumption rate, body weight, risk level, exposure period, and contaminant loss during preparation or cooking.	Use same SVs as in screening study.	Use same SVs as in screening study.
Sampling sites (see Section 6)	Sample target species at sites in each harvest area that have a high probability of contamination and at presumed clean sites or given areas as resources allow (see Appendix A).	Sample target species at each site identified in the screening study where fish/shellfish tissue concentrations exceed SVs to assess the magnitude of contamination.	Sample at additional sites in the harvest area 3 size classes of the target species found to be contaminated in Phase I study to assess the geographic extent of the contamination in the waterbody.

See notes at end of table.

(continued)

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Table 2-1. (continued)

Program element	Tier 1 Screening study	Tier 2 Intensive study (Phase I)	Tier 2 Intensive study (Phase II)
Sampling times (see Section 6)	Sample during legal harvest season when target species are most available to consumers. Ideally, sampling time should not include the spawning period for target species unless the target species can be legally harvested during this period.	Same as screening study.	Same as screening study.
Sample type (see Sections 6 and 7)	Collect composite fillet samples (skin on, belly flap included) for each target fish species and composite samples of edible portions of target shellfish species. The exceptions to the "skin on, belly flap included" recommendation is to use skin-off fillets for catfish and other scaleless species.	Same as screening study.	Same as screening study but collect composite samples for three size classes of each target species as appropriate.
	OPTIONAL: States **may** use individual fish samples, whole fish, or other sample types, if necessary, to improve exposure estimates of local fish-, shellfish-, or turtle-consuming populations. Sample type should reflect dietary and fish preparation methods of the target population of concern.	Same as screening study.	Same as screening study.
Sample replicates (see Section 6)	Collect one composite sample for each target species. **Collection of replicate composite samples is encouraged but is optional**. If resources allow, collect a minimum of one replicate composite sample for each target species at 10% of the screening sites for QC.	Collect replicate composites for each target species at each Phase I site.	Collect replicate composites of three size classes for each target species at each Phase II site.
Sample analysis (see Section 8)	Use standardized and quantitative analytical methods with limits of detection adequate to allow reliable quantitation of selected target analytes at or below SVs.	Use same analytical methods as in screening study.	Use same analytical methods as in screening study.

See notes at end of table.

(continued)

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Table 2-1. (continued)

Program element	Tier 1 Screening study	Tier 2 Intensive study (Phase I)	Tier 2 Intensive study (Phase II)
Data analysis and reporting (see Sections 6, 7, 8, and 9)	For each target species, compare target analyte concentrations of composite sample with SVs to determine which sites require Tier 2, Phase I, intensive study.	For each target species, compare target analyte arithmetic mean concentrations of replicate composite samples with SVs to determine which sites require Phase II intensive study. If resources are insufficient to conduct Phase II intensive study, conduct a risk assessment and assess the need for issuing a preliminary fish or shellfish consumption advisory.	For each of three size classes within each target species, compare target analyte arithmetic mean concentrations of replicate composite samples at each Phase II site with SVs to determine geographic extent of fish or shellfish contamination. Assess the need for issuing a final fish or shellfish consumption advisory.
Data analysis and reporting (see Sections 6, 7, 8, and 9) (continued)	The following information should be reported for each target species at each site:	The following information should be reported for each target species at each site:	The following information should be reported for each of three size classes within each target species at each site:
	• Site location (e.g., sample site name, water-body name, type of waterbody, and latitude/longitude)	• Same as screening study.	• Same as screening study.
	• Scientific and common name of target species	• Same as screening study	• Same as screening study
	• Sampling date and time	• Same as screening study	• Same as screening study
	• Sampling gear type used	• Same as screening study	• Same as screening study
	• Sampling depth	• Sampling depth	• Sampling depth
	• Number of QC replicates (optional)	• Number of replicates	• Same as Phase I study
	• Number of individual organisms used in the composite sample and in the QC replicate composite sample if applicable	• Number of individual organisms used in each replicate composite sample	• Same as Phase I study

See notes at end of table.

(continued)

Table 2-1. (continued)

Program element	Tier 1 Screening study	Tier 2 Intensive study (Phase I)	Tier 2 Intensive study (Phase II)
	• Predominant characteristics of specimens used in the composite sample and in the QC replicate if applicable (e.g., life stage, age, sex, total length or body size) and description of fish fillet or edible parts of shellfish (tissue type) used	• Predominant characteristics of specimens used in each replicate composite sample (e.g., life stage, age, sex, total length or body size) and description of fish fillet or edible parts of shellfish (tissue type) used	• Same as Phase I study
	• Analytical methods used (including a method for lipid analysis) and method detection and quantitation limits for each target analyte.	• Same as screening study	• Same as screening study
<u>Data analysis and reporting</u> (see Sections 6, 7, 8, and 9) (continued)	• Sample cleanup procedures	• Same as screening study.	• Same as screening study.
	• Data qualifiers	• Same as screening study.	• Same as screening study.
	• Percent lipid in each composite sample.	• Same as screening study.	• Same as screening study.
	• For each target analyte:	• For each target analyte:	• For each target analyte:
	– Total wet weight of composite sample (g) used in analysis	– Total wet weight of each replicate composite sample (g) used in analysis	– Same as Phase I study
	– Measured concentration (wet weight) in composite sample including units of measurement for target analyte	– Measured concentration (wet weight) in each replicate composite sample and units of measurement for target analyte	– Same as Phase I study
	– Measured concentration (wet weight) in the QC replicate, if applicable	– Range of concentrations (wet weight) for each set of replicate composite samples	– Same as Phase I study
		– Mean (arithmetic) concentration (wet weight) for each set of replicate composite samples	– Same as Phase I study
		– Standard deviation of mean concentration (wet weight)	– Same as Phase I study

See notes at end of table.

(continued)

Table 2-1. (continued)

Program element	Tier 1 Screening study	Tier 2 Intensive study (Phase I)	Tier 2 Intensive study (Phase II)
	– Evaluation of laboratory performance (i.e., description of all QA and QC samples associated with the sample(s) and results of all QA and QC analyses)	– Same as screening study	– Same as screening study
	– Comparison of measured concentration of composite sample with SV and clear indication of whether SV was exceeded	– Comparison of target analyte arithmetic mean concentration of replicate composite samples with SV using hypothesis testing and clear indication of whether the SV was exceeded	– Same as Phase I study

QA = Quality assurance.
QC = Quality control.
RfDs = Reference doses.
SVs = Screening values.

Because the sampling sites in screening studies are focused primarily on the most likely problem areas and the numbers of commonly consumed target species and samples collected are limited, relatively little detailed information is obtained on the magnitude and geographic extent of contamination in a wide variety of harvestable fish and shellfish species of concern to consumers. More information is obtained through additional intensive followup studies (**Tier 2, Phases I and II**) conducted at potentially contaminated sites identified in screening studies.

Although the EPA Office of Water recommends that screening study results not be used as the sole basis for conducting a risk assessment, EPA recognizes that this practice may be unavoidable if monitoring resources are limited or if the state must issue an advisory based on detection of elevated concentrations in one composite sample. States have several options for collecting samples during the **Tier 1** screening study (see Figure 2-1), which can provide additional information on contamination without necessitating additional field monitoring expenditures as part of the **Tier 2** intensive studies.

The following assumptions are made in this guidance document for sampling fish and shellfish and for calculating human health SVs for recreational and subsistence fishers:

- Use of commonly consumed target species that are dominant in the catch and have high bioaccumulation potential (see Section 3, Target Species)
- Use of fish fillets (with skin on and belly flap tissue included) for scaled finfish species, use of skinless fillets for scaleless finfish species, and use of edible portions of shellfish (see Section 6.1.1.6, Sample Type)
- Use of fish and shellfish above legal size to maximum size in the target species
- Use of a 10^{-5} risk level, a human body weight of 70 kg (average adult), a consumption rate of 17.5 g/d for recreational fishers and 142.4 g/d for subsistence fishers, and a 70-yr lifetime exposure period to calculate SVs for carcinogens.
- Use of a human body weight of 70 kg (average adult) and a consumption rate of 17.5 g/d for recreational fishers and 142.4 g/d for subsistence fishers to calculate SVs for noncarcinogens (see Section 5, Screening Values for Target Analytes).
- Use of no contaminant loss during preparation and cooking or from incomplete absorption in the intestines.

For certain site-specific situations, states may wish to use one or more of the following exposure assumptions to protect the health of high-end fish consumers such as subsistence fishers at potentially greater risk:

- Use of commonly consumed target species that are dominant in the catch and have the highest bioaccumulation potential

- Use of whole fish or whole body of shellfish (excluding shell of bivalves), which may provide a better estimate of contaminant exposures in ethnic or Native American subsistence populations that consume whole fish or shellfish

- Use of the largest (oldest) individuals in the target species to represent the highest likely exposure levels

- Use of a 10^{-6} or 10^{-7} risk level, body weights less than 70 kg for women and children, site-specific consumption rates for sport fishers or for subsistence fishers or other consumption rates based on dietary studies of local fish-consuming populations, and a 70-yr exposure period to calculate SVs for carcinogens. **Note:** EPA has reviewed national data on the consumption rate for sport and subsistence fishers and the recommended default values for these populations are 17.5 and 142.4 g/d, respectively (USDA/ARS, 1998; U.S. EPA, 2000c).

- Use of body weights less than 70 kg for women and children and site-specific consumption rates for sport fishers or for subsistence fishers or other consumption rates based on dietary studies of local fish-consuming populations to calculate SVs for noncarcinogens. **Note:** EPA has reviewed national data on the consumption rate for sport and subsistence fishers and the recommended default values for these populations are 17.5 and 142.4 g/d, respectively (USDA/ARS, 1998; U.S. EPA, 2000c).

There are additional aspects of the screening study design that states should review because they affect the statistical analysis and interpretation of the data. These include

- Use of composite samples, which results in loss of information on the distribution of contaminant concentrations in the individual sampled fish and shellfish. Maximum contaminant concentrations in individual sampled fish, which can be used as an indicator of potentially harmful levels of contamination (U.S. EPA, 1989d), are not available when composite sampling is used.

- Use of a single sample per screening 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 analyte SVs.

- Uncertainty factors affecting the numerical calculation of quantitative health risk information (i.e., references doses and cancer slope factors) as well as human health SVs.

The use of composite samples is often the most cost-effective method for estimating average tissue concentrations of analytes in target species populations to assess chronic human health risks. However, there are some situations in which individual sampling can be more appropriate from both ecological and risk assessment perspectives. Individual sampling provides a direct measure of the range and variability of contaminant levels in target fish populations. Information on maximum contaminant concentrations in individual fish is useful in evaluating acute human health risks. Estimates of the variability of contaminant levels among individual fish can be used to ensure that studies meet desired statistical objectives. For example, the population variance of a contaminant can be used to estimate the sample size needed to detect statistically significant differences in contaminant screening values compared to the mean contaminant concentration. Finally, the analysis of individual samples may be desirable, or necessary, when the objective is to minimize the impacts of sampling on certain vulnerable target populations, such as predators in headwater streams and aquatic turtles, and in cases where the cost of collecting enough individuals for a composite sample is excessive. For states that wish to consider use of individual sampling during either the screening or intensive studies, additional information on collecting and analyzing individual samples is provided in Appendix C. States should consider the potential effects of these study design features when evaluating screening study results.

Note: As part of screening studies, states may wish to issue information not only on restricting or avoiding consumption of certain species from certain waterbodies, but on promoting unrestricted fish consumption in those waterbodies where the levels of contamination are below the SVs for all 25 of the target analytes. Waterbodies in which target analyte concentrations (see Section 5) are below the selected target analyte SVs are known as "green areas" where states can promote fish consumption to specified fisher populations. Guidance to assist states in designating these safe or green areas is provided in detail in Appendix B.

2.2 INTENSIVE STUDIES (TIER 2)

The primary aims of intensive studies are to assess the magnitude of tissue contamination at screening sites, to determine the size class or classes of fish within a target species whose contaminant concentrations exceed the SVs, and to assess the geographic extent of the contamination for the target species in the waterbody under investigation. With respect to the design of intensive studies, EPA recommends a sampling strategy that may not be feasible for some site-specific environments. Specifically, EPA recognizes that some waterbodies cannot sustain the same intensity of sampling (i.e., number of replicate composite samples per site and number of individuals per composite sample) that others (i.e., those used for commercial harvesting) can sustain. In such cases, state fisheries personnel may consider modifying the sampling strategy (e.g., analyzing individual fish) for intensive studies to protect the fishery resource. Although one strategy cannot cover all situations, these sampling guidelines are reasonable for the majority of environmental conditions, are scientifically defensible, and provide information that can be used to assess the risk to public health. Regardless of the final study design and protocol chosen for a fish contaminant monitoring program, state fisheries, environmental, and health personnel should always evaluate and document the procedures used to ensure that results obtained meet state objectives for protecting human health.

The allocation of limited funds to screening studies or to intensive studies should always be guided by the goal of conducting adequate sampling of state fish and shellfish resources to ensure the protection of public health. The amount of sampling that can be performed by a state will be determined by available economic resources. Ideally, state agencies will allocate funds for screening as many sites as is deemed necessary while reserving adequate resources to conduct subsequent intensive studies at sites where excessive fish tissue contamination is detected. State environmental and health personnel should use all information collected in both screening and intensive studies to (1) conduct a risk assessment to determine whether the issuance of an advisory is warranted, (2) use risk management to determine the nature and extent of the advisory, and then (3) effectively communicate this risk to the fish-consuming public. Additional information on risk assessment, risk management, and risk communication procedures will be provided in subsequent volumes in this series.

SECTION 3

TARGET SPECIES

The primary objectives of this section are to: (1) discuss the purpose of using target species, (2) describe the criteria used by the 1993 EPA Fish Contaminant Workgroup to select target species, and (3) provide lists of recommended target species. Target species recommended for freshwater and estuarine/marine ecosystems are discussed in Sections 3.3 and 3.4, respectively.

3.1 PURPOSE OF USING TARGET SPECIES

The use of target species allows comparison of fish, shellfish, and turtle tissue contaminant monitoring data among sites over a wide geographic area. Differences in habitat, food preferences, and rate of contaminant uptake among various fish, shellfish, and turtle species make comparison of contaminant monitoring results within a state or among states difficult unless the contaminant data are from the same species. It is virtually impossible to sample the same species at every site, within a state or region or nationally, due to the varying geographic distributions and environmental requirements of each species. However, a limited number of species can be identified that are distributed widely enough to allow for collection and comparison of contaminant data from many sites.

Three aims are achieved by using target species in screening studies. First, states can cost-effectively compare contaminant concentrations in their state waters and then prioritize sites where tissue contaminants exceed human health screening values. In this way, limited monitoring resources can be used to conduct intensive studies at sites exhibiting the highest degree of tissue contamination in screening studies. By resampling target species used in the screening study in Phase I intensive studies and sampling additional size classes and additional target species in Phase II intensive studies as resources allow, *states can assess the magnitude and geographic extent of contamination in* species of commercial, recreational, or subsistence value. Second, the use of common target species among states allows for more reliable comparison of sampling information. Such information allows states to design and evaluate their own contaminant monitoring programs more efficiently, which should further minimize overall monitoring costs. For example, monitoring by one state of fish tissue contamination levels in the upper reaches of a particular river can provide useful information to an adjacent state on tissue contamination levels that might be anticipated in the same target species at sampling sites downstream. Third, *the use of a select group of target fish, shellfish, and freshwater turtle species will* allow for the development of a national database for tracking the magnitude and geographic extent of pollutant contamination in these target species nationwide and will permit analyses of trends in fish, shellfish, and turtle contamination over time.

3.2 CRITERIA FOR SELECTING TARGET SPECIES

The appropriate choice of target species is a key element of any chemical contaminant monitoring program. Criteria for selecting target species used in the following national fish and shellfish contaminant monitoring programs were reviewed by the 1993 EPA Fish Contaminant Workgroup to assess their applicability for use in selecting target species for state fish contaminant monitoring programs:

- National Study of Chemical Residues in Fish (U.S. EPA)
- National Dioxin Study (U.S. EPA)
- 301(h) Monitoring Program (U.S. EPA)
- National Pesticide Monitoring Program (U.S. FWS)
- National Contaminant Biomonitoring Program (U.S. FWS)
- National Status and Trends Program (NOAA).
- National Water Quality Assessment Program (USGS).

The criteria used to select target species in many of these programs are similar although the priority given each criterion may vary depending on program aims.

According to the 1993 EPA Fish Contaminant Workgroup, the most important criterion for selecting target fish, shellfish, and turtle species for state contaminant monitoring programs assessing human consumption concerns was that the species were commonly consumed in the study area and were of commercial, recreational, or subsistence fishing value. Two other criteria of major importance are that the species have the potential to bioaccumulate high concentrations of chemical contaminants and have a wide geographic distribution. EPA recommends that states use the same criteria to select species for both screening and intensive site-specific studies.

In addition to the three primary criteria for target species selection, it is also important that the target species be easy to identify taxonomically because there are significant species-specific differences in bioaccumulation potential. Because many closely related species can be similar in appearance, reliable taxonomic identification is essential to prevent mixing of closely related species with the target species. **Note:** Under no circumstance should individuals of more than one species be mixed to create a composite sample (U.S. EPA, 1991e). It is also both practical and cost-effective to sample target species that are abundant, easy to capture, and large enough to provide adequate tissue samples for chemical analyses.

It cannot be overemphasized that final selection of target species will require the expertise of state fisheries biologists with knowledge of local species that best meet the selection criteria and knowledge of local human consumption patterns. Although, ideally, all fish, shellfish, or turtle species consumed from a given waterbody by the local population should be monitored, resource constraints may dictate that only a few of the most frequently consumed species be sampled.

In the next two sections, lists of recommended target species are provided for freshwater ecosystems (inland fresh waters and the Great Lakes) and estuarine/marine ecosystems (Atlantic, Gulf, and Pacific waters), and the methods used to develop each list are discussed.

3.3 FRESHWATER TARGET SPECIES

As part of the two-tiered sampling strategy proposed for state fish contaminant monitoring programs, EPA recommends that states collect one bottom-feeding fish species and one predator fish species at each freshwater screening study site. Some suggested target species for use in state fish contaminant monitoring programs are shown in Table 3-1 for inland fresh waters and in Table 3-2 for Great Lakes waters.

The lists of target species recommended by the 1993 EPA Fish Contaminant Workgroup for freshwater ecosystems were developed based on a review of species used in the following national monitoring programs:

- National Study of Chemical Residues in Fish (U.S. EPA)
- National Dioxin Study (U.S. EPA)
- National Pesticide Monitoring Program (U.S. FWS)
- National Contaminant Biomonitoring Program (U.S. FWS)
- National Water Quality Assessment Program (USGS)

and on a review of fish species cited in state fish consumption advisories or bans (RTI, 1993). Separate target species lists were developed for inland fresh waters (Table 3-1) and Great Lakes waters (Table 3-2) because of the distinct ecological characteristics of these waters and their fisheries. Each target species list has been reviewed by regional and state fisheries experts.

Use of two distinct ecological groups of finfish (i.e., bottom-feeders and predators) as target species in freshwater systems is recommended. This permits monitoring of a wide variety of habitats, feeding strategies, and physiological factors that might result in differences in bioaccumulation of contaminants. Bottom-feeding species may accumulate high contaminant concentrations from direct physical contact with contaminated sediment and/or by consuming benthic invertebrates and epibenthic organisms that live in contaminated sediment. Predator species are also good indicators of persistent pollutants (e.g., mercury or DDT and its metabolites) that may be biomagnified through several trophic levels of the food web. Species used in several federal programs to assess the

Table 3-1. Recommended Target Species for Inland Fresh Waters

Family name	Common name	Scientific name
Percichthyidae	White bass	*Morone chrysops*
Centrarchidae	Largemouth bass	*Micropterus salmoides*
	Smallmouth bass	*Micropterus dolomieui*
	Black crappie	*Pomoxis nigromaculatus*
	White crappie	*Pomoxis annularis*
Percidae	Walleye	*Stizostedion vitreum*
	Yellow perch	*Perca flavescens*
Cyprinidae	Common carp	*Cyprinus carpio*
Catostomidae	White sucker	*Catostomus commersoni*
Ictaluridae	Channel catfish	*Ictalurus punctatus*
	Flathead catfish	*Pylodictis olivaris*
Esocidae	Northern pike	*Esox lucius*
Salmonidae	Lake trout	*Salvelinus namaycush*
	Brown trout	*Salmo trutta*
	Rainbow trout	*Oncorhynchus mykiss*[a]

[a]Formerly *Salmo gairdneri.*

Table 3-2. Recommended Target Species for Great Lakes Waters

Family name	Common name	Scientific name
Percichthyidae	White bass	*Morone chrysops*
Centrarchidae	Smallmouth bass	*Micropterus dolomieui*
Percidae	Walleye	*Stizostedion vitreum*
Cyprinidae	Common carp	*Cyprinus carpio*
Catostomidae	White sucker	*Catostomus commersoni*
Ictaluridae	Channel catfish	*Ictalurus punctatus*
Esocidae	Muskellunge	*Esox masquinongy*
Salmonidae	Chinook salmon	*Oncorhynchus tschawytscha*
	Lake trout	*Salvelinus namaycush*
	Brown trout	*Salmo trutta*
	Rainbow trout	*Oncorhynchus mykiss*[a]

[a]Formerly *Salmo gairdneri.*

extent of freshwater fish tissue contamination nationwide are compared in Table 3-3.

In addition to finfish species, states should consider monitoring the tissues of freshwater turtles for environmental contaminants in areas where turtles are consumed by recreational, subsistence, or ethnic populations. Interest has been increasing in the potential transfer of environmental contaminants from the aquatic food chain to humans via consumption of freshwater turtles. Turtles may bioaccumulate environmental contaminants in their tissues from exposure to contaminated sediments or via consumption of contaminated prey. Because some turtle species are long-lived and occupy a medium to high trophic level of the food chain, they have the potential to accumulate high concentrations of chemical contaminants from their diets (Hebert et al., 1993). Some suggested target turtle species for use in state contaminant monitoring programs are listed in Table 3-4.

The list of target turtle species recommended for freshwater ecosystems was developed based on a review of turtle species cited in state consumption advisories or bans (RTI, 1993) and a review of the recent scientific literature. The recommended target species list has been reviewed by regional and state experts.

3.3.1 Target Finfish Species

3.3.1.1 Bottom-Feeding Species

EPA recommends that, whenever practical, states use common carp (*Cyprinus carpio*), channel catfish (*Ictalurus punctatus*), and white sucker (*Catostomus commersoni*) in that order as bottom-feeding target species in both inland fresh waters (Table 3-1) and in Great Lakes waters (Table 3-2). These bottom-feeders have been used consistently for monitoring a wide variety of contaminants including dioxins/furans (Crawford and Luoma, 1993; U.S. EPA, 1992c, 1992d; Versar Inc., 1984), organochlorine pesticides (Crawford and Luoma, 1993; Schmitt et al., 1983, 1985, 1990; U.S. EPA, 1992c, 1992d), and heavy metals (Crawford and Luoma, 1993; Lowe et al., 1985; May and McKinney, 1981; Schmitt and Brumbaugh, 1990; U.S. EPA, 1992c, 1992d). These three species are commonly consumed in the areas in which they occur and have also demonstrated an ability to accumulate high concentrations of environmental contaminants in their tissues as shown in Tables 3-5 and 3-6. **Note:** The average contaminant concentrations shown in Tables 3-5 and 3-6 for fish collected for the EPA National Study of Chemical Residues in Fish (U.S. EPA, 1992c, 1992d) were derived from concentrations in fish from undisturbed areas and from areas expected to have elevated tissue contaminant concentrations. The mean contaminant concentrations shown, therefore, may be higher or lower than those found in the ambient environment because of site selection criteria used in this study.

Table 3-3. Comparison of Freshwater Finfish Species Used in Several National Fish Contaminant Monitoring Programs

	U.S. EPA National Dioxin Study	U.S. FWS NPMP and NCBP	U.S. EPA NSCRF	USGS NWQAP
BOTTOM FEEDERS				
Family *Cyprinidae* Carp *(Cyprinus carpio)*	●	●	●	●
Family *Icataluridae* Channel catfish (*Ictalurus punctatus)*	●	● Or other ictalurid	●	●
Family *Catostomidae* White sucker *(Catastomus commersoni)*	●	● Or other catostomid	●	●
Longnose sucker *(C. catostromus)*				●
Largescale sucker *(C.macrochellus)*				
Spotted sucker *(Minytrema melanops)*			●	
Redhorse sucker *(Moxostoma sp.)* included variety of species: Silver redhorse *(M. anisurum)* Grey redhorse *(M. congetum)* Black redhorse *(M. duquesnei)* Golden redhorse *(M. erythrurum)* Shorthead redhorse *(M. macrolepidotum)* Blacktail redhorse *(M. poecilurum)*			●	
PREDATORS				
Family *Salmoridae* Rainbow trout *(Oncortynchus mykiss)* [formerly *Salmo gairdneni]*	●	●		
Brown trout *(Salmo trutta)*	●	●	●	●
Brook trout *(Salvelinus fontinalis)*	●	●		●
Lake trout *(Salmo namaycush)*	●	●		
Family *Percidae* Walleye *(Stizostedion vitreum)*	● Or other pericid	● Or other pericid	●	
Sauger *(Stizostedion canadense)*	○	○		
Yellow perch *(Perca flavescans)*	○	○		
Family *Percichthyidae* White bass *(Morone chrysops)*			●	
Family *Centrarchidae* Largemouth bass *(Micropterus salmoides)*	● Or other centrarchid	● Or other centrarchid	●	●
Smallmouth bass *(Micropterus dolomieui)*			●	
Black crappie *(Pomoxis nigromaculatus)*	○	○		
White crappie *(Pomoxis annularis)*	○	○	●	
Bluegill sunfish *(Lepomis macrochirus)*	○	○		●
Family *Esocidae* Northern pike *(Esox lucius)*			●	
Family *Ictaluridae* Flathead catfish *(Pylodictis olivaris)*			●	

● Recommended target species
○ Alternate target species

NPMP = National Pesticide Monitoring Program
NCBP = National Contaminant Biomonitoring Program
NSCRF = National Study of Chemical Residues in Fish
NWQAP = National Water Quality Assessment Program

Sources: Versar, Inc., 1984; Schmitt et al., 1990; Schmitt et al., 1983; May and McKinney, 1981; U.S. EPA, 1992c, 1992d; Crawford and Luoma, 1993.

Table 3-4. Freshwater Turtles Recommended for Use as Target Species

Family name	Common name	Scientific name
Chelydridae	Snapping turtle	*Chelydra serpentina*
Emydidae	Yellow-bellied turtle	*Trachemys scripta scripta*
	Red-eared turtle	*Trachemys scripta elegans*
	River cooter	*Pseudemys concinna concinna*
	Suwanee cooter	*Pseudemys concinna suwanniensis*
	Slider	*Pseudemys concinna hieroglyphica*
	Texas slider	*Pseudemys concinna texana*
	Florida cooter	*Pseudemys floridana floridana*
	Peninsula cooter	*Pseudemys floridana penisularis*
Trionychidae	Smooth softshell	*Apalone muticus*
	Eastern spiny softshell	*Apalone spinifera spinifera*
	Western spiny softshell	*Apalone spinifera hartwegi*
	Gulf Coast spiny softshell	*Apalone spinifera aspera*
	Florida softshell	*Apalone ferox*

In addition, these three species are relatively widely distributed throughout the continental United States, and numerous states are already sampling these species in their contaminant monitoring programs. A review of the database *National Listing of State Fish and Shellfish Consumption Advisories and Bans* (RTI, 1993) indicated that the largest number of states issuing advisories for specific bottom-feeding species did so for carp (21 states) and channel catfish (22 states), with eight states issuing advisories for white suckers (see Table 3-7). Appendix D lists the freshwater fish species cited in consumption advisories for each state as of 1998.

3.3.1.2 Predator Species

EPA recommends that, whenever practical, states use predator target species listed in Tables 3-1 and 3-2 for inland fresh waters and Great Lakes waters, respectively. Predator species, because of their more definitive habitat and water temperature preferences, generally have a more limited geographic distribution. Thus, a greater number of predator species than bottom feeders have been used in national contaminant monitoring programs (Table 3-3) and these are recommended for use as target species in freshwater ecosystems. Predator fish that prefer relatively cold freshwater habitats include many members of the following families: *Salmonidae* (trout and salmon), *Percidae* (walleye and yellow perch), and *Esocidae* (northern pike and muskellunge). Members of the *Centrarchidae* (large- and smallmouth bass, crappie, and sunfish), *Percichthyidae* (white bass), and *Ictaluridae* (flathead catfish) families prefer relatively warm water habitats. Only two predator species (brown trout and largemouth bass) were used in all four of the national monitoring programs reviewed by the 1993 EPA Fish Contaminant Workgroup (Table 3-3). However, most of the other predator species recommended as target species have been used in at least one national monitoring program. To identify those predator species with a known ability to bioaccumulate contaminants in their tissues, the 1993 EPA Workgroup reviewed average tissue concentrations of xenobiotic contaminants for major

Table 3-5. Average Fish Tissue Concentrations (ppb) of Xenobiotics for Major Finfish Species Sampled in the National Study of Chemical Residues in Fish[a]

Fish Species	Alpha-BHC	Gamma-BHC	Biphenyl	Chlorpyrifos	Dicofol	Dieldrin	Endrin	Heptachlor epoxide	Mercury	Mirex	Oxychlordane	PCBs
Bottom Feeders[b]												
Carp	3.10	4.34	4.38	8.23	0.88	44.75	1.40	4.00	0.11	3.70	8.20	2941.13
White sucker	3.31	1.66	1.28	1.75	0.48	22.75	0.24	1.09	0.11	4.35	3.10	1697.81
Channel cat	2.87	3.17	1.24	6.97	0.59	15.44	9.07	0.50	0.09	14.59	6.41	1300.52
Redhorse sucker	0.82	0.41	1.25	0.35	ND	5.35	0.97	ND	0.27	0.57	2.37	487.72
Spotted sucker	1.45	2.63	3.35	0.56	0.05	5.52	ND	ND	0.12	1.79	0.05	133.90
Predators[b]												
Largemouth bass	0.15	0.07	0.38	0.23	0.20	5.01	ND	0.30	0.46	0.21	0.47	232.26
Smallmouth bass	0.36	0.15	0.33	0.08	ND	2.34	ND	0.07	0.34	1.99	0.54	496.22
Walleye	ND	ND	0.40	0.04	ND	3.73	ND	0.21	0.51	0.08	1.11	368.65
Brown trout	1.59	ND	0.81	ND	0.94	20.13	ND	2.08	0.14	43.98	5.38	2434.07
White bass	0.34	0.79	0.62	1.32	ND	9.35	ND	1.40	0.35	0.11	0.84	288.35
Northern pike	0.55	ND	0.59	11.43	0.31	9.04	ND	ND	0.34	2.39	4.00	788.40
Flathead cat	0.92	0.58	0.60	22.57	1.28	37.38	3.45	0.57	0.27	ND	0.63	521.19
White crappie	0.23	ND	0.21	ND	ND	ND	ND	ND	0.22	ND	ND	22.34
Bluefish	0.38	0.12	0.20	ND	ND	2.87	ND	ND	0.22	0.13	ND	368.06

Fish Species	Penta-chloro-anisole	Penta-chloro-benzene	DDE	Total Chlordane	Total Nonachlor	123 TCB	124 TCB	135 TCB	1234 TECB	Trifluralin	Hexa-chloro-benzene
Bottom Feeders[b]											
Carp	16.50	1.04	415.43	67.15	63.15	1.54	4.77	0.08	0.30	12.55	3.58
White sucker	9.06	0.39	78.39	18.43	20.83	0.16	0.30	0.14	0.15	ND	3.62
Channel cat	39.60	1.32	627.77	54.39	66.28	0.14	0.37	ND	0.88	1.00	2.36
Redhorse sucker	2.87	0.02	87.25	16.48	30.73	0.55	6.48	0.08	0.09	ND*	0.58
Spotted sucker	17.68	0.02	75.31	12.33	15.00	3.34	12.00	1.00	0.09	ND	0.02
Predators[b]											
Largemouth bass	0.57	0.02	55.72	2.89	4.21	0.22	0.19	0.03	0.01	ND	0.20
Smallmouth bass	0.23	0.02	33.63	4.01	7.82	0.70	0.59	0.04	0.04	ND	0.36
Walleye	0.76	ND	34.00	3.62	8.04	0.29	0.38	ND	0.004	ND	0.11
Brown trout	0.09	0.60	158.90	7.25	32.60	1.10	0.98	ND	0.09	ND	3.06
White bass	0.93	ND	17.44	10.67	16.00	0.21	0.10	ND	0.01	ND	0.83
Northern pike	1.51	0.09	59.50	5.45	13.88	0.30	0.23	ND	0.01	ND	0.20
Flathead catfish	0.31	ND	755.18	16.07	14.04	0.10	0.18	ND	ND	44.37	0.85
White crappie	0.33	ND	10.04	0.34	0.28	0.08	0.08	0.08	ND	ND	ND
Bluefish	0.05	ND	29.13	2.74	2.56	6.25	4.66	4.66	ND	ND	ND

[a] These average fish tissue concentrations may be higher or lower than those found in the ambient environment because of site selection criteria used in this study.

[b] Values were calculated using whole-body samples for bottom feeders and fillet samples for predators. Individual values below detection limit were set at zero. Asterisk indicates all values below the detection limit. Units = ppb, (µg/g) wet weight basis. ND = Not detected.

Source: US. EPA, 1991h.

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Table 3-6. Average Fish Tissue Concentrations (ppt) of Dioxins and Furans for Major Finfish Species Sampled in the National Study of Chemical Residues in Fish[a]

Fish Species	2378 TCDD	12378 PeCDD	123478 HxCDD	123678 HxCDD	123789 HxCDD	1234678 HpCDD	2378 TCDF	12378 PeCDF	23478 PeCDF	123478 HxCDF	123678 HxCDF	123789 HxCDF	234678 HxCDF	1234678 HpCDF	1234789 HpCDF	TEQ
Bottom Feeders[b]																
Carp	7.76	3.63	2.16	6.81	1.54	22.29	10.15	1.31	4.01	2.54	1.91	1.16	1.20	2.49	1.22	13.06
White sucker	8.08	2.05	1.03	1.96	0.88	3.72	22.89	1.10	2.64	2.21	1.29	1.06	1.09	1.23	1.13	12.79
Channel catfish	11.56	2.37	1.61	5.62	1.29	9.40	2.22	0.52	2.91	2.41	1.41	1.38*	1.62	2.55	1.26	14.80
Redhorse sucker	4.65	1.50	1.40	2.36	0.84	4.94	30.09	0.75	1.28	2.10	1.16	1.19*	1.50	1.57	1.36*	9.22
Spotted sucker	1.73	2.34	1.70	12.08	1.14	17.48	7.49	2.12	2.06	2.22	1.79	1.28*	1.78	1.77	1.08	6.23
Predators[b]																
Largemouth bass	1.73	0.59	1.12	1.28	0.64	2.48	2.18	0.37	0.47	1.24	1.23	1.21*	0.88	0.82	1.21*	1.91
Smallmouth bass	0.72	0.50*	1.13*	0.79	0.64*	0.67	1.93	0.36*	0.51	1.28	1.23	1.26*	0.89*	0.69	1.30*	0.65*
Walleye	0.88	0.54*	0.99*	0.73	0.62*	0.88	1.83	0.35*	0,38	1.04	1.09*	1.07*	0.75	0.74	1.21*	0.79*
Brown trout	2.52	1.01	1.07*	0.98	0.68*	1.18	3.74	0.60	1.36	1.47	1.12*	1.09*	0.94*	0.67*	1.16*	3.31
White bass	3.00	0.66	1.05*	0.78	0.61*	1.01	5.07	0.40	0.49	1.04	1.16*	1.13*	0.81*	0.63	1.17*	3.44
Northern pike	0.77	0.46*	1.23*	0.91	0.69*	0.73	1.01	0.44	0.66	1.41*	1.42*	1.38*	0.98*	0.56	1.30*	0.66
Flathead cat	0.78	0.43	0.90	1.06	0.50	1.67	1.63	0.40	0.56	1.05	1.20*	1.17*	0.61*	0.56	1.10*	0.99
White crappie	2.13	0.60	1.29*	1.03*	0.83*	1.33	10.46	0.54	0.67	1.33*	1.33*	1.30*	0.95*	0.96*	1.34*	3.80
Bluefish	0.85	0.56	1.23*	0.98*	0.69*	0.65	2.11	0.41	0.59	1.42*	1.42*	1.39*	0.98*	0.72*	1.31*	1.41

[a] These average fish tissue concentrations may be higher or lower than those found in the ambient environment because of site selection criteria used in this study.

[b] Values were calculated using whole-body samples for bottom feeders and fillet samples for predators. Values below detection limit have been replaced by one-half detection limit for the given sample. Asterisk indicates all values below detection limit. Units = ppt (pg/g) wet weight basis.

TEQ = Toxicity equivalency was based on TEF-89 toxicity weighting values; however, octachlorodibenzo-*p*-dioxin and octachlorodibenzofurans were not analyzed; therefore, the TEQ value does not include these two compounds.

Source: U.S. EPA, 1991h.

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predator fish species sampled in the National Study of Chemical Residues in Fish. Unlike the bottom feeders (common carp, channel catfish, and white suckers), no single predator species or group of predator species consistently exhibited the highest tissue concentrations for the contaminants analyzed (Tables 3-5 and 3-6). However, average fish tissue concentrations for some contaminants (i.e., mercury, mirex, chlorpyrifos, DDE, 1,2,3-trichlorobenzene [123-TCB], and trifluralin) were higher for some predator species than for the bottom feeders despite the fact that only the fillet portion rather than the whole body was analyzed for predator species. This finding emphasizes the need for using two types of fish (i.e., bottom feeders and predators) with different habitat and feeding strategies as target species.

The existence of fish consumption advisories for these predator target species was further justification for their recommended use. As was shown for the bottom-feeder target species, states were already sampling the recommended predator target species listed in Table 3-7. The largest number of states issuing advisories in 1993 for specific predator species did so for largemouth bass (15), lake trout (10), white bass (10), smallmouth bass (9), brown trout (9), walleye (9), rainbow trout (8), yellow perch (8), chinook salmon (7), northern pike (7), black crappie (5), flathead catfish (4), and muskellunge (4) (RTI, 1993). For comparison, the number of states reporting advisories for each species in 1998 is also presented in Table 3-7.

Because some freshwater finfish species (e.g., several Great Lake salmonids) are highly migratory, harvesting of these species may be restricted to certain seasons because sexually mature adult fish (i.e., the recommended size for sampling) may make spawning runs from the Great Lakes into tributary streams. EPA recommends that spawning populations not be sampled in fish contaminant monitoring programs. Sampling of target finfish species during their spawning period should be avoided because contaminant tissue concentrations may decrease during this time (Phillips, 1980) and because the spawning period is generally outside the legal harvest period. **Note:** Target finfish may be sampled during their spawning period, however, if the species can be legally harvested at this time.

State personnel, with their knowledge of site-specific fisheries and human consumption patterns, must be the ultimate judge of the species selected for use in freshwater fish contaminant monitoring programs within their jurisdiction.

3.3.2 Target Turtle Species

EPA recommends that states in which freshwater turtles are consumed by recreational, subsistence, or ethnic populations consider monitoring turtles to assess the level of environmental contamination and whether they pose a human health risk. In all cases, the primary criterion for selecting the target turtle species is whether it is commonly consumed. To identify those turtle species with a known ability to bioaccumulate contaminants in their tissues, the 1993 EPA Workgroup reviewed turtle species cited in state consumption advisories and those species identified

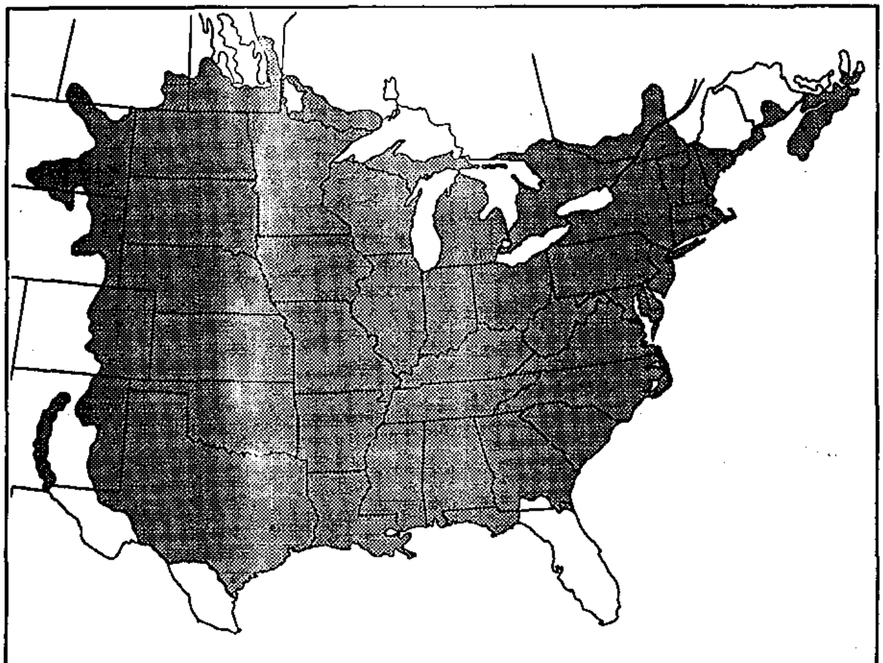
Table 3-7. Principal Freshwater Fish Species Cited in State Fish Consumption Advisories[a]

Family name	Common name	Scientific name	Number of states with advisories[b] 1993	1998
Percichthyidae	**White bass**	***Morone chrysops***	10	17
	Striped bass	*Morone saxatilis*	6	12
	White perch	*Morone americana*	4	7
Centrarchidae	**Largemouth bass**	***Micropterus salmoides***	15	33
	Smallmouth bass	***Micropterus dolomieui***	9	18
	Black crappie	***Pomoxis nigromaculatus***	5	18
	White crappie	***Pomoxis annularis***	2	11
	Bluegill sunfish	*Lepomis macrochirus*	5	11
	Rock bass	*Ambloplites rupestris*	3	5
Percidae	**Yellow perch**	***Perca flavescens***	8	12
	Sauger	*Stizostedion canadense*	4	9
	Walleye	*Stizostedion vitreum*	9	12
Cyprinidae	**Common carp**	***Cyprinus carpio***	21	25
Acipenseridae	Shovelnose sturgeon	*Scaphirhynchus platorynchus*	1	3
	Lake sturgeon	*Acipenser fulvescens*	2	3
Catostomidae	Smallmouth buffalo	*Ictiobus bubalus*	4	5
	Bigmouth buffalo	*Ictiobus cyprinellus*	4	6
	Shorthead redhorse	*Moxostoma macrolepidotum*	2	3
	White sucker	***Catostomus commersoni***	8	11
	Quillback carpsucker	*Carpiodes cyprinus*	2	5
Ictaluridae	White catfish	*Ictalurus catus*	5	6
	Channel catfish	***Ictalurus punctatus***	22	26
	Flathead catfish	***Pylodictis olivaris***	4	11
	Black bullhead	*Ictalurus melas*	2	3
	Brown bullhead	*Ictalurus nebulosus*	7	10
	Yellow bullhead	*Ictalurus natalis*	2	8
Sciaenidae	Freshwater drum	*Aplodinotus grunniens*	3	13
Esocidae	**Northern pike**	***Esox lucius***	7	10
	Muskellunge	***Esox masquinongy***	4	4
Salmonidae	Coho salmon	*Oncorhynchus kisutch*	6	8
	Chinook salmon	***Oncorhynchus tschawytscha***	7	7
	Brown trout	*Salmo trutta*	9	11
	Lake trout	***Salvelinus namaycush***	10	12
	Rainbow trout	*Oncorhynchus mykiss*[c]	8	12
	Brook trout	*Salvelinus fontinalis*	3	4
	Lake whitefish	*Coregonus clupea formis*	2	7
Anguillidae	American eel	*Anguilla rostrata*	6	7

[a] Species in boldface are EPA-recommended target species for inland fresh waters (see Table 3-1) and the Great Lakes waters (Table 3-2).
[b] Many states did not identify individual species of finfish in their advisories.
[c] Formerly *Salmo gairdneri.*
Sources: RTI, 1993; U.S. EPA, 1999c (NLFWA).

in the scientific literature as having accumulated high concentrations of environmental contaminants.

Based on information in state advisories and a number of environmental studies using turtles as biological indicators of pollution, one species stands out as an obvious choice for a target species, the common snapping turtle (*Chelydra serpentina*). This turtle has been recommended by several researchers as an important bioindicator species (Bishop et al., 1996; Bonin et al., 1995; Olafsson et al., 1983; Stone et al., 1980) and has the widest geographic distribution of any of the North American aquatic turtles (see Figure 3-1). In addition, this species is highly edible, easily identified, easily collected, long-lived (>20 years), grows to a large size, and has been extensively studied with respect to a variety of environmental contaminants. Other turtle species that should be considered for use as target species are listed in Table 3-4.



Source: Conant and Collins, 1991.

Figure 3-1. Geographic range of the common snapping turtle (*Chelydra serpentina*).

Four states (Arizona, Massachusetts, Minnesota, and New York) currently have consumption advisories in force for various turtle species (U.S. EPA, 1999c; New York State Department of Health, 1994). The species cited in the state advisories and the pollutants identified in turtle tissues as exceeding acceptable levels of contamination with respect to human health are listed in Table 3-8. New York

Table 3-8. Principal Freshwater Turtle Species Cited in State Consumption Advisories

Family name	Common name	Scientific name	Pollutant	State
Chelydridae	Snapping turtle[a]	*Chelydra serpentina*	Mercury	MN
	Snapping turtle[a] (and other unspecified turtle species)	*Chelydra serpentina*	PCBs	MA
	Snapping turtle[b]	*Chelydra serpentina*	PCBs	NY
Trionychidae	Western spiny softshell[a]	*Apalone spiniferus*	DDT toxaphene, chlordane, dieldrin	AZ

PCB = Polychlorinated biphenyls. DDT = 1,1,1-trichloro-2,2 bis(p-chlorophenyl)ethane.
[a]Source: U.S. EPA 1999c (NLFWA).
[b]Source: New York State Department of Health, 1994.

state has a statewide advisory directed specifically at women of childbearing age and children under 15 and advises these groups to avoid eating snapping turtles altogether. The advisory also recommends that members of the general population who wish to consume turtle meat should trim away all fat and discard the liver tissue and eggs of the turtles prior to cooking the meat or preparing other dishes. These three tissues (fat, liver, and eggs) have been shown to accumulate extremely high concentrations of a variety of environmental contaminants in comparison to muscle tissue (Bishop et al., 1996; Bonin et al., 1995; Bryan et al., 1987; Hebert et al., 1993; Olafsson et al 1983; 1987; Ryan et al., 1986; Stone et al., 1980). The Minnesota advisory also recommends that consumers remove all fat from turtle meat prior to cooking as a risk-reducing strategy (Minnesota Department of Health, 1994). States should consider monitoring pollutant concentrations in all three tissues (fat, liver, and eggs) in addition to muscle tissue if resources allow. If residue analysis reveals the presence of high concentrations of any environmental contaminant of concern, the state should consider making the general recommendation to consumers to discard these three highly lipophilic tissues (fat, liver, and eggs) to reduce the risk of exposure particularly to many organic chemical contaminants.

To identify those freshwater turtle species with a known ability to bioaccumulate chemical contaminants in their tissues, several studies were reviewed that identified freshwater turtle species as useful biomonitors of PCBs (Bishop et al., 1996; Bonin et al., 1995; Bryan et al., 1987; Hebert et al., 1993; Helwig and Hora, 1983; Olafsson et al., 1983; 1987; S[illegible]e, 1985; and Stone et al., 1980), dioxins and dibenzofurans (Bishop et al., 19[illegible]; Rappe et al., 1981; Ryan et al., 1986), organochlorine pesticides (Bishop et al., 1996; Bonin et al., 1995; Hebert et al., 1993; Stone et al., 1980), heavy metals (Bonin et al., 1995; Helwig and Hora, 1983; Stone et al., 1980), and radioactive nuclides (cesium-137 and strontium-90) (Lamb et al., 1991; Scott et al., 1986). The turtle species used in these studies, the pollutants monitored, and the reference sources are summarized in Table 3-9.

Table 3-9. Studies Using Freshwater Turtles as Biomonitors of Environmental Contamination

Species	Pollutant monitored	Source
Snapping turtle (*Chelydra serpentina)*	PCBs, total DDT, mirex	Hebert et al., 1993
Snapping turtle (*Chelydra serpentina)*	PCBs	Olafsson et al., 1987 Olafsson et al., 1983
Snapping turtle (*Chelydra serpentina)*	PCBs	Safe, 1987
Snapping turtle (*Chelydra serpentina)*	PCBs	Bryan et al., 1987
Snapping turtle (*Chelydra serpentina)*	Dioxins/Furans	Ryan et al., 1986
Snapping turtle (*Chelydra serpentina)*	PCBs, mercury, cadmium	Helwig and Hora, 1983
Snapping turtle (*Chelydra serpentina)*	Furans	Rappe et al., 1981
Snapping turtle (*Chelydra serpentina)*	Organochlorine pesticides (DDE, dieldrin, hexachlorobenzene, heptachlor epoxide, mirex), PCBs, cadmium, mercury	Stone et al., 1980
Snapping turtle (*Chelydra serpentina)*	29 Organochlorine pesticides, 39 PCB congeners, mercury	Bonin et al., 1995
Snapping turtle eggs	4 Organochlorine pesticides (DDE, dieldrin, mirex, hexachloro-benzene), PCBs, dioxins/furans	Bishop et al., 1996
Yellow-bellied turtle (*Trachemys scripta)*	Cesium-137 Strontium-90	Lamb et al., 1991
Yellow-bellied turtle (*Trachemys scripta)*	Cesium-137 Strontium-90	Scott et al., 1986

PCBs = Polychlorinated biphenyls.
DDT = 1,1,1-Trichloro-2,2 bis(p-chlorophenyl)ethane.
DDE = 1,1-Dichloro-2,2-bis(p-chlorophenyl)-ethylene.

State personnel, with their knowledge of site-specific fisheries and human consumption patterns, must be the ultimate judge of the turtle species selected for use in contaminant monitoring programs within their jurisdictions. Because several turtle species are becoming less common as a result of habitat loss or degradation or overharvesting, biologists need to ensure that the target species selected for the state toxics monitoring program is not of special concern within their jurisdiction or designated as a threatened or endangered species. For example, two highly edible turtle species, the Alligator snapping turtle (*Macroclemys temmincki*) and the Northern diamondback terrapin (*Malaclemys terrapin terrapin*) are protected in some states or designated as species of concern within portions of their geographic range and are also potential candidates for federal protection (Sloan and Lovich, 1995). Although protected to varying degrees by several states, George (1987) and Pritchard (1989) concluded that the Alligator snapping turtle should receive range-wide protection from the federal government as a threatened species under the Endangered Species Act. Unfortunately, basic ecological and life history information necessary to make environmental management decisions (i.e., federal listing as endangered or threatened species) is often not available for turtles and other reptiles (Gibbons, 1988).

Several species of freshwater turtles already have been designated as endangered or threatened species in the United States including the Bog turtle (*Clemmys muhlenbergii*), Plymouth red-bellied turtle (*Pseudemys rubriventris bangsi*), Alabama red-bellied turtle (*Pseudemys alabamensis*), Flattened musk turtle (*Stemotherus depressus*), Ringed map (=sawback) turtle (*Graptemys oculifera*), and the Yellow-blotched map (=sawback) turtle (*Graptemys flavimaculata*) (U.S. EPA, 1994; U.S. Fish and Wildlife Service, 1994). In addition, all species of marine sea turtles including the Green sea turtle (*Chelonia mydas*), Hawksbill sea turtle (*Eretmochelys imbricata*), Kemp's ridley sea turtle (*Lepidochelys kempii*), Olive ridley sea turtle (*Lepidochelys olivacea*), Loggerhead sea turtle (*Caretta caretta*), and the Leatherback sea turtle (*Dermochelys coriacea*) have been designated as endangered (U.S. EPA, 1994; U.S. Fish and Wildlife Service, 1994).

3.4 ESTUARINE/MARINE TARGET SPECIES

EPA recommends that states collect either one shellfish species (preferably a bivalve mollusc) and one finfish species **or** two finfish species at each estuarine/marine screening site. In all cases, the primary criterion for selecting the target species is that it is commonly consumed. Ideally, one shellfish species and one finfish species should be sampled; however, if no shellfish species from the recommended target species list meets the primary criterion, EPA recommends that states use two finfish species selected from the appropriate regional estuarine/marine target species lists. If two finfish are selected as the target species, one should be a bottom-feeding species.

EPA recommends that, whenever practical, states use target species selected from fish and shellfish species identified in Tables 3-10 through 3-16 for the following specific estuarine/marine coastal areas:

- Northeast Atlantic region (Maine through Connecticut)—Table 3-10
- Mid-Atlantic region (New York through Virginia)—Table 3-11
- Southeast Atlantic region (North Carolina through Florida)—Table 3-12
- Gulf Coast region (west coast of Florida through Texas)—Table 3-13
- Pacific Northwest region (Alaska through Oregon)—Table 3-14
- Northern California waters (Klamath River through Morro Bay)—Table 3-15
- Southern California waters (Santa Monica Bay to Tijuana Estuary)—Table 3-16.

Table 3-10. Recommended Target Species for Northeast Atlantic Estuaries and Marine Waters (Maine through Connecticut)

Family name	Common name	Scientific name
Finfish Species		
Anguillidae	American eel	*Anguilla rostrata*
Percichthyidae	Striped bass	*Morone saxatilis*
Pomatomidae	Bluefish	*Pomatomus saltatrix*
Sparidae	Scup	*Stenotomus chrysops*
Sciaenidae	Weakfish	*Cynoscion regalis*
Bothidae	Summer flounder	*Paralichthys dentatus*
	Four-spotted flounder	*Paralichthys oblongus*
Pleuronectidae	Winter flounder	Pseudopleuronectes americanus
	Yellowtail flounder	*Limanda ferruginea*
	American dab	*Hippoglossoides platessoides*
Shellfish Species		
Bivalves	*Soft-shell clam*	*Mya arenaria* *Mercenaria mercenaria* *Arctica islandica* *Spisula solidissima* *Mytilus edulis*
Crustaceans	*American lobster* *Eastern rock crab*	*Homarus americanus* *Cancer irroratus*

Table 3-11. Recommended Target Species for Mid-Atlantic Estuaries and Marine Waters (New York through Virginia)

Family name	Common name	Scientific name
Finfish Species		
Anguillidae	American eel	*Anguilla rostrata*
Ictaluridae	Channel catfish	*Ictalurus punctatus*
	White catfish	*Ictalurus catus*
Percichthyidae	White perch	*Morone americana*
	Striped bass	*Morone saxatilis*
Pomatomidae	Bluefish	*Pomatomus saltatrix*
Sparidae	Scup	*Stenotomus chrysops*
Sciaenidae	Weakfish	*Cynoscion regalis*
	Spot	*Leistomus xanthurus*
	Atlantic croaker	*Micropogonias undulatus*
	Red drum	*Sciaenops ocellatus*
Bothidae	Summer flounder	*Paralichthys dentatus*
Pleuronectidae	Winter flounder	*Pseudopleuronectes americanus*
Shellfish Species		
Bivalves	Hard clam	*Mercenaria mercenaria*
	Soft-shell clam	*Mya arenaria*
	Ocean quahog	*Arctica islandica*
	Surf clam	*Spisula solidissima*
	Blue mussel	*Mytilus edulis*
	American oyster	*Crassostrea virginica*
Crustaceans	Blue crab	*Callinectes sapidus*
	American lobster	*Homarus americanus*
	Eastern rock crab	*Cancer irroratus*

Table 3-12. Recommended Target Species for Southeast Atlantic Estuaries and Marine Waters (North Carolina through Florida)

Family name	Common name	Scientific name
Finfish Species		
Anguillidae	American eel	*Anguilla rostrata*
Ictaluridae	Channel catfish White catfish	*Ictalurus punctatus* *Ictalurus catus*
Percichthyidae	White perch Striped bass	*Morone americana* *Morone saxatilis*
Sciaenidae	Spot	*Leistomus xanthurus*
	Atlantic croaker	*Micropogonias undulatus*
	Red drum	*Sciaenops ocellatus*
Bothidae	Southern flounder	*Paralichthys lethostigma*
	Summer flounder	*Paralichthys dentatus*
Shellfish Species		
Bivalves	Hard clam	*Mercenaria mercenaria*
	American oyster	*Crassostrea virginica*
Crustaceans	West Indies spiny lobster	*Panulirus argus*
	Blue crab	*Callinectes sapidus*

Table 3-13. Recommended Target Species for Gulf of Mexico Estuaries and Marine Waters (West Coast of Florida through Texas)

Family name	Common name	Scientific name
Finfish Species		
Ictaluridae	Blue catfish	*Ictalurus furcatus*
	Channel catfish	*Ictalurus punctatus*
Ariidae	Hardhead catfish	*Arius felis*
Sciaenidae	Spotted seatrout	*Cynoscion nebulosus*
	Spot	*Leistomus xanthurus*
	Atlantic croaker	*Micropogonias undulatus*
	Red drum	*Sciaenops ocellatus*
Bothidae	Gulf flounder	*Paralichthys albigutta*
	Southern flounder	*Paralichthys lethostigma*
Shellfish Species		
Bivalves	American oyster	*Crassostrea virginica*
	Hard clam	*Mercenaria mercenaria*
Crustaceans	White shrimp	*Penaeus setiferus*
	Blue crab	*Callinectes sapidus*
	Gulf stone crab	*Menippe adina*
	West Indian spiny lobster	*Panulirus argus*

Table 3-14. Recommended Target Species for Pacific Northwest Estuaries and Marine Waters (Alaska through Oregon)

Family name	Common name	Scientific name
Finfish Species		
Embiotocidae	Redtail Surfperch	*Amphistichus rhodoterus*
Scorpaenidae	Copper rockfish	*Sebastes caurinus*
	Black rockfish	*Sebastes melanops*
Bothidae	Speckled sanddab	*Citharichthys stigmaeus*
	Pacific sanddab	*Citharichthys sordidus*
Pleuronectidae	Starry flounder	*Platichthys stellatus*
	English sole	*Parophrys vetulus*
Salmonidae	Coho salmon	*Onchorhynchus kisutch*
	Chinook salmon	*Onchorhynchus tshawytscha*
Shellfish Species		
Bivalves	Blue mussel	*Mytilus edulis*
	California mussel	*Mytilus californianus*
	Pacific oyster	*Crassostrea gigas*
	Horseneck clam	*Tresus capax*
	Pacific littleneck clam	*Protothaca staminea*
	Soft-shell clam	*Mya arenaria*
	Manila clam	*Venerupis japonica*
Crustaceans	Dungeness crab	*Cancer magister*
	Red crab	*Cancer productus*

Table 3-15. Recommended Target Species for Northern California Estuaries and Marine Waters (Klamath River through Morro Bay)

Family name	Common name	Scientific name
Finfish Species		
Triakidae	Leopard shark	*Triakis semifasciata*
Sciaenidae	White croaker	*Genyonemus lineatus*
Embiotocidae	Redtailed surfperch	*Amphistichus rhodoterus*
	Striped seaperch	*Embiotoca lateralis*
Scorpaenidae	Black rockfish	*Sebastes melanops*
	Yellowtail rockfish	*Sebastes flavidus*
	Bocaccio	*Sebastes paucispinis*
Bothidae	Pacific sanddab	*Citharichthys sordidus*
	Speckled sanddab	*Citharichthys stigmaeus*
Pleuronectidae	Starry flounder	*Platichthys stellatus*
	English sole	*Parophrys vetulus*
Salmonidae	Coho salmon	*Onchorhynchus kisutch*
	Chinook salmon	*Onchorhynchus tshawytscha*
Shellfish Species		
Bivalves	Blue mussel	*Mytilus edulis*
	California mussel	*Mytilus californianus*
	Pacific littleneck clam	*Protothaca staminea*
	Soft-shell clam	*Mya arenaria*
Crustaceans	Dungeness crab	*Cancer magister*
	Red crab	*Cancer productus*
	Pacific rock crab	*Cancer antennarius*

Table 3-16. Recommended Target Species for Southern California Estuaries and Marine Waters (Santa Monica Bay to Tijuana Estuary)

Family name	Common name	Scientific name
Finfish Species		
Serranidae	Kelp bass	*Paralabrax clathratus*
	Barred sand bass	*Paralabrax nebulifer*
Sciaenidae	White croaker	*Genyonemus lineatus*
	Corbina	*Menticirrhus undulatus*
Embiotocidae	Black perch	*Embiotoca jacksoni*
	Walleye surf perch	*Hyperprosopan argenteum*
	Barred surfperch	*Amphistichus argenteus*
Scorpaenidae	California scorpionfish	*Scorpaena guttata*
	Widow rockfish	*Sebastes entomelas*
	Blue rockfish	*Sebastes mystinus*
	Bocaccio	*Sebastes paucispinis*
Pleuronectidae	Diamond turbot	*Hypsopetta guttulata*
	Dover sole	*Microstomus pacificus*
Shellfish Species		
Bivalves	Blue mussel	Mytilus edulis
	California mussel	*Mytilus californianus*
	Pacific littleneck clam	*Protothaca staminea*
Crustaceans	Pacific rock crab	*Cancer antennarius*
	Red crab	*Cancer productus*
	California rock lobster	*Panulirus interruptus*

The seven separate regional lists of target species recommended by the 1993 EPA Workgroup for estuarine/marine ecosystems were developed because of differences in species' geographic distribution and abundance and the nature of the regional fisheries and were developed based on a review of species used in the following national monitoring programs:

- National Dioxin Study (U.S. EPA)
- Section 301(h) Monitoring Program (U.S. EPA)
- National Status and Trends Program (NOAA)
- National Study of Chemical Residues in Fish (U.S. EPA).

Because some of these programs identified some fish and shellfish species that are not of commercial, sportfishing, or subsistence value, several additional literature sources identifying commercial and sportfishing species were also reviewed (Table 3-17). Some sources included information on seasonal distribution and abundance of various life stages (i.e., adults, spawning adults, juveniles) of fish and shellfish species. This information was useful in delineating seven regional estuarine/marine areas nationwide. The 1993 EPA Workgroup also reviewed fish and shellfish species cited in state consumption advisories for estuarine/marine waters (Appendix D). Each of the final regional lists of target species has been reviewed by state, regional, and national fisheries experts.

Use of two distinct ecological groups of organisms (shellfish and finfish) as target species in estuarine/marine systems is recommended. This permits monitoring of a wide variety of habitats, feeding strategies, and physiological factors that might result in differences in bioaccumulation of contaminants. Estuarine/marine species used in several national contaminant monitoring programs reviewed by the 1993 EPA Workgroup are compared in Table 3-18.

3.4.1 Target Shellfish Species

Selection of shellfish species (particularly bivalve molluscs) as target species received primary consideration by the 1993 EPA Workgroup because of the commercial, recreational, and subsistence value of shellfish in many coastal areas of the United States. Bivalve molluscs (e.g., oysters, mussels, and clams) are filter feeders that accumulate contaminants directly from the water column or via ingestion of contaminants adsorbed to phytoplankton, detritus, and sediment particles. Bivalves are good bioaccumulators of heavy metals (Cunningham, 1979) and polycyclic aromatic hydrocarbons (PAHs) and other organic compounds (Phillips, 1980; NOAA, 1987) and, because they are sessile, they may reflect local contaminant concentrations more accurately than more mobile crustacean or finfish species.

Three bivalve species—the blue mussel (*Mytilus edulis*), the California mussel (*Mytilus californianus*), and the American oyster (*Crassostrea virginica*)—were recommended and/or used in three of the national monitoring programs reviewed by the 1993 EPA Workgroup. Two other bivalve species—the soft-shell clam (*Mya arenaria*) and the Pacific oyster (*Crassostrea gigas*)—were also recommended and/or used in two national programs. Although no bivalve species was identified by name in state fish and shellfish consumption advisories (Appendix D), seven coastal states issued advisories in 1993 for unspecified bivalves or shellfish species that may have included these and other bivalve species. All three species are known to bioaccumulate a variety of environmental contaminants (Phillips, 1988). The wide distribution of these three species makes them useful for comparison within a state or between states sharing coastal waters (Figure 3-2). Because these three species met all of the selection criteria, they were recommended as target species for use in geographic areas in which they occur.

Table 3-17. Sources of Information on Commercial and Sportfishing Species in Various Coastal Areas of the United States

Geographic area	Source
Atlantic Coast	National Marine Fisheries Service. 1987. *Marine Recreational Fishery Statistics Survey, Atlantic and Gulf Coasts, 1986.* Current Fishery Statistics Number 8392. National Oceanic and Atmospheric Administration, U.S. Department of Commerce, Rockville, MD.
	Leonard, D.L., M.A. Broutman, and K. Harkness. 1989. *The Quality of Shellfish Growing Waters on the East Coast of the United States.* Strategic Assessment Branch, National Oceanic and Atmospheric Administration, U.S. Department of Commerce, Rockville, MD.
	Nelson, D.M., M.E. Monaco, E.A. Irlandi, L.R. Settle, and L. Coston-Clements. 1991. *Distribution and Abundance of Fishes and Invertebrates in Southeast Estuaries.* ELMR Report No. 9. Strategic Assessment Division. National Oceanic and Atmospheric Administration, U.S. Department of Commerce, Rockville, MD.
	Stone, S.L., T.A. Lowery, J.D. Field, C.D. Williams, D.M. Nelson, S.H. Jury, M.E. Monaco, and L. Andreasen. 1994. *Distribution and Abundance of Fishes and Invertebrates in Mid-Atlantic Estuaries.* ELMR Rep. No. 12. NOAA/NOS Strategic Environmental Assessments Division, Silver Spring, MD.
	Jury, S.H., J.D. Field, S.L. Stone, D.M. Nelson, and M.E. Monaco. 1994. *Distribution and Abundance of Fishes and Invertebrates in North Atlantic Estuaries.* ELMR Rep. No. 13. NOAA/NOS Strategic Environmental Assessments Division, Silver Spring, MD.
Gulf Coast	National Marine Fisheries Service. 1987. *Marine Recreational Fishery Statistics Survey, Atlantic and Gulf Coasts, 1986.* Current Fishery Statistics Number 8392. National Oceanic and Atmospheric Administration, U.S. Department of Commerce, Rockville, MD.
	Broutman, M.A., and D.L. Leonard. 1988. *The Quality of Shellfish Growing Waters in the Gulf of Mexico.* Strategic Assessment Branch, National Oceanic and Atmospheric Administration, Rockville, MD.
	Monaco, M.E., D.M. Nelson, T.C. Czapla, and M.E. Patillo. 1989. *Distribution and Abundance of Fishes and Invertebrates in Texas Estuaries.* ELMR Report No. 3. Strategic Assessment Branch, National Oceanic and Atmospheric Administration, U.S. Department of Commerce, Rockville, MD.
	Williams, C.D., D.M. Nelson, M.E. Monaco, S.L. Stone, C. Iancu, L. Coston-Clements, L.R. Settle, and E.A. Irlandi. 1990. *Distribution and Abundance of Fishes and Invertebrates in Eastern Gulf of Mexico Estuaries.* ELMR Report No. 6. Strategic Assessment Branch, National Oceanic and Atmospheric Administration, U.S. Department of Commerce, Rockville, MD.
	Czapla, T.C., M.E. Patillo, D.M. Nelson, and M.E. Monaco. 1991. *Distribution and Abundance of Fishes and Invertebrates in Central Gulf of Mexico Estuaries.* ELMR Report No. 7. Strategic Assessment Branch, National Oceanic and Atmospheric Administration, U.S. Department of Commerce, Rockville, MD.
	Nelson, D.M. (editor). 1992. *Distribution and Abundance of Fishes and Invertebrates in Gulf of Mexico Estuaries,* Volume I: Data Summaries. ELMR Rep. No. 10. NOAA/NOS Strategic Environmental Assessments Division, Rockville, MD.
	Patillo, M.E., T.E. Czapla, D.M. Nelson, and M.E. Monaco. 1997. *Distribution and Abundance of Fishes and Invertebrates in Gulf of Mexico Estuaries, Vol. II: Species Life History Summaries.* ELMR Rep. No. 14. NOAA/NOS Strategic Environmental Assessments Division, Silver Spring, MD.
West Coast	National Marine Fisheries Service. 1987. *Marine Recreational Fishery Statistics Survey, Pacific Coast, 1986.* Current Fishery Statistics Number 8393. National Oceanic and Atmospheric Administration, U.S. Department of Commerce, Rockville, MD.
	Leonard, D.L., and E.A. Slaughter. 1990. *The Quality of Shellfish Growing Waters on the West Coast of the United States.* Strategic Assessment Branch, National Oceanic and Atmospheric Administration, U.S. Department of Commerce, Rockville, MD.
	Monaco, M.E., D.M. Nelson, R.L. Emmett, and S.A. Hinton. 1990. *Distribution and Abundance of Fishes and Invertebrates in West Coast Estuaries.* Volume I: Data Summaries. ELMR Report No. 4. Strategic Assessment Branch, National Oceanic and Atmospheric Administration, Rockville, MD.
	Emmett, R.L., S.A. Hinton, S.L. Stone, and M.E. Monaco. 1991. *Distribution and Abundance of Fishes and Invertebrates in West Coast Estuaries.* Volume II: Life History Summaries. ELMR Report No. 8. Strategic Environmental Assessment Division, Rockville, MD.

Table 3-18. Estuarine/Marine Species Used in Several National Fish and Shellfish Contaminant Monitoring Programs

	U.S. EPA National Dioxin Study[a]	NOAA Status and Trends	U.S. EPA 301(h) Program	U.S. EPA NSCRF[b]
FINFISH				
Family *Acipenseridae*				
White sturgeon *(Acipenser transmontanus)*				●
Family *Ariidae*				
Hardhead catfish *(Arius felis)*				●
Family *Percichthyidae*				
White perch *(Morone americana)*				●
Family *Pomatomidae*				
Bluefish *(Pomatomus saltatrix)*				●
Family *Lutjanidae*				
Red snapper *(Lutjanus campechanus)*				●
Family *Sparidae*				
Sheepshead *(Archosargus probatocephalus)*				●
Family *(Sciaenidae)*				
Spotted seatrout *(Cynoscion nebulosus)*				●
Weakfish *(Cynoscion regalis)*				●
Spot *(Leiostomus xanthurus)*		●	●	●
White croaker *(Genyonemus lineatus)*		●		
Atlantic croaker (*Micropogonias undulatus*)		●		●
Black drum *(Pogonias cromis)*				●
Red drum *(Sciaenops ocellatus)*				●
Family *Serranidae*				
Barred sand bass *(Paralabrax nebulifer)*		●		
Family *Mugilidae*				
Striped mullet *(Mugil cephalus)*				●
Family *Bothidae*				
Southern flounder *(Paralichthys lethostigma)*				●
Windowpane flounder *(Scophthalmus aquosus)*		●		
Family *Pleuronectidae*				
Pacific sanddab *(Citharichthys sordidus)*			●	
Flathead sole *(Hippoglossoides elassodon)*				●
Diamond turbot *(Hypsopsetta guttulata)*		●		
Starry flounder *(Platichthys stellatus)*		●		●
Hornyhead turbot *(Pleuronichthys verticalis)*		●		
Winter flounder *(Pseudopleuronectes americanus)*		●	●	●
English sole *(Parophrys vetulus)*		●	●	
Dover sole *(Microstomus pacificus)*		●	●	

See notes at end of table. *(continued)*

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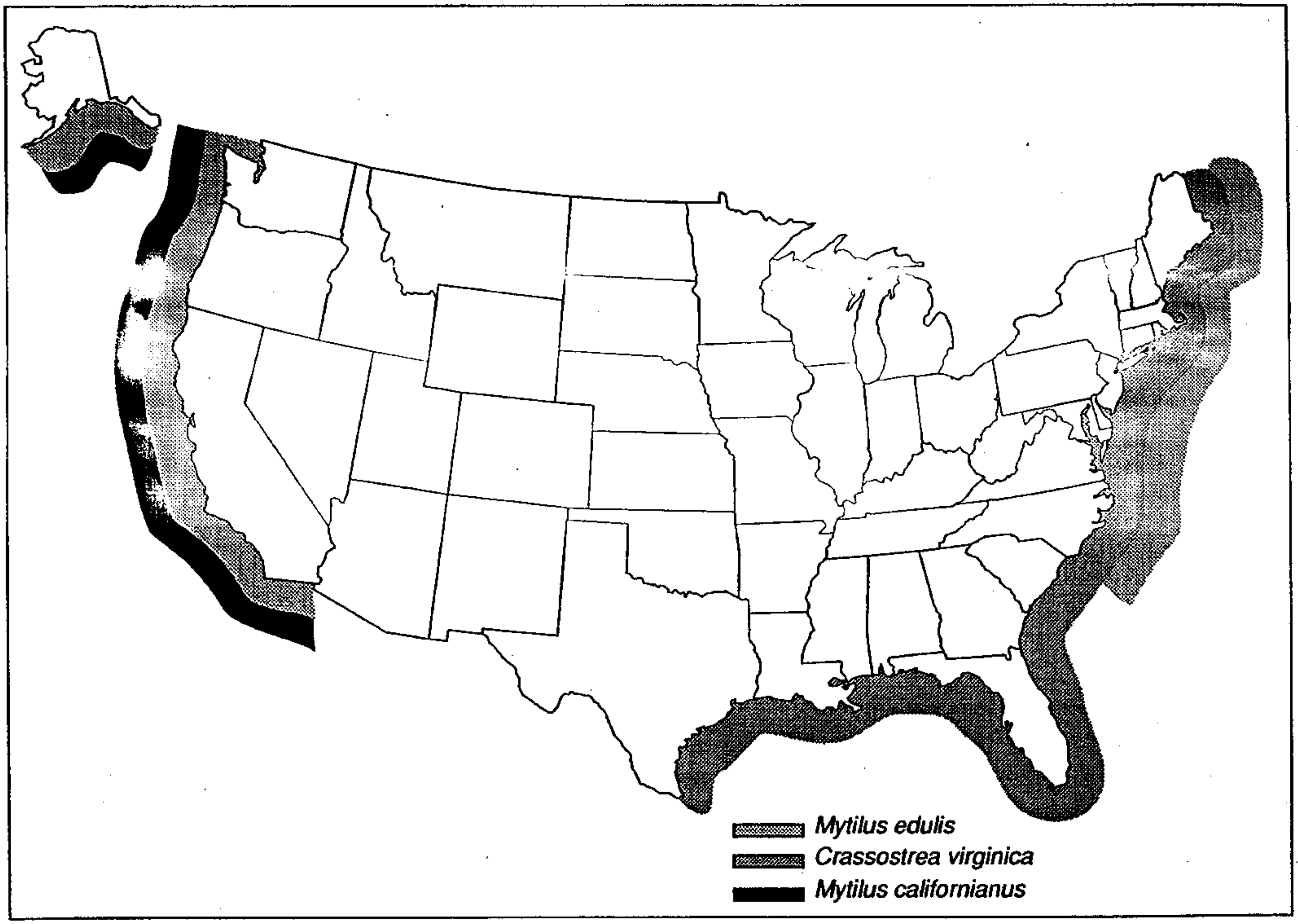
Table 3-18. (continued)

	U.S. EPA National Dioxin Study[a]	NOAA Status and Trends	U.S. EPA 301(h) Program	U.S. EPA NSCRF[b]
SHELLFISH				
Bivalves				
Hard clam *(Mercenaria mercanaria)*			●	
Soft-shell clam *(Mya arenaria)*			●	●
Ocean quahog *(Arctica islandia)*			●	
Surf clam *(Spisula solidissima)*			●	
Blue mussel *(Mytilus edulis)*	●	●	●	
California mussel *(Mytilus californianus)*	●	●	●	
American oyster *(Crassostrea virginica)*	●	●	●	
Hawaiian oyster *(Ostrea sandwichensis)*		●		
Pacific oyster *(Crassostrea gigas)*			●	●
Bent-nosed macoma *(Macoma nasuta)*			●	
Baltic macoma *(Macoma baltica)*			●	
White sand macoma *(Macoma secta)*			●	
Crustaceans				
American lobster *(Homarus americanus)*			●	
West Indies spiny lobster *(Panulirus argus)*			●	
California rock lobster *(Panulirus interruptus)*			●	
Hawaiian spiny lobster *(Panulirus penicillatus)*			●	
Eastern rock crab *(Cancer irroratus)*			●	
Dungeness crab *(Cancer magister)*			●	●
Pacific rock crab *(Cancer antennarius)*			●	
Yellow crab *(Cancer anthonyi)*			●	
Red crab *(Cancer productus)*			●	

NSCRF = National Study of Chemical Residues in Fish.

[a] Only freshwater finfish were identified as target species; bivalves were identified as estuarine/marine target species.

[b] Species listed were those collected at more than one site nationally; *Salmonidae* were not listed because they were included on freshwater lists.



Source: Abbott, 1974.

Figure 3-2. Geographic distributions of three bivalve species used extensively in national contaminant monitoring programs.

In addition, several species of edible clams were added to the various estuarine/marine target species lists based on recommendations received from specific state and regional fisheries experts.

Crustaceans are also recommended as target species for estuarine/marine sampling sites. Many crustaceans are bottom-dwelling and bottom-feeding predator and/or scavenger species that are good indicators of contaminants that may be biomagnified through several trophic levels of the food web. Several species of lobsters and crabs were recommended in one national monitoring program, and the Dungeness crab was recommended in two national monitoring programs (Table 3-18). These crustaceans, although of fishery value in many areas, are not as widely distributed nationally as the three bivalve species (Figure 3-2). However, they should be considered for selection as target species in states where they are commonly consumed.

Only two crustaceans—the American lobster (*Homarus americanus*) and the blue crab (*Callinectes sapidus*)—were specifically identified in state advisories (RTI, 1993). However, in 1993, seven coastal states reported advisories in estuarine/marine waters for unspecified shellfish species that may have included these and other crustacean species (Table 3-19). All of the shellfish species cited in state advisories are included as EPA-recommended target species on the appropriate estuarine/marine regional lists.

3.4.2 Target Finfish Species

Two problems were encountered in the selection of target finfish species for monitoring fish tissue contamination at estuarine/marine sites regionally and nationally. First is the lack of finfish species common to both Atlantic and Gulf Coast waters as well as Pacific Coast waters. Species used in several federal fish contaminant monitoring programs are compared in Table 3-18. Members of the families *Sciaenidae* (seven species), *Bothidae* (two species), and *Pleuronectidae* (eight species) were used extensively in these programs. Bottom-dwelling finfish species (e.g., flounders in the families *Bothidae* and *Pleuronectidae*) may accumulate high concentrations of contaminants from direct physical contact with contaminated bottom sediments. In addition, these finfish feed on sedentary infaunal or epifaunal organisms and are at additional risk of accumulating contaminants via ingestion of these contaminated prey species (U.S. EPA, 1987a). For finfish species, two Atlantic coast species, spot (*Leiostomus xanthurus*) and winter flounder (*Pseudopleuronectes americanus*), are recommended and/or used in three of the national monitoring programs, and the Atlantic croaker (*Micropogonias undulatus*) is recommended and/or used in two national monitoring programs. Three Pacific coast species, Starry flounder (*Platichthys stellatus*), English sole (*Parophrys vetulus*), and Dover sole (*Microstomus pacificus*), are recommended or used in two of the national monitoring programs.

Table 3-19. Principal Estuarine/Marine Fish and Shellfish Species Cited in State Consumption Advisories[a,b]

Species group name	Common name	Scientific name	Number of states with advisories in 1993	Number of states with advisories in 1998
Finfish				
Percichthyidae	**Striped bass**	***Morone saxatilis***	**5**	**6**
	White perch	*Morone americana*	3	3
Centrarchidae	Largemouth bass	*Micropterus salmoides*	0	3
	Smallmouth bass	*Micropterus dolomieui*	0	1
Ictaluridae	White catfish	*Ictalurus catus*	4	2
	Channel catfish	*Ictalurus punctatus*	5	2
Anguillidae	**American eel**	***Anguilla rostrata***	**6**	**5**
Elopidae	Ladyfish	*Elops saurus*	0	1
Carangidae	Crevalle jack	*Caranx hippos*	0	1
Pomatomidae	**Bluefish**	***Pomatomus saltatrix***	**4**	**6**
Labridae	Tautog	*Tautoga onitis*	0	1
Sparidae	**Scup**	***Stenotomus chrysops***	**0**	**1**
Sciaenidae	**Spotted sea trout**	***Cynoscion nebulosus***	**0**	**2**
	A[illegible]ker	***Micropogonias undulatus***	**0**	**1**
	Red drum	***Sciaenops ocellatus***	**0**	**1**
	Black drum	***Pogonias cromis***	**0**	**1**
	Silver perch	*Bairdiella chrysoura*	0	1
Scombridae	King mackerel	*Scomberomorus cavalla*	0	5
	Spanish mackerel	*Scomberomorus maculatus*	0	1
Ariidae	Gafftopsail catfish	*Bagre marinus*	0	1
Belonidae	Atlantic needlefish	*Strongylura marina*	1	1
Serranidae	**Kelp bass**	***Paralabrax clathratus***	**1**	**1**
Sciaenidae	Black cro[illegible]r	*Cheilotrema saturnum*	1	1
	Whit[illegible]r	***Genyonemus lineatus***	**1**	**1**
	Que[illegible]	***Seriphus politus***	**1**	**1**
	Co[illegible]	***Menticirrhus undulatus***	**1**	**1**
Shellfish				
Crustaceans[c]	**American lobster**	***Homarus americanus***	**1**	**5**
	B[illegible]e	***Callinectes sapidus***	**3**	**4**

[a] Species in boldface are EPA-recommended target species for regional estuarine/marine waters (see Tables 3-10 through 3-16).

[b] Many coastal states issued advisories for fish and shellfish species and thus did not identify specific finfish and shellfish species in their advisories.

[c] Eight coastal states (California, Georgia, Hawaii, Louisiana, Massachusetts, North Carolina, Texas, and Washington) and the U.S. territory [illegible]can Samoa report advisories for unspecified shellfish or bivalve species.

Sources: RTI, 1993, EPA 1999a (N[illegible]).

Second, because some estuarine/marine finfish species are highly migratory, harvesting of these species may be restricted to certain seasons because sexually mature adult fish (i.e., the recommended size for sampling) may enter the estuaries only to spawn. EPA recommends that neither spawning populations nor undersized juvenile stages be sampled in fish contaminant monitoring programs. Sampling of target finfish species during their spawning period should be avoided as contaminant tissue concentrations may decrease during this time (Phillips, 1980) and because the spawning period is generally outside the legal harvest period. **Note:** Target finfish species may be sampled during their spawning period if the species can be legally harvested at this time. Sampling of undersized juveniles of species that use estuaries as nursery areas is precluded by EPA's recommended monitoring strategy because juveniles may not have had sufficient time to bioaccumulate contaminants or attain harvestable size.

Because of these problems, the 1993 EPA Workgroup consulted with regional and state fisheries experts and reviewed the list of state fish consumption advisories and bans to determine which estuarine/marine finfish species should be recommended as target species. As shown in Table 3-19, the largest number of states issuing advisories in 1993 for specific estuarine and marine waters did so for the American eel (6), channel catfish (5), striped bass (5), bluefish (4), white catfish (4), and white perch (3). Several other estuarine/marine species were cited in advisories for one state each (Table 3-19). Many coastal states did not identify individual finfish species by name in their advisories (see Appendix D); however, almost all of the species that have been cited in state advisories are recommended as target species by EPA (see Tables 3-10 through 3-16). The listing of estuarine fish and shellfish cited in state advisories in 1998 is also shown in Table 3-19.

These seven regional lists of recommended estuarine/marine target species are provided to give guidance to states on species commonly consumed by the general population. state personnel, with their knowledge of site-specific fisheries and human consumption patterns, must be the ultimate judge of the species selected for use in estuarine/marine fish contaminant monitoring programs within their jurisdiction.

SECTION 4

TARGET ANALYTES

The selection of appropriate target analytes in fish and shellfish contaminant monitoring programs is essential to the adequate protection of the health of fish and shellfish consumers. The procedures used for selecting target analytes for screening studies and a list of recommended target analytes are presented in this section.

4.1 RECOMMENDED TARGET ANALYTES

Recommended target analytes for screening studies in fish and shellfish contaminant monitoring programs are listed in Table 4-1. This list was developed by the EPA 1993 Fish Contaminant Workgroup from a review of the following information:

1. **Pollutants analyzed in several national or regional fish contaminant monitoring programs**—The monitoring programs reviewed included

   - National Study of Chemical Residues in Fish (U.S. EPA)
   - National Dioxin Study (U.S. EPA)
   - 301(h) Monitoring Program (U.S. EPA)
   - National Pollutant Discharge Elimination System (U.S. EPA)
   - National Pesticide Monitoring Program (U.S. FWS)
   - National Contaminant Biomonitoring Program (U.S. FWS)
   - National Status and Trends Program (NOAA)
   - Great Lakes Sportfish Consumption Advisory Program
   - National Water Quality Assessment Program (USGS).

   Criteria for selection of the target analytes in these programs varied widely depending on specific program objectives. The target analytes used in these major fish contaminant monitoring programs are compared in Appendix E. Over 200 potential contaminants are listed, including metals, pesticides, base/neutral organic compounds, dioxins, dibenzofurans, acidic organic compounds, and volatile organic compounds.

Table 4-1. Recommended Target Analytes

Metals	**Organophosphate Pesticides**
Arsenic (inorganic)	Chlorpyrifos
Cadmium	Diazinon
Mercury (methylmercury)	Disulfoton
Selenium	Ethion
Tributyltin	Terbufos
Organochlorine Pesticides	**Chlorophenoxy Herbicides**
Chlordane, total (cis- and trans-chlordane, cis- and trans-nonachlor, oxychlordane)	Oxyfluorfen
DDT, total (2,4'-DDD, 4,4'-DDD, 2,4'-DDE, 4,4'-DDE, 2,4'-DDT, 4,4'-DDT)	**PAHs**[d]
Dicofol	**PCBs**
Dieldrin	
Endosulfan (I and II)	Total PCBs[e] (sum of PCB cogeners or Aroclor equivalents)
Endrin	
Heptachlor epoxide[a]	
Hexachlorobenzene	**Dioxins/furans**[f,g]
Lindane (γ-hexachlorocyclohexane; γ-HCH)[b]	
Mirex[c]	
Toxaphene	

PAHs = Polycyclic aromatic hydrocarbons; PCBs = Polychlorinated biphenyls; DDT = p,p'-dichlorodiphenyl trichloroethane; DDE = p,p'-dichlorodiphenyl dichloroethylene; and DDD = dichlorodiphenyldichloro ethane.

[a] Heptachlor epoxide is not a pesticide but is a metabolite of two pesticides, heptachlor and chlordane.
[b] Also known as γ-benzene hexachloride (γ-BHC).
[c] Mirex should be regarded primarily as a regional target analyte in the Southeast and Great Lakes states, unless historic tissue, sediment, or discharge data indicate the likelihood of its presence in other areas.
[d] It is recommended that tissue samples be analyzed for benzo[*a*]pyrene, and 14 other PAHs and that the order-of-magnitude relative potencies given for these PAHs be used to calculate a potency equivalency concentration (PEC) for each sample for comparison with the recommended SVs for benzo[*a*]pyrene (see Section 5.3.2.5).
[e] Analysis of total PCBs (as the sum of Aroclors or PCB congeners is recommended for conducting human health risk assessments for total PCBs (see Sections 4.3.6 and 5.3.2.6). A standard method for Aroclor analysis is available (EPA Method 608). A standard method for congener analysis (EPA Method 1668) is currently under development; however, it has not been finalized. States that currently do congener-specific PCB analysis should continue to do so and other states are encouraged to develop the capability to conduct PCB congener analysis. When standard methods for congener analysis are verified and peer reviewed, the Office of Water will evaluate the use of these methods.
[f] Note: The EPA Office of Research and Development is currently reassessing the human health effects of dioxins/furans.
[g] It is recommended that the 17 2,3,7,8-substituted tetra- through octa-chlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) and 12 dioxin-like PCBs be determined and a toxicity-weighted total concentration calculated for each sample (Van den Berg et al., 1998) (see Sections 4.3.7, 5.3.2.6, and 5.3.2.7).

2. **Pesticides with active registrations**—The EPA Office of Pesticide Programs (OPP) Fate One Liners Database (U.S. EPA, 1993a) containing information for more than 900 registered pesticides was reviewed to identify pesticides and herbicides with active registrations that met four criteria. The screening criteria used were

   - Oral toxicity, Class I or II
   - Bioconcentration factor greater than 300
   - Half-life value of 30 days or more
   - Initial use application profile.

At the time of this review, complete environmental fate information was available for only about half of the registered pesticides. As more data become available, additional pesticides will be evaluated for possible inclusion on the target analyte list.

Use of the OPP database was necessary because many pesticides and herbicides with active registrations have not been monitored extensively either in national or state fish contaminant monitoring programs.

3. **Contaminants that have triggered states to issue fish and shellfish consumption advisories or bans**—The database, *National Listing of State Fish and Shellfish Consumption Advisories and Bans* (RTI, 1993), was reviewed to identify specific chemical contaminants that have triggered issuance of consumption advisories by the states. As shown in Table 4-2, four contaminants (PCBs, mercury, chlordane, and dioxins/furans) triggered advisories in the largest number of states in 1993. As a comparison, the number of states issuing advisories for each pollutant in 1998 has also been presented while the total number of states issuing advisories for most pollutants generally has increased, the number of states issuing advisories for two major pollutants, chlordane and dioxin, has decreased over the past 5 years.

4. **Published literature on the chemistry and health effects of potential contaminants**—The physical, chemical, and toxicologic factors considered to be of particular importance in developing the recommended target analyte list were

   - Oral toxicity
   - Potential of the analyte to bioaccumulate
   - Prevalence and persistence of the analyte in the environment
   - Biochemical fate of the analyte in fish and shellfish
   - Human health risk of exposure to the analyte via consumption of contaminated fish and shellfish
   - Analytical feasibility.

Final selection of contaminants by the EPA 1993 Workgroup for the recommended target analyte list (Table 4-1) was based on their frequency of inclusion in national monitoring programs, on the number of states issuing consumption advisories for them in 1993 (Table 4-2), and on their origins, chemistry, potential to bioaccumulate, estimated human health risk, and feasibility of analysis. Primary consideration was also given to the recommendations of the Committee on Evaluation of the Safety of Fishery Products, published in *Seafood Safety* (NAS, 1991).

4.2 SELECTION AND PRIORITIZATION OF TARGET ANALYTES

The decision to conduct a fish tissue monitoring study is normally the result of the discovery of specific contaminants during water quality or sediment studies and/or

Table 4-2. Contaminants Resulting in Fish and Shellfish Advisories

Contaminant	Number of states issuing advisories 1993	1998
Metals		
Arsenic (total)	1	3
Cadmium	2	3
Chromium	1	1
Copper	1	1
Lead	4	5
Mercury	29	40
Selenium	5	5
Tributyltin	1	0
Zinc	1	1
Organometallics	1	1
Unidentified metals	3	1
Pesticides		
Chlordane	24	22
DDT and metabolites	9	12
Dieldrin	3	6
Heptachlor epoxide	1	1
Hexachlorobenzene	2	2
Kepone	1	1
Mirex	3	3
Photomirex	1	0
Toxaphene	2	4
Unidentified pesticides	2	2
Polycyclic aromatic hydrocarbons (PAHs)	3	4
Polychlorinated biphenyls (PCBs)	32	36
Dioxins/furans	20	19
Other chlorinated organics		
Dichlorobenzene	1	1
Hexachlorobutadiene	1	1
Pentachlorobenzene	1	0
Pentachlorophenol	1	2
Tetrachlorobenzene	2	0
Tetrachloroethane	1	0
Others		
Creosote	2	2
Gasoline	1	1
Multiple pollutants	2	1
Phthalate esters	1	0
Polybrominated biphenyls (PBBs)	1	1
Unspecified pollutants	3	0

Sources: RTI, 1993; U.S. EPA, 1999c.

the identification of pollutant sources in waters routinely used by recreational or subsistence fishers. EPA recognizes that measuring all 25 target analytes in fish tissues collected at all state monitoring sites is expensive and that cost is an important consideration that states must evaluate in designing and implementing their fish monitoring programs. Ideally, if resources are available to conduct sampling and analysis of all 25 target analytes, the state should consider this option because it provides the greatest amount of information for fishers in the state on levels of contamination statewide. Also, this approach can better detect the presence of those contaminants that are transported long distances from their points of release (e.g., methylmercury, dioxins/furans, toxaphene), often outside the state's borders, and contaminate relatively pristine areas devoid of any obvious pollutant sources.

If the cost of this approach is prohibitive, however, the state may wish to use a watershed-based approach as a way to reduce sampling and analysis costs (Table 4-3). The selection and prioritization recommendations discussed below are watershed-based and take into consideration land use categories (rural, agricultural, suburban/urban, and industrial) as well as geological characteristics, regional differences, and national pollution trends. Land use patterns (both current and historic) are often the most important factors in deciding what analytes to select for analysis. The watershed-based approach gives the highest priority (XXX) to analysis of contaminants that are widely dispersed nationally and relatively inexpensive to analyze, such as mercury. This approach gives a lower priority (X) to monitoring organochlorine pesticides (e.g., chlordane, DDT, and dieldrin) at rural and suburban sites, but a higher priority (XX) to monitoring these same chemicals in agricultural watersheds where their use has been extensive or in industrial watersheds where they may have been released during manufacturing, formulation, packaging, or disposal. Because of the very high cost of analysis for some contaminants (e.g., PCBs and dioxins/furans and dioxin-like PCBs), this watershed approach also allows money for these analyses to be directed toward analysis primarily in suburban/urban and industrial watersheds where sources either from historic manufacturing or historic and/or current practices (combustion or incineration sources) have been identified or where water and/or sediment data in the watershed have detected these chemicals at elevated concentrations.

States should use all available environmental data and their best scientific judgment when developing their fish monitoring programs. Using the watershed approach gives states the flexibility to tailor their sampling and analysis programs to obtain needed information as cost-effectively as possible by directing limited resources to obtaining information on contaminant levels most likely to be found in fish tissue at a given site. To be most effective, states need to recognize and carefully evaluate all existing data when assessing which target analytes to monitor at a particular site. States should include any of the recommended EPA target analytes and any additional target analytes in their screening programs when site-specific information (e.g., tissue, water, or sediment data; discharge monitoring data from municipal and industrial sources; or pesticide use data) suggests that these contaminants may be present at levels of concern for human health.

Table 4-3. Selection and Prioritization of Target Analytes by Watershed Type

Analyte	Rural	Agricultural	Suburban/ Urban	Industrial	Sources/Uses
Metals					
Arsenic	XX[a]	X[a,b]	X[a,b]	XX[b]	Naturally occurring as a sulfide in mineral ores; fossil fuel combustion; mining/smelting; wood preservative; insecticide, herbicide, and algacide; hazardous waste site leachate
Cadmium	XX[a]	X[a,b]	X[a,b]	XX[b]	Smelting/mining; surface mine drainage; uses in paints, alloys, batteries, plastics, pesticides, herbicides; waste disposal operations.
Mercury	XXX[c]	XXX[c]	XXX[c]	XXX[c]	Naturally occurring; atmospheric transport from fossil fuel combustion; mining/smelting; chlorine alkali production; historic use in pulp and paper and paints; Hazardous waste site leachate; statewide freshwater and/or coastal advisories in 15 states
Selenium	XX[a]	X[a]	X[a]	XX[d]	Naturally occurring in west and southwest soils; emissions from fossil fuel combustion; leachate from coal fly ash disposal areas
Tributyltin			X[d]	XX[d]	Shipyards and marinas; uses in antifouling paint, cooling tower disinfectants, wood preservatives, pulp and paper industry, and textile mills.
Organochlorine Pesticides					
Chlordane		XX[b]	X[b]	XX[b]	Domestic termite control; pesticide manufacturing/ packaging/formulation sites
DDT		XX[b]	X[b]	XX[b]	Broad spectrum pesticide use; pesticide manufacturing/ packaging/formulation sites
Dicofol[e]		XX[b]		XX[b]	Miticide/pesticide for cotton, apples, and citrus primarily in FL and CA; lesser use in turf, ornamentals, pears, apricots, and cherries; pesticide manufacturing/ packaging/formulation sites
Dieldrin		XX[b]	X[b]	XX[b]	Broad spectrum pesticide for termites/soil insects and for cotton, corn, and citrus; pesticide manufacturing/ packaging/formulation sites
Endosulfan[e]		XX[b]		XX[b]	Noncontact insecticide for seed and soil treatments; pesticide manufacturing/packaging/formulation sites
Endrin		XX[b]		XX[b]	Broad spectrum pesticide; pesticide manufacturing/ packaging/formulation sites
Heptachlor epoxide		XX[b]	X[b]	XX[b]	Degradation product of heptachlor used as a contact and ingested soil insecticide for termites and household pesticide and chlordane also used as a termiticide; pesticide manufacturing/packaging/formulation sites for heptachlor and chlordane

Table 4-3. (continued)

Analyte	Rural	Agricultural	Suburban/ Urban	Industrial	Sources/Uses
Hexachlorobenzene		XX[b]		XX[b]	Fungicide used as seed protectant, used as chemical intermediate in production of many other organochlorine pesticides; pesticide manufacturing/packaging/formulation sites for a wide variety of organochlorine pesticides
Lindane[e]		XX[b]	X[b]	XX[b]	Seed and soil treatments for tobacco; foliage applications for fruit and nut trees and vegetables; wood preservative. pesticide manufacturing/packaging/formulation sites
Mirex		XX[b]	X[b]	XX[b]	Used extensively in Southeast and Gulf Coast states against fire ants; used in fire retardants and plastic polymerizer; pesticide manufacturing/packaging/ formulation sites
Toxaphene		XX[b]		XX[b]	Insecticide for cotton; piscicide for rough fish; pesticide manufacturing/packaging/formulation sites
Organophosphate Pesticides					
Chlorpyrifos[e]		XX[b]	X[b]	XX[b]	Widely used on cotton, peanuts, and sorghum as well as fruits and vegetables; domestic household insecticide with lawn and garden applications. Use applications will change by the end of 2001. All residential use will end as will use on tomatoes. Use on apples and grapes will be greatly reduced (U.S. EPA, 2000b). Used as a termiticide in California; pesticide manufacturing/packaging/ formulation sites
Diazinon[e]		XX[b]	X[b]	XX[b]	Widely used on a broad variety of fruits and vegetables, field crops, and pastureland; domestic household insecticide used for lawn and garden applications; pesticide manufacturing/packaging/formulation sites
Disulfoton[e]		XX[b]		XX[b]	Widely used as a side dressing, broadcast, and foliar spray and as a seed dressing; pesticide manufacturing/ packaging/formulation sites
Ethion[e]		XX[b]	X[b]	XX[b]	Major use on citrus, fruit and nut trees, and vegetables. Domestic outdoor use around homes and lawns; pesticide manufacturing/packaging/formulation sites
Terbufos[e]		XX[b]		XX[b]	Used principally on corn, sugar beets, and grain sorghum; pesticide manufacturing/packaging/formulation sites
Chlorophenoxy Herbicides					
Oxyfluorfen[e]		XX[b]		XX[b]	Widely used to control grass and weeds in corn, cotton, soybeans, fruit and nut trees, and ornamental crops; pesticide manufacturing/packaging/formulation sites

Table 4-3. (continued)

Analyte	Rural	Agricultural	Suburban/ Urban	Industrial	Sources/Uses
Polycyclic Aromatic Hydrocarbons (PAHS)			X[d]	X[d]	Components of crude and refined petroleum and coal products; waste incineration, wood preservatives; creosote; coal tar; coal coking; urban runoff from asphalt, automobile tires and exhaust emissions, and petroleum spills; coal gasification sites, and petroleum refineries
Polychlorinated Biphenyls (PCBs)			X[d]	X[d]	Produced as Arochlors for use as dielectric fluid in electrical transformers and as hydraulic fluid; leachate from land fills and Superfund sites
Dioxins and Dibenzofurans			X[d]	X[d]	Industrial sites including bleached kraft paper mills, facilities handling 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2,4,5-trichlorophenol (2,4,5-TCP), silvex, hexachlorobenzene, pentachlorophenol, and PCBs; Industrial and municipal combustors and incinerators

[a] Tissue residue analysis is recommended if geologic characteristics suggest potential for elevated metal concentrations in water or sediment or if sources are identified in the watershed suggesting the presence of this target analyte at the sampling site.
[b] Tissue residue analysis is recommended if use application of this pesticide has been reported in the watershed either from historic or current use data, if sources like pesticide production/packaging/formulation facilities exist in the watershed, or if the state has water and/or sediment data indicating the presence of this target analyte at the sampling site.
[c] Tissue residue analysis is highly recommended at all sites.
[d] Tissue residue analysis is recommended if sources as described in Sources/Uses column are identified in suburban/urban or industrial watershed or the state has water and/or sediment data indicating the presence of this analyte at the sampling site.
[e] Pesticide with currently active registration

X = Analysis for target analyte should be considered if water and or sediment analysis results detect the target analyte or if historic or current use information provide evidence for the potential presence of this target analyte in the watershed.
XX = Analysis for target analyte is recommended for this land use type if historic or current use information provides evidence of the potential presence of this target analyte in the watershed.
XXX = Analysis for target analyte is highly recommended at all stations in all watershed types.

Rural. The major analytes of concern in rural waterbodies (i.e., watersheds with no past or current urban/suburban, industrial, or agricultural uses) are the metals, including arsenic, cadmium, mercury, and selenium. Weathering processes in certain geologic areas can result in elevated levels of arsenic, cadmium, mercury, and selenium in water and sediments. State agencies should also be aware of past land use patterns in what are now considered rural areas of their states. For example, abandoned mining sites may be a source of metal contamination via leaching from mine drainage or slag piles. Large areas east of the Appalachians were agricultural watersheds during the early to mid twentieth century. While some of this agriculture land is now suburban/urban in its use, other areas, particularly in the South, are reverting to forests that might at first glance be classified as rural use. Arsenic compounds were used as pesticides in the early

1900s, and, along with organochlorine pesticides, may still be present in farmland abandoned after the 1940s. States should also be aware that mercury has been identified in fish collected from what would be classified as rural or pristine areas of the Great Lakes basins and waterbodies in the northeastern and southeastern states remote from any obvious point sources of pollution. Mercury contamination in these areas seems to be facilitated through the atmospheric transport of this metal. Because mercury is the target analyte that has triggered issuance of the largest number of advisories in the United States (nearly 68 percent of all advisories nationwide) and because of the relatively low cost of chemical analysis for this analyte, EPA recommends that this metal be monitored at all rural sites, especially those where little or no monitoring data are available.

Depending on site-specific conditions and considerations, states may opt to analyze for mercury as well as a suite of other heavy metals that can be analyzed as a group at relatively low cost. The only target analyte metal that should not be analyzed for routinely in rural areas without other supporting data is tributyltin, which is typically found near boatyards and marinas or near wood preservative production facilities. States may include any of the recommended EPA target analytes and any additional target analytes in their screening programs when site-specific information on a rural watershed suggests that these contaminants may be present at levels of concern for human health.

Agricultural. The major analytes of concern in agricultural waterbodies (i.e., watersheds where past or current land use is dominated by agriculture) are the organochlorine and organophosphate pesticides and the chlorophenoxy herbicide, oxyfluorfen. These analytes fall into two categories, those with inactive registrations (i.e. banned or withdrawn from the market) and those with active registrations (endosulfan, lindane, dicofol, chlorpyrifos, diazinon, terbufos, ethion, disulfoton, and oxyfluorfen). Although use of some of the organochlorine pesticides was terminated more than 20 years ago in the United States (e.g., DDT, dieldrin, endrin, and mirex) , these compounds still need to be monitored. Many of the organochlorine pesticides that are now banned were used in large quantities for over a decade and are still present in high concentrations at some sites. On a nationwide basis, chlordane and DDT, for example, are responsible for 3 and 1 percent, respectively, of the advisories currently in effect. For the pesticides with active registrations, use and rate application information maintained by the state's Department of Agriculture should be reviewed to identify watersheds where these pesticides are currently used and are likely to be present in aquatic systems as a result of agricultural runoff or drift. Unlike many of the historically used organochlorine pesticides, the pesticides in current use degrade relatively rapidly in the environment. In addition, federal regulations are in effect that set maximum application rates and minimize use near waterbodies. At the time of this writing, no fish consumption advisories for these analytes have yet been issued; however, state agencies should be aware of special circumstances that could result in accumulation in fish. In addition to accidental spills and misapplication, heavy and repeated rainfall shortly after application may wash these pesticides into streams. Signs of pesticide pollution may include erratic swimming behavior in fish as well as fish kills.

It is also important to note that pesticide uses and labels may change over time. All pesticides with active registrations are currently being reviewed by EPA under provisions of the Food Quality Protection Act of 1996. The state agency responsible for designing the fish contaminant monitoring program should be aware of all historic and current uses of each pesticide within its state, including the watersheds, application rates, and acreage where the pesticide has been or currently is applied to ensure that all potentially contaminated sites are included in the sampling plan. Because mercury contamination seems to be facilitated through atmospheric transport, because it has triggered issuance of the largest number of U.S. advisories, and because of the relatively low cost of chemical analysis for this analyte, EPA recommends that this metal be monitored at all agricultural sites, especially those for which little or no monitoring data are available. Additionally, states may also want to analyze for other metals (arsenic, cadmium, and selenium). States may include any of the recommended EPA target analytes and any additional target analytes in their screening programs when site-specific information on an agricultural watershed suggests that these contaminants may be present at levels of concern for human health.

Suburban/Urban. Water and sediment quality are often regularly monitored in suburban and urban areas, and selection of target analytes should be based on these data when available. Some suburban watersheds of today were agricultural watersheds during the early twentieth century. Arsenic compounds were widely used as pesticides in the early 1900s, as were organochlorine pesticides. These contaminants may still be present in farmland abandoned after the 1940s. As a result of the rapid population growth in recent years, other suburban areas have been built on former industrial sites, so historical information on land use should be obtained by states whenever possible and reviewed carefully during the target analyte selection process.

Several of the organophosphates as well as organochlorine pesticides have had wide use in control of pests around domestic structures as well as in lawn and garden applications (see Table 4-3). Chlorpyrifos and diazinon are currently used by pest control applicators and the general public (Robinson et al., 1994), and diazinon has been reported at high concentrations in effluents from POTWs in some suburban/urban areas (Amato et al., 1992; Burkhard and Jensen, 1993). Historically, chlordane was used extensively in termite control around homes and DDT was used as a general all-purpose insecticide. Nationally, chlordane and DDT are responsible for 3 and 1 percent, respectively, of the advisories currently in effect, and their use within suburban/urban watersheds should be considered as should the use of any of the pesticides registered for use around domestic structures or in lawn and garden applications. Depending on the proximity of some suburban/urban sites to industrial areas, states may also wish to review historic or current information on production sites associated with any of the pesticides, PAHs, PCBs, and dioxin/furans. Because of the historic and current uses of mercury in a variety of industrial processes, because it has triggered issuance of the largest number of U.S. advisories, and because of the relatively low cost of chemical analysis, EPA recommends that this metal be monitored at all surburban/urban sites, especially those where either little or no monitoring data are available. States should include any of the recommended EPA target analytes and any additional target analytes in their screening programs when site-specific information on a suburban/urban watershed suggests that these contaminants may be present at levels of concern for human health.

Industrial. All of the recommended target analytes can enter waterbodies through releases from industrial processes, Superfund sites, or landfills. Often water and sediment data are available to help guide the selection of the target analytes that should be given high priority with respect to analysis. Selection of analytes for analysis in industrial watersheds should be guided by knowledge of the type of industrial production that has existed in the past or is currently present in the watershed. Historical information is particularly important since potential contaminants may still be present at abandoned industrial sites or contained in sediments in receiving waterbodies. Sources of these target analytes are listed in Section 4.3, which contains the individual target analyte profiles and descriptions of the types of industries that may contribute to releases of these specific pollutants. Again, the states should review all existing water and sediment quality data available before selecting the specific target analytes for analysis at each site. Because of the historic and current uses of mercury in a variety of industrial processes, because it has triggered issuance of the largest number of U.S. advisories, and because of the relatively low cost of chemical analysis, EPA recommends that this metal be monitored at all industrial sites, especially those where little or no monitoring data are available. The other metals, including tributyltin, should also be considered for analysis based on existence of industrial production facilities, waste disposal facilities (e.g., Superfund or hazardous waste sites, and landfills), or shipyards where these target analytes may have been released to the environment. With respect to the pesticides, sites of production, formulation, and packaging facilities can all potentially be sites for release of these contaminants into the surrounding environment. Petroleum refining and coal gasification and processing facilities can also be sites for discharges of PAHs. PCBs can be released from historic landfills where PCB-containing equipment was disposed of or from sites of historic PCB production or use. Dioxins and dibenzofurans are likely to be found in proximity to historic or current industrial sites such as bleached kraft paper mills or production facilities for 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2,4,5-trichlorophenol (2,4,5-TCP), and/or silvex and medical, municipal, or industrial combustors or incinerators. States should include any of the recommended EPA target analytes and any additional target analytes in their screening programs when site-specific information on an industrial watershed suggests that these contaminants may be present at levels of concern for human health.

Specific factors that have been considered in the selection of the recommended 25 target analytes and sources for their release into the environment are summarized in the next section. Chemical pollutants that are currently under review by EPA's Office of Water for inclusion as recommended target analytes are discussed in Section 4.4.

4.3 TARGET ANALYTE PROFILES

4.3.1 Metals

Five metals—arsenic, cadmium, mercury, selenium, and tributyltin—are recommended as target analytes in screening studies. Arsenic, cadmium, and mercury have been included in at least five of the eight major fish contaminant monitoring programs reviewed by the 1993 Workgroup (see Appendix E). It should be noted, however, that with respect to arsenic, all monitoring programs measured total arsenic rather than inorganic arsenic. Selenium was monitored in four national monitoring programs. Tributyltin, a constituent in antifouling paints was not recommended for analysis in any of the national programs evaluated by the 1993 Workgroup. As of 1993, fish consumption advisories were in effect for arsenic, cadmium, mercury, selenium, and tributyltin in 1, 2, 29, 5, and 1 states, respectively (Table 4-2). As of 1998, fish advisories were in effect for arsenic, cadmium, mercury, and selenium in 3, 3, 40, and 11 states, respectively. No states had active advisories for tributyltin (U.S. EPA, 1999c). Also, with the exception of tributyltin, these metals have been identified as having the greatest potential toxicity resulting from ingestion of contaminated fish and shellfish (NAS, 1991).

4.3.1.1 Arsenic—

Arsenic is the twentieth most abundant element in the earth's crust and naturally occurs as a sulfide in a variety of mineral ores containing copper, lead, iron, nickel, cobalt, and other metals (Eisler, 1988; Merck Index, 1989; Woolson, 1975). Arsenic is released naturally to the atmosphere from volcanic eruptions and forest fires (Walsh et al., 1979) and to water via natural weathering processes (U.S. EPA, 1982b). Arsenic also has several major anthropogenic sources including industrial emissions from coal-burning electric generating facilities, releases, as a byproduct of nonferrous metal (gold, silver, copper, lead, uranium, and zinc) mining and smelting operations (Eisler, 1988; May and McKinney, 1981; NAS, 1977), releases associated with its production and use as a wood preservative (primarily as arsenic trioxide), and application as an insecticide, herbicide, algicide, and growth stimulant for plants and animals (Appendix F) (Eisler, 1988). Arsenic releases are also associated with leaching at hazardous waste disposal sites and discharges from sewage treatment facilities. Arsenic trioxide is the arsenic compound of chief commercial importance (U.S. EPA, 1982b) and was produced in the United States until 1985 at the ASARCO smelter near Tacoma, Washington. Arsenic is no longer produced commercially within the United States in any significant quantities, but arsenic compounds are imported into the United States primarily for use in various wood preservative and pesticide formulations.

The toxicity of arsenicals is highly dependent upon the nature of the compounds, and particularly upon the valency state of the arsenic atom (Frost, 1967; Penrose, 1974; Vallee et al., 1960). Typically, compounds containing trivalent (+3) arsenic are much more toxic than those containing pentavalent (+5) arsenic. The valency of the arsenic atom is a more important factor in determining toxicity than the organic or inorganic nature of the arsenic-containing compound (Edmonds and Francesconi, 1993). With respect to inorganic arsenic compounds, salts of arsenic acid (arsenates) with arsenic in the pentavalent state are less toxic than arsenite compounds with arsenic in the trivalent state (Penrose, 1974). Because some reduction of arsenate (pentavalent arsenic) to arsenite (trivalent arsenic) might occur in the mammalian body (Vahter and Envall, 1983), it would be unwise to disregard the possible toxicity of inorganic arsenic ingested in either valency state (Edmonds and Francesconi, 1993).

Seafood is a major source of trace amounts of arsenic in the human diet. However, arsenic in the edible parts of fish and shellfish is predominantly present as the arsenic-containing organic compound arsenobetaine (Cullen and Reimer, 1989; Edmonds and Francesconi, 1987a; NAS, 1991). Arsenobetaine is a stable compound containing a pentavalent arsenic atom, which has been shown to be metabolically inert and nontoxic in a number of studies (Cannon et al., 1983; Bos et al., 1985; Kaise et al., 1985; Sabbioni et al., 1991; Vahter et al., 1983) and is not generally considered a threat to human health (ATSDR, 1998a). Inorganic arsenic, although a minor component of the total arsenic content of fish and shellfish when compared to arsenobetaine, presents potential toxicity problems. To the degree that inorganic forms of arsenic are either present in seafood or, upon consumption, may be produced as metabolites of organic arsenic compounds in seafood, some human health risk, although small, would be expected (NAS, 1991).

Inorganic arsenic is very toxic to mammals and has been assigned to Toxicity Class I based on oral toxicity tests (U.S. EPA, 1998d). Use of several arsenical pesticides has been discontinued because of the health risks to animals and man. Inorganic arsenic also has been classified as a human carcinogen (A), and long-term effects include dermal hyperkeratosis, dermal melanosis and carcinoma, hepatomegaly, and peripheral neuropathy (IRIS, 1999) (Appendix G).

Total arsenic (inclusive of both inorganic and organic forms) has been included in five of the eight national monitoring programs evaluated by the 1993 Workgroup (Appendix E). Arsenic and arsenic-containing organic compounds have not been shown to bioaccumulate to any great extent in aquatic organisms (NAS, 1977). Experimental evidence indicates that inorganic forms of both pentavalent and trivalent arsenic bioaccumulate minimally in several species of finfish including rainbow trout, bluegill, and fathead minnows (ASTER, 1999). A bioconcentration factor (BCF) value of 350 was reported for the American oyster (*Crassostrea virginica*) exposed to trivalent arsenic (Zaroogian and Hoffman, 1982).

In 1984 and 1985, the U.S. Fish and Wildlife Service collected 315 composite samples of whole fish from 109 stations nationwide as part of the National Contaminant Biomonitoring Program (Schmitt and Brumbaugh, 1990). The authors reported the the maximum, geometric mean, and 85th percentile concentrations for total arsenic were 1.5, 0.14, and 0.27 ppm (wet weight), respectively. No information, however, was available on the percentage of inorganic arsenic in the fish sampled in the NCBP study. Kidwell et al. (1995)

conducted an analysis of total arsenic levels in bottom-feeding and predator fish using the 1984-1985 data from the NCBP study. These authors reported that the mean total arsenic tissue concentrations of 0.16 ± 0.23 ppm in bottom feeders and 0.16 ± 0.14 ppm in predator fish were not significantly different.

Edmonds and Francesconi (1993) summarized existing data from studies conducted outside the United States comparing concentrations of total arsenic, organic arsenic, and inorganic arsenic in marine fish and shellfish. Inorganic arsenic was found to represent from 0 to 44 percent of the total arsenic in marine fish and shellfish species surveyed. Residue concentrations of inorganic arsenic in the tissues typically ranged from 0 to 5.6 ppm (wet weight basis); but were generally less than 0.5 ppm for most species. In a study of six species of freshwater fish monitored as part of the Lower Columbia River study, inorganic arsenic represented from 0.1 to 27 percent of the total arsenic, and tissue residues of inorganic arsenic ranging from 0.001 to 0.047 ppm (wet weight) were 100 times lower than those reported for marine species (Tetra Tech, 1995).

In 1993, only one state (Oregon) had an advisory in effect for arsenic contamination (RTI, 1993). As of 1998, there were three advisories in effect in three states (Louisiana, Oregon, and Washington) for this metal (U.S. EPA, 1999c). Because it is the concentration of inorganic arsenic in fish and shellfish that poses the greatest threat to human health, EPA recommends that total inorganic arsenic (not total arsenic) be analyzed in contaminant monitoring programs. A chemical analysis procedure for determining total inorganic arsenic residues in fish and shellfish tissues is provided in Appendix H. Total inorganic arsenic should be considered for inclusion in state fish and shellfish monitoring programs in areas where it occurs in geologic formations, sites where mining or smelter operations have occurred, or where its use is or has been extensive. States should contact their appropriate state agencies to obtain information on the historic and current uses of arsenic particularly as a wood preservative and in agricultural pesticides.

4.3.1.2 Cadmium—

Cadmium is commonly found in zinc, lead, and copper deposits (May and McKinney, 1981). It is released into the environment from several anthropogenic sources: smelting and refining of ores, electroplating, application of phosphate fertilizers, surface mine drainage (Farag et al., 1998; U.S. EPA, 1978), and waste disposal operations (municipal incineration and land application) (U.S. EPA, 1979a, 1987c). Cadmium is also used in the manufacture of paints, alloys, batteries, and plastics and has been used in the control of moles and plant diseases in lawns.

Cadmium is a cumulative human toxicant; it has been shown to cause renal dysfunction and a degenerative bone disease, Itai-Itai, in Japanese populations exposed via consumption of contaminated rice, fish, and water. Because cadmium is retained in the kidney, older individuals (over 40-50 years of age) typically have both the highest renal concentrations of cadmium and the highest prevalence of renal dysfunction (U.S. EPA, 1979a). Cadmium is a known carcinogen in animals, and there is limited evidence of the carcinogenicity of cadmium or cadmium compounds in humans. It has been classified by EPA as a probable human carcinogen by inhalation (B1) (IRIS, 1999).

Cadmium has been found to bioaccumulate in fish and shellfish tissues in fresh water (Schmitt and Brumbaugh, 1990) and in estuarine/marine waters (NOAA, 1987, 1989a) nationwide. In 1984 and 1985, the U.S. Fish and Wildlife Service collected 315 composite samples of whole fish from 109 stations nationwide as part of the NCBP (Schmitt and Brumbaugh, 1990). The authors reported the maximum, geometric mean, and 85th percentile concentrations for cadmium were 0.22, 0.03, and 0.05 ppm (wet weight), respectively. In the NCBP study, geometric mean concentrations of cadmium in freshwater fish were found to have declined from 0.07 ppm in 1976 to 0.03 ppm in 1984 (Schmitt and Brumbaugh, 1990). This trend contradicts the general trend of increasing cadmium concentrations in surface waters, which Smith et al. (1987) attribute to increasing U.S. coal combustion (Schmitt and Brumbaugh, 1990). Kidwell et al. (1995) conducted an analysis of cadmium concentrations in bottom-feeding and predatory fish species using the 1984-1985 data from the NCBP study. These authors found that mean cadmium tissue concentration (whole fish samples) of 0.04 ± 0.05 ppm in bottom feeders (e.g., carp, white sucker, and channel catfish) was significantly higher than the mean cadmium tissue concentration of 0.01 ± 0.02 ppm found in predator fish (e.g., trout, walleye, largemouth bass).

In 1993, only two states (New York and Ohio) had issued fish advisories for cadmium contamination (RTI, 1993). As of 1998, there were seven advisories in effect in three states (Maine, New Jersey, and New York) for this heavy metal (U.S. EPA, 1999c). Two of these states, New York and New Jersey, have issued advisories for this metal in all of their marine coastal waters. Maine has a statewide wildlife advisory in effect for cadmium in moose liver and kidney tissue (U.S. EPA, 1999c). Cadmium should be considered for inclusion in all state fish and shellfish contaminant monitoring programs in areas where it occurs in geologic formations, where mining or smelter operations have occurred, or where its use is or has been extensive.

4.3.1.3 Mercury—

A major source of atmospheric mercury is the natural degassing of the earth's crust, amounting to 2,700 to 6,000 tons per year (WHO, 1990) Primary points of entry of mercury into the environment from anthropogenic sources include mining and smelting, industrial processes including chlorine-alkali production facilities and atmospheric deposition resulting from combustion of coal and other fossil fuels and municipal and medical refuse incinerators (U.S. EPA, 1997c; Glass et al., 1990). Primary industrial uses of mercury are in the manufacture of batteries, vapor discharge lamps, rectifiers, fluorescent bulbs, switches, thermometers, and industrial control instruments (May and McKinney, 1981), and these products ultimately end up in landfills or incinerators. Mercury has also been used as a slimicide in the pulp and paper industry, as an antifouling and mildew-proofing agent in paints, and as an antifungal seed dressing (ATSDR, 1998; *Farm Chemicals Handbook*, 1989; Friberg and Vostal, 1972).

Although mercury use and losses from industrial processes in the United States have been reduced significantly since the 1970s, mercury contamination associated with increased fossil fuel combustion is of concern in some areas and may pose more widespread contamination problems in the future. An estimated 5,000 tons of mercury per year is released into the environment from fossil fuel burning (Klaassen et al., 1986). The best estimate of annual anthropogenic U.S. emissions of mercury in 1994-1995 was 158 tons. Of this, about 87 percent was released from combustion sources, including waste and fuel combustion. (U.S. EPA, 1997). There is also increasing evidence of elevated mercury concentrations in areas where acid rain is believed to be a factor (NESCAUM, 1998; Sheffy, 1987; Wiener, 1987). Volatilization from surfaces painted with mercury-containing paints, both indoors and outdoors, may have been a significant source in the past (Agocs et al., 1990; Sheffy, 1987). The United States estimated that 480,000 pounds of mercuric fungicides were used in paints and coatings in 1987 (NPCA, 1988). In July 1990, EPA announced an agreement with the National Paint and Coatings Association to cancel all registrations for use of mercury or mercury compounds in interior paints and coatings. In May 1991, the paint industry voluntarily canceled all remaining registrations for mercury in exterior paints.

Cycling of mercury in the environment is facilitated by the volatile character of its metallic form and by bacterial transformation of metallic and inorganic forms to stable alkyl mercury compounds, particularly in bottom sediments, which leads to bioaccumulation of mercury (Wood, 1974). Practically all mercury in fish tissue is in the form of methylmercury (Bache et al., 1971; Bloom, 1992; Kannan et al., 1998; Spry and Wiener, 1991), which is toxic to humans (NAS, 1991; Tollefson, 1989), with the percentage of methylmercury to total mercury in the muscle tissue increasing as the fish ages (Bache et al., 1971). Several studies have shown that mercury concentrations in fish tissue generally increase with age, and therefore size (length or weight), owing to methylmercury accumulation with increasing duration of exposure (Driscoll et al., 1994; Jackson, 1990; Johnson, 1987; Lange et al., 1993); however this relationship is not as strongly correlated in all environmental situations or for all fish species (Goldstein et al., 1996; Neumann et al., 1997).

EPA has classified methylmercury as a Group C, possible human carcinogen, based on inadequate data in humans and limited evidence in animals (Appendix G). No persuasive evidence of increased carcinogenicity attributable to methylmercury exposure was observed in three human studies; however, interpretation of these studies was limited by poor study design and other problems. Animal studies have shown significant increases in the incidences of kidney tumors in male, but not in female, mice (IRIS, 1999).

Both inorganic and organic forms of mercury are neurotoxicants. Fetuses exposed to organic mercury have been found to be born mentally retarded and with symptoms similar to those of cerebral palsy (Marsh, 1987; U.S. EPA, 1997c). Individuals exposed to mercury via long-term ingestion of mercury-contaminated fish have been found to exhibit a wide range of symptoms, including numbness of the extremities, tremors, spasms, personality and behavior changes, difficulty in walking, deafness, blindness, and death (U.S. EPA, 1997c). Organomercury compounds were the causative agents of Minamata Disease, a neurological disorder reported in Japan during the 1950s among individuals consuming contaminated fish and shellfish (Kurland et al., 1960), with infants exposed prenatally found to be at significantly higher risk than adults. Another methylmercury poisoning incident involving fish and shellfish occurred in 1965 in Niigata, Japan. A third methylmercury poisoning incident occurred in the late 1960s and early 1970s in Iraq; however, this last incident was associated with the accidental consumption of seed grain treated with organomercury fungicide (U.S. EPA, 1997c). The EPA is especially concerned about evidence that the fetus is at increased risk of adverse neurological effects from exposure to methylmercury (e.g., Marsh et al., 1987; Piotrowski and Inskip, 1981; Skerfving, 1988; WHO, 1976, 1990; U.S. EPA, 1997c).

The EPA has set an interim Reference Dose (RfD) for methylmercury of 0.1 μg/kg-d (IRIS 1999). The National Academy of Sciences (NAS) conducted an independent assessment of the interim RfD. They concluded "On the basis of its evalution, the committee's consensus is that the value of EPA's current RfD for methylmercury, 0.1 µg/kg per day, is a scientifically justifiable level for the protection of public health". However, the NAS recommended that the Iraqi study no longer be used as the scientific basis for the RfD. In addition, the NAS recommended that the developmental neurotoxic effects of methylmercury reported in the Faroe Islands study should be used as the basis for the derivation of the RfD." (NAS, 2000)

Mercury has been found in both fish and shellfish from estuarine/marine (NOAA, 1987, 1989a) and fresh waters (Schmitt and Brumbaugh, 1990) at diverse locations nationwide. In 1984 and 1985, the U.S. Fish and Wildlife Service collected 315 composite samples of whole fish from 109 stations nationwide as part of the National Contaminant Biomonitoring Program (NCBP) (Schmitt and Brumbaugh, 1990). The authors reported that the maximum, geometric mean, and 85th percentile concentrations for mercury were 0.37, 0.10, and 0.17 ppm (wet weight), respectively. In contrast to cadmium and selenium, concentrations of mercury in freshwater fish tissue did not decline between 1976 and 1984 (Schmitt and Brumbaugh, 1990). Kidwell et al. (1995) conducted an analysis of mercury levels in bottom-feeding and predator fish using the 1984-1985 data from the NCBP study. These authors reported that the mean mercury tissue concentration (whole fish samples) of 0.12 ± 0.08 ppm in predator fish (e.g., trout, walleye, largemouth bass) was significantly higher than the mean tissue concentration of 0.08 $\pm$ 0.006 ppm in bottom feeders (e.g., carp, white sucker, and channel catfish).

Mercury, the only metal analyzed as part of the EPA National Study of Chemical Residues in Fish, was detected at 92 percent of 374 sites surveyed. Maximum, arithmetic mean, and median concentrations in fish tissue were 1.77, 0.26, and 0.17 ppm (wet weight), respectively (U.S. EPA, 1991h, 1992c, 1992d). Bahnick et al. (1994) analyzed the NSCRF data by fish species and reported that mean mercury concentrations in bottom feeders (whole body samples) were generally lower than concentrations for predator fish (fillet samples). Carp, white sucker, and channel catfish (bottom feeders) had average tissue concentrations of 0.11, 0.11, and 0.09 ppm, respectively. Largemouth bass, smallmouth bass, and walleye (predator species) had average tissue concentrations of 0.46, 0.34, and 0.52 ppm, respectively (Bahnick et al., 1994). With regard to the source of the mercury contamination, Bahnick et al. (1994) reported that the highest mean concentration of mercury was detected in fish sampled near public treatment works (0.59 ppm); however, background sites and sites near wood preserving facitities exhibited the second (0.34 ppm) and third (0.31 ppm) highest mean mercury concentrations. The authors also reported that most of the higher tissue concentrations of mercury were detected in freshwater fish samples collected in the Northeast.

Recently, the northeastern states and eastern Canadian provinces issued their own mercury study, including a comprehensive analysis of mercury concentrations in a variety of freshwater sportfish (NESCAUM, 1998). This study involved a large number of sampling sites, including remote lake sites that did not receive point source discharges. Top-level piscivores (i.e., predator fish), such as walleye, chain pickerel, and large and smallmouth bass, were typically found to exhibit the highest concentrations, with mean tissue residues greater than 0.5 ppm and maximum residues exceeding 2 ppm. One largemouth bass sample was found to contain 8.94 ppm of mercury, while a smallmouth bass sampled contained 5 ppm. A summary of the range and the mean concentrations found in eight species of sportfish sampled is shown in Table 4-4 (NESCAUM, 1998).

Table 4-4. Total Mercury and Methylmercury Concentrations in Estuarine Fish from South Florida

Species	Mean mercury concentration[a] (ppm) and range	Mean methylmercury[a] concentration (ppm) and range
Hardhead catfish	1.94 (0.44-4.64)	1.54 (0.18-4.42)
Gafftopsail catfish	3.0 (0.76-10.10)	1.86 (0.72-4.50)
Sand seatrout	2.41 (2.21-2.61)	2.04 (1.60-2.47)
Sand seaperch	0.48 (0.40-0.54)	0.42 (0.40-0.49)
Pinfish	0.54 (0.32-1.06)	0.44 (0.20-0.90)
White grunt	0.49 (0.28-1.03)	0.49 (0.31-0.99)
Lane snapper	0.57 (0.22-1.03)	0.58 (0.19-1.27)
Spot	0.29 (0.11-0.43)	0.24 (0.06-0.40)

[a]Concentrations are in ppm (µg/g) wet weight basis.

Source: Kannan et al., 1998.

EPA's Office of Water also recently published results of a national survey of mercury concentrations in fish (U.S. EPA, 1999d). This survey compiled state data on tissue residue levels of mercury in fish analyzed by 39 states between 1990 and 1995. The range of mean mercury concentrations (ppm) for the nine major fish species reported were as follows: largemouth bass, 0.001-8.94; smallmouth bass, 0.008-3.34; walleye, 0.008-3.0; northern pike, 0.10-4.4; channel catfish, 0.001-2.57; bluegill sunfish, 0.001-1.68; common carp, 0.001-1.8; white sucker, 0.002-1.71; and yellow perch, 0.01-2.14. All mercury concentrations used in the study were expressed on a wet weight and fillet basis. While the majority of the finfish sampled were freshwater species, some estuarine and marine species were also included; however, the report excluded all nonfish species such as turtles, molluscs, and crustaceans. Although comparison of data between states was difficult because of differences in sampling strategies (representative versus targeted), differences in analytical procedures, and the fact that mercury concentrations may vary with age of the fish, the analysis did indicate that both the magnitude and variability of mercury concentrations were greater in higher trophic level fish species.

Another recent study was conducted to assess total mercury and methylmercury concentrations in estuarine fish from south Florida coastal waters (Kannan et al., 1998). The authors reported that concentrations of total mercury in fish muscle tissue ranged between 0.03 and 2.22 ppm (mean: 0.31 ppm) (wet weight basis), with methylmercury contributing 83 percent of the total mercury. The mean concentrations and range of total mercury and methylmercury in muscle tissue of different species collected from south Florida's coastal waters are shown in Table 4-4.

In another study, methylmercury concentrations in muscle tissue of nine species of sharks were analyzed from four different locations along the coast of Florida (Hueter et al., 1995). Muscle tissue methylmercury concentrations averaged 0.88 ppm (wet weight) and ranged from 0.06 to 2.87 ppm, with 31 percent of the samples tested exceeding 1 ppm. A positive correlation was found between methylmercury concentration and the body length (size) of the shark, such that sharks larger than 2 m in total length contained methylmercury concentrations >1 ppm. Sharks collected off the southern and southwestern coastal areas contained significantly higher concentrations than those caught in the northeast coastal region (Cape Canaveral and north). Methylmercury concentrations were highest in the Caribbean reef shark (*Carcharhinus perezi*). The two most abundant shark species in the U.S. East Coast commercial shark fishery, the sandbar (*C. plumbeus*) and blacktip (*C. limbatus*) sharks, are of special public health concern. Although the mean methylmercury concentration in the sandbar shark (0.77 ppm) was below the average for all sharks, sandbar shark tissues contained up to 2.87 ppm methylmercury, and 20.9 percent of the sampled fish exceeded 1 ppm. Of more concern is that 71.4 percent of the blacktip shark samples (mean, 1.3 ppm) exceeded 1 ppm methylmercury. The authors suggest that continued monitoring of methylmercury concentrations in various shark species is warranted, since these fish are taken in both recreational and commercial fisheries. Similarly, on the West Coast, Fairey et al. (1997) reported that the highest concentrations of mercury found in all of the fish species sampled as part of a fish monitoring effort in the San Franscico Bay and Estuary were detected in leopard shark muscle tissue (1.26 ppm wet weight basis).

In 1993, 898 fish advisories had been issued in 29 states as a result of mercury contamination (see Figure 4-1). In particular, mercury was included in a large number of the fish advisories in effect for lakes in Minnesota, Wisconsin, and Michigan and for rivers and lakes in Florida (RTI, 1993). As of 1998, 1,931 advisories had been issued in 40 states for this metal, and mercury is responsible for more than 68 percent of all fish advisories issued in the United States. In addition, 10 states have statewide advisories in effect for mercury in freshwater lakes and/or rivers and 5 Gulf Coast states have statewide mercury advisories in effect for their coastal marine waters (U.S. EPA, 1999c).

Because of its widespread occurrence in fish across the United States, mercury should be monitored in all state fish and shellfish contaminant monitoring programs at all stations. Only one national program reviewed by the 1993 Workgroup—EPA 301(h) monitoring program—recommended analyzing specifically for methylmercury; however, six programs recommended analyzing for total mercury (Appendix E). Because of the higher cost of methylmercury analysis two to three times greater than for total mercury analysis). EPA recommends that total mercury be determined in state fish contaminant monitoring programs and the conservative assumption be made that all mercury is present as methylmercury so as to be most protective of human health. It should be noted that Bache et al. (1971) analyzed methylmercury concentrations in lake trout of known ages and found that methylmercury concentration and the ratio of methylmercury to total mercury increased with age. Relative proportions of methylmercury in fish varied between 30 and 100 percent, with methylmercury concentrations lower than 80 percent occurring in fish 3 years of age or younger. Thus, when high concentrations of total mercury are detected, and if resources are sufficient, states may wish to repeat sampling and obtain more specific information on actual concentrations of methylmercury in various age or size classes of fish.

4.3.1.4 Selenium—

Selenium is a natural component of many soils, particularly in the west and southwest regions of the United States (NAS, 1991). It enters the environment primarily via emissions from oil and coal combustion (May and McKinney, 1981; Pillay et al., 1969). Selenium is an essential nutrient but is toxic to both humans and animals at high concentrations (NAS, 1991). Long-term adverse effects from ingestion by humans have not been studied thoroughly. EPA has determined that the evidence of carcinogenicity of selenium in both humans and animals is inadequate and, therefore, has assigned this metal a D carcinogenicity classification (IRIS, 1999).

Selenium is frequently detected in ground and surface waters in most regions of the United States and has been detected in marine fish and shellfish (NOAA,

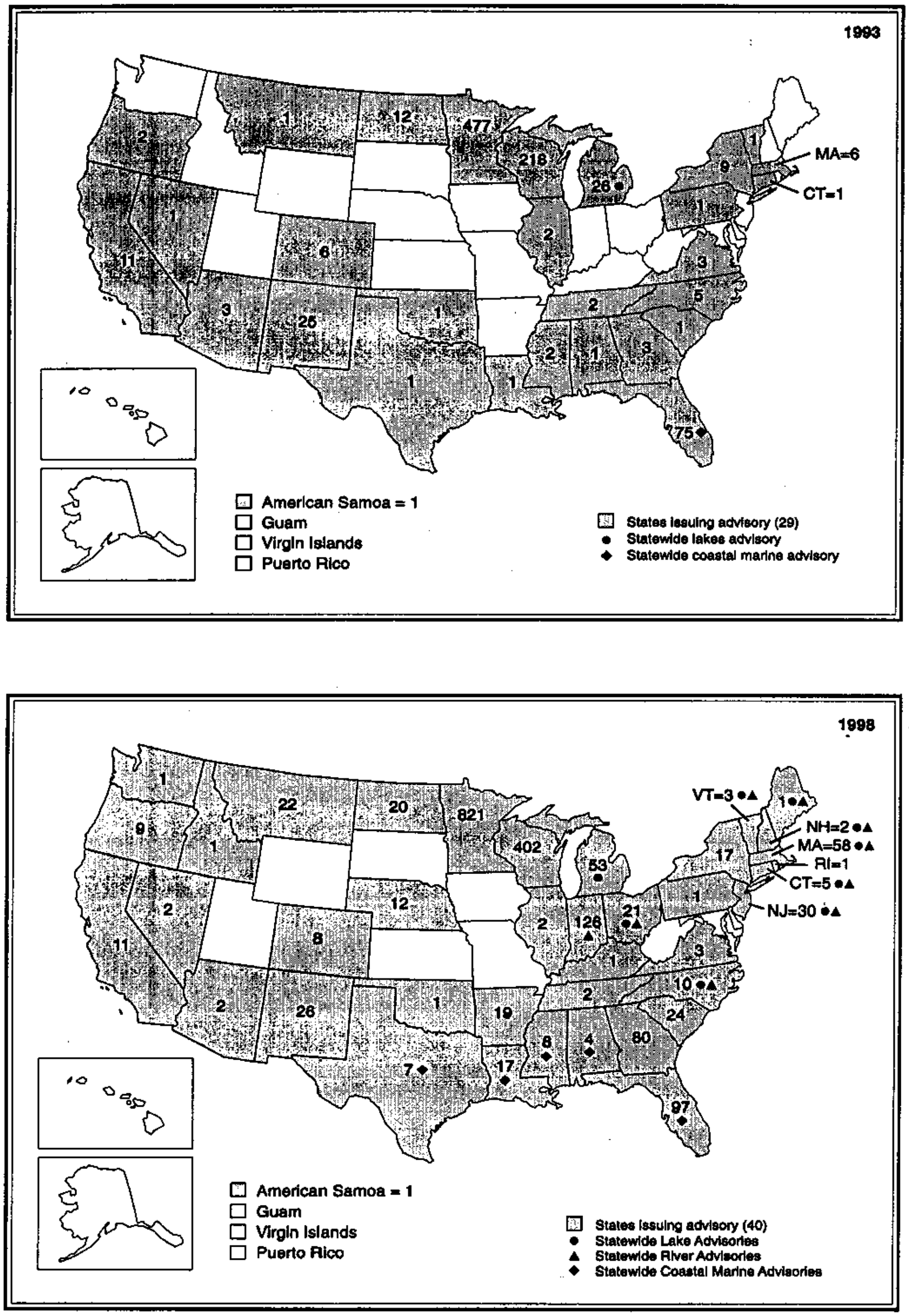


Figure 4-1. States issuing fish and shellfish advisories for mercury.

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1987, 1989a) and in freshwater fish (Schmitt and Brumbaugh, 1990) from several areas nationwide. In 1984 and 1985, the U.S. Fish and Wildlife Service collected 315 composite samples of whole fish from 109 stations nationwide as part of the National Contaminant Biomonitoring Program (Schmitt and Brumbaugh, 1990). The authors reported the maximum, geometric mean, and 85th percentile concentrations for selenium were 2.30, 0.42, and 0.73 ppm (wet weight), respectively. Kidwell et al. (1995) conducted an analysis of selenium concentrations in bottom-feeding and predator fish using the 1984-1985 data from the NCBP study. Mean selenium tissue concentrations (whole fish samples) were not significantly different in bottom feeders (0.50 ± 0.41 ppm) as compared to predator fish (0.50 ± 0.42 ppm). Like cadmium, concentrations of selenium declined in fish tissues between 1976 and 1984 (Schmitt and Brumbaugh, 1990).

In a more recent study (May 1993 to January 1994), selenium concentrations in the tissues of fish from the Pigeon River and Pigeon Lake in Michigan were examined. Mean selenium concentrations in white sucker fillets were 0.49 ± 0.19, 1.8 ± 0.96, and 1.7 ± 0.80 ppm (wet weight) in samples taken from the Upper Pigeon River, Lower Pigeon River, and Pigeon Lake, respectively. At these same locations, northern pike fillets contained selenium concentrations of 0.88 ± 0.22, 1.1 ± 0.91, and 2.2 ± 0.90 ppm (wet weight), respectively (Besser et al., 1996). This study was conducted to assess the potential hazard of selenium leaching from a coal fly ash disposal area.

Selenium was monitored in four national fish contaminant monitoring programs reviewed by the EPA 1993 Workgroup (Appendix E). Definitive information concerning the chemical forms of selenium found in fish and shellfish is not available (NAS, 1976, 1991).

In 1993, five states (California, Colorado, North Carolina, Texas, and Utah) had issued advisories for selenium contamination in fish (RTI, 1993). As of 1998, there were 11 advisories in effect in these same five states for this heavy metal (U.S. EPA, 1999c). These advisories include one wildlife advisory in Nevada for selenium in several species of waterfowl. Selenium should be considered for inclusion in all state fish and shellfish monitoring programs in areas where it occurs in geologic formations (particularly in the western and southwestern states) and near sites where oil or coal combustion currently occurs or historically has occurred.

4.3.1.5 Tributyltin Compounds—

Tributyltin compounds belong to the organometallic family of tin compounds that have been used as biocides, disinfectants, and antifoulants. Antifoulant paints containing tributyltin compounds were first registered for use in the United States in the early 1960s (Appendix F). Tributyltin compounds are used in paints applied to boat and ship hulls as well as to crab pots, fishing nets, and buoys to retard the growth of fouling organisms. These compounds were also registered for use as wood preservatives, disinfectants, and biocides in cooling towers, pulp and paper mills, breweries, leather processing facilities, and textile mills (U.S. EPA, 1988c).

Tributyltin compounds are acutely toxic to aquatic organisms at concentrations below 1 ppb and are chronically toxic to aquatic organisms at concentrations as low as 0.002 ppb (U.S. EPA, 1988c). EPA initiated a Special Review of tributyltin compounds used as antifoulants in January of 1986 based on concerns over its adverse effects on nontarget aquatic species. Shortly thereafter the Organotin Antifouling Paint Control Act (OAPCA) was enacted in June 1988, which contained interim and permanent tributyltin use restrictions as well as environmental monitoring, research, and reporting requirements. The Act established interim release rate restrictions under which only tributyltin-containing products that do not exceed an average daily release rate of 4 micrograms organotin/cm^2-d can be sold or used. The OAPCA also contained a permanent provision to prohibit the application of tributyltin antifouling paints to non-aluminum vessels under 25 meters (82 feet) long (U.S. EPA, 1988c).

Tributyltin oxide appears to be toxic to animals, with oral LD_{50}s ranging between 52 and 194 mg/kg (ATSDR, 1992; HSDB, 1999; WHO, 1999). Immunotoxicity is the critical effect produced by chronic exposure to tributyltin. Insufficient data are available to evaluate the carcinogenicity of tributyltin oxide compounds; therefore, EPA has listed this compound in Group D (Appendix G) (IRIS, 1999).

Tributyltins have been found to bioaccumulate in fish, bivalve mollusks, and crustaceans. Bioconcentration factors have been reported to range from 200 to 4,300 for finfish, from 2,000 to 6,000 for bivalves, and a BCF value of 4,400 was reported for crustaceans (U.S. EPA, 1988c). Tributyltin used to control marine fouling organisms in an aquaculture rearing pen has been found to bioaccumulate in fish tissue (Short and Thrower, 1987a and 1987b). Tsuda et al. (1988) reported a BCF value of 501 for tributyltin in carp (*Cyprinus carpio*) muscle tissue. Martin et al. (1989) reported a similar BCF value of 406 for tributyltin in rainbow trout (*Salmo gairdneri*) and Ward et al. (1981) reported a BCF value of 520 for the sheepshead minnow (*Cyprinodon variegatus*). In an environmental monitoring study conducted in England, a BCF value of 1,000 was reported for tributyltin in seed oysters (*Crassostrea gigas*) (Ebdon et al., 1989).

Tributyltin was not monitored in any national fish contaminant monitoring program evaluated by the EPA 1993 Workgroup (Appendix E). In 1993, only one state, Oregon, had an advisory in effect for tributyltin contamination in shellfish (RTI, 1993). As of 1998, there were no active fish advisories in effect for tributyltin, since the advisory in Oregon was rescinded (U.S. EPA, 1999c).

Tributyltin compounds should be considered for inclusion in all state fish and shellfish contaminant monitoring programs, particularly in states with coastal waters, states bordering the Great Lakes, or states with large rivers where large ocean-going vessels are used for commerce. Tributyltin concentrations have been reported to be highest in areas of heavy boating and shipping activities including shipyards, drydocks, and marinas where tributyltin-containing antifouling paints are often removed and reapplied. Before recoating, old paint containing tributyltin residues is scraped from the vessel hull and these paint scrapings are sometimes washed into the water adjacent to the boat or shipyard despite the tributyltin label prohibiting this practice (U.S. EPA, 1988c). Tributyltin should be considered for inclusion in state fish and shellfish monitoring programs in areas where its use is or has been extensive. States should contact their appropriate agencies to obtain information on the historic and current uses of tributyltin, particularly with respect to its uses in antifouling paints and wood preservatives.

4.3.2 Organochlorine Pesticides

The following organochlorine pesticides and metabolites are recommended as target analytes in screening studies: total chlordane (sum of cis- and trans-chlordane, cis- and trans-nonachlor, and oxychlordane), total DDT (sum of 2,4'- and 4,4'-homologues of DDT, DDD, and DDE), dicofol, dieldrin, endosulfan I and II, endrin, heptachlor epoxide, hexachlorobenzene, lindane (γ-hexachlorocyclohexane), mirex, and toxaphene (see Appendix F). Mirex is of particular concern in the Great Lakes states and the southeast states (NAS, 1991). All of these compounds are neurotoxins and most are known or suspected human carcinogens (IRIS, 1999; Sax, 1984).

With the exception of endosulfan I and II, dicofol, and total DDT, each of the pesticides on the recommended target analyte list (Table 4-1) had been included in at least four major fish contaminant monitoring programs (Appendix E), and seven of the compounds had triggered at least one state fish consumption advisory in 1993 (Table 4-2). Although use of some of these pesticides has been terminated or suspended within the United States for over 25 years (Appendix F), these compounds still require long-term monitoring. Many of the organochlorine pesticides that are now banned were used in large quantities for over a decade and are still present in sediments at high concentrations. These organochlorine pesticides are not easily degraded or metabolized and, therefore, persist in the environment. These compounds are either insoluble or have relatively low solubility in water, but are quite lipid-soluble. Because these compounds are not readily metabolized or excreted from the body and are readily stored in fatty tissues, they can bioaccumulate to high concentrations through aquatic food chains to secondary consumers (e.g., fish, piscivorous birds, and mammals including humans).

Pesticides may enter aquatic ecosystems from point source industrial discharges or from nonpoint sources such as aerial drift and/or runoff from agricultural use areas, leaching from landfills, or accidental spills or releases. Agricultural runoff from crop and grazing lands is considered to be the major source of pesticides in water, with industrial waste (effluents) from pesticide manufacturing the next most common source (Li, 1975). Significant atmospheric transport of pesticides to aquatic ecosystems can also result from aerial drift of pesticides, volatilization from applications in terrestrial environments, and wind erosion of treated soil (Li, 1975). Once in water, pesticide residues may become adsorbed to suspended material, deposited in bottom sediment, or absorbed by organisms in which they are detoxified and eliminated or accumulated (Nimmo, 1985).

The reader should note that three of the organochlorine pesticides still have active registrations: endosulfan, lindane, and dicofol. These pesticides are much less persistent in the environment and have a lower bioaccumulation potential than the banned organochlorines. However, agricultural runoff particularly during the period immediately after field application could result in significant levels of these pesticides in fish and shellfish tissues. States should contact their appropriate state agencies to obtain information on both the historic and current uses of these pesticides.

4.3.2.1 Chlordane (Total)—

Chlordane is a multipurpose insecticide that has been used extensively in home and agricultural applications in the United States for the control of termites and many other insects (Appendix F). This pesticide is similar in chemical structure to dieldrin, although less toxic (Toxicity Class II), and has been classified as a probable human carcinogen (B2) by EPA (Appendix G) (IRIS, 1999; Worthing, 1991).

Although the last labeled use of chlordane as a termiticide was phased out in the United States beginning in 1975, it has been monitored in seven national fish contaminant programs evaluated by the EPA 1993 Workgroup (Appendix E) and has been widely detected in freshwater fish (Schmitt et al., 1990) and in both estuarine/marine finfish (NOAA, 1987) and marine bivalves (NOAA, 1989a) at concentrations of human health concern. In 1984 and 1985, the U.S. Fish and Wildlife Service collected 321 composite samples of whole fish from 112 stations nationwide as part of the National Contaminant Biomonitoring Program (Schmitt et al., 1990). These authors reported the maximum and geometric mean concentrations for the five major degradation products of chlordane (*cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, and oxychlordane) were 0.66 and 0.03 ppm, 0.35 and 0.02 ppm, 0.45 and 0.02 ppm, 1.00 and 0.30 ppm, and 0.29 and 0.01 ppm (wet weight), respectively. Kidwell et al. (1995) conducted an analysis of all 1984-1985 data from the NCBP study on the major constituents of chlordane (including *cis*- and *trans*-chlordane, *cis*- and *trans*-nonachlor, and oxychlordane) in bottom-feeding and predator fish species. The authors reported there was no significant difference in residues in these two trophic groups of fish except for concentrations of *trans*-chlordane, which were significantly higher in the tissues of bottom feeders. Mean tissue concentrations of *cis*- and *trans*-chlordane, *cis*- and *trans*-nonachlor, and oxychlordane were 0.03 $\pm$ 0.06, 0.02 $\pm$ 0.04, 0.02 $\pm$ 0.04, 0.03 $\pm$ 0.01, and 0.01 $\pm$ 0.02 ppm, respectively, for bottom feeders as compared to 0.02 $\pm$ 0.04, 0.01 $\pm$ 0.02, 0.02 $\pm$ 0.03, 0.03 $\pm$ 0.06, and 0.01 $\pm$ 0.01 ppm, respectively, for predator species (Kidwell et al., 1995).

The *cis*- and *trans*-isomers of chlordane and *cis*- and *trans*-isomers of nonachlor, which are primary constituents of technical-grade chlordane, and oxychlordane, the major metabolite of chlordane, were also monitored as part of the EPA National Study of Chemical Residues in Fish (U.S. EPA, 1992c, 1992d). These compounds were detected in fish tissue at the following percentage of the 362 sites surveyed: *cis*-chlordane (64 percent), *trans*-chlordane (61 percent), *cis*-nonachlor (35 percent), *trans*-nonachlor (77 percent), and oxychlordane (27 percent) (U.S. EPA, 1992c, 1992d). The maximum, arithmetic mean, and median concentrations (wet weight) of *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, and oxychlordane are summarized in Table 4-5. Mean total chlordane residues from the NSCRF study were highest in bottom feeders such as carp (0.067 ppm), white sucker (0.018 ppm), and channel catfish (0.054 ppm) as compared to predator fish such as largemouth bass (0.029 ppm), smallmouth bass (0.004 ppb), and walleye (0.004 ppm) (Kuehl et al., 1994).

Table 4-5. Chlordane Constituent Concentrations[a] Detected in the EPA National Study of Chemical Residues in Fish

Chlordane constituent or metabolite	Maximum	Arithmetic mean	Median
cis-Chlordane	0.378	0.021	0.004
trans-Chlordane	0.310	0.017	0.003
cis-Nonachlor	0.127	0.009	ND
trans-Nonachlor	0.477	0.031	0.009
Oxychlordane	0.243	0.005	ND

ND = Not detected.

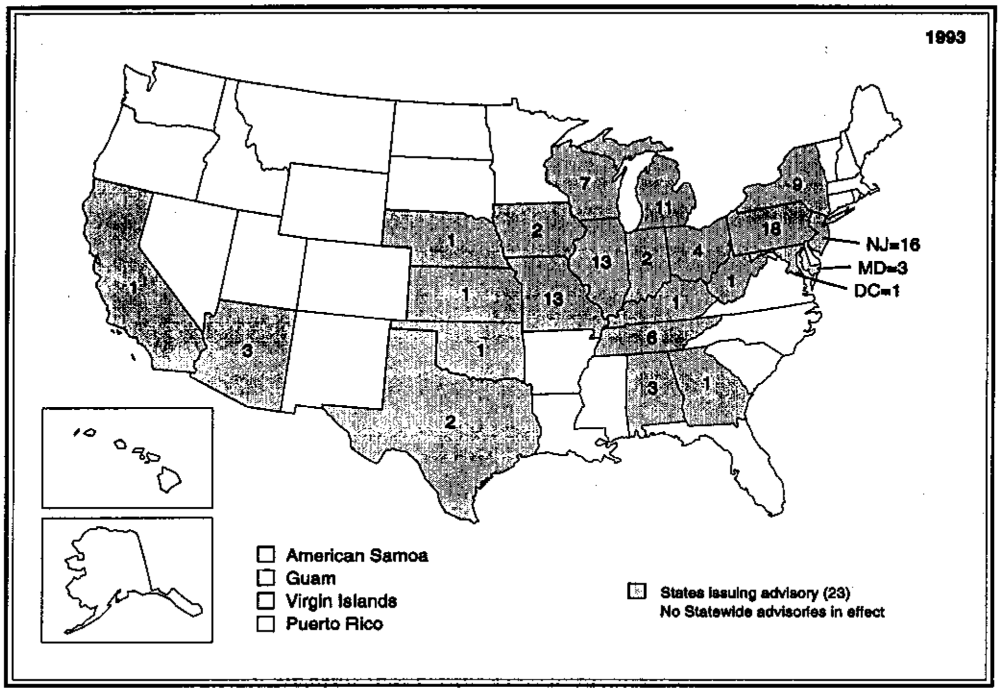
[a]Concentrations are in ppm (micrograms/g) on a wet weight basis.

Source: U.S. EPA, 1992c,1992d.

In 1993, 120 fish advisories in 24 states had been issued as a result of chlordane contamination (see Figure 4-2). As of 1998, there were 104 advisories in effect in 22 states for this pesticide, and New York currently has a statewide advisory for chlordane in all waterfowl (U.S. EPA, 1999c). Because of its extensive use in termite control and its widespread detection in fish tissues, total chlordane (i.e., sum of *cis*- and *trans*-chlordane, *cis*- and *trans*-nonachlor, and oxychlordane) should be considered for inclusion in all state fish and shellfish contaminant monitoring programs (NAS, 1991). Monitoring sites in agricultural watersheds should be reviewed to determine the application rate and acreage where chlordane was used historically. In suburban/urban watersheds, the degree of historic use of chlordane as a termiticide around domestic structures should also be evaluated. Sites in industrial watersheds should be reviewed to identify historic sites of chlordane production, formulation, or packaging facilities.

4.3.2.2 DDT (Total)—

Although the use of DDT was terminated in the United States in 1972, DDT and its DDE and DDD metabolites persist in the environment and are known to bioaccumulate (Ware, 1978). DDT, DDD, and DDE have all been classified by EPA as probable human carcinogens (B2) (Appendix G) (IRIS, 1999).



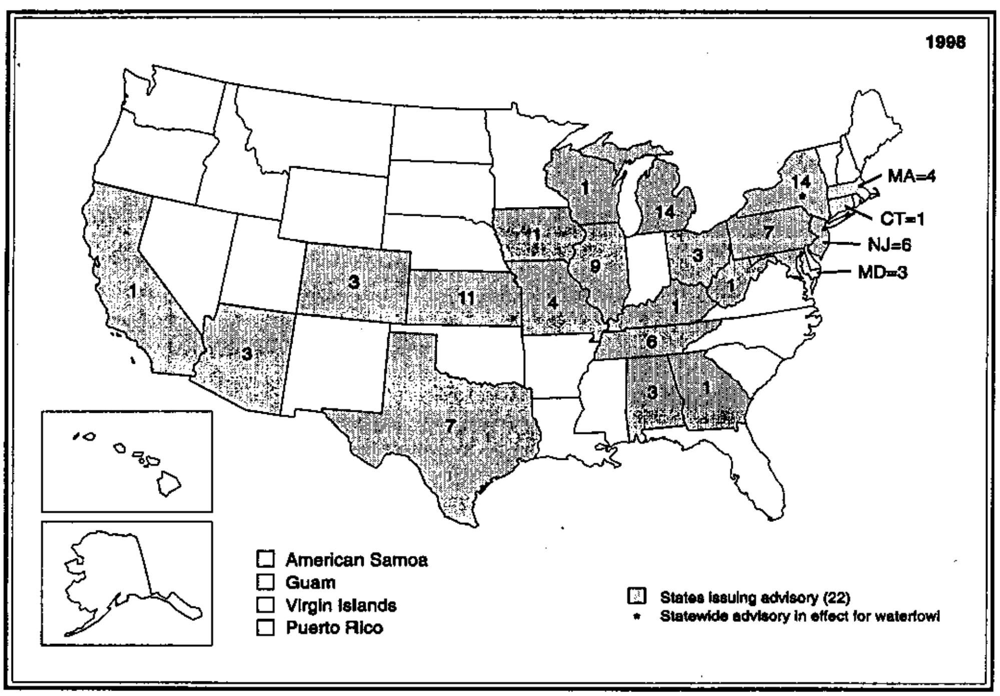


Figure 4-2. States issuing fish and shellfish advisories for chlordane.

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DDT or its metabolites have been included as target analytes in as many as seven major fish and shellfish monitoring programs (Appendix E) and contamination has been found to be widespread (NOAA, 1987, 1989a; Schmitt et al., 1990). In 1984 and 1985, the U.S. Fish and Wildlife Service collected 321 composite samples of whole fish from 112 stations nationwide as part of the National Contaminant Biomonitoring Program (Schmitt et al., 1990). Maximum and geometric mean tissue concentrations of DDT, DDE, and DDD in 1984 were 1.79 and 0.03 ppm, 4.74 and 0.19 ppm, and 2.55 and 0.06 ppm (wet weight), respectively (Schmitt et al., 1990). Kidwell et al. (1995) conducted an analysis of all 1984-1985 data from the NCBP study on DDT and its major metabolites (DDE and DDD) in bottom-feeding and predator fish. The authors reported that there was no significant difference in residues in these two trophic groups of fish. Mean tissue concentrations of DDT, DDE, and DDD were 0.03 ± 0.14, 0.21 ± 0.46, and 0.07 ± 0.21 ppm for bottom feeders as compared to 0.03 ± 0.06, 0.24 ± 0.55, and 0.06 ± 0.14 ppm for predator species, respectively. DDE, the only DDT metabolite surveyed in fish tissue in the EPA National Study of Chemical Residues in Fish, was detected at more sites than any other single chemical pollutant (99 percent of the 362 sites sampled) (U.S. EPA, 1992c, 1992d). Maximum, arithmetic mean, and median concentrations of DDE were 14, 0.295, and 0.058 ppm (wet weight), respectively. Mean DDE residues from the NSCRF study were highest in bottom feeders such as carp (0.42 ppm), white sucker (0.08 ppm), and channel catfish (0.63 ppm) as compared to predator species such as largemouth bass (0.06 ppm), smallmouth bass (0.03 ppb), and walleye (0.03 ppm) (Kuehl et al., 1994). In 1993, eight states (Alabama, Arizona, California, Delaware, Massachusetts, Nebraska, New York, and Texas) and the territory of American Samoa had fish consumption advisories in effect for DDT or its metabolites (RTI, 1993). As of 1998, there were 34 advisories in effect in 11 states and the territory of American Samoa for DDT and/or one of its metabolites, DDE or DDD (U.S. EPA, 1999c). In addition, New York has a statewide DDT advisory in effect for mergansers. Because of the extensive national use of this compound and its widespread detection in fish tissues, total DDT (i.e., sum of the 4,4'- and 2,4'-homologues of DDT and of its metabolites, DDE and DDD) should be considered for inclusion in all state fish and shellfish contaminant monitoring programs. Monitoring sites in agricultural watersheds should be reviewed to determine the application rate and acreage where DDT was applied historically. In suburban/urban watersheds, the degree of historic use of DDT in domestic home and garden applications should be evaluated. Sites in industrial watersheds should be reviewed to identify historic sites of DDT production, formulation, or packaging facilities.

4.3.2.3 Dicofol—

Dicofol, one of the three organochlorine target analytes with an active registration, is a miticide/pesticide that was first registered for use in 1957. Currently, dicofol is used primarily on cotton, apples, and citrus crops, mostly in California and Florida (U.S. EPA, 1998c). Dicofol is considered a DDT analog based on its structure and activity (Hayes and Laws, 1991). In the past, dicofol often contained 9 to 15 percent DDT and its analogs. In 1989, EPA required that these contaminants constitute less than 0.1 percent of dicofol (HSDB, 1993).

Historically, dicofol has been used to control mites on cotton and citrus (60 percent), on apples (10 percent), on ornamental plants and turf (10 percent), and on a variety of other agricultural products (20 percent) including pears, apricots, and cherries (*Farm Chemical Handbook*, 1989), as a seed crop soil treatment, on vegetables (e.g., beans and corn), and on shade trees (U.S. EPA, 1992c, 1992d).

Dicofol is moderately toxic to laboratory rats and has been assigned to EPA Toxicity Class III based on an oral LD_{50} of 587 mg/kg in rats (U.S. EPA, 1998d) (Appendix F). Technical-grade dicofol induced hepatocellular (liver) carcinomas in male mice; however, results were negative in female mice and in rats (NCI, 1978) and in a second 2-year feeding study in both sexes of rats (U.S. EPA, 1998d). EPA has classified dicofol as a possible human carcinogen (C) (Appendix G) (U.S. EPA, 1998c).

Dicofol was recommended for monitoring by the EPA Office of Water as part of the Assessment and Control of Bioconcentratable Contaminants in Surface Waters Program and has been included in two other national monitoring programs (see Appendix E). Experimental evidence indicates this compound bioaccumulates extensively in bluegill sunfish (BCF from 6,600 to 17,000) (U.S. EPA, 1993a).

In the EPA National Study of Chemical Residues in Fish, dicofol was detected at 16 percent of the 374 sites monitored (U.S. EPA, 1992c, 1992d). Maximum, arithmetic mean, and median dicofol concentrations (wet weight basis) were 0.074 ppm, 0.001 ppm, and ND (not detectable). Dicofol concentrations were greater than the quantification limit (0.0025 ppm) in samples from only 7 percent of the sites. Most of the sites where dicofol was detected were in agricultural areas where citrus and other fruits and vegetables are grown (U.S. EPA, 1992c, 1992d). It should be noted that this national study did not specifically target agricultural sites where this pesticide historically had been or currently was used. Dicofol residues in fish could be much higher if sampling were targeted for pesticide runoff, particularly during the period immediately after field application. Mean dicofol residues from the NSCRF study were highest in bottom feeders such as carp (0.88 ppm), white sucker (0.48 ppm), and channel catfish (0.59 ppm) as compared to predator species such as largemouth bass (0.20 ppm), smallmouth bass (not detected), and walleye (not detected) (Kuehl et al., 1994).

In 1993, however, no consumption advisories were in effect for dicofol (RTI, 1993). As of 1998, there were no advisories in effect for this pesticide (U.S. EPA, 1999c). Dicofol should be considered for inclusion in state fish and shellfish contaminant monitoring programs, in areas where its use is or has been extensive. States should contact their appropriate state agencies to obtain information on the historic and current uses of this pesticide. Monitoring sites in agricultural watersheds should be reviewed to determine the application rate and acreage where dicofol is currently used and was used historically. Sites in industrial watersheds should be reviewed to identify historic and current sites of dicofol production, formulation, or packaging facilities.

4.3.2.4 Dieldrin—

Dieldrin is a chlorinated cyclodiene that was widely used in the United States from 1950 to 1974 as a broad spectrum pesticide, primarily on termites and other soil-dwelling insects and on cotton, corn, and citrus crops. Because the toxicity of this persistent pesticide posed an imminent danger to human health, EPA banned the production and most major uses of dieldrin in 1974, and, in 1987, all uses of dieldrin were voluntarily canceled by industry (see Appendix F).

Dieldrin has been classified by EPA as a probable human carcinogen (B2) (Appendix G) (IRIS, 1999) and has been identified as a human neurotoxin (ATSDR, 1991). Dieldrin has been included in seven national monitoring programs (Appendix E) and has been detected nationwide in freshwater finfish (Schmitt et al., 1990) and estuarine/marine finfish and shellfish (NOAA, 1987, 1989a). Because it is a metabolite of aldrin, the environmental concentrations of dieldrin are a cumulative result of the historic use of both aldrin and dieldrin (Schmitt et al., 1990).

In 1984 and 1985, the U.S. Fish and Wildlife Service collected 321 composite samples of whole fish from 112 stations nationwide as part of the National Contaminant Biomonitoring Program. Maximum and geometric mean tissue concentrations of dieldrin in 1984 were 1.39 and 0.04 ppm (wet weight), respectively (Schmitt et al., 1990). Kidwell et al. (1995) conducted an analysis of all 1984-1985 data from the NCBP study on dieldrin in bottom-feeding and predator fish. These authors reported there was no significant difference in residues in these two trophic groups of fish. Mean tissue concentrations of dieldrin were 0.05 ± 0.14 ppm for bottom feeders as compared to 0.04 ± 0.10 ppm for predator species. Dieldrin was also detected in fish tissue at 60 percent of the 362 sites surveyed as part of the EPA National Survey of Chemical Residues in Fish (U.S. EPA, 1992c, 1992d). Maximum, arithmetic mean, and median concentrations of dieldrin in fish tissues were 0.450, 0.028, and 0.004 ppm (wet weight), respectively. Mean dieldrin residues from the NSCRF study were highest in bottom feeders such as carp (0.045 ppm), white sucker (0.023 ppm), and channel catfish (0.015 ppm) as compared to predator species such as largemouth bass (0.005 ppm), smallmouth bass (0.002 ppm), and walleye (0.002 ppm) (Kuehl et al., 1994).

In 1993, three states (Arizona, Illinois, and Nebraska) had issued advisories for dieldrin contamination in fish (RTI, 1993). As of 1998, there were 23 advisories in effect in six states (Arizona, California, Colorado, Hawaii, Nebraska, and Texas) for this pesticide (U.S. EPA, 1999c). Dieldrin should be considered for inclusion in all state fish and shellfish contaminant monitoring programs in areas where its use as well as the use of aldrin have been extensive. States should contact their appropriate state agencies to obtain information on the historic uses of these two pesticides. Monitoring sites in agricultural watersheds should be reviewed to determine the application rate and acreage where dieldrin and aldrin were applied since dieldrin is a degradation product of aldrin. In suburban/urban watersheds, the degree of historic use of dieldrin and aldrin in domestic home and garden applications should be evaluated. Sites in industrial watersheds should be reviewed to identify historic sites of dieldrin and aldrin production, formulation, or packaging facilities.

4.3.2.5 Endosulfan—

Endosulfan is a chlorinated cyclodiene pesticide that is currently in wide use primarily as a noncontact insecticide for seed and soil treatments (Appendix F). Two stereohomologues (I and II) exist and exhibit approximately equal effectiveness and toxicity (Worthing, 1991).

Endosulfan is highly toxic to laboratory animals and has been assigned to EPA Toxicity Class I (U.S. EPA, 1998d). To date, no studies have been found concerning carcinogenicity in humans after oral exposure to endosulfan (ATSDR, 1998c). EPA has classified endosulfan as Group E, evidence of noncarcinogenicity for humans (U.S. EPA, 1999b).

Agricultural runoff is the primary source of this pesticide in aquatic ecosystems. Endosulfan has been shown to be highly toxic to fish and marine invertebrates and is readily absorbed in sediments. It therefore represents a potential hazard in the aquatic environment (Sittig, 1980). However, data are insufficient to assess nationwide endosulfan contamination (NAS, 1991). Endosulfan has been included in one national fish contaminant monitoring program—the U.S. EPA 301(h) Program—the (U.S. EPA 301(h) Program—evaluated by the 1993 EPA Workgroup (Appendix E); however, no information was located related to its concentrations in fish or shellfish tissue.

In 1993, no consumption advisories were in effect for endosulfan I or II (RTI, 1993). As of 1998, there were no advisories in effect for this pesticide (U.S. EPA, 1999c). Endosulfan I and II should be considered for inclusion in all state fish and shellfish contaminant monitoring programs in areas where its use is or has been extensive. States should contact their appropriate agencies to obtain information on the historic and current uses of this pesticide. Monitoring sites in agricultural watersheds should be reviewed to determine the application rate and acreage where endosulfan currently is used and was used historically. Sites in industrial watersheds should be reviewed to identify historic and current sites of endosulfan production, formulation, or packaging facilities.

4.3.2.6 Endrin—

Endrin is a chlorinated cyclodiene that historically was widely used as a broad spectrum pesticide. Endrin was first registered for use in the United States in 1951. However, recognition of its long-term persistence in soil and its high levels of mammalian toxicity led to restriction of its use beginning in 1964 and 1979 (U.S. EPA, 1980a; 44 FR 43632) and to final cancellation of its registration in 1984 (U.S. EPA, 1984a) (Appendix F).

Endrin is highly toxic to humans (EPA Toxicity Class I) (U.S. EPA, 1998d), with acute exposures affecting the central nervous system primarily (Sax, 1984). At present, evidence of both animal and human carcinogenicity of endrin is considered inadequate, and EPA has classified endrin in Group D, not classifisable as to human carcinogenicity insufficient information available (Appendix G) (IRIS, 1999).

Although endrin has been included in five national fish contaminant monitoring programs (Appendix E), it has not been found widely throughout the United States. In 1984 and 1985, the U.S. Fish and Wildlife Service collected 321 composite samples of whole fish from 112 stations nationwide as part of the National Contaminant Biomonitoring Program (Schmitt et al., 1990). Endrin was detected in freshwater fish at only 29 percent of 112 stations sampled in the NCBP study. Maximum and geometric mean tissue concentrations of endrin in 1984 were 0.22 and <0.01 ppm (wet weight), respectively (Schmitt et al. 1990). Endrin was also detected in freshwater and marine species at 11 percent of the 362 sites surveyed in the EPA National Study of Chemical Residues in Fish (U.S. EPA, 1992c, 1992d). Maximum, arithmetic mean, and median concentrations of endrin in fish tissues were 0.162 ppm, 0.002 ppm, and not detectable (wet weight), respectively. Mean endrin residues from the NSCRF study were highest in bottom feeders such as carp (0.0014 ppm), white sucker (0.0002 ppm), and channel catfish (0.009 ppm) as compared to predatory species such as largemouth bass (not detectable), smallmouth bass (not detectable), and walleye (not detectable) (Kuehl et al., 1994).

In 1993, no state had issued a fish advisory for endrin (RTI, 1993). As of 1998, there were no advisories in effect for this pesticide (U.S. EPA, 1999c). Endrin should be considered for inclusion in all state fish and shellfish contaminant monitoring programs in areas where its use has been extensive. States should contact their appropriate agencies to obtain information on the historic uses of this pesticide. Monitoring sites in agricultural watersheds should be reviewed to determine the application rate and acreage where endrin was used historically. Sites in industrial watersheds should be reviewed to identify historic sites of endrin production, formulation, or packaging facilities.

4.3.2.7 Heptachlor Epoxide—

Heptachlor epoxide is not a formulated pesticide but is a metabolic degradation product of the pesticides heptachlor and chlordane. It is also found as a contaminant in heptachlor and chlordane formulations (Appendix F). Heptachlor epoxide is also more toxic than either parent compound (ATSDR, 1993). Heptachlor has been used as a persistent, nonsystemic contact and ingested insecticide on soils (particularly for termite control) and seeds and as a household insecticide (Worthing, 1991). EPA suspended the major uses of heptachlor in 1978 (ATSDR, 1993). Acute exposures to high doses of heptachlor epoxide in humans can cause central nervous system effects (e.g., irritability, dizziness, muscle tremors, and convulsions (U.S. EPA, 1986c). In animals, liver, kidney, and blood disorders can occur (IRIS, 1999). Exposure to this compound produced an increased incidence of liver carcinomas in rats and mice and hepatomas in female rats (IRIS, 1999). Heptachlor epoxide has been classified by EPA as a probable human carcinogen (B2) (Appendix G) (IRIS, 1999).

Heptachlor epoxide has been included in six national fish monitoring programs (Appendix E) and has been detected widely in freshwater finfish (Schmitt et al., 1990), but infrequently in bivalves and marine fish (NOAA, 1987, 1989a). In 1984 and 1985, the U.S. Fish and Wildlife Service collected 321 composite samples of whole fish from 112 stations nationwide as part of the National Contaminant Biomonitoring Program (Schmitt et al., 1990). Heptachlor epoxide was detected in freshwater fish at 49 percent of 112 stations sampled in the NCBP study. Maximum and geometric mean tissue concentrations of heptachlor epoxide in 1984 were 0.29 and 0.01 ppm (wet weight), respectively (Schmitt et al., 1990). Heptachlor epoxide also was detected in fish tissue at 16 percent of the 362 sites where it was surveyed in the EPA National Study of Chemical Residues in Fish (U.S. EPA, 1992c, 1992d). Maximum, arithmetic mean, and median concentrations of heptachlor epoxide were 0.063 ppm, 0.002 ppm, and not detectable (wet weight). It should be noted that one of the parent compounds, heptachlor was detected at only 2 percent of the 362 sites where it was surveyed at a maximum, arithmetic mean, and median concentration of 0.076, 0.0004 ppm, and not detectable, respectively. The five degradation products of chlordane were detected at from 27 to 77 percent of these same sites (see Section 4.3.2.1 for a discussion of chlordane). Mean heptachlor epoxide residues from the NSCRF study were highest in bottom feeders such as carp (0.004 ppm), white sucker (0.001 ppm), and channel catfish (0.0005 ppm) as compared to predator species such as largemouth bass (0.0003 ppm), smallmouth bass (0.00007 ppm), and walleye (0.0002 ppm) (Kuehl et al., 1994).

In 1993, only Nebraska had fish advisories for heptachlor epoxide contamination (RTI, 1993). As of 1998, there was only one advisory in effect, in Texas, for this pesticide degradation product (U.S. EPA, 1999c). Heptachlor epoxide should be considered for inclusion in all state fish and shellfish monitoring programs in areas where the use of heptachlor or chlordane have been extensive. States should contact their appropriate agencies to obtain information on the historic uses of these pesticides. Monitoring sites in agricultural watersheds should be reviewed to determine the application rate and acreage where heptachlor and chlordane were historically used since both of these pesticides degrade to heptachlor epoxide. In suburban/urban watersheds, the degree of historic use of heptachlor and chlordane in domestic home and garden applications should be evaluated. Sites in industrial watersheds also should be reviewed to identify historic sites of heptachlor and chlordane production, formulation, or packaging facilities.

4.3.2.8 Hexachlorobenzene—

Hexachlorobenzene is a fungicide that was widely used as a seed protectant in the United States until 1984 (Appendix F). The use of hexachlorobenzene and the presence of hexachlorobenzene residues in food are banned in many countries including the United States (Worthing, 1991). Registration of hexachlorobenzene as a pesticide was voluntarily canceled in 1984 (Morris and Cabral, 1986).

The toxicity of this compound is minimal; it has been given an EPA toxicity classification of IV (i.e., oral LD_{50} greater than 5,000 ppm in laboratory animals (U.S. EPA, 1998d). However, nursing infants are particularly susceptible to hexachlorobenzene poisoning as lactational transfer can increase infant tissue levels to two to five times maternal tissue levels (ATSDR, 1996). Hexachlorobenzene is a known animal carcinogen (ATSDR, 1996) and has been classified by EPA as a probable human carcinogen (B2) (Appendix G) (IRIS, 1999).

Of the chlorinated benzenes, hexachlorobenzene is the most widely monitored (Worthing, 1991). It was included as a target analyte in seven of the major monitoring programs reviewed by the 1993 Workgroup (Appendix E). In 1984 and 1985, the U.S. Fish and Wildlife Service collected 321 composite samples of whole fish from 112 stations nationwide as part of the National Contaminant Biomonitoring Program (Schmitt et al., 1990). Hexachlorobenzene was detected in freshwater fish at 19 percent of 112 stations sampled in the NCBP study. Maximum and geometric mean tissue concentrations of hexachlorobenzene in 1984 were 0.41 and <0.01 ppm (wet weight), respectively (Schmitt et al., 1990). Kidwell et al. (1995) conducted an analysis of all 1984-1985 data from the NCBP on hexachlorobenzene in bottom-feeding and predator fish. The authors reported that there was no significant difference in residues in these two trophic groups. Mean tissue concentrations of HCB were 0.00 ± 0.01 and 0.01 ± 0.04 ppm, respectively, for bottom feeders and predator species. Hexachlorobenzene also was detected in fish tissue at 46 percent of the 362 sites where it was surveyed in the EPA National Study of Chemical Residues in Fish (U.S. EPA, 1992c, 1992d). Maximum, arithmetic mean, and median concentrations were 0.913 ppm, 0.006 ppm, and not detectable (wet weight), respectively. Mean hexachlorobenzene residues from the NSCRF study were highest in bottom feeders such as carp (0.0036 ppm), white sucker (0.0036 ppm), and channel catfish (0.0024 ppm) as compared to predator species such as largemouth bass (0.0002 ppm), smallmouth bass (0.0004 ppm), and walleye (0.0001 ppm) (Kuehl et al., 1994).

In 1993, Louisiana and Ohio had issued advisories for hexachlorobenzene contamination in fish and shellfish (RTI, 1993). As of 1988, there were three advisories in effect in these two states for this pesticide (U.S. EPA, 1999c). Hexachlorobenzene should be considered for inclusion in all state fish and shellfish monitoring programs. Monitoring sites in agricultural watersheds should be reviewed to determine the application rate and acreage where hexachlorobenzene was historically used. Sites in industrial watersheds also should be reviewed to identify historic sites of hexachlorobenzene as well as other organochlorine pesticide production, formulation, or packaging facilities since hexachlorobenzene was used as an intermediate in the chemical synthesis of many organochlorine pesticides.

4.3.2.9 Lindane—

Lindane is a mixture of homologues of hexachlorocyclohexane ($C_6H_6Cl_6$), whose major component (≥99 percent) is the gamma isomer. It is commonly referred to as either γ-HCH (hexachlorocyclohexane) or γ-BHC (benzene hexachloride). Lindane is used primarily in seed treatments, soil treatments for tobacco transplants, foliage applications on fruit and nut trees and vegetables, and wood and timber protection. Lindane is used as a therapeutic scabicide, pediculicide, and ectoparasiticide for humans and animals (Merck Index 1989). Since 1985, many uses of lindane have been banned or restricted (see Appendix F) and its application is permitted only under supervision of a certified applicator (U.S. EPA, 1985c). In 1993, EPA issued a "Notice of Receipt of a Request for Amendments to Delete Uses" for several formulations of lindane provider, 99.5 percent technical, and dust concentrate, which would delete from the pesticide label most uses of lindane for agricultural crops and use on animals and humans (EPA 1993).

Lindane is a neurotoxin (assigned to EPA Toxicity Class II) (U.S. EPA, 1998d) and has been found to cause aplastic anemia in humans (Worthing, 1991). Lindane has been classified by EPA as a probable/possible human carcinogen (B2/C) (Appendix G) (U.S. EPA, 1999b).

Lindane has been included in seven major fish contaminant monitoring programs (Appendix E). This pesticide has been detected in freshwater fish (Schmitt et al., 1990) and in marine fish and bivalves (NOAA, 1987, 1989a) nationwide. In 1984 and 1985, the U.S. Fish and Wildlife Service collected 321 composite samples of whole fish from 112 stations nationwide as part of the National Contaminant Biomonitoring Program (Schmitt et al., 1990). Lindane was detected in freshwater fish at 47 percent of 112 stations sampled in the NCBP study. Maximum and geometric mean tissue concentrations of lindane in 1984 were 0.40 and <0.01 ppm (wet weight), respectively (Schmitt et al., 1990). Kidwell et al. (1995) conducted an analysis of all 1984-1985 data from the NCBP study on lindane in bottom-feeding and predator fish. These authors reported there was no significant difference in residues in these two trophic groups of fish. Lindane also was detected in fish tissue at 42 percent of 362 sites surveyed in the EPA National Study of Chemical Residues in Fish (U.S. EPA, 1992c, 1992d). Maximum, arithmetic mean, and median lindane concentrations were 0.083 ppm, 0.003 ppm, and not detectable (wet weight), respectively. Mean lindane residues from the NSCRF study were highest in bottom feeders such as carp (0.0043 ppm), white sucker (0.0017 ppm), and channel catfish (0.0032 ppm) as compared to predator species such as largemouth bass (0.00007 ppm), smallmouth bass (0.00015 ppm), and walleye (not detectable) (Kuehl et al., 1994).

In 1993, although it had been widely monitored and widely detected, no consumption advisories were in effect for lindane (RTI, 1993). As of 1998, there were no advisories in effect for this pesticide (U.S. EPA, 1999c). Lindane should be considered for inclusion in all state fish and shellfish monitoring programs in areas where its use has been extensive. States should contact their appropriate agencies to obtain information on the historic and current uses of this pesticide. Monitoring sites in agricultural watersheds should be reviewed to determine the application rate and acreage where lindane was used historically. In suburban/ urban watersheds, the degree of historic use of lindane in domestic home and garden applications should be evaluated. Sites in industrial watersheds should be reviewed to identify historic and current sites of lindane production, formulation, or packaging facilities.

4.3.2.10 Mirex—

Mirex is a chlorinated cyclodiene pesticide that was used in large quantities in the United States from 1962 through 1975 primarily for control of fire ants in the Southeast and Gulf Coast states and, more widely, under the name Dechlorane as a fire retardant and polymerizing agent in plastics (Kaiser, 1978; Kutz et al., 1985) (Appendix F).

Mirex has been assigned to EPA Toxicity Class II on the basis of an oral LD_{50} in rats of 368 mg/kg (ATSDR, 1995; U.S. EPA, 1998d) (Appendix F). Mirex has been assigned a carcinogenicity classification of group B2, probable human carcinogen (HEAST, 1997). EPA instituted restrictions on the use of mirex in 1975, and, thereafter, the U.S. Department of Agriculture (USDA) suspended the fire ant control program (Hodges, 1977).

Mirex has been included in seven major fish contaminant monitoring programs (Appendix E). It has been found primarily in the Southeast, Gulf Coast, and the Great Lakes regions (Kutz et al., 1985; NAS, 1991; Schmitt et al., 1990). In 1984 and 1985, the U.S. Fish and Wildlife Service collected 321 composite samples of whole fish from 112 stations nationwide as part of the National Contaminant Biomonitoring Program (NCBP) (Schmitt et al., 1990). Mirex was detected in freshwater fish at 13 percent of 112 stations sampled in the NCBP study. Maximum and geometric mean tissue concentrations of mirex in 1984 were 0.44 and <0.01 ppm (wet weight), respectively (Schmitt et al., 1990). Kidwell et al. (1995) conducted an analysis of all 1984-1985 data from the NCBP study on mirex in bottom-feeding and predator fish. These authors reported there was no significant difference in residues in these two trophic groups of fish. Mean tissue concentrations of mirex were 0.00 ± 0.04 and 0.01 ± 0.05 ppm, respectively, for bottom feeders and predator species. Mirex also was detected in fish tissue at 38 percent of 362 sites surveyed in the EPA National Study of Chemical Residues in Fish (NSCRF) (U.S. EPA, 1992c, 1992d). Maximum, arithmetic mean, and median mirex concentrations were 0.225 ppm, 0.004 ppm, and not detectable (wet weight), respectively. Mean mirex residues from the EPA NSCRF study were highest in bottom feeders such as carp (0.0037 ppm), white sucker (0.0044 ppm), and channel catfish (0.0146 ppm) as compared to predator species such as largemouth bass (0.0002 ppm), smallmouth bass (0.002 ppm), and walleye (0.00008 ppm) (Kuehl et al., 1994).

In 1993, three states (New York, Ohio, and Pennsylvania) had issued fish advisories for mirex (RTI, 1993). As of 1998, there were 11 advisories in effect in these same three states for this pesticide (U.S. EPA, 1999c). New York has a statewide advisory in effect for mergansers. Mirex should be considered for inclusion in all state fish and shellfish monitoring programs in areas where its use has been extensive. States should contact their appropriate agencies to obtain information on the historic uses of this pesticide. Monitoring sites in agricultural watersheds should be reviewed to determine the application rate and acreage where mirex was used historically. In suburban/urban watersheds, the degree of historic use of mirex in domestic home and garden applications should be evaluated. Sites in industrial watersheds should be reviewed to identify historic sites of mirex production, formulation, or packaging facilities.

4.3.2.11 Toxaphene—

Toxaphene is an organochlorine pesticide composed of a complex mixture of chlorinated camphenes (chlorinated bornanes and some bornenes) that was first registered for use in the United States in 1947. It was commercially produced by the chlorination of camphenes derived from pine trees. It has been estimated that the commercial mixture of toxaphene contained at least 670 congeners with the majority of these having 6 to 10 chlorines (Jansson and Wideqvist, 1983). Historically, this compound was used in the United States as an insecticide primarily on cotton (Hodges, 1977). In addition, toxaphene was used as a piscicide for rough fish in the 1950s and 1960s in North America and was the replacement for DDT after DDT's use was severely restricted in 1972 (Saleh, 1991). Partly as a consequence of the ban on the use of DDT imposed in 1972, toxaphene was for many years the most heavily used pesticide in the United States (Eichers et al., 1978). In 1982, toxaphene's registration for most uses was canceled (47 FR 53784) and all uses were banned in 1990 (55 FR 31164-31174). Toxaphene is a global pollutant whose chemical-physical properties make it a candidate for long-range atmospheric transport via the cold condensation effect once it is released into the environment (Wania and Mackay, 1993, 1996).

Like many of the other organochlorine pesticides, toxaphene has been assigned to EPA Toxicity Class II (U.S. EPA, 1998d) (Appendix F). Some components of toxaphene may accumulate in body fat. Toxaphene has been classified by EPA as a probable human carcinogen (B2) (Appendix G) (IRIS, 1999).

Toxaphene has been included in four major fish contaminant monitoring programs (Appendix E). It has been detected frequently in both freshwater fish (Schmitt et al., 1990) and estuarine species (NOAA, 1989a) but is only consistently found in Georgia, Texas, and California (NAS, 1991). In 1984 and 1985, the U.S. Fish and Wildlife Service collected 321 composite samples of whole fish from 112 stations nationwide as part of the National Contaminant Biomonitoring Program (Schmitt et al., 1990). Toxaphene was detected in freshwater fish at 69 percent of 112 stations sampled in the NCBP study. Maximum and geometric mean tissue concentrations of toxaphene in 1984 were 8.2 and 0.14 ppm (wet weight), respectively (Schmitt et al., 1990). Kidwell et al. (1995) conducted an analysis of all 1984-1985 data from the NCBP study on toxaphene in bottom-feeding and predatory fish species. These authors reported there was no significant difference in residues in these two trophic groups of fish. Mean tissue concentrations of toxaphene were 0.19 ± 0.63 and 0.17 ± 0.35 ppm, respectively, for bottom feeders and predator species.

In 1993, two states (Arizona and Texas) had fish advisories in effect for toxaphene (RTI, 1993). As of 1988, there were six advisories in effect in four states (Arizona, Georgia, Oklahoma, and Texas) for this pesticide (U.S. EPA, 1999c). Toxaphene should be considered for inclusion in all state fish and shellfish monitoring programs in areas where its use has been extensive. States should contact their appropriate agencies to obtain information on the historic uses of this pesticide. Monitoring sites in agricultural watersheds should be reviewed to determine the application rate and acreage where toxaphene was used historically. Sites in industrial watersheds should be reviewed to identify historic sites of toxaphene production, formulation, or packaging facilities.

4.3.3 Organophosphate Pesticides

The following organophosphate pesticides are recommended as target analytes in screening studies: chlorpyrifos, diazinon, disulfoton, ethion, and terbufos (Appendix E). These pesticides share two distinct features that differentiate them from the organochlorines. Organophosphate pesticides are generally more acutely toxic to vertebrates than organochlorine pesticides and exert their toxic action by inhibiting the activity of cholinesterase (ChE), one of the vital nervous system enzymes. In addition, organophosphates are chemically unstable (they are all slowly hydrolyzed by water) and thus are less persistent in the environment. It is this latter feature that made them attractive alternatives to the organochlorine pesticides that were used extensively in agriculture from the 1940s to the early 1970s.

With the exception of chlorpyrifos, none of the organophosphates has been included in any of the national fish contaminant monitoring programs evaluated by the EPA 1993 Workgroup and none of these pesticides (including chlorpyrifos) has triggered state fish consumption advisories. All of the organophosphate pesticides have active pesticide registrations and have been recommended for monitoring because they have an EPA Toxicity Classification of I or II (Appendix F), BCFs >300, and a half-life of 30 days or more in the environment and their use profiles suggest they could be potential problems in some agricultural watersheds.

The target organophosphates are used in agriculture throughout the United States, particularly in areas under intensive cultivation (row crops, orchards, fruits, and vegetables). Bioconcentration studies indicate they can accumulate in fish and, because they are known human neurotoxins, the potential exists for human health effects from consuming contaminated fish. For this reason, federal regulations are in effect that set maximum application rates and minimize use near waterbodies. At the time of this writing, no fish consumption advisories for these target analytes have yet been issued; however, state agencies should be aware of special circumstances that could result in their accumulation in fish. In addition to chemical spills and misapplications, heavy and repeated rainfall shortly after application may wash pesticides off of plants and into streams. Signs of acute organophosphate pollution may include erratic swimming behavior in fish or fish kills.

States should contact their appropriate agencies to obtain information on both the historic and current uses of these pesticides. With the exception of ethion, which is used almost exclusively on citrus, the target organophosphates are used on a wide variety of crops. In addition, chlorpyrifos and diazinon have significant uses in domestic and commercial pest control in suburban/urban areas (Robinson et al., 1994). If a state determines that high concentrations of these pesticides may be present in its agricultural watersheds, sampling should be conducted during late spring or early summer within 1 to 2 months following pesticide application to maximize detection of these compounds in fish tissues. In general, the organophosphates are degraded relatively rapidly in the environment and metabolized relatively rapidly by fish, so timing of the sampling program is a more important consideration for this class of pesticides. Additional discussion of appropriate sampling times for fish contaminant monitoring programs is provided in Section 6.1.1.5.

All of the target organophosphates are members of the organothiophosphate group of insecticides. They are all metabolized in the liver to their active form, referred to as an "oxon" (e.g., chlorpyrifos is activated to chlorpyrifos oxon) (Klaasen, 1996). The oxons are approximately 300- to 1,000-fold more toxic than the parent compounds; however, they are also less lipid-soluble than the parent compounds and, therefore, are expected to be less likely to bioaccumulate in fish tissue. In another laboratory study where chlorpyrifos was fed to channel catfish, only chlorpyrifos and its inactive metabolites were found; the oxon was not detected in any tissue (Barron et al., 1991). No information is available on the presence of the oxon metabolites in fish tissue for the other organophosphates.

Note: The potential human toxicity of the organophosphates is undergoing reassessment by EPA at this time as a result of the provisions of the Food Quality Protection Act of 1996. For more information, consult the EPA Office of Pesticide Programs webpage available on the Internet at:
http://www.epa.gov.pesticides/op.

4.3.3.1 Chlorpyrifos—

This organophosphate pesticide was first introduced in 1965 to replace the more persistent organochlorine pesticides (e.g., DDT) (U.S. EPA, 1986c) and has been used for a broad range of insecticide applications (Appendix F). Chlorpyrifos is used primarily to control soil and foliar insects on cotton, peanuts, and sorghum (Worthing, 1991; U.S. EPA, 1986c). Chlorpyrifos is also used to control root-infesting and boring insects on a variety of fruits (e.g., apples, bananas, citrus, grapes), nuts (e.g., almonds, walnuts), vegetables (e.g., beans, broccoli, brussel sprouts, cabbage, cauliflower, peas, and soybeans), and field crops (e.g., alfalfa and corn) (U.S. EPA, 1984c). As a household insecticide, chlorpyrifos has been used to control ants, cockroaches, fleas, and mosquitoes (Worthing, 1991) and is registered for use in controlling subsurface termites in California (U.S. EPA, 1983a). Based on use application, 48 percent of chlorpyrifos use is agricultural and 52 percent is nonagricultural (U.S. EPA, 2000b). Chlorpyrifos is also used by the general public for home, lawn, and garden insect control (ATSDR, 1997).

Note: As a result of the reassessment conducted under the Food Quality Act of 1996, use patterns of chlorpyrifos will change significantly by the end of 2001. In particular, virtually all indoor and outdoor residential use will end, as well as all agricultural use on tomatoes. Agricultural use of chlorpyrifos on apples and grapes will be reduced substantially (U.S. EPA, 2000b).

Chlorpyrifos has a moderate mammalian toxicity and has been assigned to EPA Toxicity Class II based on oral feeding studies (U.S. EPA, 1998d). No carcinogenicity was found in chronic feeding studies with rats, mice, and dogs (U.S. EPA, 1983a). Because chlorpyrifos did not increase the incidence of cancer in feeding studies on rats and mice (U.S. EPA, 1999b, U.S. EPA, 2000b) EPA has classified chlorpyrifos in Group E (Appendix G) (U.S. EPA, 2000b). Experimental evidence indicates this compound bioaccumulates in rainbow trout (BCF from 1,280 to 3,903) (U.S. EPA, 1993a).

Chlorpyrifos has been included in one national monitoring program reviewed by the EPA 1993 Workgroup, the EPA National Study of Chemical Residues in Fish (NSCRF) (see Appendix E). In this study, chlorpyrifos was detected at 26 percent of sites sampled nationally (U.S. EPA, 1992c, 1992d). Eighteen percent of the sites with relatively high concentrations (0.0025 to 0.344 ppm) were scattered throughout the East, Midwest, and in California; the highest mean concentrations detected (0.060 to 0.344 ppm) were found either in agricultural areas or in urban areas with a variety of nearby industrial sources. Maximum, arithmetic mean, and median tissue concentrations (wet weight) of chlorpyrifos were 0.344 ppm, 0.004 ppm, and not detectable, respectively. Mean chlorpyrifos residues from the NSCRF study were highest in bottom feeders such as carp (0.0082 ppm), white sucker (0.0018 ppm), and channel catfish (0.007 ppm) as compared to predator species such as largemouth bass (0.00028 ppm), smallmouth bass (0.00008 ppm), and walleye (0.00004 ppm) (Kuehl et al., 1994). It should be noted that this national study did not specifically target agricultural sites where this pesticide historically had been used or is currently used. Chlorpyrifos residues in fish could be much higher if sampling were targeted for pesticide runoff, especially during the period immediately after field application.

In 1993, no consumption advisories were in effect for chlorpyrifos (RTI, 1993). As of 1998, there were no advisories in effect for this pesticide (U.S. EPA, 1999c). Chlorpyrifos should be considered for inclusion in state fish and shellfish contaminant monitoring programs in areas where its use is or has been extensive. States should contact their appropriate agencies to obtain information on the historic and current uses of this pesticide. Monitoring sites in agricultural watersheds should be reviewed to determine the application rate and acreage where chlorpyrifos is currently used or was used historically. In suburban/urban watersheds, the degree of historic and current use of chlorpyrifos in domestic home and garden applications should be evaluated. Sites in industrial watersheds also should be reviewed to identify historic and current sites of chlorpyrifos production, formulation, or packaging facilities.

4.3.3.2 Diazinon—

Diazinon is a phosphorothiate insecticide and nematicide that was first registered in 1952 for control of soil insects and pests of fruits, vegetables, tobacco, forage, field crops, range, pasture, grasslands, and ornamentals; for control of cockroaches and other household insects; for control of grubs and nematodes in turf; as a seed treatment; and for fly control (U.S. EPA, 1986d). Diazinon is also used by the general public for home, lawn, and garden insect control (Appendix F) (ATSDR, 1996).

Diazinon is moderately toxic to mammals and has been assigned to EPA Toxicity Class II based on oral toxicity tests (U.S. EPA, 1998d) (Appendix F). Diazinon was not found to be carcinogenic in rats and mice (ATSDR, 1996). Because of inadequate evidence of carcinogenicity, EPA has classified diazinon as "not likely to be a human carcinogen") (Appendix G) (U.S. EPA, 1998d). This compound is also highly toxic to birds, fish, and other aquatic invertebrates (U.S. EPA, 1986d).

Diazinon was not included in any national fish contaminant monitoring program evaluated by the EPA 1993 Workgroup (Appendix E). Experimental evidence indicates this compound accumulates in trout (BCF of 542) (U.S. EPA, 1993a).

In 1993, no consumption advisories were in effect for diazinon (RTI, 1993). As of 1998, there were no advisories in effect for this pesticide (U.S. EPA, 1999c). Diazinon should be considered for inclusion in state fish and shellfish contaminant monitoring programs in areas where its use is or has been extensive. States should contact their appropriate agencies to obtain information on the historic and current uses of this pesticide. Monitoring sites in agricultural watersheds should be reviewed to determine the application rate and acreage where diazinon is currently used or was used historically. In suburban/urban watersheds, the degree of historic and current use of diazinon in domestic home and garden applications should be evaluated. Sites in industrial watersheds should be reviewed to identify historic and current sites of diazinon production, formulation, or packaging facilities.

4.3.3.3 Disulfoton—

Disulfoton is a multipurpose systemic insecticide and acaricide first registered in 1958 for use as a side dressing, broadcast, or foliar spray in the seed furrow to control many insect and mite species and as a seed treatment for sucking insects (Appendix F) (*Farm Chemicals Handbook*, 1989).

Disulfoton is highly toxic to all mammalian systems and has been assigned to EPA Toxicity Class I on the basis of all routes of exposure (U.S. EPA, 1998d).

Disulfoton was not found to be carcinogenic in dogs, rats, or mice (ATSDR, 1995). Because of inadequate evidence of carcinogenicity, EPA has classified disulfoton as Group E, evidence of noncarcinogenicity for humans (Appendix G) (U.S. EPA, 1999b).

Disulfoton was not included in any national fish contaminant monitoring program evaluated by the EPA 1993 Workgroup (Appendix E). Experimental evidence indicates this compound accumulates in fish (BCF from 460 to 700) (U.S. EPA, 1993a).

In 1993, no consumption advisories were in effect for disulfoton (RTI, 1993). As of 1998, there were no advisories in effect for this pesticide (U.S. EPA, 1999c). Disulfoton should be considered for inclusion in state fish and shellfish contaminant monitoring programs in areas where its use is or has been extensive. States should contact their appropriate agencies to obtain information on the historic and current uses of this pesticide. Monitoring sites in agricultural watersheds should be reviewed to determine the application rate and acreage where disulfoton currently is used or was used historically. Sites in industrial watersheds also should be reviewed to identify historic and current sites of disulfoton production, formulation, or packaging facilities.

4.3.3.4 Ethion—

Ethion is a multipurpose insecticide and acaricide that has been registered since 1965 for use on a wide variety of nonfood crops (turf, evergreen plantings, and ornamentals), food crops (seed, fruit, nut, fiber, grain, forage, and vegetables), and for domestic outdoor uses around dwellings and for lawns (Appendix F) (*Farm Chemicals Handbook,* 1989). Application to citrus crops accounts for 86 to 89 percent of the ethion used in the United States. The remaining 11 to 14 percent is applied to cotton and a variety of fruit and nut trees and vegetables. Approximately 55 to 70 percent of all domestically produced citrus fruits are treated with ethion (U.S. EPA, 1989e).

Acute oral toxicity studies have shown that technical-grade ethion is moderately toxic to mammals (EPA Toxicity Class II) (U.S. EPA, 1998d). Ethion was not found to be carcinogenic in rats and mice (U.S. EPA, 1989e). EPA has classified ethion in Group E–evidence of noncarcinogenicity for humans (Appendix G) (U.S. EPA, 1999b).

Ethion was not included in any national fish contaminant monitoring program evaluated by the EPA 1993 Workgroup (Appendix E). Experimental evidence indicates this compound accumulates in bluegill sunfish (BCF from 880 to 2,400) (U.S. EPA, 1993a).

In 1993, no consumption advisories were in effect for ethion (RTI, 1993). As of 1998, there were no advisories in effect for this pesticide (U.S. EPA, 1999c). Ethion should be considered for inclusion in state fish and shellfish contaminant monitoring programs in areas where its use is or has been extensive. States should contact their appropriate agencies to obtain information on the historic and current uses of this pesticide. Monitoring sites in agricultural watersheds should be reviewed to determine the application rate and acreage where ethion currently is used or was used historically. In suburban/urban watersheds, the degree of historic and current use of ethion in domestic home and garden applications should be evaluated. Sites in industrial watersheds also should be reviewed to identify historic and current sites of ethion production, formulation, or packaging facilities.

4.3.3.5 Terbufos—

Terbufos is a systemic organophosphate insecticide and nematicide registered in 1974 principally for use on corn, sugar beets, and grain sorghum. The primary method of application involves direct soil incorporation of a granular formulation (*Farm Chemicals Handbook*, 1989). Two soil metabolites of terbufos, terbufos sulfoxide and terbufos sulfone, are also toxic to humans and are found at sites where terbufos has been applied (U.S. EPA, 1995)

Terbufos is highly toxic to humans and has been assigned to EPA Toxicity Class I (U.S. EPA, 1998d) (Appendix F). Terbufos was not found to be carcinogenic in rats and mice (U.S. EPA, 1995j). EPA has assigned terbufos to carcinogenicity classification E, evidence of noncarcinogenicity for humans (U.S. EPA, 1998d) (Appendix G). Terbufos is also highly toxic to birds, fish, and other aquatic invertebrates (U.S. EPA, 1985d).

Terbufos was not included in any national fish contaminant monitoring program evaluated by the EPA 1993 Workgroup (Appendix E). Experimental evidence indicates this compound accumulates in fish (BCF from 320 to 1,400) (U.S. EPA, 1993a).

In 1993, no consumption advisories were in effect for terbufos (RTI, 1993). As of 1998, there were no advisories in effect for this pesticide (U.S. EPA, 1999c). Terbufos and its toxic metabolites should be considered for inclusion in state fish and shellfish contaminant monitoring programs in areas where its use is or has been extensive. States should contact their appropriate agencies to obtain information on the historic and current uses of this pesticide. Monitoring sites in agricultural watersheds should be reviewed to determine the application rate and acreage where terbufos currently is used or was used historically. Sites in industrial watersheds also should be reviewed to identify historic and current sites of terbufos production, formulation, or packaging facilities.

4.3.4 Chlorophenoxy Herbicides

Chlorophenoxy herbicides, which include oxyfluorfen, are nonselective foliar herbicides that are most effective in hot weather (Ware, 1978).

4.3.4.1 Oxyfluorfen—

Oxyfluorfen is a pre- and postemergence herbicide with an active registration that has been registered since 1979 for use to control a wide spectrum of annual broadleaf weeds and grasses in apples, artichokes, corn, cotton, jojoba, tree fruits, grapes, nuts, soybeans, spearmint, peppermint, and certain tropical plantation and ornamental crops (Appendix F) (*Farm Chemicals Handbook*, 1989).

Oxyfluorfen is of low toxicity to mammals (oral LD_{50} in rats >5,000 mg/kg) and has been assigned to EPA Toxicity Class IV (U.S. EPA, 1998d) (Hayes and Lawes, 1991). There is also evidence of carcinogenicity (liver tumors) in mice (U.S. EPA, 1993a) and therefore oxyfluorfen has been classified by EPA as a possible human carcinogen (C) (Appendix G) (U.S. EPA, 1999b).

Oxyfluorfen was not included in any national fish contaminant monitoring program evaluated by the EPA 1993 Workgroup (Appendix E). Experimental evidence indicates this herbicide accumulates in bluegill sunfish (BCF from 640 to 1,800) (U.S. EPA, 1993a).

In 1993, no consumption advisories were in effect for oxyfluorfen (RTI, 1993). As of 1998, there were no advisories in effect for this herbicide (U.S. EPA, 1999c). Oxyfluorfen should be considered for inclusion in state fish and shellfish contaminant monitoring programs in areas where its use is or has been extensive. States should contact their appropriate agencies to obtain information on the historic and current uses of this pesticide. Monitoring sites in agricultural watersheds should be reviewed to determine the application rate and acreage where oxyfluorfen currently is used or was used historically. Sites in industrial watersheds also should be reviewed to identify historic and current sites of oxyfluorfen production, formulation, or packaging facilities.

4.3.5 Polycyclic Aromatic Hydrocarbons

PAHs are base/neutral organic compounds that have a fused ring structure of two or more benzene rings. PAHs are also commonly referred to as polynuclear aromatic hydrocarbons (PNAs). PAHs with two to five benzene rings (i.e., 10 to 24 skeletal carbons) are generally of greatest concern for environmental and human health effects (Benkert, 1992). These PAHs have been identified as the most important with regard to human exposure (ATSDR, 1995):

- Acenaphthene
- Acenaphthylene
- Anthracene
- Benz[*a*]anthracene
- Benzo[*a*]pyrene
- Benzo[e]pyrene
- Benzo[*b*]fluoranthene
- Benzo[*k*]fluoranthene
- Benzo[j]fluoranthene
- Benzo[*g,h,i*]perylene
- Chrysene
- Dibenz[*a,h*]anthracene
- Fluoranthene
- Fluorene
- Indeno[*1,2,3-cd*]pyrene
- Phenanthrene

- Pyrene.

The metabolites of many of the high-molecular-weight PAHs (e.g., benz[*a*] anthracene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, chrysene, dibenz[*a,h*]anthracene, indeno[*1,2,3-cd*]pyrene, and benzo[*g,h,i*]perylene) have been shown in laboratory test systems to be carcinogens, cocarcinogens, teratogens, and/or mutagens (Moore and Ramamoorthy, 1984; ATSDR 1995). Benzo[*a*]pyrene, one of the most widely occurring and potent PAHs, and six other PAHs (e.g., benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, chrysene, dibenz[*a,h*]anthracene, indeno[*1,2,3-cd*]pyrene) have been classified by EPA as probable human carcinogens (B2) (IRIS, 1999). Evidence for the carcinogenicity of PAHs in humans comes primarily from epidemiologic studies that have shown an increased mortality due to lung cancer in humans exposed to PAH-containing coke oven emissions, roof-tar emissions, and cigarette smoke (ATSDR, 1995).

PAHs are ubiquitous in the environment and usually occur as complex mixtures with other toxic chemicals. They are components of crude and refined petroleum products and of coal. They are also produced by the incomplete combustion of organic materials. Many domestic and industrial activities involve pyrosynthesis of PAHs, which may be released into the environment in airborne particulates or in solid (ash) or liquid byproducts of the pyrolytic process. Domestic activities that produce PAHs include cigarette smoking, home heating with wood or fossil fuels, waste incineration, broiling and smoking foods, and use of internal combustion engines. Industrial activities that produce PAHs include wood preserving, coal coking; production of carbon blacks, creosote, and coal tar; petroleum refining; synfuel production from coal; and use of Soderberg electrodes in aluminum smelters and ferrosilicum and iron works (ATSDR, 1995; Neff, 1985). Historic coal gasification sites have also been identified as significant sources of PAH contamination (ATSDR, 1995).

Major sources of PAHs found in marine and fresh waters include biosynthesis (restricted to anoxic sediments), spillage and seepage of fossil fuels, discharge of domestic and industrial wastes, atmospheric deposition, and runoff (Neff, 1985). Urban stormwater runoff contains PAHs from leaching of asphalt roads, wearing of tires, deposition from automobile exhaust, and oiling of roadsides and unpaved roadways with crankcase oil (ATSDR, 1995; MacKenzie and Hunter, 1979). Solid PAH-containing residues from activated sludge treatment facilities have been disposed of in landfills or in the ocean (ocean dumping was banned in 1989). Although liquid domestic sewage contains <1 µg/L total PAH, the total PAH content of industrial sewage is 5 to 15 µg/L (Borneff and Kunte, 1965) and that of sewage sludge is 1 to 30 mg/kg (Grimmer et al., 1978; Nicholls et al., 1979).

In most cases, there is a direct relationship between PAH concentrations in river water and the degree of industrialization and human activity in the surrounding watersheds. Rivers flowing through heavily industrialized areas may contain 1 to

5 ppb total PAH, compared to unpolluted river water, ground water, or seawater that usually contains less than 0.1 ppb PAH (Neff, 1979).

PAHs can accumulate in aquatic organisms from water, sediments, and food. BCFs of PAHs in fish, crustaceans, and bivalves have frequently been reported to be in the range of 12 to 9,200 for fish, 200 to 134,248 for crustaceans, and 8 to 242 for bivalves based on short-term exposure studies typically less than 7 days duration (Eisler, 1987). In general, bioconcentration was greater for the higher molecular weight PAHs than for the lower molecular weight PAHs. Biotransformation by the mixed function oxidase system in the fish liver can result in the formation of carcinogenic and mutagenic intermediates, and exposure to PAHs has been linked to the development of tumors in fish (Eisler, 1987). The ability of fish to metabolize PAHs probably explains why benzo[*a*]pyrene frequently is not detected or is found only at very low concentrations in fish from areas heavily contaminated with PAHs (Varanasi and Gmur, 1980, 1981).

Sediment-associated PAHs can be accumulated by bottom-dwelling invertebrates and fish (Eisler, 1987). For example, Great Lakes sediments containing elevated levels of PAHs were reported by Eadie et al. (1983) to be the source of the body burdens of the compounds in bottom-dwelling invertebrates. Similarly, Varanasi et al. (1985) found that benzo[*a*]pyrene was accumulated in fish, amphipod crustaceans, shrimp, and clams when estuarine sediment was the source of the compound. Approximate tissue-to-sediment ratios were 0.6 to 1.2 for amphipods, 0.1 for clams, and 0.05 for fish and shrimp. Although fish and most crustaceans evaluated to date have the mixed function oxidase system required for biotransformation of PAHs, many molluscs lack this system and are unable to metabolize PAHs efficiently (Varanasi et al., 1985). More important, PAHs induce mixed function oxidase enzymes (and thus their own biotransformation) in fish and other vertebrates, but not in molluscs and crustaceans (Stegeman and Lech, 1991). The resulting dramatic difference in biotransformation means that in PAH-contaminated waters, fish may show little or no accumulation of PAHs, while bivalve molluscs and crustaceans are heavily contaminated. Varanasi et al. (1985) ranked benzo[*a*]pyrene metabolism by aquatic organisms as follows: fish > shrimp > amphipod crustaceans > clams. Half-lives for elimination of PAHs in fish ranged from less than 2 days to 9 days (Niimi, 1987). NAS (1991) reported that PAH contamination in bivalves has been found in all areas of the United States. If PAHs are selected as a target analyte to be monitored at a site, primary preference should be given to selection of a bivalve mollusc (clam, oyster, mussel) as the target species, secondary preference should be given to a crustacean (shrimp, lobster, crab) (if available), and finfish should be given the lowest priority for selection as the target species. This ranking of the preferred target species for PAH analysis assumes that a bivalve mollusc and crustacean are available at the sampling site and that these species are eaten by the consumer population of concern.

In 1993, three states (Massachusetts, Michigan, and Ohio) had issued advisories for PAH contamination in finfish (RTI, 1993). As of 1998, there were five advisories in effect in four states (Massachusetts, Michigan, Ohio, and

Washington) for PAHs (U.S. EPA, 1999c). Monitoring sites in industrial and suburban/urban watersheds should be reviewed to identify current and historic sites of waste incinerators, coal gasification facilities, petroleum refineries, and creosote, coal tar, coal coking, and wood preservative facilities that are potential sources for PAH releases to the environment. Sites of petroleum spills should also be reviewed.

The EPA and others have developed a relative potency estimate approach for the PAHs (Nisbet and LaGoy, 1992; U.S. EPA, 1993c). Using this approach, the cancer potency of 14 carcinogenic PAHs can be estimated based on their relative potency to benzo[*a*]pyrene. Toxicity equivalence factors (TEF) for benzo[a]pyrene and the other 14 PAHs based on carcinogenicity are discussed in Section 5.3.2.4.

Although several PAHs have been classified as probable human carcinogens (Group B2), benzo[*a*]pyrene is the only PAH for which an oral CSF is currently available in IRIS (1999). It is recommended that, in both screening and intensive studies, tissue samples be analyzed for benzo[*a*]pyrene and the other 14 PAHs for which TEFs are available and that the relative potencies given for these PAHs (Nisbet and LaGoy, 1992; U.S. EPA, 1993c) be used to calculate a potency equivalency concentration for each sample for comparison with the recommended SVs for benzo[*a*]pyrene (see Section 5.3.2.4).

4.3.6 Polychlorinated Biphenyls (Total)

PCBs are base/neutral compounds that are formed by the direct chlorination of biphenyl. PCBs are closely related to many chlorinated hydrocarbon pesticides (e.g., DDT, dieldrin, and aldrin) in their chemical, physical, and toxicologic properties and in their widespread occurrence in the aquatic environment (Nimmo, 1985). There are 209 different PCB compounds, termed congeners, based on the possible chlorine substitution patterns. In the United States, mixtures of various PCB congeners were formulated for commercial use under the trade name Aroclor on the basis of their percent chlorine content. For example, a common PCB mixture, Aroclor 1254, has an average chlorine content of 54 percent by weight (Nimmo, 1985).

Unlike the organochlorine pesticides, PCBs were never intended to be released directly into the environment; most uses were in closed industrial systems. Important properties of PCBs for industrial applications include thermal stability, fire and oxidation resistance, and solubility in organic compounds (Hodges, 1977). PCBs were used as insulating fluids in electrical transformers and capacitors, as plasticizers, as lubricants, as fluids in vacuum pumps and compressors, and as heat transfer and hydraulic fluids (Hodges, 1977; Nimmo, 1985). Although use of PCBs as a dielectric fluid in transformers and capacitors was generally considered a closed-system application, the uses of PCBs, especially during the 1960s, were broadly expanded to many open systems where losses to the environment were likely. Heat transfer systems, hydraulic fluids in die cast machines, and uses in specialty inks are examples of more open-ended applications that resulted in serious contamination in fish near industrial discharge points (Hesse, 1976).

Although PCBs were once used extensively by industry, their production and use in the United States were banned by the EPA in July 1979 (Miller, 1979). Prior to 1979, the disposal of PCBs and PCB-containing equipment was not subject to federal regulation. Prior to regulation, of the approximately 1.25 billion pounds purchased by U.S. industry, 750 million pounds (60 percent) were still in use in capacitors and transformers, 55 million pounds (4 percent) had been destroyed by incineration or degraded in the environment, and over 450 million pounds (36 percent) were either in landfills or dumps or were available to biota via air, water, soil, and sediments (Durfee et al., 1976).

PCBs are extremely persistent in the environment and are bioaccumulated throughout the food chain (Eisler, 1986; Worthing, 1991). There is evidence that PCB health risks increase with increased chlorination because more highly chlorinated PCBs are retained more efficiently in fatty tissues (IRIS, 1999). However, individual PCB congeners have widely varying potencies for producing a variety of adverse biological effects including hepatotoxicity, cardiovascular toxicity, developmental toxicity, immunotoxicity, neurotoxicity, and carcinogenicity. The non-ortho-substituted coplanar PCB congeners, and some of the mono-ortho-substituted congeners, have been shown to exhibit "dioxin-like" effects (Golub et al., 1991; Kimbrough and Jensen, 1989; McConnell, 1980; Poland and Knutson, 1982; Safe, 1985, 1990; Tilson et al., 1990; U.S. EPA 1993c; Van den Berg et al., 1998). The neurotoxic effects of PCBs appear to be associated with some degree of ortho-chlorine substitution. There is increasing evidence that many of the toxic effects of PCBs result from alterations in hormonal function. Because PCBs can act directly as hormonal agonists or antagonists, PCB mixtures may have complex interactive effects in biological systems (Korach et al., 1988; Safe et al., 1991; Shain et al., 1991; U.S. EPA, 1993c). Because of the lack of sufficient toxicologic data, EPA has not developed quantitative estimates of health risk for specific congeners; however, 12 dioxin-like congeners have been assigned TEFs and may be evaluated as contributing to dioxin health risk (Van den Berg et al., 1998). PCB mixtures have been classified as probable human carcinogens (Group B2) (Appendix G) (IRIS, 1999; U.S. EPA, 1988a).

PCB mixtures have been shown to cause adverse developmental effects in experimental animals (ATSDR, 1998b). Data are inconclusive in regard to developmental effects in humans. Several studies in humans have suggested that PCB exposure may cause adverse developmental effects in children and in developing fetuses (ATSDR, 1998b) These include lower IQ scores (Jacobson and Jacobson, 1996), low birth weight (Rylander et al., 1998), and lower behavior assessment scores (Lonky et al., 1996). However, study limitations, including lack of control for confounding variables, deficiencies in the general areas of exposure assessment, selection of exposed and control subjects, and the comparability of exposed and control samples obscured interpretation of these results (ATSDR, 1998b).

PCBs, total or as Aroclors, have been included in seven major fish contaminant monitoring programs evaluated by the 1993 EPA Workgroup (Appendix E). A summary of the U.S. Fish and Wildlife Service National Contaminants Biomonitoring Program (NCBP) data from 1976 through 1984 indicated a significant downward trend in the geometric mean concentration (wet weight basis) of total PCBs (from 0.89 ppm in 1976 to 0.39 ppm in 1984); however, PCB residues in fish tissue remain widespread, being detected at 91 percent of the sites monitored in 1984 (Schmitt et al., 1990). Maximum total PCB tissue residue concentrations during this same period also declined, from 70.6 ppm in 1976 to 6.7 ppm in 1984. Coinciding declines in tissue residue concentrations of three Aroclors (1248, 1254, and 1260) were also observed. Kidwell et al. (1995) conducted an analysis of all 1984-1985 data from the NCBP study on the three Aroclors in bottom-feeding and predatory fish species. These authors reported there was no significant difference in residues in these two trophic groups of fish for Aroclor 1248 and 1254; however, there were significantly higher concentrations of Aroclor 1260 in predator species as compared to bottom feeders. Mean tissue concentrations of Aroclor 1248, 1254, and 1260 were 0.06 ± 0.32, 0.21 ± 0.39, and 0.14 ± 0.24 ppm, respectively, for bottom feeders (e.g., carp, white suckers, and channel catfish) and 0.08 ± 0.31, 0.35 ± 0.69, and 0.23 ± 0.38 ppm, respectively, for predator species (e.g., rainbow, brown, brook, and lake trout, largemouth bass, and walleye).

Total PCBs also were detected at 91 percent of 374 sites surveyed in the EPA National Study of Chemical Residues in Fish (NSCRF) (U.S. EPA, 1992c, 1992d). Maximum, arithmetic mean, and median total PCB concentrations (wet weight) reported were 124, 1.89, and 0.209 ppm, respectively. As is shown in Table 4-6, the tri-, tetra-, penta-, hexa-, and heptachlorobiphenyls were detected in fish tissue samples at >50 percent of the NSCRF sites. Mean tissue concentrations were highest for the tetra- and pentachlorobiphenyls with concentrations of 0.696, 0.565, and 0.356 ppm, respectively. The median fish tissue concentrations were highest for the hexa- followed by the pentachlorobiphenyls with concentrations of 0.077 and 0.072 ppm, respectively.

With respect to sources of these compounds, PCBs were detected in all parts of the country with the highest concentrations being associated with paper mills, refinery/other industry sites, Superfund sites, wood preserving facilities, and industrial/urban areas. Mean total PCB concentrations from the NSCRF study were highest in bottom feeders (whole fish) such as carp (2.94 ppm), white sucker (1.7 ppm), and channel catfish (1.3 ppm) as compared to predator species (fillet samples) such as largemouth bass (0.23 ppm), smallmouth bass (0.5 ppm), and walleye (0.37 ppm) (Kuehl et al., 1994).

In 1993, PCB contamination in fish and shellfish resulted in the issuance of 328 advisories in 31 states and the U.S. territory of American Samoa (Figure 4-3) (RTI, 1993). As of 1998, there were 679 advisories in effect in 36 states and the U.S. territory of American Samoa for this compound (Figure 4-3) (U.S. EPA, 1999c.). In addition, two states (Indiana and New York) and the District of Columbia had statewide advisories for PCBs in freshwater rivers and/or lakes.

Table 4-6. Summary of PCBs Detected in Fish Tissue[a] as Part of the National Study of Chemical Residues in Fish

Congener group	% sites where detected	Maximum	Mean	Median
Monochlorobiphenyl	13.8	0.235	0.001	ND
Dichlorobiphenyl	30.7	5.072	0.021	ND
Trichlorobiphenyl	57.5	18.344	0.150	0.002
Tetrachlorobiphenyl	72.4	60.764	0.696	0.023
Pentachlorobiphenyl	86.7	29.578	0.565	0.072
Hexachlorobiphenyl	88.7	8.862	0.356	0.077
Heptachlorobiphenyl	69.1	1.850	0.097	0.017
Octachlorobiphenyl	34.8	0.593	0.017	ND
Nonachlorobiphenyl	9.7	0.413	0.003	ND
Decachlorobiphenyl	3.3	0.038	0.001	0.003
Total PCBs*	91.4	-----	1.898	0.209

* The sum of the concentrations of compounds with 1 to 10 chlorines.

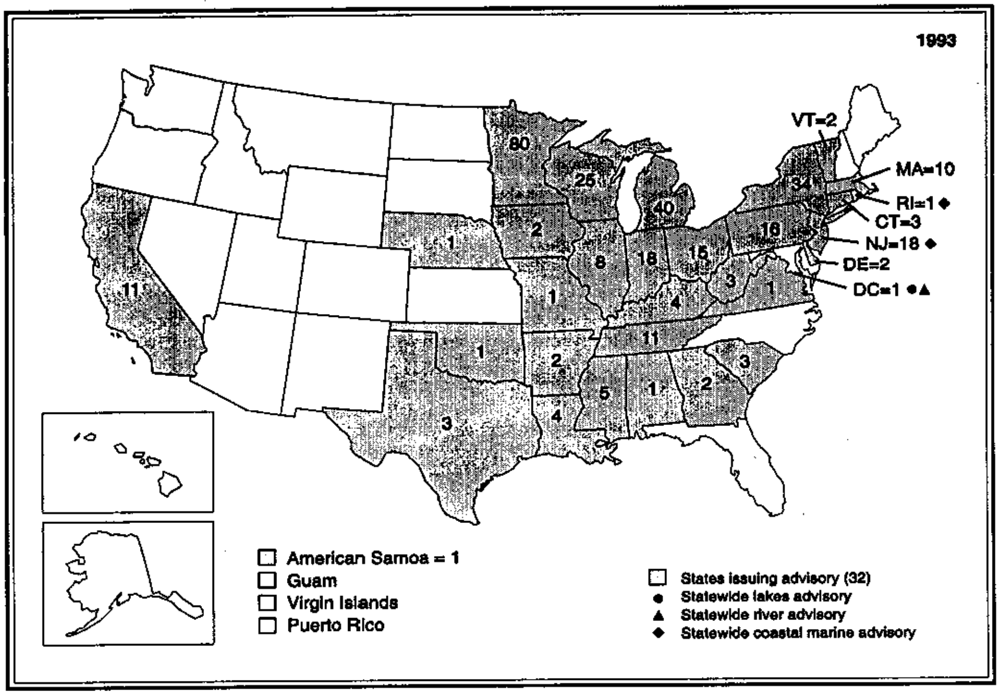
[a] Concentrations are in ppm (µg/g) wet weight basis.

Source: U.S. EPA, 1992c, 1992d.

One state, Connecticut, had an advisory for all its coastal estuarine waters (Long Island Sound), and five states (Massachusetts, New Hampshire, New Jersey, New York, and Rhode Island) had advisories in effect for all of their coastal marine waters (U.S. EPA, 1999c). Monitoring sites in industrial and suburban/urban watersheds should be reviewed to identify sites of historical Aroclor production facilities, current and historic transformer manufacturing or refurbishing facilities, current and historic landfill and Superfund sites, and current and historic incineration or combustion facilities that are potential sources for PCB releases to the environment.

PCBs may be analyzed quantitatively as Aroclor equivalents, as homologue groups, or as individual congeners. Historically, Aroclor analysis has been performed by most laboratories. This procedure can, however, result in significant error in determining total PCB concentrations (Schwartz et al., 1987; Cogliano, 1998; U.S. EPA, 1996) and in assessing the toxicologic significance of PCBs, because it is based on the assumption that distribution of PCB congeners in environmental samples and parent Aroclors is similar.

The distribution of PCB congeners in Aroclors is, in fact, altered considerably by physical, chemical, and biological processes after release into the environment, particularly when the process of biomagnification is involved (Norstrom, 1988; Oliver and Niimi, 1988; Smith et al., 1990; U.S. EPA, 1996). Aquatic environmental studies indicate that the chlorine content of PCBs increases at higher trophic levels (Bryan et al., 1987; Kubiak et al., 1989; Oliver and Niimi, 1988).



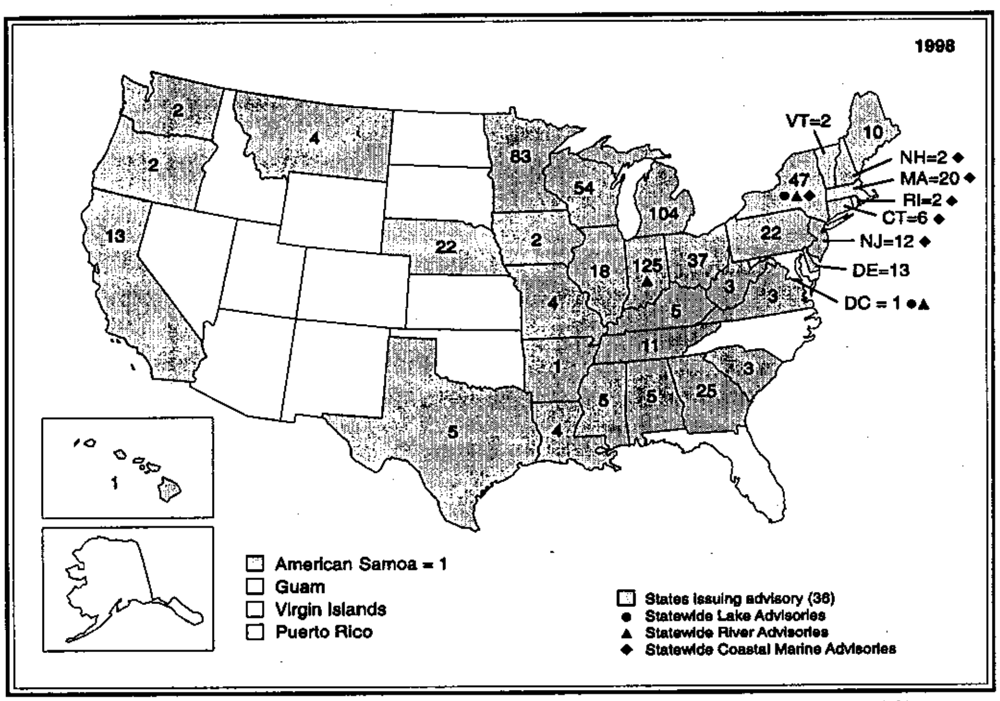


Figure 4-3. States issuing fish and shellfish advisories for PCBs.

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The available data indicate that bioaccumulated PCBs are more toxic and more persistent than the original Aroclor mixtures (Cogliano, 1998). Consequently, analysis of homologue groups or congeners should provide a more accurate determination of total PCB concentrations than Aroclor analysis. PCB concentrations derived from Aroclor methods may underestimate total PCBs. In one study, the Delaware Department of National Resources and Environmental Control (DDNREC) compared results of PCBs in six fish samples as determined by Aroclor analysis (Method 608) and homologue analysis (Method 680) (Greene, 1992). On the average, the homologue method gave PCB estimates that were 230 percent higher than the results from the Aroclor method.

The major advantage to analyzing PCBs as Aroclor equivalents is that the analysis is relatively inexpensive (approximately $200 - $500) compared to analyzing PCBs as individual congeners (approximately $800-$2000). Another disadvantage to analyzing PCBs as individual congeners is that the large number of PCB congeners presents analytical difficulties. Quantitation of individual PCB congeners is relatively time-consuming. EPA has not issued a standard method for PCB congener analysis but has developed a draft method (1668) for dioxin-like congeners (U.S. EPA 1997a). This method is likely to be revised to include the capability to detect all 209 PCB congeners. Currently, only a few laboratories have the capability or expertise to perform congener analyses. Both NOAA (MacLeod et al., 1985; NOAA, 1989b) and the EPA Narragansett Research Laboratory conduct PCB congener analyses. Some states currently conduct both congener and Aroclor analysis; however, most states routinely perform only Aroclor analysis. Analytical methods for congener analysis are discussed in the following references: Cogliano, 1998; Huckins et al., 1988; Kannan et al., 1989; Lake et al., 1995; MacLeod et al., 1985; Maack and Sonzogni, 1988; Mes and Weber, 1989; NOAA, 1989b; Skerfving et al., 1994; Smith et al., 1990; Tanabe et al., 1987; U.S. EPA, 1996.

For the purposes of conducting a risk assessment to determine whether tissue residues exceed potential levels of public health concern in fish and shellfish monitoring programs, analysis of PCB congener or Aroclor equivalents is acceptable. However, because of their lower cost, Aroclor analyses may be the more cost-effective method to use if a large number of samples are analyzed for PCB contamination.

States are encouraged to develop the capability to perform PCB congener analysis. When congener analysis is conducted, at a minimum the 18 congeners recommended by NOAA (shown in Table 4-7) should be analyzed and summed to determine a total PCB concentration according to the approach used by NOAA (1989b). States may wish to consider including additional congeners based on site-specific considerations. PCB congeners of potential environmental importance identified by McFarland and Clarke (1989) and dioxin-like congeners identified by Van den Berg et al. (1998) also are listed in Table 4-7. Lake et al. (1995) and Oliver and Niimi (1988) included more than 80 congeners in their analyses of PCB patterns in water, sediment, and aquatic organisms. A recent study conducted by the DDNREC (Greene, 1999) analyzed for 75 congeners in

Table 4-7. Polychlorinated Biphenyl (PCB) Congeners Recommended for Quantitation as Potential Target Analytes

PCB Congener[a,b]	NOAA[c]	McFarland and Clarke (1989) Highest priority[d]	McFarland and Clarke (1989) Second priority[e]	Dioxin-Like PCBs[f]
2,4' diCB	8			
2,2',5 triCB	18		18	
2,4,4' triCB	28		37	
3,4,4' triCB				
2,2'3,5' tetraCB	44		44	
2,2'4,5' tetraCB	52	77	49	77
2,2',5,5' tetraCB	66		52	81
2,3',4,4' tetraCB	77		70	
2,3',4',5 tetraCB			74	
2,4,4',5 tetraCB			81	
3,3',4,4' tetraCB				
3,4,4',5 tetraCB				
2,2',3,4,5' pentaCB		87		
2,2',3,4',5 pentaCB	101	90	114	105
2,2',4,5,5' pentaCB	105	101	119	114
2,3,3',4,4' pentaCB	118	105	123	118
2,3,4,4',5 pentaCB	126	118		123
2,3',4,4',5 pentaCB		126		126
2,3',4,4',6 pentaCB				
2',3,4,4',5 pentaCB				
3,3',4,4',5 pentaCB				
2,2',3,3',4,4' hexaCB	128	128		
2,2',3,4,4',5' hexaCB	138	138	151	156
2,2',3,5,5',6 hexaCB	153	153	157	157
2,2',4,4',5,5' hexaCB	169	156	158	167
2,3,3',4,4',5 hexaCB		169	167	169
2,3,3',4,4',5 hexaCB			168	
2,3,3',4,4',6 hexaCB				
2,3',4,4',5,5' hexaCB				
2,3',4,4',5',6 hexaCB				
3,3',4,4',5,5' hexaCB				
2,2',3,3',4,4',5 heptaCB	170	170		
2,2',3,4,4',5,5' heptaCB	180	180	187	189
2,2',3,4,4',5',6 heptaCB	187	183	189	
2,2',3,4,4',6,6' heptaCB		184	201	
2,2',3,4',5,5',6 heptaCB		195		
2,3,3',4,4',5,5' heptaCB				
2,2',3,3',4,4',5,6 octaCB				
2,2',3,3',4,5',6,6' octaCB				
2,2',3,3',4,4',5,5',6 nonaCB		206		
2,2',3,3',4,4',5,5',6,6' decaCB		209		

[a] Congeners recommended for quantitation, from dichlorobiphenyl (diCB) through decachlorobiphenyl (decaCB).
[b] Congeners are identified in each column by their International Union of Pure and Applied Chemistry (IUPAC) number, as referenced in Ballschmitter and Zell (1980) and Mullin et al. (1984).
[c] EPA recommends that these 18 congeners be summed to determine total PCB concentration (NOAA, 1989b).
[d] PCB congeners having highest priority for potential environmental importance based on potential for toxicity, frequency of occurrence in environmental samples, and relative abundance in animal tissues.
[e] Congeners having second priority for potential environmental importance based on potential for toxicity, frequency of occurrence in environmental samples, and relative abundance in animal tissues.
[f] Van den Berg et al., 1998.

fish tissue. Of the 75 congeners, 40 were detected in every fish sample and 20 other congeners were detected in at least half the samples. The DDNREC concluded that a comprehensive target congener list is needed to account for total PCBs in environmental samples because most of the congeners contributed less than 5 percent of the total PCBs.

The EPA Office of Water recommends that PCBs be analyzed as either congeners or Aroclors, with total PCB concentrations reported as the sum of the individual congeners or the sum of the individual Aroclors. If a congener analysis is conducted, the 12 dioxin-like congeners identified in Table 4-7 may be evaluated separately as part of the dioxin risk (see Section 4.3.7). The recommendation is intended to allow states flexibility in PCB analysis and to encourage the continued development of reliable databases of PCB congener and Aroclor equivalents concentrations in fish and shellfish tissue in order to increase our understanding of the mechanisms of action and toxicities of these chemicals. The rationale for, and the uncertainties of, this recommended approach are discussed further in Section 5.3.2.6.

4.3.7 Dioxins and Dibenzofurans

Note: At this time, EPA's Office of Research and Development is reevaluating the potency of dioxins and dibenzofurans. Information provided here as well as information in Section 5.3.2.7 related to calculating TEQs and SVs for dioxins/furans has been modified since the second edition of this Volume 1 guidance was published, but is subject to change pending the results of this reevaluation.

The polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are included as target analytes primarily because of the extreme potency of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD). Extremely low doses of this homologue have been found to elicit a wide range of toxic responses in animals, including carcinogenicity, teratogenicity, fetotoxicity, reproductive dysfunction, and immunotoxicity (U.S. EPA, 1987d). This compound is the most potent animal carcinogen evaluated by EPA, and EPA has determined that there is sufficient evidence to conclude that 2,3,7,8-TCDD is a probable human carcinogen (B2) (HEAST, 1997). Concern over the health effects of 2,3,7,8-TCDD is increased because of its persistence in the environment and its high potential to bioaccumulate (U.S. EPA, 1987d). As of 1998, the TEF value for 1,2,3,7,8-PeCDD was changed from 0.5 to 1.0, giving 1,2,3,7,8-PeCDD and 2,3,7,8-TCDD the same toxicity equivalency factor (Van den Berg et al., 1998). 1,2,3,7-8-PeCDD is also one of the congeners that is bioaccumulated by fish (U.S. EPA, 1992c, 1992d).

Because dioxin/furan contamination is found in proximity to industrial sites (e.g., bleached kraft paper mills or facilities handling 2,4,5-trichlorophenoxyacetic acid [2,4,5-T], 2,4,5-trichlorophenol [2,4,5-TCP], and/or silvex), and municipal or industrial combustors and incinerators (U.S. EPA, 1987d), it is recommended that each state agency responsible for monitoring include these compounds as target analytes on a site-specific basis based on the presence of potential sources and results of any environmental (water, sediment, soil, air) monitoring performed in areas adjacent to these sites. All states should maintain a current awareness of potential dioxin/furan contamination, including contamination from the 12 coplanar PCBs that exhibit dioxin-like effects.

Fifteen dioxin and dibenzofuran congeners have been included in two major fish contaminant monitoring programs; however, one congener, 2,3,7,8-TCDD, has been included in six national monitoring programs (Appendix E). Six dioxin congeners and nine dibenzofuran congeners were measured in fish tissue samples in the EPA National Study of Chemical Residues in Fish. The various dioxin congeners were detected at 32 to 89 percent of the 388 sites surveyed, while the furan congeners were detected at 1 to 89 percent of the 388 sites surveyed (U.S. EPA, 1992c, 1992d). As shown in Table 4-8, the dioxin/furan congeners detected at more than 50 percent of the sites included four CDD compounds and three CDF compounds: 1,2,3,4,6,7,8 HpCDD (89 percent), 2,3,7,8 TCDF (89 percent), 2,3,7,8 TCDD (70 percent), 1,2,3,6,7,8 HxCDD (69 percent), 2,3,4,7,8 PeCDF (64 percent), 1,2,3,4,6,7,8 HpCDF (54 percent), and 1,2,3,7,8 PeCDD (54 percent). The most frequently detected CDD/CDF compounds (1,2,3,4,6,7,8-HpCDD and 2,3,7,8-TCDF) were also detected at the highest concentrations–249 ppt and 404 ppt (wet weight), respectively. The mean concentrations of these two compounds were considerably lower, at 10.5 and 13.6 ppt, respectively. The dioxin congener (2,3,7,8-TCDD) believed to be one of the two most toxic congeners to mammals was detected at 70 percent of the sites at a maximum concentration of 204 ppt and a mean concentration of 6.8 ppt. The other toxic congener, 1,2,3,7,8-PeCDD, was detected at 54 percent of the sites at a maximum and mean concentration of 53.95 and 2.38 ppt, respectively.

The NSCRF data showed that pulp and paper mills using chlorine bleach pulp were the dominant source of 2,3,7,8-TCDD and 2,3,7,8-TCDF and that these sites had the highest median 2,3,7,8-TCDD concentrations (5.66 ppt), compared to other source categories studied, including refinery/other industrial sites (1.82 ppt), industrial/urban sites (1.40 ppt), Superfund sites (1.27 ppt), and background sites (0.5 ppt). Source categories that had the highest 2,3,7,8-TCDD concentrations in fish also had the highest TEQ values. It should be noted that OCDD and OCDF were not analyzed in fish tissues because the TEFs were zero for these compounds at the initiation of the NSCRF study. In 1989, TEFs for OCDD and OCDFs were given a TEF value of 0.001. Therefore, TEQ values presented in the NSCRF report may be underreported for samples collected at sites with sources of OCDD/OCDF contamination (e.g., wood preservers) (U.S. EPA, 1992, 1992d). It is noted that the latest TEFs for OCDD and OCDF are 0.0001 (Van den Berg et al., 1998) (see Table 5-6).

In 1993, 20 states had issued 67 fish advisories for dioxins/furans (Figure 4-4) (RTI, 1993). As of 1998, there were 59 advisories in effect in 19 states for this chemical contaminant (Figure 4-4) (U.S. EPA, 1999c). In addition, three states (Maine, New Jersey, and New York) had dioxin advisories in effect for all coastal marine waters (U.S. EPA, 1999c).

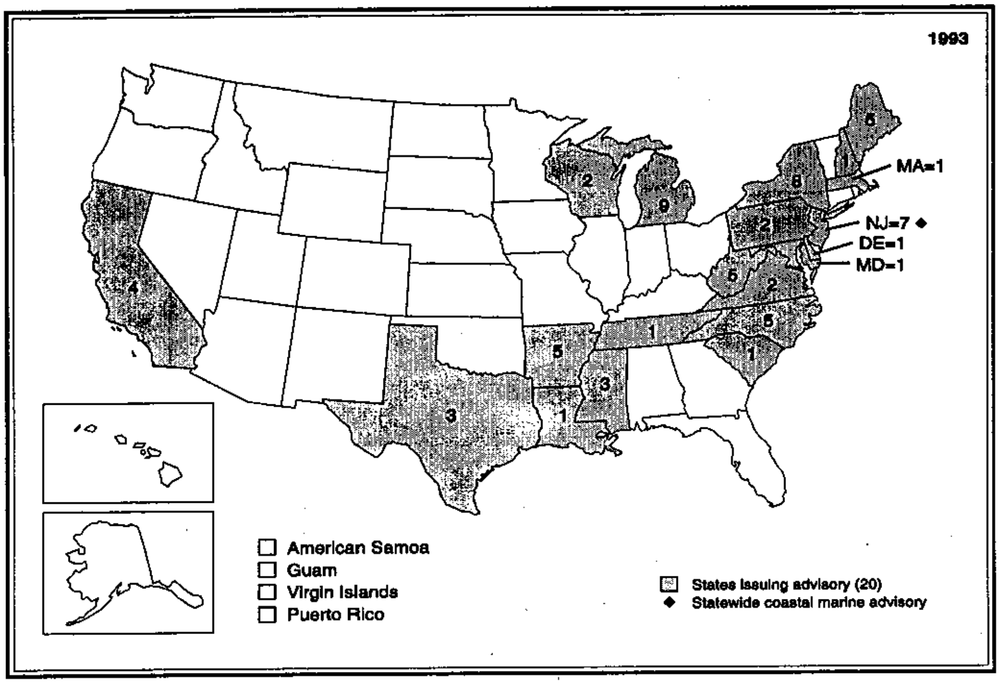
Table 4-8. Summary of Dioxins/Furans Detected in Fish Tissue as Part of the EPA National Study of Chemical Residues in Fish[a]

Congener	% Sites where detected	Maximum	Mean	Standard deviation	Median
Dioxins					
2,3,7,8-TCDD	70	203.6	6.89	19.41	1.38
1,2,3,7,8-PeCDD	54	53.95	2.38	4.34	0.93
1,2,3,4,7,8-HxCDD	32	37.56	1.67	2.39	1.24
1,2,3,6,7,8-HxCDD	69	100.9	4.30	9.25	1.32
1,2,3,7,8,9-HxCDD	38	24.76	1.16	1.74	0.69
1,2,3,4,6,7,8-HpCDD	89	249.1	10.52	25.30	2.83
Furans					
2,3,7,8-TCDF	89	403.9	13.61	40.11	2.97
1,2,3,7,8-PeCDF	47	120.3	1.71	7.69	0.45
2,3,4,7,8-PeCDF	64	56.37	3.06	6.47	0.75
1,2,3,4,7,8-HxCDF	42	45.33	2.35	4.53	1.42
1,2,3,6,7,8-HxCDF	21	30.86	1.74	2.34	1.42
1,2,3,7,8,9-HxCDF	1	0.96[b]	1.22	0.41	1.38
2,3,4,6,7,8-HxCDF	32	19.3	1.24	1.51	0.98
1,2,3,4,6,7,8-HpCDF	54	58.3	1.91	4.41	0.72
1,2,3,4,7,8,9-HpCDF	4	2.57	1.24	0.33	1.30
EPA-TEQ[c]	NA	213	11.1	23.8	2.80

[a] Concentrations are given in picograms per gram (pg/g) or parts per trillion (ppt) by wet weight. The mean, median, and standard deviation were calculated using one-half the detection limit for samples that were below the detection limit. In cases where multiple samples were analyzed per site, the value used represents the highest concentration.
[b] Detection limits were higher than the few quantified values for 1,2,3,4,7,8,9-HpCDF and 1,2,3,7,8,9-HxCDF. Maximum values listed are measured values.
[c] This EPA study used TEF-89 toxicity weighting values but did not analyze concentrations of octachlorodibenzo-*p*-dioxin or octachlorodibenzofurans in fish tissues; therefore, the TEQ value does not include these two compounds or the 12 coplanar PCB congeners.

EPA = U.S. Environmental Protection Agency
HpCDD = Heptachlorodibenzo-*p*-dioxin
HpCDF = Heptachlorodibenzofuran
HxCDD = Hexachlorodibenzo-*p*-dioxin
HxCDF = Hexachlorodibenzofuran
NA = Not applicable
PeCDD = Pentachlorodibenzo-*p*-dioxin
PeCDF = Pentachlorodibenzofuran
TCDD = Tetrachlorodibenzo-*p*-dioxin
TCDF = Tetrachlorodibenzofuran
TEQ = Toxicity equivalency concentration.

Source: U.S. EPA, 1992c and 1992d.



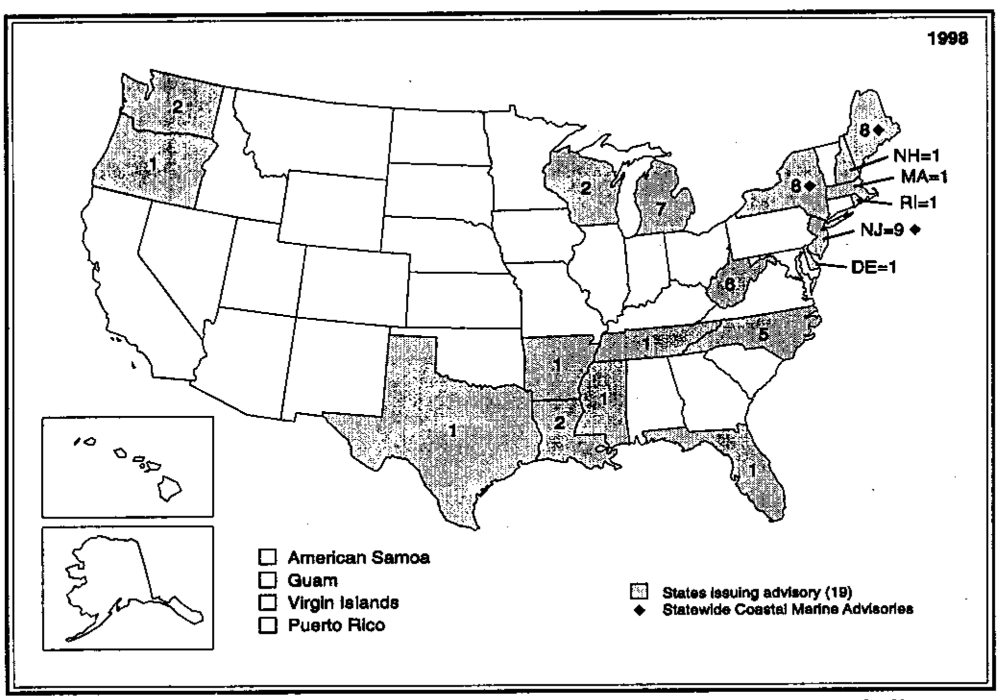


Figure 4-4. States issuing fish and shellfish advisories for dioxin/furans.

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Dioxins/furans should be considered for analysis primarily in suburban/urban and industrial watersheds at sites of pulp and paper mills using a chlorine bleaching process and at industrial sites where the following organic compounds have been or are currently produced: herbicides (containing 2,4,5-trichlorophenoxy acids and 2,4,5-trichlorophenol), silvex, hexachlorophene, pentachlorophenol, and PCBs as well as at sites of municipal and industrial waste incinerators and combustors (U.S. EPA, 1987d). EPA recommends that all of the 17 2,3,7,8-substituted tetra- through octachlorinated dioxin and dibenzofuran congeners shown in Table 4-9 as well as the 12 dioxin-like PCB congeners shown in Table 4-7 be included as target analytes.

Table 4-9. Dibenzo-p-Dioxins and Dibenzofurans Recommended for Analysis as Target Analytes

Dioxins	Furans
2,3,7,8-TCDD	2,3,7,8-TCDF
1,2,3,7,8-PeCDD	1,2,3,7,8-PeCDF 2,3,4,7,8-PeCDF
1,2,3,4,7,8-HxCDD 1,2,3,6,7,8-HxCDD 1,2,3,7,8,9-HxCDD	1,2,3,4,7,8-HxCDF 1,2,3,6,7,8-HxCDF 1,2,3,7,8,9-HxCDF 2,3,4,6,7,8-HxCDF
1,2,3,4,6,7,8-HpCDD	1,2,3,4,6,7,8-HpCDF 1,2,3,4,7,8,9-HpCDF
OCDD	OCDF

Source: Van den Berg et al., 1998.

4.4 TARGET ANALYTES UNDER EVALUATION

At present, the EPA Office of Water is evaluating one metal (lead) for possible inclusion as a recommended target analyte in state fish and shellfish contaminant monitoring programs. A toxicologic profile for this metal and the status of the evaluation are provided in this section. Other contaminants will be evaluated and may be recommended as target analytes as additional toxicologic data become available.

Note: Any time a state independently deems that an analyte currently under evaluation and/or other contaminants are of public health concern within its jurisdiction, the state should include these contaminants in its fish and shellfish contaminant monitoring program.

4.4.1 Lead

Lead is derived primarily from the mining and processing of limestone and dolomite deposits, which are often sources of lead, zinc, and copper (May and McKinney, 1981). It is also found as a minor component of coal. Historically, lead has had a number of industrial uses, including use in paints, in solder used in plumbing and food cans, and as a gasoline additive. In the past, the primary source of lead in the environment was the combustion of gasoline; however, use of lead in U.S. gasoline has fallen sharply in recent years due to an EPA phase-down program to minimize the amount of lead in gasoline over time. By 1988, the total lead usage in gasoline had been reduced to less than 1 percent of the amount used in the peak year of 1970 (ATSDR, 1997). At present, lead is used primarily in batteries, electric cable coverings, ammunition, electrical equipment, and sound barriers. Currently, the major points of entry of lead into the environment are from industrial processes, including metals processing, waste disposal and recycling, and chemical manufacturing and from the leachates of landfills (ATSDR, 1997; May and McKinney, 1981).

Lead has been included in five national monitoring programs (Appendix E). Lead has been shown to bioaccumulate, with the organic forms, such as tetraethyl lead, appearing to have the greatest potential for bioaccumulation in fish tissues. High concentrations of lead have been found in marine bivalves and finfish from both estuarine and marine waters (NOAA, 1987, 1989a). In 1984 and 1985, the U.S. Fish and Wildlife Service collected 315 composite samples of whole fish from 109 stations nationwide as part of the National Contaminant Biomonitoring Program (Schmitt and Brumbaugh, 1990). The authors reported that the maximum, geometric mean, and 85th percentile concentrations for lead were 4.88, 0.11, and 0.22 ppm (wet weight), respectively. Lead concentrations in freshwater fish declined significantly from a geometric mean concentration of 0.28 ppm in 1976 to 0.11 ppm in 1984. This trend has been attributed primarily to reductions in the lead content of U.S. gasoline (Schmitt and Brumbaugh, 1990). Kidwell et al. (1995) conducted an analysis of lead levels in tissues from bottom-feeding and predatory fish using the 1984-1985 data from the NCBP study. These authors reported that the mean lead tissue concentrations of 0.18 ± 0.37 ppm in bottom feeders and 0.15 ± 0.43 ppm in predator fish were not significantly different.

In 1993, three states (Massachusetts, Missouri, and Tennessee) and the U.S. territory of American Samoa had fish advisories for lead contamination (RTI, 1993). As of 1998, there were 10 advisories in effect in four states (Hawaii, Louisiana, Missouri, and Ohio) and the U.S. territory of American Samoa for this heavy metal (U.S. EPA, 1999c).

Lead is particularly toxic to children and fetuses. Subtle neurobehavioral effects (e.g., fine motor dysfunction, impaired concept formation, and altered behavior profile) occur in children exposed to lead at concentrations that do not result in clinical encephalopathy (ATSDR, 1997). A great deal of information on the health effects of lead has been obtained through decades of medical observation and scientific research. By comparison to most other environmental toxicants, the degree of uncertainty about the health effects of lead is quite low. It appears that some of these effects, particularly changes in the levels of certain blood enzymes and in aspects of children's neurobehavioral development, may occur at blood lead levels so low as to be essentially without a threshold. EPA's Reference Dose (RfD) Work Group discussed inorganic lead (and lead compounds) in 1985 and considered it inappropriate to develop an RfD for inorganic lead (IRIS, 1999). Lead and its inorganic compounds have been classified as probable human carcinogens (B2) by EPA (IRIS, 1999). However, EPA has not derived a quantitative estimate of carcinogenic risk from oral exposure to lead because age, health, nutritional status, body burden, and exposure duration influence the absorption, release, and excretion of lead. In addition, current knowledge of lead pharmacokinetics indicates that an estimate derived by standard procedures would not truly describe the potential risk (IRIS, 1999).

Because of the lack of quantitative health risk assessment information for oral exposure to inorganic lead, the EPA Office of Water has not included lead as a recommended target analyte in fish and shellfish contaminant monitoring programs at this time. **Note:** *Because of the observation of virtually no-threshold* neurobehavioral developmental effects of lead in children, states should include lead as a target analyte in fish and shellfish contaminant programs if there is any evidence that this metal may be present at detectable levels in fish or shellfish in their jurisdictional waters.

SECTION 5

SCREENING VALUES FOR TARGET ANALYTES

For the purpose of this guidance document, screening values are defined as concentrations of target analytes in fish or shellfish tissue that are of potential public health concern and that are used as threshold values against which levels of contamination in similar tissue collected from the ambient environment can be compared. *Exceedance of these SVs should be taken as an indication that more intensive site-specific monitoring and/or evaluation of human health risk should be conducted.*

The EPA-recommended risk-based method for developing SVs (U.S. EPA, 1989d) is described in this section. This method is considered to be appropriate for protecting the health of fish and shellfish consumers for the following reasons (Reinert et al., 1991):

- It gives full priority to protection of public health.
- It provides a direct link between fish consumption rate and risk levels (i.e., between dose and response).
- It generally leads to conservative estimates of increased risk.
- It is designed for protection of consumers of locally caught fish and shellfish, including susceptible populations such as sport and subsistence fishers who are at potentially greater risk than the general adult population because they tend to consume greater quantities of fish and because they frequently fish the same sites repeatedly.

At this time, the EPA Office of Water is recommending use of this method because it is the basis for developing current water quality criteria. A detailed discussion of the flexibility of the EPA risk-based method and the use of EPA's SVs as compared to FDA action levels is provided in Section 1.2. Further discussion of the EPA Office of Water risk-based approach, including a detailed description of the four steps involved in risk assessment (hazard identification, dose-response assessment, exposure assessment, and risk characterization) is provided in the second guidance document in this series, *Volume 2: Risk Assessment and Fish Consumption Limits.*

5.1 GENERAL EQUATIONS FOR CALCULATING SCREENING VALUES

Risk-based SVs are derived from the general model for calculating the effective ingested dose of a chemical *m* (E_m) (U.S. EPA, 1989d):

$$E_m = (C_m \cdot CR \cdot X_m) / BW \qquad (5\text{-}1)$$

where

E_m = Effective ingested dose of chemical *m* in the population of concern averaged over a 70-yr lifetime (mg/kg-d)

C_m = Concentration of chemical *m* in the edible portion of the species of interest (mg/kg; ppm)

CR = Mean daily consumption rate of the species of interest by the general population or subpopulation of concern averaged over a 70-yr lifetime (kg/d)

X_m = Relative absorption coefficient, or the ratio of human absorption efficiency to test animal absorption efficiency for chemical *m* (dimensionless)

BW = Mean body weight of the general population or subpopulation of concern (kg).

Using this model, the SV for the chemical *m* (SV_m) is equal to C_m when the appropriate measure of toxicologic potency of the chemical *m* (P_m) is substituted for E_m. Rearrangement of Equation 5-1, with these substitutions, gives

$$SV_m = (P_m \cdot BW) / (CR \cdot X_m) \qquad (5\text{-}2)$$

where

P_m = Toxicologic potency for chemical *m*; the effective ingested dose of chemical *m* associated with a specified level of health risk as estimated from dose-response studies; **dose-response variable.**

In most instances, relative absorption coefficients (X_m) are assumed to be 1.0 (i.e., human absorption efficiency is assumed to be equal to that of the test animal), so that

$$SV_m = (P_m \cdot BW) / CR . \qquad (5\text{-}3)$$

However, if X_m is known, Equation 5-2 should be used to calculate SV_m.

Dose-response variables for noncarcinogens and carcinogens are defined in Sections 5.1.1 and 5.1.2, respectively. These variables are based on an assessment of the occurrence of a critical toxic or carcinogenic effect via a specific route of exposure (i.e., ingestion, inhalation, dermal contact). Oral dose-response variables for the recommended target analytes are given in Appendix G. Because of the fundamental differences between the noncarcinogenic and carcinogenic dose-response variables used in the EPA risk-based method, SVs must be calculated separately for noncarcinogens and potential carcinogens as shown in the following subsections.

5.1.1 Noncarcinogens

The dose-response variable for noncarcinogens is the **reference dose.** The RfD is an estimate of a daily exposure to the human population (including sensitive subpopulations) that is likely to be without appreciable risk of deleterious effects during a lifetime. The RfD is derived by applying uncertainty or modifying factors to a subthreshold dose (i.e., lowest observed adverse effects level [LOAEL] if the no observed adverse effect level [NOAEL] is indeterminate) observed in chronic animal bioassays. These uncertainty or modifying factors range from 1 to 10 for each factor and are used to account for uncertainties in:

- Sensitivity differences among human subpopulations
- Interspecies extrapolation from animal data to humans
- Short-term to lifetime exposure extrapolation from less-than-chronic results on animals to humans when no long-term human data are available
- Deriving an RfD from a LOAEL instead of a NOAEL
- Incomplete or inadequate toxicity or pharmacokinetic databases.

The uncertainty (UF) and modifying (MF) factors are multiplied to obtain a final UF•MF value. This factor is divided into the NOAEL or LOAEL to derive the RfD (Barnes and Dawson, 1988; U.S. EPA, 1989d).

The following equation should be used to calculate SVs for noncarcinogens:

$$SV_n = (RfD \cdot BW)/CR \qquad (5\text{-}4)$$

where

SV_n = Screening value for a noncarcinogen (mg/kg; ppm)
RfD = Oral reference dose (mg/kg-d)

and BW and CR are defined as in Equation 5-1.

5.1.2 Carcinogens

According to *The Risk Assessment Guidelines of 1986* (U.S. EPA, 1987f), the default model for low-dose extrapolation of carcinogens is a version (GLOBAL 86) of the linearized multistage no-threshold model developed by Crump et al. (1976). This extrapolation procedure provides an upper 95 percent bound risk estimate (referred to as a q1*), which is considered by some to be a conservative estimate of cancer risk. Other extrapolation procedures may be used when justified by the data.

Screening values for carcinogens are derived from: (1) a carcinogenicity potency factor or **cancer slope factor,** which is generally an upper bound risk estimate; and (2) a **risk level** (RL), an assigned level of maximum acceptable individual lifetime risk (e.g., RL = 10^{-5} for a level of risk not to exceed one excess case of cancer per 100,000 individuals exposed over a 70-yr lifetime) (U.S. EPA, 1997b). The following equation should be used to calculate SVs for carcinogens:

$$SV_c = [(RL / CSF) \cdot BW] / CR \qquad (5\text{-}5)$$

where

SV_c = Screening value for a carcinogen (mg/kg; ppm)
RL = Maximum acceptable risk level (dimensionless)
CSF = Oral cancer slope factor $(mg/kg\text{-}d)^{-1}$

and BW and CR are defined as in Equation 5-1.

5.1.3 Recommended Values for Variables in Screening Value Equations

The default values for variables used in Equations 5-4 and 5-5 to calculate SVs are based on assumptions for the general adult population. These default values are consistent with values included in the *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000)* (EPA-822-B-00-004). For risk management purposes (e.g., to protect sensitive populations such as pregnant and nursing women), states may choose to use alternative values for consumption rates, etc. different from those recommended in this section.

5.1.3.1 Dose-Response Variables—

EPA has developed oral RfDs and/or CSFs for all of the recommended target analytes in Section 4 (see Appendix G). These are maintained in the EPA Integrated Risk Information System (IRIS, 1999), an electronic database containing health risk and EPA regulatory information on approximately 400 different chemicals. IRIS is available online at:

http://www.epa.gov/iris/subst/index.html

The IRIS RfDs and CSFs are reviewed regularly and updated as necessary when new or more reliable information on the toxic or carcinogenic potency of chemicals becomes available.

When IRIS values for oral RFDs and CSFs are available, they should be used to calculate SVs for target analytes from Equations 5-4 and 5-5, respectively. It is important that the most current IRIS values for oral RfDs and CSFs be used to calculate SVs for target analytes unless otherwise recommended.

In cases where IRIS values for oral RFDs or CSFs are not available for calculating SVs for target analytes, estimates of these variables may be derived from the most recent water quality criteria (U.S. EPA, 1992e) according to procedures described in U.S. EPA (1991a, p. IV-12), or from the Classification

List of Chemicals Evaluated for Carcinogenicity Potential (U.S. EPA 1999b) from the Office of Pesticide Programs Health Effects Division.

5.1.3.2 Body Weight and Consumption Rate—

Values for the variables BW and CR in Equations 5-4 and 5-5 are given in Table 5-1 for various subpopulations including recreational and subsistence fishers. **Note:** In this third edition of this document, EPA's Office of Water uses a BW of 70 kg, a default CR of 17.5 g/d to calculate the SV for the general populations and recreational fishers, and a default CR of 142.4 g/d to calculate the SV for subsistence fishers. The CR values have been revised since the release of the previous edition.

Table 5-1. Recommended Values for Mean Body Weights (BWs) and Fish Consumption Rates (CRs) for Selected Subpopulations

Variable	Recommended value	Subpopulation
BW	70 kg	All adults (U.S. EPA, 1999a)
	78 kg	Adult males (U.S. EPA, 1985b, 1990a)
	65 kg	Adult females (U.S. EPA, 1985b, 1990a)
	12 kg	Children <3 yr (U.S. EPA, 1985b, 1990a)
	17 kg	Children 3 to <6 yr (U.S. EPA, 1985b, 1990a)
	25 kg	Children 6 to <9 yr (U.S. EPA, 1985b, 1990a)
	36 kg	Children 9 to <12 yr (U.S. EPA, 1985b, 1990a)
	51 kg	Children 12 to <15 yr (U.S. EPA, 1985b, 1990a)
	61 kg	Children 15 to <18 yr (U.S. EPA, 1985b, 1990a)
CR[a]	17.5 g/d (0.0175 kg/d)	Estimate of the 90th percentile of recreational or sport fishers (USDA/ARS, 1998) and of the average consumption of uncooked fish and shellfish from estuarine and fresh waters by recreational fishers (U.S. EPA, 2000c)
	142.4 g/d (0.1424 kg/d)	Estimate of the 99th percentile of subsistence fishers (USDA/ARS, 1998) and of the average consumption of uncooked fish and shellfish from estuarine and fresh waters by subsistence fishers (U.S. EPA, 2000c)

[a] These are recommended default consumption rates only. **Note:** When local consumption rate data are available for recreational and subsistence fishers, they should be used to calculate SVs for noncarcinogens and carcinogens by subsistence fishers, as described in Sections 5.1.1 and 5.1.2, respectively.

The default CR of 6.5 g/d used in the previous edition of Volume I was based on data from a fish consumption survey conducted in 1973 and 1974 by the National Purchase Diaries and funded by the Tuna Institute. This value represented the estimated mean per capita freshwater/estuarine finfish and shellfish consumption rate for the general U.S. population (Jacobs et al., 1998). This value has been revised based on new data from the combined 1994, 1995, and 1996 *Continuing Survey of Food Intake by Individuals* (CSFII) survey (USDA/ARS, 1998). The

CSFII survey is a national food consumption survey conducted by the U.S. Department of Agriculture, consisting of multistage, stratified-cluster area probability samples from all states except Alaska and Hawaii.

These data are collected over 3 consecutive days. On the first day of the survey, participants give information to an in-home interviewer, and on the second and third days, data are taken from self-administered dietary records. Meals consumed both at home and away from home are recorded. Average daily individual consumptions of fish in a given fish-by-habitat category were calculated by summing the amount of fish eaten by the individual across 3 reporting days for all fish-related food codes in a given fish-by-habitat category. The total individual consumption was then divided by three to obtain an average daily consumption rate. The 3-day individual food consumption data collection period is one during which a majority of sampled individuals did not consume any finfish or shellfish. The nonconsumption of finfish or shellfish by a majority of individuals, combined with consumption data from high-end consumers, resulted in a wide range of observed fish consumption rates. This range of fish consumption data would tend to produce distributions of fish consumption with larger variances than would be associated with a longer survey period, such as 30 days. The larger variances would reflect greater dispersion, which results in larger upper-percentile estimates, as well as upper confidence intervals associated with parameter estimates. It follows that estimates of the upper percentiles (90th and 99th percentiles) of per capita fish consumption based on 3 days of data will be consecutive with regard to risk (U.S. EPA, 1998a).

If states and tribes do not have site-specific fish consumption information concerning their recreational and subsistence fishers, it is EPA's preference that they use as fish intake assumptions the default values from the most recent 1994-1996 CSFII study (USDA/ARS, 1998). The fish consumption default values of 17.5 g/d for the general adult population and recreational fishers and 142.4 g/d for subsistence fishers used in this document are representative of fish intake for these different population groups. These values are based on risk management decisions that EPA has made after evaluating numerous fish consumption surveys (U.S. EPA, 2000c). These default values represent the uncooked weight intake of freshwater/estuarine finfish and shellfish. EPA recognizes the data gaps and uncertainties associated with the analysis of the 1994-1996 CSFII survey conducted in the process of making its default consumption rate recommendations. The estimated mean of freshwater/estuarine fish ingestion for adults is 7.50 g/d, and the median is 0 g/d. The estimated 90th percentile is 17.53 g/d; the estimated 95th percentile is 49.59 g/d; and the estimated 99th percentile is 142.41 g/d. The median value of 0 g/d may reflect the portion of individuals in the population who never eat fish as well as the limited reporting period (2 days) over which intake was actually measured. By applying as a default consumption rate the 17.5-g/d value for the general adult population, EPA intends to select a consumption rate that is protective of the majority of the population (the 90th percentile of consumers and nonconsumers according to the 1994-1996 CSFII survey data). EPA further considers this rate to be indicative of the average consumption among recreational fishers based on averages in the studies reviewed (U.S. EPA, 2000c). Similarly, EPA believes that the assumption of 142.4 g/d is within the range of average consumption estimates for subsistence fishers based on the studies reviewed. Experts at a 1992 National Water Quality Workshop acknowledged, however, that the national survey high-end values are representative of average rates for highly exposed groups such as subsistence fishers, specific ethnic groups, or other high-risk populations. EPA is aware that some local and regional studies indicate greater fish consumption among Native Americans, Pacific Asian Americans, and other subsistence consumers and recommends the use of those studies in appropriate cases. States and tribes have the flexibility to choose fish consumption rates higher than an average value for these populations groups. If a state has not identified a separate well-defined population of high-end consumers and believes that the national data from the 1994-1996 CSFII are representative, they may choose these consumption rates.

With respect to consumption rates, EPA recommends that states always evaluate any type of consumption pattern they believe could reasonably be occurring at a site. Evaluating additional consumption rates involves calculating additional SVs only and does not add to sampling or analytical costs.

EPA has published a review and analysis of survey methods that can be used by states to determine fish and shellfish consumption rates of local populations (U.S. EPA, 1992b, 1998b). States should consult these documents to ensure that appropriate values are selected to calculate SVs for site-specific exposure scenarios.

For any given population, there can be a sensitive subpopulation composed of individuals who may be at higher-than-average risk due to their increased exposure or their increased sensitivity to a contaminant or both. For Native American subsistence fishers, there are several exposure issues of concern that should be addressed as part of a comprehensive exposure assessment:

- **Consumption rates and dietary preferences.** Harris and Harper (1997) surveyed traditional tribal members in Oregon with a subsistence lifestyle and determined a consumption rate of 540 g/d, which included fresh, dried, and smoked fish. They also confirmed that the parts of the fish (heads, fins, tails, skeleton, and eggs) eaten by this group were not typically eaten by other groups. Another study conducted of four tribes in the Northwest that also surveyed tribal members in Oregon but did not target subsistence fishers, reported a 99th percentile ingestion rate of 390 g/d for tribal members (CRITFC, 1994). These consumption rates are much higher than the default consumption rates provided in this document for subsistence fishers and emphasize the need for identifying the consumption rate of the Native American subsistence population of concern.

- **Community characteristics** - It is important to consider family-specific fishing patterns in any exposure scenario, and attention should be paid to the role of the fishing family with respect to the tribal distribution of fish, the sharing ethic, and providing fish for ceremonial religious events. Entire communities are exposed if fish are contaminated, and the community contaminant burden as a whole must be considered, not just the maximally exposed individual.

- **Multiple contaminant exposure** - Multiple contaminant exposure is significant for Native American subsistence fishers. A large number of contaminants are often detected in fish tissues and their combined risk associated with the higher consumption rates and dietary preferences for certain fish parts could be very high even if individual contaminants do not exceed the EPA reference dose (Harper and Harris, 1999).

- **Other exposure pathways** - For Native American subsistence fishers, overall exposure to a contaminant may be underestimated if it fails to take into account nonfood uses of fish and other animal parts that may contribute to overall exposure, such as using teeth and bones for decorations and whistles, animal skins for clothing, and rendered fish belly fat for body paint (Harper and Harris, 1999). If other wildlife species (e.g., feral mammals, turtles, waterfowl) that also live in or drink from the contaminated waterbody are eaten, or if the contaminated water is used for irrigation of crops or for livestock watering or human drinking water, the relative source contribution of these other pathways of exposure must also be considered. As with fish and wild game, plants are used by Native Americans for more than just nutrition. Daily cleaning, preparation, and consumption of plants and crafting of plant materials into household goods occurs throughout the year (Harris and Harper, 1997).

As in the general population, increased sensitivity to a chemical contaminant for Native Americans can result from factors such as an individual's underlying health status and medications, baseline dietary composition and quality, genetics, socioeconomic status, access to health care, quality of replacement protein, age, gender, pregnancy, and lactation. These factors are only partially considered in the uncertainty factor(s) used to develop the RfD (Harper and Harris, 1999).

Other important issues that need to be considered concern risk characterization and risk management. For Native American subsistence fishers, the use of an acceptable risk level of 1 in 100,000 (10^{-5}) may not be acceptable to all tribes. Each tribe has the right to decide for themselves what an acceptable level of risk is, and, in some cases, it may be zero risk (zero discharge) to protect cultural resources and uses. Ecological well-being or health is another key issue. Human and ecological health are connected in many ways and the ripple effects are often not recognized. For example, human health may be affected by injury to the environment, which affects the economy and the culture (Harper and Harris, 1999).

Native American subsistence fishers should be treated as a special high-risk group of fish consumers distinct from fishers in the general population and distinct even from other Native American fish consumers living in more suburbanized communities. Table 5-2 compares fish consumption rates for various fisher populations within the general population and in several surveys of specific Native American tribal populations. EPA currently recommends default fish consumption rates of 17.5 g/d for the general and recreational fishers and 142.4 g/d for subsistence fishers. However, the tribal population fish consumption studies show that some Native American tribal members living in river-based communities (CRITFC, 1994) eat from 3 to 22 times more fish (from 59 g/d up to 390 g/d) than do recreational fishers, but that traditional Native American subsistence fishing families may eat up to 30 times more fish, almost 1.2 lb/d (540 g/d) (Harris and Harper, 1997). The fish consumption rate from Harris and Harper (1997) for Native American subsistence fishers is also 3.8 times higher than the EPA default consumption rate for subsistence fishers (142.4 g/d) in the general population. The difference in fish consumption is due to the fact that the Native American subsistence fisher's lifestyle is not the same as a recreational fisher's lifestyle with additional fish consumption added, nor is it the same as the "average" Native American tribal member living in a fairly suburbanized tribal community. In addition to exposures from direct consumption of contaminated fish, Native American subsistence fishers also receive more exposure to the water and sediments associated with catching and preparing fish and possibly from drinking more unfiltered river water than more suburbanized tribal community members as well. The Native American subsistence fishing population should be treated as a separate group with a unique lifestyle, distinct from recreational and subsistence fishers in the general U.S. population and also distinct from other Native American fisher populations.

5.1.3.3 Risk Level (RL)—

In this guidance document, EPA's Office of Water uses an RL of 10^{-5} to calculate screening values for the general adult population. However, states have the flexibility to choose to use an appropriate RL value typically ranging from 10^{-4} to 10^{-7}. This is the range of risk levels employed in various U.S. EPA programs. Selection of the appropriate RL is a risk management decision that is made by the state.

5.2 SCREENING VALUES FOR TARGET ANALYTES

Target analyte SVs, and the dose-response variables used to calculate them, are given in Tables 5-3 and 5-4. The SVs are provided as default values for the states to use when site-specific information on variables such as consumption rates are not available for local recreational or subsistence fisher populations.

Table 5-2. Fish Consumption Rates for Various Fisher Populations

Source	Recreational Fishers (g/d)	Subsistence Fishers (g/d)	Native American Subsistence Fishers (g/d)	Native Americans (g/d)	Basis for Consumption Rate
U.S. EPA	17.5[a]	142.4[a]	70 (mean)[b] 170 (95th percentile)[b]	NA	Fish consumption rate from 1994 and 1996 Continuing Survey of Food Intake by Individuals (CSFII)
Harris and Harper (1997)	NA	NA	540 (fresh, smoked and dried)	NA	Surveyed members of the Confederated Tribes of the Umatilla Indian Reservation
CRITFC (1994)	NA	NA	NA	59 (mean) 170 (95th percentile) 390 (99th percentile)	Surveyed members of the Umatilla, Nez Perce, Yakama, and Warm Springs Tribes
Toy et al. (1996)	NA	NA	NA	53 (median, males) 34 (median, females)	Surveyed members of the Tulalip Tribe
				66 (median, males) 25 (median, females)	Surveyed members of the Squaxin Island Tribe

[a] These values were revised in this 3rd edition of Volume 1 of this series (USDA/ARS, 1998)
[b] These values are from EPA's Exposure Factors Handbook (U.S. EPA, 1997b)

These SVs were calculated from Equations 5-4 or 5-5 using the following values for BW, CR, and RL and the most current IRIS values for oral RfDs and CSFs (IRIS, 1999) unless otherwise noted:

- **For noncarcinogens:**

  BW = 70 kg, average adult body weight
  CR = 17.5 g/d (0.0175 kg/d), estimate of average consumption of uncooked fish and shellfish from estuarine and fresh waters by recreational fishers, or
  = 142.4 g/d (0.1424 kg/d), estimate of average consumption of uncooked fish and shellfish from estuarine and freshwaters by subsistence fishers.

- **For carcinogens:**

  BW and CR, as above

  RL = 10^{-5}, a risk level corresponding to one excess case of cancer per 100,000 individuals exposed over a 70-yr lifetime.

If both oral RfD and CSF values are available for a given target analyte, SVs for both noncarcinogenic and carcinogenic effects are listed in Table 5-2 for recreational fishers and Table 5-3 for subsistence fishers. Unless otherwise indicated,

Table 5-3. Dose-Response Variables and Recommended Screening Values (SVs) for Target Analytes - Recreational Fishers[a]

Target analyte	Noncarcinogens RfD (mg/kg-d)	Carcinogens CSF $(mg/kg\text{-}d)^{-1}$	SV[b] (ppm) Noncarcinogens[b]	SV[b] (ppm) Carcinogens[b] (RL=10^{-5})
Metals				
Arsenic (inorganic)[c]	3×10^{-4}	1.5	1.2	0.026
Cadmium	1×10^{-3}	NA	4.0	-
Mercury (methylmercury)[d]	1×10^{-4}	NA	0.4	-
Selenium	5×10^{-3}	NA	20	-
Tributyltin[e]	3×10^{-4}	NA	1.2	-
Organochlorine Pesticides				
Total chlordane (sum of cis- and trans-chlordane, cis- and trans-nonachlor, and oxychlordane)[f]	5×10^{-4}	0.35	2.0	0.114
Total DDT (sum of 4,4'- and 2,4'- isomers of DDT, DDE, and DDD)[g]	5×10^{-4}	0.34	2.0	0.117
Dicofol[h]	4×10^{-4}	NA[i]	1.6	2.5
Dieldrin	5×10^{-5}	16	0.2	2.50×10^{-3}
Endosulfan (I and II)[j]	6×10^{-3}	NA	24	-
Endrin	3×10^{-4}	NA	1.2	-
Heptachlor epoxide	1.3×10^{-5}	9.1	5.2×10^{-2}	4.39×10^{-3}
Hexachlorobenzene	8×10^{-4}	1.6	3.2	2.50×10^{-2}
Lindane (g-hexachlorocyclohexane; g-HCH)[k]	3×10^{-4}	1.3	1.2	3.07×10^{-2}
Mirex	2×10^{-4}	NA[i]	0.8	-
Toxaphene[l,m]	2.5×10^{-4}	1.1	1.0	3.63×10^{-2}
Organophosphate Pesticides				
Chlorpyrifos[n]	3×10^{-4}	NA	1.2	-
Diazinon[o]	7×10^{-4}	NA	2.8	-
Disulfoton	4×10^{-5}	NA	0.16	-
Ethion	5×10^{-4}	NA	2.0	-
Terbufos[p]	2×10^{-5}	NA	0.08	-
Chlorophenoxy Herbicides				
Oxyfluorfen[q]	3×10^{-3}	7.32×10^{-2}	12	5.46×10^{-1}
PAHs[r]	NA	7.3	-	5.47×10^{-3}
PCBs				
Total PCBs[s]	2×10^{-5}	2.0	0.08	0.02
Dioxins/furans[t]	NA	1.56×10^{5}	-	2.56×10^{-7}

NA = Not available in EPA's Integrated Risk Information System (IRIS, 1999).
DDD = p,p'-dichlorodiphenyldichloroethane
DDT = p,p'-dichlorodiphenyltrichloroethane
DDE = p,p'-dichlorodiphenlydichloroethylene
PAH = Polycyclic aromatic hydrocarbon
PCB = Polychlorinated biphenyl
RfD = Oral reference dose (mg/kg-d)
CSF = Cancer slope factor $(mg/kg\text{-}d)^{-1}$

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Table 5-3. (continued)

[a] Based on fish consumption rate of 17.5 g/d, 70kg body weight and, for carcinogens, 10^{-5} risk level and 70-yr lifetime. Unless otherwise noted, values listed are the most current oral RfDs and CSF in EPA's IRIS database (IRIS, 1999).
[b] The shaded screening value (SV) is the recommended SV for each target analyte. States should note that the screening values listed may be below analytical detection limits achievable for some of the target analytes. Please see Table 8-4 for detection limits.
[c] Total inorganic arsenic rather than total arsenic should be determined.
[d] Because most mercury in fish and shellfish tissue is present primarily as methylmercury (NAS, 1991;Tollefson, 1989) and because of the relatively high cost of analyzing for methylmercury, it is recommended that total mercury be analyzed and the conservative assumption be made that all mercury is present as methylmercury. This approach is deemed to be most protective of human health and most cost-effective. The National Academy of Sciences conducted an independent assessment of the RfD for methylmercury. They concluded that "On the basis of its evaluation, the committee's consensus is that the value of EPA's current RfD for methylmercury, 0.1μg/kg per day, is a scientifically justifiable level for the protection of human health".
[e] The RfD value listed is for tributyltin oxide (IRIS, 1999).
[f] The RfD and CSF values listed are derived from studies using technical-grade chlordane (IRIS, 1999) for the *cis*- and *trans*-chlordane isomers or the major chlordane metabolite, oxychlordane, or for the chlordane impurities *cis*- and *trans*-nonachlor. It is recommended that total chlordane be determined by summing the concentrations of *cis*- and *trans*-chlordane, *cis*- and *trans*-nonachlor, and oxychlordane.
[g] The RfD value listed is for DDT. The CSF value (0.34) is for total DDT sum of DDT, DDE and DDD); the CSF value for DDD is 0.24. It is recommended that the total concentration of DDT include the 2,4'- and 4,4'-isomers of DDT and its metabolites, DDE and DDD.
[h] The RfD value is from Office of Pesticide Programs Reregistration Eligibility Decision (RED) for Dicofol (EPA, 1998c).
[i] *The CSF for dicofol was withdrawn from IRIS pending further review by the CRAVE Agency Work Group (IRIS, 1999).*
[j] The RfD value listed is from the Office of Pesticide Program's Reference Dose Tracking Report (U.S. EPA, 1997).
[k] IRIS (1999) has not provided a CSF for lindane. The CSF value listed for lindane was calculated from the water quality criteria (0.063 mg/L) (U.S. EPA, 1992f).
[l] No CSF or cancer classification is available for mirex. This compound is undergoing further review by the CRAVE Agency Work Group (IRIS, 1999)
[m] The RfD value has been agreed upon by the Office of Pesticide Programs and the Office of Water.
[n] Because of the potential for adverse neurological developmental effects from chlorpyrifos, EPA recommends the use of a Population Adjusted Dose (PAD) of 3 x 10^{-5} for infants, children under the age of 6 years, and women ages 13 to 50 years (U.S. EPA, 2000b).
[o] The RfD value is from a memorandum dated April 1, 1998, Diazinon:-Report of the Hazard Identification Assessment Review Committee. HED Doc. No. 012558.
[p] *The RfD value listed is from a memorandum* dated September 25, 1997; Terbufos-FQPA Requirement- Report of the Hazard Idenification Review.
[q] The CSF value is from the Office of Pesticide Programs List of Chemicals Evaluated for Carcinogenic Potential (U.S. EPA, 1999b).
[r] The CSF value listed is for benzo[*a*]pyrene. Values for other PAHs are not currently available in IRIS (1999). It is recommended that tissue samples be analyzed for benzo[*a*]pyrene and 14 other PAHs, and that the order-of-magnitude relative potencies given for these PAHs (Nisbet and LaGoy, 1992; U.S. EPA, 1993c) be used to calculate a potency equivalency concentration (PEC) for each sample (see Section 5.3.2.4).
[s] Total PCBs may be determined as the sum of congeners or Aroclors. The RfD is based on Aroclor 1254 and should be applied to total PCBs. The CSF is based on a carcinogenicity assessment of Aroclors 1260, 1254, 1242, and 1016. The CSF presented is the upper-bound slope factor for food chain exposure. The central estimate is 1.0 (IRIS, 1999).
[t] The CSF value listed is for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (HEAST, 1997). It is recommended that the 17 2,3,7,8-substituted *tetra*- through *octa*-chlorinated dibenzo-*p*-dioxins and dibenzofurans and the 12 dioxin-like PCBs be determined and a toxicity-weighted total concentration be calculated for each sample, using the method for estimating toxicity equivalency concentrations (TEQs) (Van den Berg et al., 1998).

Table 5-4. Dose-Response Variables and Recommended Screening Values (SVs) for Target Analytes - Subsistence Fishers[a]

Target analyte	Noncarcinogens RfD (mg/kg-d)	Carcinogens CSF (mg/kg-d)$^{-1}$	SV[b] (ppm) Noncarcinogens[b]	SV[b] (ppm) Carcinogens[b] (RL=10^{-5})
Metals				
Arsenic (inorganic)[c]	3×10^{-4}	1.5	0.147	3.27×10^{-3}
Cadmium	1×10^{-3}	NA	0.491	-
Mercury (methylmercury)[d]	1×10^{-4}	NA	0.049	-
Selenium	5×10^{-3}	NA	2.457	-
Tributyltin[e]	3×10^{-4}	NA	0.147	-
Organochlorine Pesticides				
Total chlordane (sum of cis- and trans-chlordane, cis- and trans-nonachlor, and oxychlordane)[f]	5×10^{-4}	0.35	0.245	1.40×10^{-2}
Total DDT (sum of 4,4'- and 2,4'- isomers of DDT, DDE, and DDD)[g]	5×10^{-4}	0.34	0.245	1.44×10^{-2}
Dicofol[h]	4×10^{-4}	NA[i]	0.196	-
Dieldrin	5×10^{-5}	16	0.024	3.07×10^{-4}
Endosulfan (I and II)[j]	6×10^{-3}	NA	2.949	-
Endrin	3×10^{-4}	NA	0.147	-
Heptachlor epoxide	1.3×10^{-5}	9.1	6.39×10^{-3}	5.40×10^{-4}
Hexachlorobenzene	8×10^{-4}	1.6	0.393	3.07×10^{-3}
Lindane (γ-hexachlorocyclohexane; γ-HCH)[k]	3×10^{-4}	1.3	0.147	3.78×10^{-3}
Mirex	2×10^{-4}	NA[i]	0.098	-
Toxaphene[l,m]	2.5×10^{-4}	1.1	0.122	4.46×10^{-3}
Organophosphate Pesticides				
Chlorpyrifos[n]	3×10^{-4}	NA	0.147	-
Diazinon[o]	7×10^{-4}	NA	0.344	-
Disulfoton	4×10^{-5}	NA	0.019	-
Ethion	5×10^{-4}	NA	0.245	-
Terbufos[p]	2×10^{-5}	NA	0.009	-
Chlorophenoxy Herbicides				
Oxyfluorfen[q]	3×10^{-3}	7.32×10^{-2}	1.474	6.71×10^{-2}
PAHs[r]	NA	7.3	-	6.73×10^{-4}
PCBs				
Total PCBs[s]	2×10^{-5}	2.0	9.83×10^{-3}	2.45×10^{-3}
Dioxins/furans[t]	NA	1.56×10^{5}	-	3.15×10^{-8}

NA = Not available in EPA's Integrated Risk Information System (IRIS, 1999).
DDD = p,p'-dichlorodiphenyldichloroethane
DDT = p,p'-dichlorodiphenyltrichloroethane
DDE = p,p'-dichlorodiphenlydichloroethylene
PAH = Polycyclic aromatic hydrocarbon
PCB = Polychlorinated biphenyl
RfD = Oral reference dose (mg/kg-d)
CSF = Cancer slope factor (mg/kg-d)$^{-1}$

Table 5-4. (continued)

[a] Based on fish consumption rate of 142.4 g/d, 70kg body weight and, for carcinogens, 10^{-5} risk level and 70-yr lifetime. Unless otherwise noted, values listed are the most current oral RfDs and CSF in EPA's IRIS database (IRIS, 1999)
[b] The shaded screening value (SV) is the recommended SV for each target analyte. States should note that the screening values listed may be below analytical detection limits achievable for some of the target analytes. Please see Table 8-4 for detection limits.
[c] Total inorganic arsenic rather than total arsenic should be determined.
[d] Because most mercury in fish and shellfish tissue is present primarily as methylmercury (NAS, 1991;Tollefson, 1989) and because of the relatively high cost of analyzing for methylmercury, it is recommended that total mercury be analyzed and the conservative assumption be made that all mercury is present as methylmercury. This approach is deemed to be most protective of human health and most cost-effective. The National Academy of Sciences conducted an independent assessment of the RfD for methylmercury. They concluded that "On the basis of its evaluation, the committee's consensus is that the value of EPA's current RfD for methylmercury, 0.1μg/kg per day, is a *scientifically justifiable level for the protection of human health*".
[e] The RfD value listed is for tributyltin oxide (IRIS, 1999).
[f] The RfD and CSF values listed are derived from studies using technical-grade chlordane (IRIS, 1999) for the *cis*- and *trans*-chlordane isomers or the major chlordane metabolite, oxychlordane, or for the chlordane impurities *cis*- and *trans*-nonachlor. It is recommended that total chlordane be determined by summing the concentrations of *cis*- and *trans*-chlordane, *cis*- and *trans*-nonachlor, and oxychlordane.
[g] The RfD value listed is for DDT. The CSF value (0.34) is for total DDT sum of DDT, DDE and DDD); the CSF value for DDD is 0.24. It is recommended that the total concentration of DDT include the 2,4'- and 4,4'-isomers of DDT and its metabolites, DDE and DDD.
[h] The RfD value is from Office of Pesticide Programs Reregistration Eligibility Decision (RED) for Dicofol (EPA, 1998c).
[i] The CSF for dicofol was withdrawn from IRIS pending further review by the CRAVE Agency Work Group (IRIS, 1999).
[j] The RfD value listed is from the Office of Pesticide Program's Reference Dose Tracking Report (U.S. EPA, 1997).
[k] IRIS (1999) has not provided a CSF for lindane. The CSF value listed for lindane was calculated from the water quality criteria (0.063 mg/L) (U.S. EPA, 1992f).
[l] No CSF or cancer classification is available for mirex. This compound is undergoing further review by the CRAVE Agency Work Group (IRIS, 1999)
[m] The RfD value has been agreed upon by the Office of Pesticide Programs and the Office of Water.
[n] Because of the potential for adverse neurological developmental effects from chlorpyrifos, EPA recommends the use of a Population Adjusted Dose (PAD) of 3 x 10^{-5} for infants, children under the age of 6 years, and women ages 13 to 50 years (U.S. EPA, 2000b).
[o] The RfD value is from a memorandum dated April 1, 1998, Diazinon:-Report of the Hazard Identification Assessment Review Committee. HED Doc. No. 012558.
[p] The RfD value listed is from a memorandum dated September 25, 1997; Terbufos-FQPA Requirement- Report of the Hazard Idenification Review.
[q] The CSF value is from the Office of Pesticide Programs List of Chemicals Evaluated for Carcinogenic Potential (U.S. EPA, 1999b).
[r] The CSF value listed is for benzo[*a*]pyrene. Values for other PAHs are not currently available in IRIS (1999). It is recommended that tissue samples be analyzed for benzo[*a*]pyrene and 14 other PAHs, and that the order-of-magnitude relative potencies given for these PAHs (Nisbet and LaGoy, 1992; U.S. EPA, 1993c) be used to calculate a potency equivalency concentration (PEC) for each sample (see Section 5.3.2.4).
[s] Total PCBs may be determined as the sum of congeners or Aroclors. The RfD is based on Aroclor 1254 and should be applied to total PCBs. The CSF is based on a carcinogenicity assessment of Aroclors 1260, 1254, 1242, and 1016. The CSF presented is the upper-bound slope factor for food chain exposure. The central estimate is 1.0 (IRIS, 1999).
[t] The CSF value listed is for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (HEAST, 1997). It is recommended that the 17 2,3,7,8-substituted tetra- through octa-chlorinated dibenzo-*p*-dioxins and dibenzofurans and the 12 dioxin-like PCBs be determined and a toxicity-weighted total concentration be calculated for each sample, using the method for estimating toxicity equivalency concentrations (TEQs) (Van den Berg et al., 1998).

the lower of the two SVs (generally, the SV for carcinogenic effects) should be used for the respective fisher population. EPA recommends that the SVs in the shaded boxes (Tables 5-3 and 5-4) be used by states when making the decision to implement Tier 2 intensive monitoring. However, states may choose to adjust these SVs for specific target analytes for the protection of sensitive populations (e.g., pregnant women, nursing mothers, and children or for recreational or subsistence fishers based on site-specific consumption rates). EPA recognizes that states may use higher CRs that are more appropriate for recreational and subsistence fishers in calculating SVs for use in their jurisdictions rather than the EPA default values of 17.5 g/d CR for recreational fishers used to calculate the SVs shown in Table 5-3 and the 142.4 g/d CR for subsistence fishers used to calculate the SVs shown in Table 5-4.

Note: States should use the same SV for a given target analyte in both screening and intensive studies. Therefore, it is critical that states clearly define their program objectives and accurately characterize the target fish-consuming population(s) of concern to ensure that appropriate SVs are selected. If the selected analytical methodology is not sensitive enough to reliably quantitate target analytes at or below selected SVs (see Section 8.2.2 and Table 8-4), program managers must determine appropriate fish consumption guidance based on the lowest detectable concentrations or provide justification for adjusting SVs to values at or above achievable method detection limits. It should be emphasized that when SVs are below method detection limits, the failure to detect a target analyte cannot be assumed to indicate that there is no cause for concern for human health effects.

States should recognize the importance of ensuring that the analytical method selected for quantification of any target analyte must have a method detection limit (MDL) lower than the risk-based screening values calculated using the EPA methodology for noncarcinogenic and carcinogenic effects of the target analyte. If the method detection limit for a specific target analyte is higher than the target analyte SV, the following procedure is recommended as a means to reduce the problem of interpreting data results for chemicals that fall in this category. For example, if fish tissue residue values for several replicate samples are above the MDL while other data values are reported as below the method detection limit (<MDL) including not detected (e.g., no observed response), the state may make a risk management decision to use a value of one-half the MDL as the residue concentration in their risk assessment for those data below the MDL rather than using a value of zero. In this way, the calculated mean target analyte concentration for a group of replicate samples may be higher than the SV. If all of the replicate samples from a particular monitoring site are below the MDL or are not detected, the state may choose to use one-half MDL value for all not detected values rather than a value of zero. The use of one-half MDL rather than zero for these data (< MDL) is a risk management policy decision that should be made by the state.

For noncarcinogens, adjusted SVs should be calculated from Equation 5-4 using appropriate alternative values of BW and/or CR. For carcinogens, adjusted SVs should be calculated from Equation 5-5 using an RL ranging from 10^{-4} to 10^{-7} and/or sufficiently protective alternative values of BW and CR. Examples of SVs calculated for selected populations of concern and for RL values ranging from 10^{-4} to 10^{-7} are given in Table 5-5.

The need to accurately characterize the target fisher population of interest in order to establish sufficiently protective SVs cannot be overemphasized. For example, the recommended consumption rate of 142.4 g/d for subsistence fishers may be an underestimate of consumption rate and exposures for some subsistence populations such as Native American subsistence fishers (see Section 5.1.3.2). In a recent study of a Native American subsistence fishing population, an average daily consumption rate for these subsistence fishers was estimated to be 540 g/d (Harris and Harper, 1997). Using this average consumption rate and an estimated average body weight of 70 kg, the SV for cadmium (RfD = 1×10^{-3} mg/kg/d) is, from Equation 5-4,

$$SV = (0.001 \text{ mg/kg-d} \cdot 70 \text{ kg}) / (0.540 \text{ kg/d}) = 0.129 \text{ mg/kg (ppm)}. \quad (5\text{-}7)$$

This value is almost four times lower than the SV of 0.491 ppm for cadmium based on the EPA default consumption rate of 142.4 g/d for subsistence fishers, as shown in Table 5-4.

5.3 COMPARISON OF TARGET ANALYTE CONCENTRATIONS WITH SCREENING VALUES

As noted previously, the same SV for a specific target analyte should be used in both the screening and intensive studies. The measured concentrations of target analytes in fish or shellfish tissue should be compared with their respective SVs in both screening and intensive studies to determine the need for additional monitoring and risk assessment.

Recommended procedures for comparing target analyte concentrations with SVs are provided below. Related guidance on data analysis is given in Section 9.1.

5.3.1 Metals

5.3.1.1 Arsenic—

Most of the arsenic present in fish and shellfish tissue is organic arsenic, primarily pentavalent arsenobetaine, which has been shown in numerous studies to be metabolically inert and nontoxic (Brown et al., 1990; Cannon et al., 1983; Charbonneau et al., 1978; Bos et al., 1985; Kaise et al. 1985; Luten et al., 1982; Sabbioni et al., 1991; Siewicki, 1981; Bryce et al., 1982; Vahter et al., 1983; Yamauchi et al., 1986). Inorganic arsenic, which is of concern for human health effects (ATSDR, 1998a; WHO, 1989), is generally found in seafood at concentra-

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Table 5-5. Example Screening Values (SVs) for Various Target Populations and Risk Levels (RLs)[a]

Chemical	Target population[b]	CR[c]	BW	RfD	CSF	RL	SV (ppm)
Noncarcinogens							
Chlorpyrifos	Recreational fisher	17.5	70	3×10^{-4}	—	—	1.2
	Children (<6 yr)	6.5	17[d]	3×10^{-5}[e]	—	—	0.078
	Subsistence fisher	142.4	70	3×10^{-4}	—	—	0.147
Cadmium	Recreational fisher	17.5	70	1×10^{-3}	—	—	4.0
	Children	6.5	17[d]	1×10^{-3}	—	—	2.6
	Subsistence fisher	142.4	70	1×10^{-3}	—	—	0.491
Carcinogens							
Lindane	Recreational fisher	17.5	70	—	1.3 1.3 1.3 1.3	10^{-4} 10^{-5} 10^{-6} 10^{-7}	3.07×10^{-1} 3.07×10^{-2} 3.07×10^{-3} 3.07×10^{-4}
	Children	6.5	17[d]	—	1.3 1.3 1.3 1.3	10^{-4} 10^{-5} 10^{-6} 10^{-7}	1.98×10^{-1} 1.98×10^{-2} 1.98×10^{-3} 1.98×10^{-4}
	Subsistence fisher	142.4	70	—	1.3 1.3 1.3 1.3	10^{-4} 10^{-5} 10^{-6} 10^{-7}	3.78×10^{-2} 3.78×10^{-3} 3.78×10^{-4} 3.78×10^{-5}
Toxaphene	Recreational fisher	17.5	70	—	1.1 1.1 1.1 1.1	10^{-4} 10^{-5} 10^{-6} 10^{-7}	3.63×10^{-1} 3.63×10^{-2} 3.63×10^{-3} 3.63×10^{-4}
	Children	6.5	17[d]	—	1.1 1.1 1.1 1.1	10^{-4} 10^{-5} 10^{-6} 10^{-7}	2.35×10^{-1} 2.35×10^{-2} 2.35×10^{-3} 2.35×10^{-4}
	Subsistence fisher	142.5	70	—	1.1 1.1 1.1 1.1	10^{-4} 10^{-5} 10^{-6} 10^{-7}	4.6×10^{-2} 4.6×10^{-3} 4.6×10^{-4} 4.6×10^{-5}

CR = Mean daily fish or shellfish consumption rate (uncooked weight), averaged over a 70-yr lifetime for the population of concern (g/d).
BW = Mean body weight, estimated for the population of concern (kg).
RfD = Oral reference dose for noncarcinogens (mg/kg-d).
CSF = Oral slope factor for carcinogens $(mg/kg\text{-}d)^{-1}$.
RL = Maximum acceptable risk level for carcinogens (dimensionless).

[a] See Equations 5-4 and 5-5.
[b] See Tables 5-1, 5-2, 5-3 and 5-4 for information on target populations.
[c] To calculate SVs, the CRs given in this table must be divided by 1,000 to convert g/d to kg/d.
[d] BW used is for children 3 to <6 yr (see Table 5-1).
e Because of the potential for adverse neurological developmental effects, EPA recommends the use of a Population Adjusted Dose for chlorpyrifos of 3×10^{-5} mg/kg-d for infants, children to the age of 6, and women ages 13 to 50 years (U.S. EPA, 2000b).

tions ranging from <1 to 20 percent of the total arsenic concentration (Edmonds and Francesconi, 1993; Nraigu and Simmons, 1990). It is recommended that, in both screening and intensive studies, total inorganic arsenic tissue concentrations be determined for comparison with the recommended SV for chronic oral exposure. This approach is more rigorous than the current FDA-recommended method of analyzing for total arsenic and estimating inorganic arsenic concentrations based on the assumption that 10 percent of the total arsenic in fish tissue is in the inorganic form (U.S. FDA, 1993). Although the cost of analysis for inorganic arsenic (see Table 8-5) may be three to five times greater than for total arsenic, the increased cost is justified to ensure that the most accurate data are obtained for quantitative assessment of human health risks.

5.3.1.2 Cadmium, Mercury, and Selenium—

For cadmium, mercury, and selenium, the total metal tissue concentration should be determined for comparison with the appropriate target population SV.

Because most mercury in fish and shellfish tissue is present as methylmercury (Kannan et al., 1998; NAS, 1991; Tollefson, 1989), and because of the relatively high analytical cost for methylmercury, it is recommended that total mercury be determined and the conservative assumption be made that all mercury is present as methylmercury. The determination of methylmercury in fish tissue is not recommended even though methylmercury is the compound of greatest concern for human health (NAS, 1991; Tollefson, 1989) and the recommended SVs are for methylmercury (see Tables 5-3 and 5-4). This approach is deemed to be most protective of human health and most cost-effective.

5.3.1.3 Tributyltin—

Tissue samples should be analyzed specifically for tributyltin for comparison with the recommended target population SVs for this compound (see Tables 5-3 and 5-4).

5.3.2 Organics

For each of the recommended organic target analytes that are single compounds, the determination of tissue concentration and comparison with the appropriate SV is straightforward. However, for those organic target analytes that include a parent compound and structurally similar compounds or metabolites (i.e., total chlordane, total DDT, endosulfan I and II) or that represent classes of compounds (i.e., PAHs, PCBs, dioxins/furans, or toxaphene), additional guidance is necessary to ensure that a consistent approach is used to determine appropriate target analyte concentrations for comparison with recommended SVs.

5.3.2.1 Chlordane—

The SVs for total chlordane are derived from technical-grade chlordane. Oral cancer slope factors are not available in IRIS (1999) for *cis*- and *trans*-chlordane, *cis*- and *trans*-nonachlor, and oxychlordane. At this time, as a conservative approach, EPA recommends that, in both screening and intensive studies, the concentrations of all chlordane constituents (*cis*- and *trans*-chlordane, *cis*- and *trans*-nonachlor) and the metabolite of chlordane (oxychlordane) be determined and summed to give a total chlordane concentration for comparison with the recommended SVs (see Tables 5-3 and 5-4).

5.3.2.2 DDT—

DDT and its metabolites (i.e., the 4,4'- and 2,4'-isomers of DDE and DDD) are all potent toxicants, DDE isomers being the most prevalent in the environment. As a conservative approach, EPA recommends that, in both screening and intensive studies, the concentrations of 4,4'- and 2,4'-DDT and their 4,4' and 2,4'-DDE and DDD metabolites be determined and a total DDT concentration be calculated for comparison with the recommended SVs for total DDT (see Tables 5-3 and 5-4).

5.3.2.3 Endosulfan—

Endosulfan collectively refers to two stereoisomers designated I and II. At this time, for both screening and intensive studies, EPA recommends that the concentrations of the two endosulfan constituents (endosulfan I and II) be determined and summed to give a total endosulfan concentration for comparison with the recommended SVs for total endosulfan.

5.3.2.4 Toxaphene—

The SVs for toxaphene are derived from technical-grade toxaphene, a mixture of approximately 670 chlorinated camphenes (ATSDR, 1996). At this time, determination of total toxaphene is recommended rather than individual congener analysis. Research is currently under way to determine the relative health risks of the toxaphene congeners. In the future, it may be possible to develop a congener-specific quantitative risk assessment approach for toxaphene similar to that for PCBs and dioxins/furans. The total toxaphene concentration should be analyzed for comparison with the recommended SVs for toxaphene (see Tables 5-3 and 5-4).

5.3.2.5 PAHs—

Although several PAHs have been classified as B2 carcinogens (probable human carcinogens), benzo[*a*]pyrene is the only PAH for which a CSF is currently available in IRIS (1999). As a result, EPA quantitative risk estimates for PAH mixtures have often assumed that all carcinogenic PAHs are equipotent to benzo[*a*]pyrene. The EPA Office of Health and Environmental Assessment has issued guidance for quantitative risk assessment of PAHs (Nisbet and LaGoy, 1992; U.S. EPA, 1993c) in which an estimated order of potential potency for 14 PAHs relative to benzo[*a*]pyrene is recommended, as shown in Table 5-6. Based on this guidance, EPA recommends that, in both screening and intensive studies, tissue samples be analyzed for the PAHs shown in Table 5-6 and that a potency-weighted total concentration be calculated for each sample for comparison with the recommended SVs for benzo[*a*]pyrene (see Tables 5-3 and 5-4). *This potency equivalency concentration should be calculated using the* following equation:

$$PEC = \sum_i (RP_i \cdot C_i) \qquad (5\text{-}8)$$

where

RP_i = Relative potency for the ith PAH (from Table 5-6)
C_i = *Concentration of the ith PAH.*

Table 5-6. Toxicity Equivalency Factors for Various PAHs

Compound	Toxicity Equivalency Factor (TEF)
Dibenz[*a,h*]anthracene	5
Benzo[*a*]pyrene	1
Benz[*a*]anthracene	0
Benzo[*b*]fluoranthene	0.1
Benzo[*k*]fluoranthene	0.1
Indeno[*1,2,3-cd*]pyrene	0.1
Anthracene	0.01
Benzo[*g,h,i*]perylene	0.01
Chrysene	0.01
Acenaphthene	0.001
Acenaphthylene	0.001
Fluoranthene	0.001
Fluorene	0.001
Phenanthrene	0.001
Pyrene	0.001

Source: Nisbet and LaGoy (1992).

5.3.2.6 PCBs—

Using the approach for PCB analysis recommended by the EPA Office of Water (see Section 4.3.6), total PCB concentrations may be determined as the sum of Aroclor equivalents in screening studies. For intensive studies, the total PCB concentration should be determined as the sum of PCB congeners or the sum of homologue groups. The total PCB concentration should be compared with the recommended SVs for PCBs (see Tables 5-3 and 5-4). The EPA Office of Water *recognizes the potential problems associated with PCB congener analysis (i.e.,* standard methods are not yet available but are under development, relatively high analytical cost, and limited number of qualified laboratories), but is recommending these methods for intensive studies because Aroclor analysis does not adequately represent bioconcentrated PCB mixtures found in fish tissue. EPA has developed a draft method for selected PCB congeners (Method 1668) (U.S. EPA, 1997a). This method is being tested and may be revised to include all PCB congeners. Currently, Method 680 is available for PCB homologue analysis.

5.3.2.7 Dioxins and Dibenzofurans—

Note: *At this time, EPA's Office of Research and Development is reevaluating the potency of dioxins/furans. Consequently, the following recommendation may change pending the results of this reevaluation.*

It is recommended in both screening and intensive studies that the 17 2,3,7,8-substituted tetra- through octa-chlorinated PCDDs and PCDFs and the 12 coplanar congeners with dioxin-like effects be determined and that a toxicity-weighted total concentration be calculated for each sample for comparison with the recommended SVs for 2,3,7,8-TCDD (see Tables 5-3 and 5-4).

The method for estimating total TEQ (Van den Berg et al., 1998) should be used to estimate TCDD equivalent concentrations according to the following equation:

$$TEQ = \sum_i (TEF_i \cdot C_i) \tag{5-9}$$

where

TEF_i = Toxicity equivalency factor for the ith congener (relative to 2,3,7,8-TCDD)

C_i = Concentration of the ith congener.

TEFs for the 2,3,7,8-substituted tetra- through octa-PCDDs and PCDFs and the 12 dioxin-like PCBs are shown in Table 5-7. Note: TEFs for five congeners have changed over those TEFs recommended by Barnes and Bellin (1989).

Table 5-7. Toxicity Equivalency Factors (TEFs) for Tetra- through Octa-Chlorinated Dibenzo-p-Dioxins and Dibenzofurans and Dioxin-Like PCBs

Analyte	Old TEF-89	TEF-98
Dioxins[a]		
2,3,7,8-TCDD	1.00	1.00
1,2,3,7,8-PeCDD	0.50	1.00*
1,2,3,4,7,8-HxCDD	0.10	0.10
1,2,3,6,7,8-HxCDD	0.10	0.10
1,2,3,7,8,9-HxCDD	0.10	0.10
1,2,3,4,6,7,8-HpCDD	0.01	0.01
OCDD	0.001	0.0001*
Furans[a]		
2,3,7,8-TCDF	0.10	0.10
1,2,3,7,8-PeCDF	0.05	0.05
2,3,4,7,8-PeCDF	0.50	0.50
1,2,3,4,7,8-HxCDF	0.10	0.10
1,2,3,6,7,8-HxCDF	0.10	0.10
1,2,3,7,8,9-HxCDF	0.10	0.10
2,3,4,6,7,8-HxCDF	0.10	0.10
1,2,3,4,6,7,8-HpCDF	0.01	0.01
1,2,3,4,7,8,9-HpCDF	0.01	0.01
OCDF	0.001	0.0001*
PCBs		
3,3',4,4'-TetraCB (77)	0.0005	0.0001*
3,4,4',5-TetraCB (81)	not available	0.0001*
2,3,3',4,4'-PentaCB (105)	0.0001	0.0001
2,3,4,4',5-PentaCB (114)	0.0005	0.0005
2,3',4,4',5-PentaCB (118)	0.0001	0.0001
2',3,4,4',5-PentaCB (123)	0.0001	0.0001
3,3',4,4',5-PentaCB (126)	0.1	0.1
2,3,3',4,4',5-HexaCB (156)	0.0005	0.0005
2,3,3',4,4',5'-HexaCB (157)	0.0005	0.0005
2,3',4,4',5,5'-HexaCB (167)	0.00001	0.00001
3,3',4,4',5,5'-HexaCB (169)	0.01	0.01
2,3,3',4,4',5,5--HexaCB (189)	0.0001	0.0001

Sources: Barnes and Bellin, 1989; Van den Berg et al., 1998.

*Note: TEF-98 value changed from TEF-89 value.

[a]TEFs for all non-2,3,7,8-substituted congeners are zero.

SECTION 6

FIELD PROCEDURES

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

6.1 SAMPLING DESIGN

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

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

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

The seven major parameters of the sampling plan should be documented on a sample request form prepared by the program manager for each sampling site. The sample request form should provide the field collection team with readily available information on the study objective, site location, site name/number, target species and alternate species to be collected, target analytes to be evaluated, anticipated sampling dates, sample type to be collected, number and size range of individuals to be collected for each composite sample, sampling method to be used, and number of replicates to be collected. An example of a sample request form is shown in Figure 6-1. The original sample request form should be filed with the program manager and a copy kept with the field logbook. The seven major parameters that must be specified in the sampling plan for screening and intensive studies are discussed in Sections 6.1.1 and 6.1.2, respectively.

6.1.1 Screening Studies (Tier 1)

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

6.1.1.1 Site Selection—

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

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

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

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

- Areas where sediments are disturbed by dredging activities

Sample Request Form

Project Objective
- ☐ Screening Study
- ☐ Intensive Study

Sample Type

Screening Study:
- ☐ 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 ________)*

Intensive Study:
- ☐ 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

Screening Study:
- ☐ All target contaminants
- ☐ Additional contaminants *(Specify ________)*

Intensive Study:
- ☐ Contaminants exceeding screening study SVs *(Specify ________ ________)*

INSTRUCTIONS TO SAMPLE COLLECTION TEAM

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

County/Parish: ________ Lat./Long.: ________

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

☐ Freshwater ________ ________

☐ Estuarine ________ ________

________ ________

Proposed Sampling Dates: ________

Proposed Sampling Method: ________

- ☐ Electrofishing
- ☐ Seining
- ☐ Trawling
- ☐ Mechanical grab or tongs
- ☐ Biological dredge
- ☐ Hand collection
- ☐ Other *(Specify* ________)

Number of Sample Replicates:
- ☐ No field replicates (1 composite sample only)
- ☐ ________ field replicates *(Specify number for each target species)*

Number of Individuals per Composite:

________Fish per composite

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

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

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

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

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

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

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

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

Availability of data on the indigenous fish and shellfish communities should be considered in final site selection. Information on preferred feeding areas and migration patterns is valuable in locating populations of the target species (Versar, 1982). Knowledge of habitat preference provided by fisheries biologists or commercial fishermen may significantly reduce the time required to locate a suitable population of the target species at a given site.

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

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

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

Each sampling site must be described accurately because state fish and shellfish contaminant monitoring data may be stored in a database available to users nationwide (see Section 9.2). For example, a sampling site may be defined as a 2-mile section of river (e.g., 1 mile upstream and 1 mile downstream of a reference point) or a 2-mile stretch of lake or estuarine/marine shoreline (U.S. EPA, 1990d). Each sampler should provide a detailed description of each site *using a 7.5-minute* USGS *map to determine the exact latitude and longitude* coordinates for the reference point of the site. This information should be documented on the sample request form and field record sheets (see Section 6.2.3).

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

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

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

Freshwater Threatened and Endangered Species

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

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

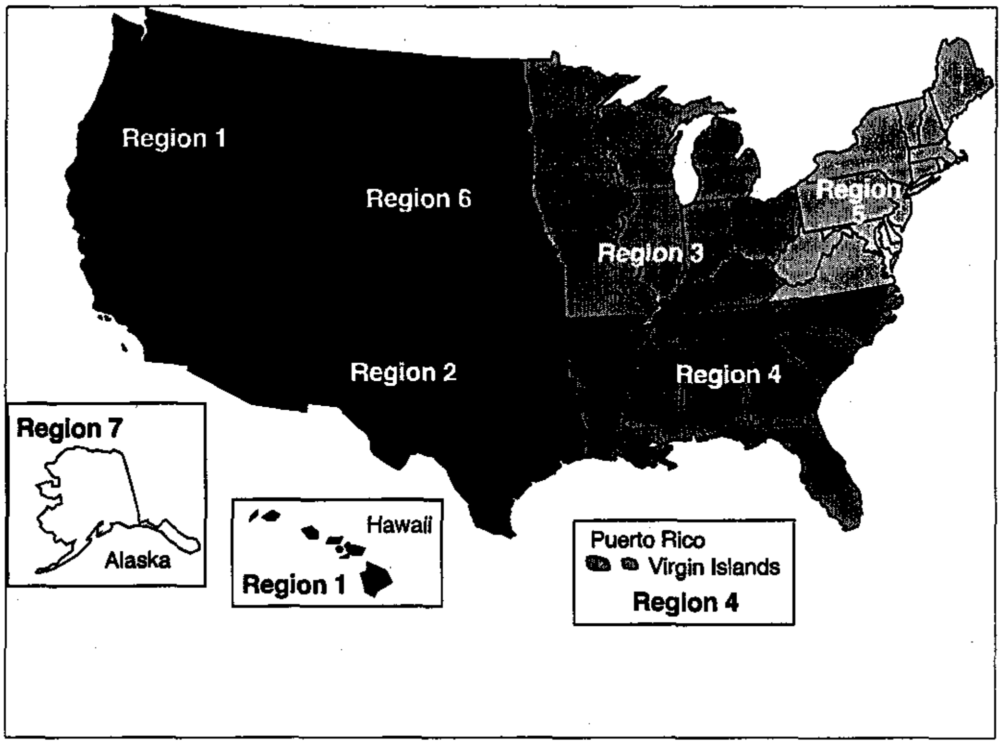

Figure 6-2. U.S. Fish and Wildlife Service Regions.

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

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

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

The application must also include a conservation plan that specifies

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

The completed application should be submitted to

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

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

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

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

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

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

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

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

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

Marine or Anadromous Threatened and Endangered Species

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

States are required to submit information about the following:

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

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

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

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

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

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

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

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

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

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

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

Threatened or Endangered Sea Turtles

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

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

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

- Type of permit

- Date of application

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

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

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

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

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

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

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

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

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

- A list of all sources of data used in preparation of the plan, including reference reports, environmental assessments and impact statements, and personal communications with recognized experts on the species or activity who may have access to data not published in the current literature.
- Other measures the Assistant Administrator of NMFS may require as necessary or appropriate for the purposes of the plan.

The following criteria are considered for permit issuance:

- *Status of the stock and/or species to be incidentally taken*
- Likely direct and indirect impacts of the activity on sea turtles
- Availability and effectiveness of monitoring and enforcement programs
- Public comments received during the 30-day public notice and comment period
- Adequate funding for the Conservation Plan
- The fact that taking will not appreciably reduce the likelihood of survival and recovery of the species in the wild.

An issued permit would

- Require regular reporting and rights of inspection
- Identify species and number of animals allowed to be taken incidentally
- Specify the authorized method of incidental taking
- Require procedures for captured sea turtles (i.e., resuscitation techniques, disposal)
- Potentially impose administrative fees
- Establish duration of the permit
- Specify any other terms or conditions that the Assistant Administrator of NMFS identifies as necessary or appropriate
- The application may be submitted electronically if possible (either by e-mail or by mailing a diskette), but one signed original of the complete application must be sent to

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

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

6.1.1.2 Target Species and Size Class Selection—

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

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

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

Option 1 (single composite analysis for each of three size classes) provides additional information on size-specific levels of contamination that may allow states to issue an advisory for only the most contaminated size classes while allowing other size classes of the target species to remain open to fishing. The state could analyze the composite sample from the largest size class first. If any SVs are exceeded, analysis of the smaller size class composite samples could be conducted. This option, however, does not provide any additional information for estimating the variability of the contamination level in any specific size class. To obtain information for estimating the variability of the contamination level in the target species, states could separately analyze each individual fish specimen in any composite that exceeded the SVs. **Note:** This option of analyzing individual fish within a composite sample is more resource-intensive with respect to analytical costs but is currently used by some Great Lakes states.

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

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

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

6.1.1.3 Target Analyte Selection—

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

6.1.1.4 Target Analyte Screening Values—

To enhance national consistency in screening study data, states should use the target analyte screening values listed in Tables 5-3 and 5-4 to evaluate tissue contaminant data. Specific methods used to calculate SVs for noncarcinogenic and carcinogenic target analytes, including examples of SVs calculated for selected subpopulations, are given in Sections 5.1 and 5.2. If target analytes different from those default SVs shown in Tables 5-3 and 5-4 are included in a screening study, these calculation procedures should be used to estimate SVs based on typical exposure assumptions for the fish-consuming public for the additional compounds. **Note:** If the state chooses to use a different risk level or consumption rate to address site-specific considerations, the corresponding SVs should be calculated prior to initiation of chemical analyses to ensure that the detection limits of the analytical procedures are sufficiently low to allow reliable quantitation at or below the chosen SV. If analytical methodology is not sensitive enough to reliably quantitate target analytes at or below selected SVs (see Sections 5.2 and 8.2.2 and Table 8-4), program managers must determine appropriate fish consumption guidance based on lowest detectable concentrations or provide justification for adjusting SVs to values at or above achievable method detection limits. It should be emphasized that when SVs are below method detection limits, the failure to detect a target analyte cannot be assumed to indicate that there is no cause for concern for human health effects.

6.1.1.5 Sampling Times—

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

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

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

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

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

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

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

6.1.1.6 Sample Type—

Composite samples of fish fillets or of the edible portions of shellfish are recommended for analysis of target analytes in screening studies (U.S. EPA 1987b; 1989d). For health risk assessments, the recommended composite sample type for chemical analysis should be based on both the study objectives and the sample type consumed by the target population of concern. For example, using skinless fillets for assessing mercury exposures for members of the general population and most recreational fishers is most conservative. Because mercury is differentially concentrated in muscle tissue, leaving the skin on the fish fillet actually results in a lower mercury concentration per gram of skin-on fillet than per gram of skin-off fillet (Gutenmann and Lisk, 1991). In addition, few consumers in the general population eat the skin of the fish, which justifies its removal for analysis, particularly when monitoring concerns are directed solely at mercury contamination. Analysis of skinless fillets may also be more appropriate for some target species such as catfish and other scaleless finfish species. In contrast, using whole fish with skin-on as the sample type for assessing PCBs, dioxins/furans, or organochlorine pesticide exposures in populations of Native Americans, Asian Americans, Caribbean-Americans, or other ethnic groups that consume whole fish in a stew or soup is warranted because these contaminants accumulate in fatty tissues of the fish. Cooking the whole fish to make a stew or soup releases the PCBs, dioxins/furans, or organochlorine contaminants into the broth; thus, the whole fish should be analyzed to mirror the way the consumer prepares the fish. Similarly, using skin-on fillets with belly-flap included for most other scaled fish to evaluate PCB, dioxin/furan, or organochlorine pesticide exposures in the general fishing population or among recreational fishers is appropriate since this is a standard filleting method (see Sections 7.2.2.6 and 7.2.2.7). This method also allows for the inclusion of the fatty belly flap tissue and skin in which organochlorines, PCBs, and dioxins/furans concentrate and takes into account the fact that some consumers may not neatly trim the more highly contaminated fatty tissue from the edible muscle fillet tissue.

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

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

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

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

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

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

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

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

For persistent chlorinated organic compounds (e.g., DDT, dioxin, PCBs, and toxaphene) and methylmercury, the larger (older) individuals within a population are generally the most contaminated (Phillips, 1980; Voiland et al., 1991). As noted earlier, this correlation between increasing size and increasing contaminant concentration is most striking in freshwater finfish species but is less evident in estuarine and marine species. Size is used as a surrogate for age, which provides some estimate of the total time the individual organism has been at risk of exposure. Therefore, the primary target size range ideally should include the larger individuals harvested at each sampling site. In this way, the states will maximize their chances of detecting high levels of chemical contamination in the single composite sample collected for each target species. If this ideal condition cannot be met, the field sampling team should retain individuals of similar length that fall within a secondary target size range.

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

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

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

Deviations from the recommended study design have implications that may make the statistical analyses more complicated. The statistical methods for analyzing composite samples are made tractable and easier-to-use by simplifying the study design. Using equal numbers of fish in replicate composite samples is one way to do this. For example, with equal numbers of fish, the arithmetic average of the replicate composite measurements is an unbiased estimator of the population mean. *When unequal numbers are used, the arithmetic average is no longer* unbiased. Instead, a weighted average of the composite measurements is calculated, where the weight for each composite reflects the number of fish it is made up of. Oftentimes fish are lost or damaged prior to compositing. When several fish are damaged or lost, the allocation of the remaining fish to composites may be reconfigured to allow equal numbers of fish in composites. If this is not possible, care should be taken to adjust the statistical procedures to account for the unequal allocations.

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

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

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

Recommended sample preparation procedures are discussed in Section 7.2.

6.1.1.7 Replicate Samples—

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

6.1.2 Intensive Studies (Tier 2)

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

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

6.1.2.1 Site Selection—

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

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

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

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

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

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

6.1.2.2 Target Species and Size Class Selection—

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

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

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

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

6.1.2.3 Target Analyte Selection—

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

6.1.2.4 Target Analyte Screening Values—

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

6.1.2.5 Sampling Times—

To the extent that program resources allow, sampling in intensive studies should be conducted during the same period or periods during which screening studies were conducted (i.e., when the target species are most frequently harvested for consumption) and should be conducted preferably within 1 year of the screening studies. In some cases, it may be best to combine Phase I and Phase II sampling to decrease both the time required to obtain adequate data for issuance of specific advice relative to species, size classes, and geographic extent and/or the monitoring costs entailed in revisiting the site (see Section 6.1.2.2).

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

6.1.2.6 Sample Type—

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

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

Recommended sample preparation procedures are discussed in Section 7.2.

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

6.1.2.7 Replicate Samples—

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

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

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

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

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

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

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

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

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

If the population variance of the target analyte concentrations at a site is small, fewer replicate composite samples and/or fewer individuals per composite sample may be required to test the null hypothesis of interest with the desired statistical power. In this case, using sample sizes that are larger than required to achieve the desired statistical power would not be cost-effective.

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

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

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

where

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

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

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

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

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

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

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

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

- Minimum detectable difference between the site-specific mean target analyte concentration and the selected SV

- Power of the hypothesis test (i.e., the probability of detecting a true difference when one exists)

- Level of significance (i.e., the probability of rejecting the null hypothesis of no difference between the site-specific mean target analyte concentration and the SV when a difference does not exist)

- Population variance, σ^2 (i.e., the variance in target analyte concentrations among individuals from the same species, which the statistician often must estimate from prior information)

- Cost components (including fixed costs and variable sample collection, preparation, and analysis costs).

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

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

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

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

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

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

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

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

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

Two assumptions are made to examine the statistical power of the test of the null hypothesis of interest. First, it is assumed that the true mean of the site-specific composite target analyte concentrations (μ) is either 10 percent, 25 percent, or 50 percent higher than the screening value. Second, it is presumed that a factor similar to a coefficient of variation, the ratio of the estimated population standard deviation to the screening value (i.e., σ/SV), is 50, 75 or 100 percent. Nine scenarios result from joint consideration of these two assumptions. The power of the test of the null hypothesis that the mean composite target analyte concentration at a site is equal to the SV versus the alternative hypothesis that the mean target analyte concentration is greater than the SV is estimated under each set of assumptions. Estimates of the statistical power for six of the nine scenarios are shown in Table 6-2.

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

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

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

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

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

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

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Table 6-2. (continued)

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

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

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

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

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

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

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

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

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

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

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

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

where the summation occurs over the n composite samples.

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

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

where the summation occurs over the n composite samples.

3. Calculate the test statistic:

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

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

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

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

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

Example

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

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

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

HCB=Hexachlorobenzene.

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

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

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

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

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

6.1.2.7.4 Issuing Statewide Advisories—In addition to issuing fish consumption advisories for individual waterbodies, 18 states have also issued blanket statewide advisories for certain types of waterbodies within their jurisdictions (U.S. EPA, 1999c). States have issued statewide advisories for their freshwater lakes and/or rivers and their coastal waters, which can include estuaries and/or coastal marine waters. States often issue statewide advisories for certain waterbody types to warn the public of the potential for widespread contamination of certain species of fish or shellfish in these waterbodies. In these cases, the state has typically found a level of contamination of a specific pollutant in a particular fish species over a relatively wide geographic area that warrants advising the public of the situation. A state often issues a statewide advisory when, for example, it has many lakes that need to be monitored but has limited resources to collect fish (can sample only four or five lakes per year). If the state has even 100 lakes that need monitoring at the level of resources available, it could take 10 to 20 years to adequately monitor all 100 lakes. As an alternative, some states monitor a small percentage of their lakes and, based on the level of contamination found, many have determined that a statewide advisory should be issued to be conservative with respect to protection of public health. Methylmercury, because it is dispersed and transported via the atmosphere, is the leading pollutant responsible for the issuance of statewide advisories in 15 states, although PCBs, dioxins/furans, cadmium, chlordane, mirex, and DDT are also responsible for statewide advisories in a smaller number of states. Assuming that the levels of contamination are determined based on the fish compositing guidelines in this document, the biggest question is determining which waterbodies to monitor. Finding a "representative" sample of waterbodies is a daunting task since there are many different ways to determine representativeness: size of waterbody, species of interest, dynamics of dispersion of pollutants of interest, or geographical location. Taking a simple random sample of lakes may not achieve sufficient coverage, whereas taking a stratified random sample approach may require more lakes be sampled than can be afforded. A conservative approach may be to look at the "worst case scenario". States may decide to sample the lakes that are believed to have the highest levels of pollutants, based on historical contaminant data, current water and sediment sampling results, or other variables. Another approach would be to select one or two of the factors described above ("representativeness"), stratify the lakes according to these factors, and select a random sample within each stratum. The set of factors for stratification may change every few years or so if it is deemed that some other factors are becoming more indicative of the levels of contamination.

6.2 SAMPLE COLLECTION

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

6.2.1 Sampling Equipment and Use

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

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

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

6.2.1.1 Active Collection—

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

Table 6-4. Summary of Fish Sampling Equipment

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

Source: Versar, 1982.

Table 6-5. Summary of Shellfish Sampling Equipment

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

(continued)

Table 6-5. (continued)

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

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

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

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

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

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

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

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

6.2.1.2 Passive Collection—

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

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

6.2.2 Preservation of Sample Integrity

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

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

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

Note: Ideally, all sample processing (e.g., resections) should be performed at a sample processing facility under cleanroom conditions to reduce the possibility of sample contamination (Schmitt and Finger, 1987; Stober, 1991). However, there may be some situations in which state staff find it necessary to fillet finfish or resect edible turtle or shellfish tissues in the field prior to packaging the samples for shipment to the processing laboratory. This practice should be avoided whenever possible. If states find that filleting fish or resecting other edible tissues must be performed in the field, a clean area should be set up away from sources of diesel exhaust and areas where gasoline, diesel fuel, or grease are used to help reduce the potential for surface and airborne contamination of the samples from PAHs and other contaminants. Use of a mobile laboratory or use of a portable resection table and enclosed hood would provide the best environment for sample processing in the field. General guidance for conducting sample processing under cleanroom conditions is provided in Section 7.2.1. States should review this guidance to ensure that procedures as similar as possible to those recommended for cleanroom processing are followed. If sample processing is conducted in the field, a notation should be made in the field records and on the sample processing record (see Figure 7-2). Procedures for laboratory processing and resection are described in Section 7.2. Procedures for assessing sources of sample contamination through the analyses of field and processing blanks are described in Section 8.3.3.6.

6.2.3 Field Recordkeeping

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

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

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

6.2.3.1 Field Record Form—

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

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

Field Record for Fish Contaminant Monitoring Program — Screening Study

Project Number: ____________ Sampling Date and Time: ____________

SITE LOCATION

Site Name/Number: ____________

County/Parish: ____________ Lat./Long.: ____________

Waterbody Name/Segment Number: ____________

Waterbody Type: ☐ RIVER ☐ LAKE ☐ ESTUARY

Site Description: ____________

____

Collection Method: ____________

Collector Name: ____________
(print and sign)

Agency: ____________ Phone: (___) ____________

Address: ____________

____

FISH COLLECTED

Bottom Feeder—Species Name: ____________

Composite Sample #: ____________ Number of Individuals: ____________

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

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

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

Predator—Species Name: ____________

Composite Sample #: ____________ Number of Individuals: ____________

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

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

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

____

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

Field Record for Shellfish Contaminant Monitoring Program — Screening Study

Project Number: ____________________ Sampling Date and Time: ____________________

SITE LOCATION

Site Name/Number: ____________________

County/Parish: ____________________ Lat./Long.: ____________________

Waterbody Name/Segment Number: ____________________

Waterbody Type: ☐ RIVER ☐ LAKE ☐ ESTUARY

Site Description: ____________________

Collection Method: ____________________

Collector Name: ____________________
(print and sign)

Agency: ____________________ Phone: (____) ____________

Address: ____________________

SHELLFISH COLLECTED

Bivalve Species Name: ____________________

Composite Sample #: ____________________ Number of Individuals: ____________________

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

$\frac{\text{Minimum size}}{\text{Maximum size}} \times 100 =$ ________ $\geq 75\%$ Composite mean size ____________ mm

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

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

Field Record for Fish Contaminant Monitoring Program — Intensive Study

Project Number: ____________________ Sampling Date and Time: ____________________

SITE LOCATION

Site Name/Number: ____________________

County/Parish: ____________________ Lat./Long.: ____________________

Waterbody Name/Segment Number: ____________________

Waterbody Type: ☐ RIVER ☐ LAKE ☐ ESTUARY

Site Description: ____________________

____

Collection Method: ____________________

Collector Name: ____________________
(print and sign)

Agency: ____________________ Phone: (____) ____________

Address: ____________________

____

FISH COLLECTED

Species Name: ____________________ **Replicate Number:** ____________

Composite Sample #: ____________________ Number of Individuals: ____________

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

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

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

____

Species Name: ____________________ **Replicate Number:** ____________

Composite Sample #: ____________________ Number of Individuals: ____________

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

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

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

____

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

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

Project Number: ______________________ Sampling Date and Time: ______________________

SITE LOCATION:

Site Name/Number: ______________________

County/Parish: ______________________ Lat./Long.: ______________________

FISH COLLECTED

Species Name: ______________________ **Replicate Number:** ________

Composite Sample #: ______________ Number of Individuals: ______________

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

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

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

Species Name: ______________________ **Replicate Number:** ________

Composite Sample #: ______________ Number of Individuals: ______________

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

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

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

Species Name: ______________________ **Replicate Number:** ________

Composite Sample #: ______________ Number of Individuals: ______________

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

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

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

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Figure 6-5. (continued)

Field Record for Shellfish Contaminant Monitoring Program — Intensive Study

Project Number: ______________________ Sampling Date and Time: ______________________

SITE LOCATION

Site Name/Number: ______________________

County/Parish: ______________________ Lat./Long.: ______________________

Waterbody Name/Segment Number: ______________________

Waterbody Type: ☐ RIVER ☐ LAKE ☐ ESTUARY

Site Description: ______________________

Collection Method: ______________________

Collector Name: ______________________
(print and sign)

Agency: ______________________ Phone: (____) ______________________

Address: ______________________

SHELLFISH COLLECTED

Species Name: ______________________ **Replicate Number:** ______________________

Composite Sample #: ______________________ Number of Individuals: ______________________

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

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

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

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

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

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

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

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

6.2.3.2 Sample Identification Label—

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

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

<table>
<tr><td colspan="2">Species Name or Code</td><td>Sample Type</td></tr>
<tr><td>Total Length or Size (mm)</td><td colspan="2">Sampling Site (name/number)</td></tr>
<tr><td colspan="2" rowspan="2">Specimen Number
☐☐☐☐☐ — ☐☐☐</td><td>Sampling Date (YYYMMDD)</td></tr>
<tr><td>Time (24-h clock)</td></tr>
</table>

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

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

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

6.2.3.3 Chain-of-Custody Label or Tag—

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

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

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

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

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

Chain-of-Custody Record

Project Number	Collecting Agency (name, address, phone)					Sampling Date	Chemical Analyses		
Samplers (print and sign)						Container ___ of ___			
Composite Number	Specimen 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 6-9. Example of a chain-of-custody record form.

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

6.2.3.4 Chain-of-Custody Form—

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

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

6.2.3.5 Field Logbook—

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

6.3 SAMPLE HANDLING

6.3.1 Sample Selection

6.3.1.1 Species Identification—

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

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

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

6.3.1.2 Initial Inspection and Sorting—

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

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

After inspection, each turtle should be placed individually in a heavy burlap sack or canvas bag tied tightly with a strong cord and then placed in an ice-filled cooler. Placing turtles on ice will slow their metabolic rate, making them easier to handle. **Note:** It is recommended that each turtle be analyzed as an individual sample, especially if the target turtle species is not abundant in the waterbody being sampled or if the collected individuals differ greatly in size or age. Analysis of individual turtles can provide an estimate of the maximum contaminant concentrations to which recreational or substistence fishers are exposed. Target analyte concentrations in composite samples represent averages for a specific target species population. The use of these values in risk assessment is appropriate if the objective is to estimate the average concentration to which consumers of the target species are exposed over a long period of time. The use of long exposure periods (e.g., 70 years) is typical for the assessment of *carcinogenic effects, which may be manifest over an entire lifetime* (see Volume II of this guidance series). Noncarcinogenic effects, on the other hand, may cause acute health effects over a relatively short period of time (e.g., hours or days) after consumption. The maximum target analyte contaminant concentration may be more appropriate than the average target analyte concentration for use with noncarginogenic target analytes (U.S. EPA, 1989d). This is especially important for those target analytes for which acute exposures to very high concentrations may be toxic to consumers.

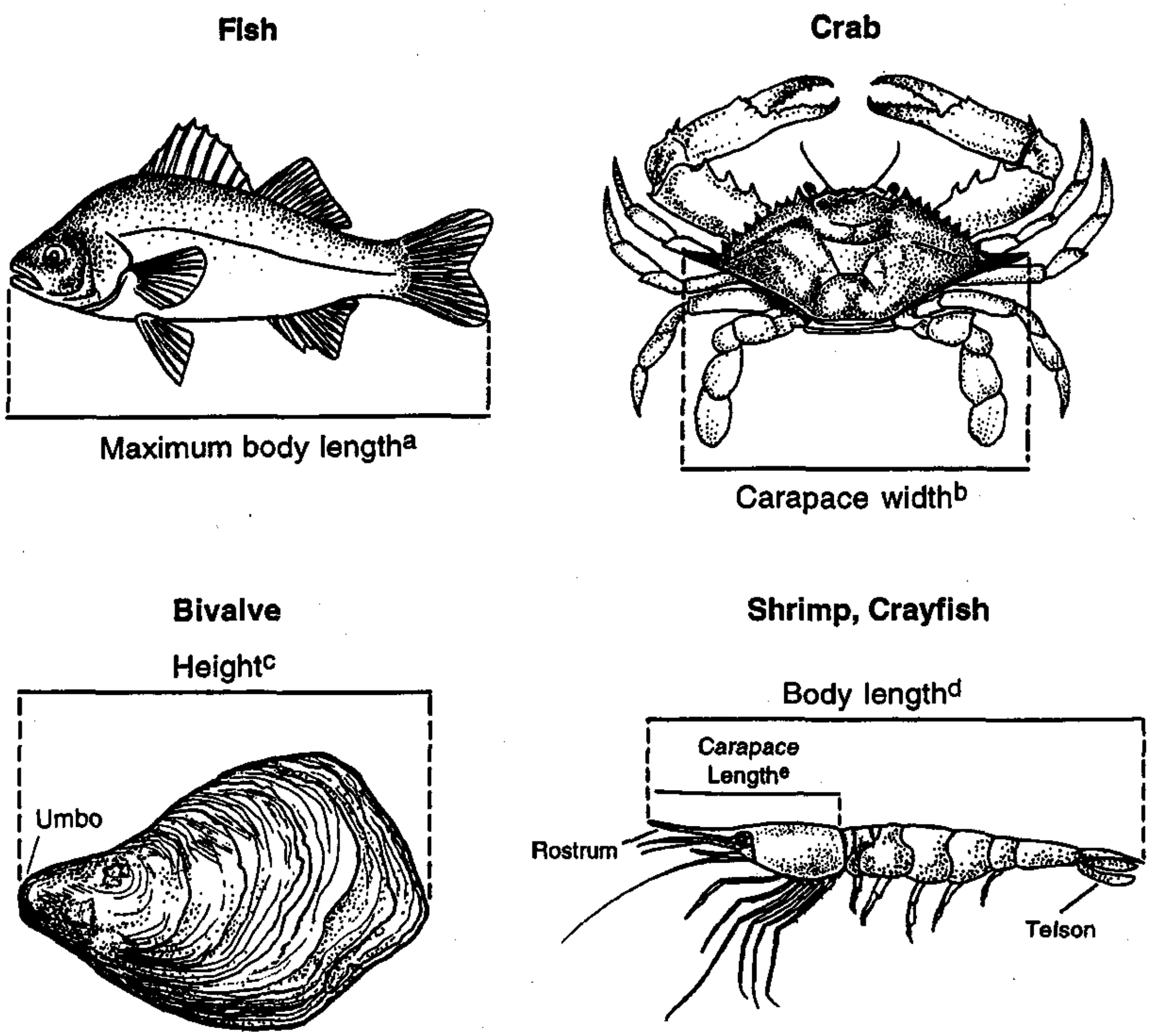
Stone et al. (1980) reported extremely high concentrations of PCBs in various tissues of snapping turtles from a highly contaminated site on the Hudson River. Contaminant analysis of various turtle tissues showed mean PCB levels of 2,991 ppm in fatty tissue, 66 ppm in liver tissue, and 29 ppm in eggs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

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

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

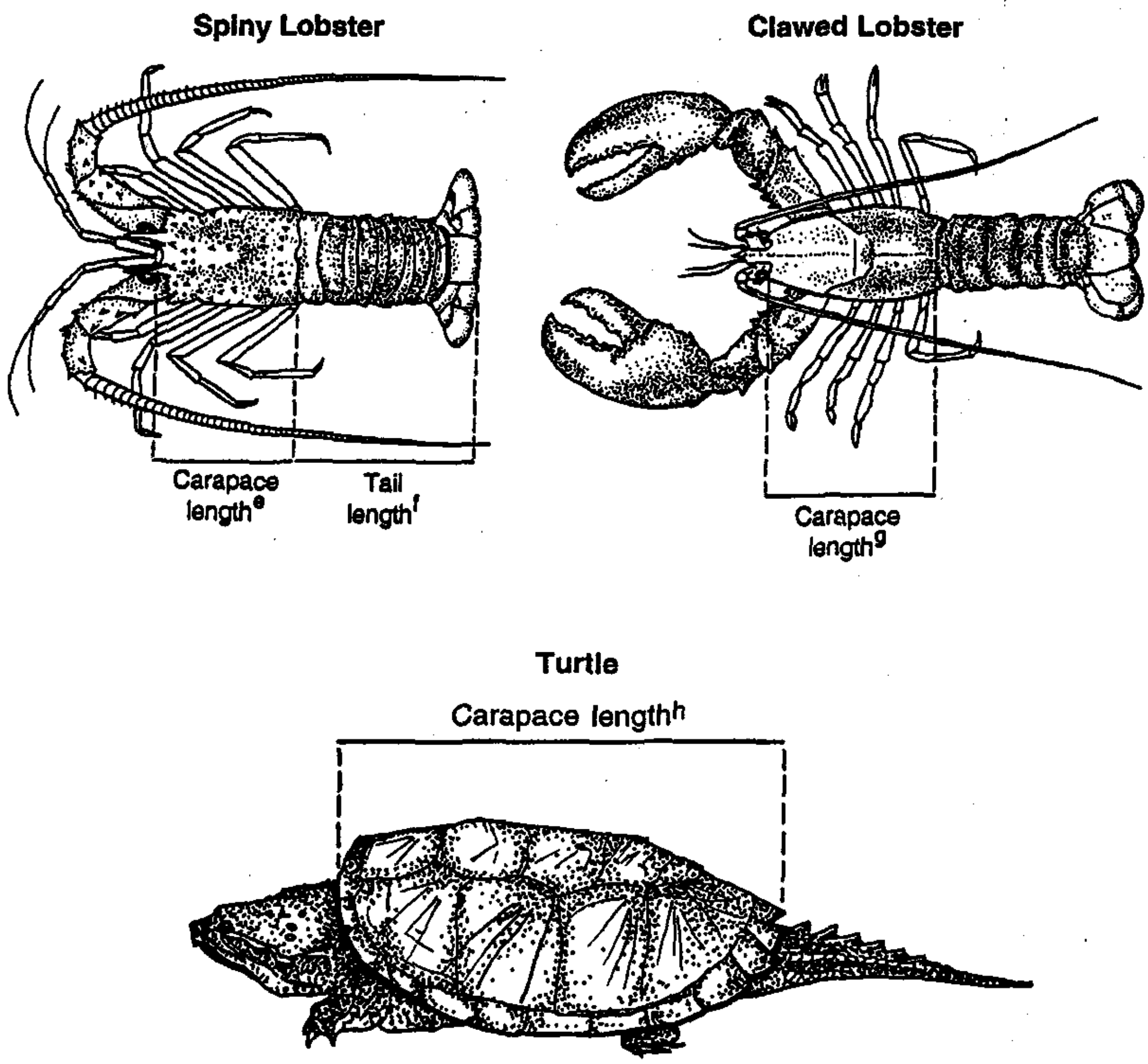
6.3.1.3 Length or Size Measurements—

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



[a] Maximum body length is the length from the anterior-most part of the fish to the tip of the longest caudal fin ray (when the lobes of the caudal fin are compressed dorsoventrally (Anderson and Gutreuter, 1983).
[b] Carapace width is the lateral distance across the carapace (from tip of spine to tip of spine (U.S. EPA, 1990c).
[c] Height is the distance from the umbo to the anterior (ventral) shell margin (Galtsoff, 1964).
[d] Body length is the distance from the tip of the rostrum to the tip of the telson (Texas Water Commission, 1990).
[e] Carapace length is distance from top of rostrum to the posterior margin of the carapace.

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



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

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

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

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

Figure 6-10. (continued)

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

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

6.3.1.4 Sex Determination (Optional)—

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

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

6.3.1.5 Morphological Abnormalities (Optional)—

If resources allow, states may wish to consider documenting external gross morphological conditions in fish from contaminated waters. Severely polluted aquatic habitats have been shown to produce a higher frequency of gross pathological disorders than similar, less polluted habitats (Krahn et al., 1986; Malins et al., 1984, 1985; Mix, 1986; Sinderman, 1983; and Sinderman et al., 1980).

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

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

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

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

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

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

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

6.3.2 Sample Packaging

6.3.2.1 Fish—

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

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

6.3.2.2 Turtles—

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

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

6.3.2.3 Shellfish—

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

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

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

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

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

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

All of the individual aluminum-foil-wrapped shellfish specimens (in the same composite sample) should be placed in the same waterproof plastic bag for transport. In this case, a COC tag or label should be completed for the composite sample and appropriate information recorded on the field record sheet and COC form. The COC label or tag should then be attached to the outside of the plastic bag with string or tape. For composite samples containing more than 10 shellfish specimens or especially large individuals, additional waterproof plastic bags may be required to ensure proper preservation. Once packaged, composite samples should be cooled on ice immediately. **Note:** When a large number of individual specimens in the same composite sample are shipped together in the same waterproof plastic bag, the samples must have adequate space in the bag to ensure that contact with ice can occur; thus ensuring proper preservation during shipping. This is especially important when samples are collected during hot weather and/or when the time between field collection and delivery to the processing laboratory approaches the maximum shipping time (Table 6-8).

6.3.3 Sample Preservation

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

6.3.3.1 Fish, Turtles, or Shellfish To Be Resected–

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

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

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

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

Dry ice requires special packaging precautions before shipping by aircraft to comply with U.S. Department of Transportation (DOT) regulations. The *Code of Federal Regulations* (49 CFR 173.217) classifies dry ice as Hazard Class 9 UN1845 (Hazardous Material). These regulations specify the amount of dry ice that may be shipped by air transport and the type of packaging required. For each shipment by air exceeding 5 pounds of dry ice per package, advance arrangements must be made with the carrier. Not more than 441 pounds of dry ice may be transported in any one cargo compartment on any aircraft unless the shipper has made special written arrangements with the aircraft operator.

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

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

6.3.4 Sample Shipping

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

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

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SECTION 7

LABORATORY PROCEDURES I — SAMPLE HANDLING

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

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

7.1 SAMPLE RECEIPT AND CHAIN-OF-CUSTODY

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

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

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

Fish, shellfish, and turtle samples should be brought or shipped to the sample processing laboratory in sealed containers accompanied by a copy of the sample request form (Figure 6-1), a chain-of-custody form (Figure 6-9), and the field records (Figures 6-3 through 6-6). Each time custody of a sample or set of samples is transferred, the Personnel Custody Record of the COC form must be completed and signed by both parties. Corrections to the COC form should be made in indelible ink by drawing a single line through the original entry, entering the correct information and the reason for the change, and initialing and dating the correction. The original entry should never be obscured.

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

- Note the shipping time. If samples have been shipped on wet or blue ice, check that the shipping time has not exceeded 24 hours.
- Check that each shipping container has arrived undamaged and that the seal is intact.
- Open each shipping container and remove the copy of the sample request form, the COC form, and the field records.
- Note the general condition of the shipping container (samples iced properly with no leaks, etc.) and the accompanying documentation (dry, legible, etc.).
- Locate individuals in each composite sample listed on the COC form and note the condition of their packaging. Individual specimens should be properly wrapped and labeled. Note any problems (container punctured, illegible labels, etc.) on the COC form.
- If individuals in a composite are packaged together, check the contents of each composite sample container against the field record for that sample to ensure that the individual specimens are properly wrapped and labeled. Note any discrepancies or missing information on the COC form.
- Initial the COC form and record the date and time of sample receipt.
- Enter the following information for each composite sample into a permanent laboratory record book and, if applicable, a computer database:
  - — Sample identification number (specify conventions for the composite sample number and the specimen number) **Note:** EPA recommends processing and analysis of turtles as individual samples.
  - — Receipt date (use Year 2000 comliant format [YYYYMMDD])
  - — Sampling date (use Year 2000 comliant format [YYYYMMDD])
  - — Sampling site (name and/or identification number)
  - — Fish, turtle, and shellfish species (scientific name or code number)
  - — Total length of each fish, carapace length of each turtle, or size of each shellfish (mm)

- If samples have been shipped on wet or blue ice, distribute them immediately to the technician responsible for resection (see Section 7.2). See Section 7.2.3 for the procedure for processing turtle samples as individual samples. If samples have been shipped on dry ice, they may be distributed immediately to the technician for processing or stored in a freezer at ≤-20 °C for later processing. Once processed, fillets or edible portions of fish, turtles, or shellfish or tissue homogenates, should be stored according to the procedures described in Section 7.2 and in Table 7-1. **Note:** Holding times in Table 7-1 are maximum times recommended for holding samples from the time they are received at the laboratory until they are analyzed. These holding times are based on guidance that is sometimes administrative rather than technical in nature; there are no promulgated holding time criteria for tissues (U.S. EPA, 1995i). If states choose to use longer holding times, they must demonstrate and document the stability of the target analyte residues over the extended holding times.

7.2 SAMPLE PROCESSING

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

7.2.1 General Considerations

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

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

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

PTFE = Polytetrafluoroethylene (Teflon).

[a] Maximum holding times recommended by EPA (1995i).
[b] This maximum holding time is also recommended by the Puget Sound Estuary Program (1990e). The California Department of Fish and Game (1990) and the USGS National Water Quality Assessment Program (Crawford and Luoma, 1993) recommend a maximum holding time of 6 months for all metals, including mercury.
[c] This maximum holding time is also recommended by the California Department of Fish and Game (1990), the 301(h) monitoring program (U.S. EPA, 1986b), and the USGS National Water Quality Assessment Program (Crawford and Luoma, 1993). The Puget Sound Estuary Program (1990e) recommends a maximum holding time of 2 years.
[d] This maximum holding time is also recommended by the Puget Sound Estuary Program (1990e). The California Department of Fish and Game (1990) and the USGS National Water Quality Assessment Program (Crawford and Luoma, 1993) recommend a more conservative maximum holding time of 6 months. U.S. EPA (1995b) recommends a maximum holding time of 1 year at $\leq$-10 °C for dioxins/furans.

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

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

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

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

7.2.1.1 Samples for Organics Analysis—

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

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

7.2.1.2 Samples for Metals Analysis—

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

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

7.2.1.3 Samples for Both Organics and Metals Analyses—

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

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

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

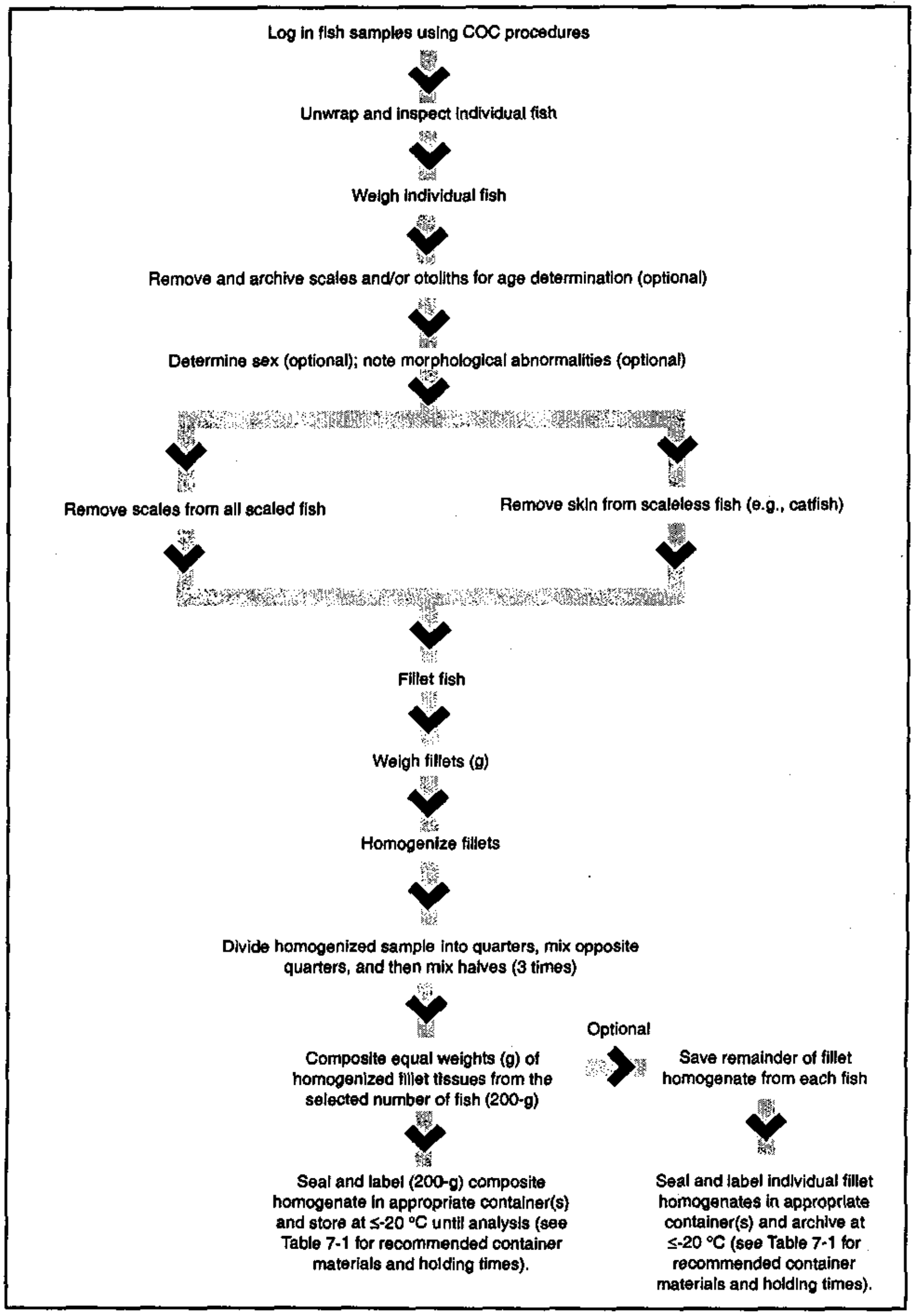
Aliquots of composite homogenates taken for metals analysis (see Section 7.3.1) may be stored in plastic containers that have been cleaned according to the procedure outlined above, with the exception that aqua regia must not be used for the acid soaking step.

7.2.2 Processing Fish Samples

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

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

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



COC = Chain of custody.

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

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

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

7.2.2.1 Sample Inspection—

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

7.2.2.2 Sample Weighing—

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

Fish shipped on wet or blue ice should be weighed directly on a foil-lined balance tray. To prevent cross contamination between individual fish, the foil lining should be replaced after each weighing. Frozen fish (i.e., those shipped on dry ice) should be weighed in clean, tared, noncontaminating containers if they will thaw before the weighing can be completed. **Note:** Liquid from the thawed whole fish sample will come not only from the fillet tissue but from the gut and body cavity, which are not part of the final fillet sample. Consequently, inclusion of this liquid with the sample may result in an overestimate of target analyte and lipid concentrations in the fillet homogenate. Nevertheless, it is recommended, as a conservative approach, that all liquid from the thawed whole fish sample be kept in the container as part of the sample.

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

7.2.2.3 Age Determination (Optional)—

Age provides a good indication of the duration of exposure to pollutants (Versar, 1982). A few scales or otoliths (Jearld, 1983) should be removed from each fish and delivered to a fisheries biologist for age determination. For most warm water inland gamefish, 5 to 10 scales should be removed from below the lateral line and behind the pectoral fin. On soft-rayed fish such as trout and salmon, the scales should be taken just above the lateral line (WDNR, 1988). For catfish and other

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

Project Number: ____________ Sampling Date and Time: ____________

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

SITE LOCATION

Site Name/Number: ____________

County/Parish: ____________ Lat./Long.: ____________

Waterbody Name/Segment Number: ____________ Waterbody Type: ____________

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

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

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

Total Composite Weight (g) (F1 or C) ________ (F2) ________

Notes: ____________

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

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

7.2.2.4 Sex Determination (Optional)—

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

7.2.2.5 Assessment of Morphological Abnormalities (Optional)—

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

7.2.2.6 Scaling or Skinning—

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

A fish is scaled by laying it flat on a clean glass or PTFE cutting board or on one that has been covered with heavy duty aluminum foil and removing the scales and adhering slime by scraping from the tail to the head using the blade edge of a clean stainless steel, ceramic, or titanium knife. Cross-contamination is controlled by rinsing the cutting board and knife with contaminant-free distilled water between fish. If an aluminum-foil-covered cutting board is used, the foil should be changed between fish. The skin should be removed from fish without scales by loosening the skin just behind the gills and pulling it off between knife blade and thumb or with pliers as shown in Figure 7-3.

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

7.2.2.7 Filleting—

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

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

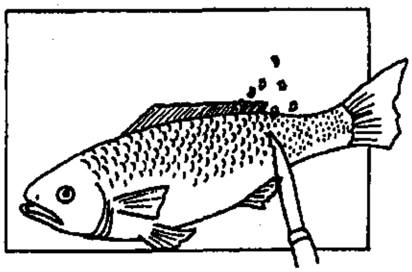
Clean, high-quality stainless steel, ceramic, or titanium utensils should be used to remove one or both fillets from each fish, as necessary. The general procedure recommended for filleting fish is illustrated in Figure 7-3 (U.S. EPA, 1991d).

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

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

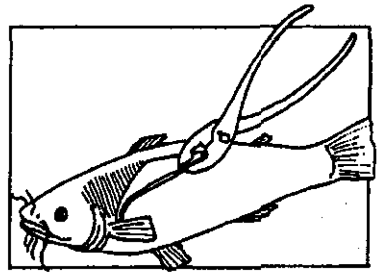
1 **Scaled Fish**

After removing the scales (by scraping with the edge of a knife) and rinsing the fish:



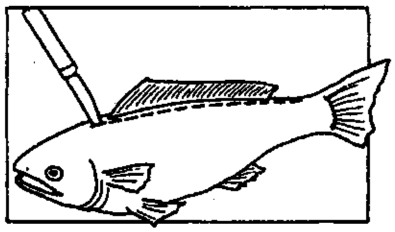
1b **Scaleless Fish**

Grasp the skin at the base of the head (preferably with pliers) and pull toward the tail.



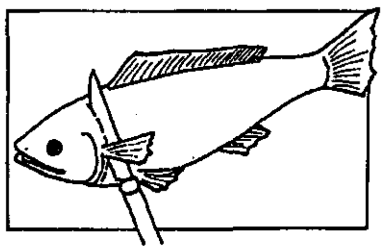
Note: This step applies only for catfish and other scaleless species.

2



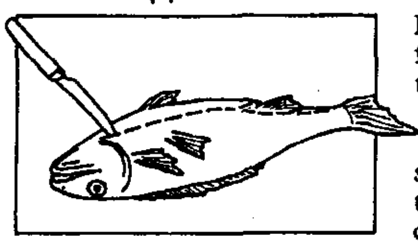
Make a shallow cut through the skin (on either side of the dorsal fin) from the top of the head to the base of the tail.

3



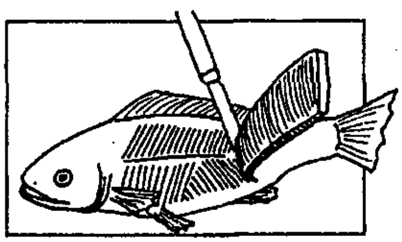
Make a cut behind the entire length of the gill cover, cutting through the skin and flesh to the bone.

4



Make a shallow cut along the belly from the base of the pectoral fin to the tail. A single cut is made from behind the gill cover to the anus and then a cut is made on both sides of the anal fin. Do not cut into the gut cavity as this may contaminate fillet tissues.

5



Remove the fillet.

Source: U.S. EPA, 1991d.

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

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

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

7.2.2.8 Preparation of Individual Homogenates—

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

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

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

The fillet sample should be ground until it appears to be homogeneous. The ground sample should then be divided into quarters, opposite quarters mixed together by hand, and the two halves mixed together. The grinding, quartering, and hand-mixing steps should be repeated at least two more times. If chunks of tissue are present at this point, the grinding and homogenization should be repeated. **Note:** Skin-on fillets are the fish fillet sample type recommended for use in state fish contaminant monitoring programs. However, skin-on fillets of some finfish species are especially difficult to homogenize completely. No chunks of tissue or skin should remain in the sample homogenate because these may not be extracted or digested efficiently and could bias the analytical results. If complete homogenization of skin-on fillets for a particular target species is a chronic problem or if local consumers are likely to prepare skinless fillets of the species, the state should consider analyzing skinless fillet samples. If the sample is to be analyzed for metals only, the ground tissue may be mixed by hand in a polyethylene bag (Stober, 1991). The preparation of each individual homogenate should be noted (marked with a check) on the sample processing record. At this time, individual homogenates may be either processed further to prepare composite homogenates or frozen separately and stored at ≤-20 °C (see Table 7-1).

7.2.2.9 Preparation of Composite Homogenates—

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

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

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

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

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

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

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

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

[a] Based on total number of fish per composite and the total composite weight required for analysis in screening studies. The total composite weight required in intensive studies may be less if the number of target analytes is reduced significantly.
[b] Individual homogenates may be prepared from one or both fillets from a fish. A composite homogenate should be prepared only from individual homogenates of the same type (i.e., **either** from individual homogenates each prepared from a single fillet **or** from individual homogenates each prepared from both fillets).

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

7.2.3 Processing Turtle Samples

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

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

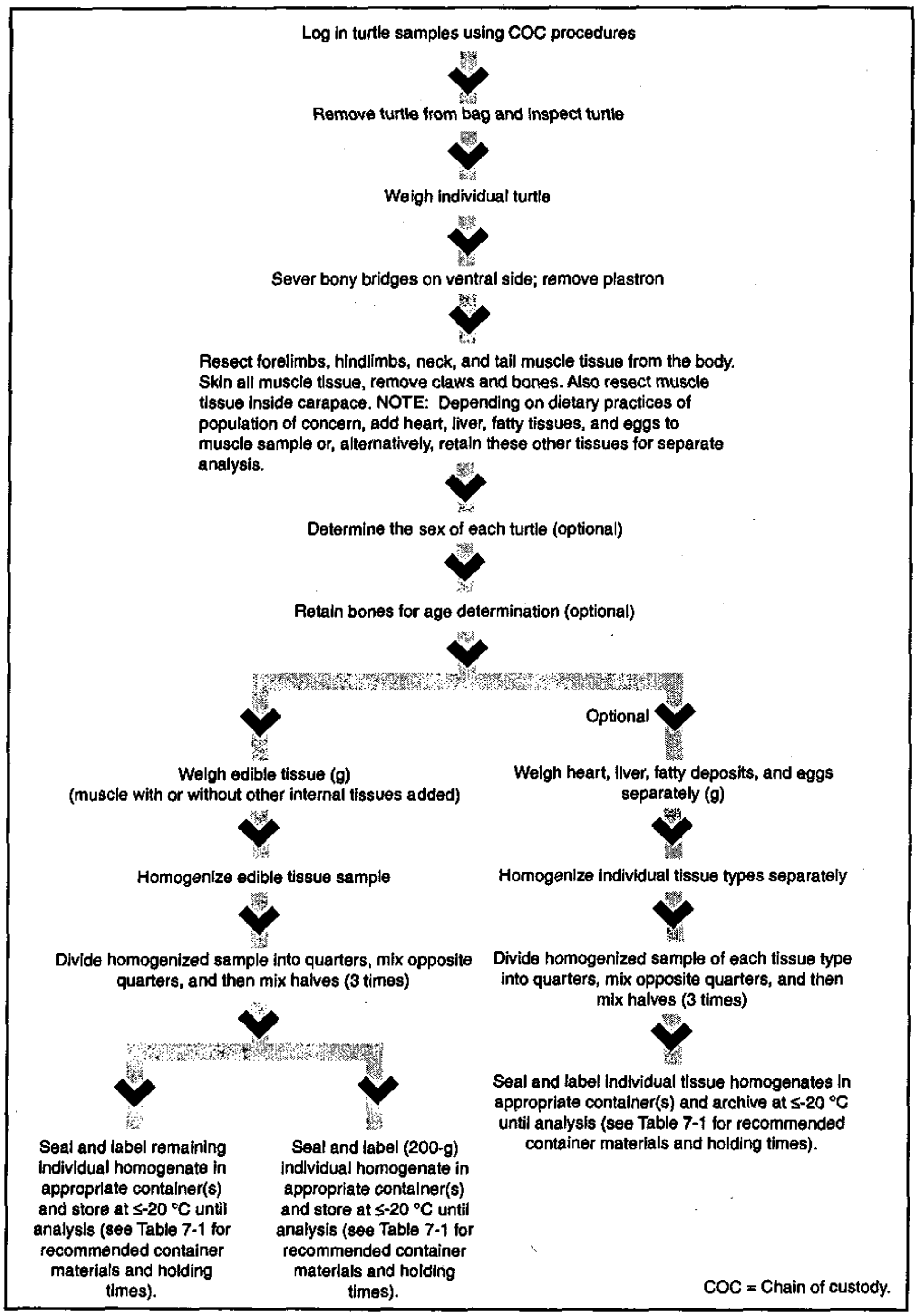


Figure 7-4. Preparation of individual turtle homogenate samples.

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

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

7.2.3.1 Sample Inspection—

Turtles received for resection should be removed from the canvas or burlap collection bags and inspected carefully to ensure that they have not been compromised in any way (i.e., not properly preserved during shipment). Any specimen deemed unsuitable for further processing and analysis should be discarded and identified on the sample processing record.

7.2.3.2 Sample Weighing—

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

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

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

Sample Processing Record for Turtle Contaminant Monitoring Program — Individual Samples

Project Number: ____________________ Sampling Date and Time: ____________________

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

SITE LOCATION

Site Name/Number: ____________________

County/Parish: ____________________ Lat./Long.: ____________________

Waterbody Name/Segment Number: ____________________ Waterbody Type: ____________________

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

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

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

Total Composite Weight (g) ____________

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

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

7.2.3.3 Removal of Edible Tissues—

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

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

Ideally, turtles should be resected while ice crystals are still present in the muscle tissue. Thawing of frozen turtles should be kept to a minimum during tissue removal to avoid loss of liquids. A turtle should be thawed only to the point where it becomes possible to make an incision into the flesh (U.S. EPA, 1991d).

Clean, high-quality stainless steel, ceramic, or titanium utensils should be used to remove the muscle tissue and, depending on dietary or culinary practices of the population of concern, some of the other edible tissues from each turtle. The general procedure recommended for resecting turtles is illustrated in Figure 7-6.

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

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

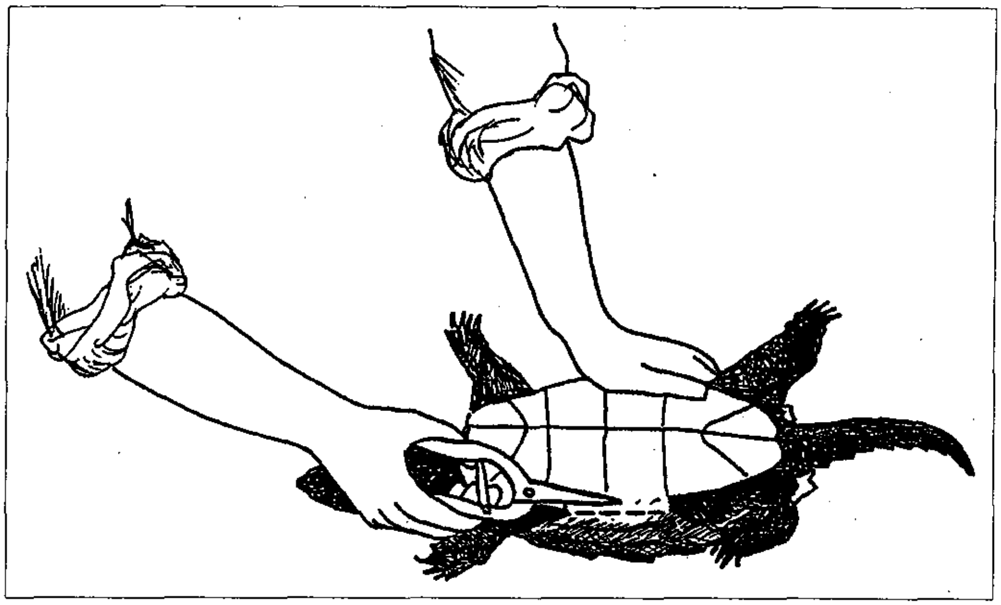


Figure 7-6. Illustration of basic turtle resection procedure.

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

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

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

If the tissues are to be homogenized later, they should be wrapped in heavy duty aluminum foil and labeled with the sample identification number, the sample type (e.g., "M" for muscle, "E" for eggs, or "FD" for fatty deposits), the weight (g), and the date of resection. The individual muscle tissue samples from each turtle should be packaged together and given an individual sample identification number. The date of resection should be recorded and the sample should be stored at ≤-20°C until homogenization. **Note:** State staff may determine that the most appropriate sample type is muscle tissue only, with internal organ tissues analyzed separately (liver, heart, fatty deposits, or eggs). Alternatively, state staff may determine that the most appropriate sample type is muscle tissue with several other internal organs included as the turtle tissue sample. This latter sample type typically will provide a more conservative estimate of contaminant residues, particularly with respect to lipophilic target analytes (e.g., PCBs, dioxins, and organochlorine pesticides).

7.2.3.4 Sex Determination (Optional)—

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

7.2.3.5 Age Determination (Optional)—

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

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

The use of bone rings is the second method that may be used to estimate age in turtles (Enlow and Brown, 1969; Peabody, 1961). Unlike the previous visual method, this method requires that the bones of the turtle be removed during resection and retained for later analysis. The growth rings appear at the surface or inside primary compacta of bone tissues. There are two primary methods for observing growth marks: either directly at the surface of the bone as in flat bones using transmitted or reflected light or inside the long bones using thin sections (Castanet, 1994; Dobie, 1971; Galbraith and Brooks, 1987; Hammer, 1969; Gibbons, 1976; Mattox, 1935; Peabody, 1961). The methods of preparation of whole bones and histological sections of fresh material for growth mark determinations are now routinely performed. Details of these methods can be found in Castanet (1974 and 1987), Castanet et al. (1993), and Zug et al. (1986). State staff interested in using either of these methods for age determination of turtles should read the review articles by Castanet (1994) and Gibbons (1976) for discussions of the advantages and disadvantages of each method, and the associated literature cited in these articles on turtle species of particular interest within their jurisdictions.

7.2.3.6 Preparation of Individual Homogenates—

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

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

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

The tissue sample should be ground until it appears to be homogeneous. The ground sample should then be divided into quarters, opposite quarters mixed together by hand, and the two halves mixed together. The grinding, quartering, and hand-mixing steps should be repeated at least two more times. If chunks of tissue are present at this point, the grinding and homogenization should be repeated. No chunks of tissue should remain because these may not be extracted or digested efficiently and could bias the analytical results. This is particularly true when lipophilic tissues (e.g., fatty deposits, liver, or eggs) are not completely homogenized throughout the sample. Portions of the tissue sample that retain unhomogenized portions of tissues may exhibit higher or lower residues of target analytes than properly homogenized samples.

If the sample is to be analyzed for metals only, the ground tissue may be mixed by hand in a polyethylene bag (Stober, 1991). The preparation of each individual homogenate should be noted (marked with a check) on the sample processing record. At this time, individual homogenates may be frozen separately and stored at ≤-20 °C (see Table 7-1).

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

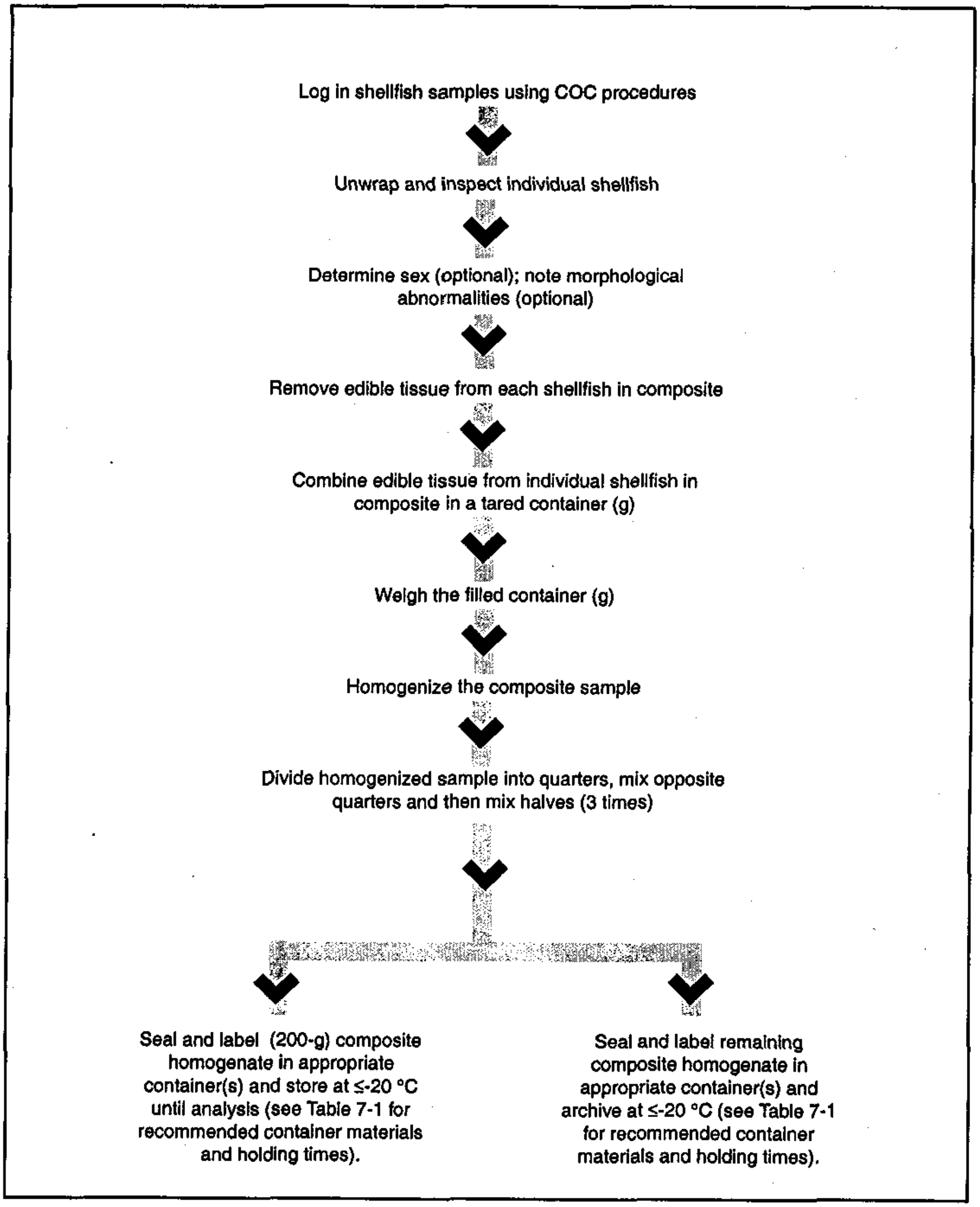
It is essential that the weight of individual homogenate samples is of adequate size to perform all necessary analyses. The recommended sample size of 200 g for screening studies is intended to provide sufficient sample material to (1) analyze for all recommended target analytes (see Table 4-1) at appropriate detection limits; (2) meet minimum QC requirements for the analyses of laboratory duplicate, matrix spike, and matrix spike duplicate samples (see Sections 8.3.3.4 and 8.3.3.5); and (3) allow for reanalysis if the QC control limits are not met or if the sample is lost. However, sample size requirements may vary among laboratories and the analytical methods used. Each program manager must consult with the analytical laboratory supervisor to determine the actual weights of homogenates required to analyze for all selected target analytes at appropriate detection limits. The total sample weight required for intensive studies may be less than that for screening studies if the number of target analytes is reduced significantly.

7.2.4 Processing Shellfish Samples

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

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

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



COC = Chain of custody.

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

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

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

7.2.4.1 Sample Inspection—

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

7.2.4.2 Sex Determination (Optional)—

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

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

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

7.2.4.3 Assessment of Morphological Abnormalities (Optional)—

Assessment of gross morphological abnormalities in shellfish is optional. This assessment may be conducted in the field (see Section 6.3.1.5) or during initial inspection at the processing laboratory prior to removal of the edible tissues. States interested in documenting morphological abnormalities should consult Sinderman and Rosenfield (1967), Rosen (1970), and Murchelano (1982) for

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

Project Number: ____________________ Sampling Date and Time: ____________________

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

SITE LOCATION

Site Name/Number: ____________________

County/Parish: ____________________ Lat./Long.: ____________________

Waterbody Name/Segment Number: ____________________ Waterbody Type: ____________________

SHELLFISH COLLECTED

Species Name: ____________________

Description of Edible Tissue ____________________

Composite Sample #: ____________________ Number of Individuals: ____________________

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

Preparation of Composite:

Weight of container + shellfish ______ g

Weight of container (tare weight) ______ g

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

Notes: ____________________

____

Analyst ____________________ Date ____________________

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

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

7.2.4.4 Removal of Edible Tissue—

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

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

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

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

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

7.2.4.5 Sample Weighing—

Edible tissue from all shellfish in a composite sample (3 to 50 individuals) should be placed in an appropriate preweighed and labeled noncontaminating container. The weight of the empty container (tare weight) should be recorded to the nearest gram on the sample processing record. All fluids accumulated during removal of edible tissue should be retained as part of the sample. As the edible portion of each shellfish is placed in the container, it should be noted on the sample processing record. When the edible tissue has been removed from all shellfish in the composite, the container should be reweighed and the weight recorded to the nearest gram on the sample processing record. The total composite weight should be approximately 200 g for screening studies. If the number of target analytes is significantly reduced in intensive studies, a smaller composite homogenate sample may suffice (see Section 7.2.2.9). At this point, the composite sample may be processed for analysis or frozen and stored at ≤-20°C (see Table 7-1).

7.2.4.6 Preparation of Composite Homogenates—

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

7.3 SAMPLE DISTRIBUTION

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

7.3.1 Preparing Sample Aliquots

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

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

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

PTFE = Polytetrafluoroethylene (Teflon).

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

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

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

Fish and Shellfish Monitoring Program
Sample Aliquot Record

Aliquot prepared by ______________________ Date ____________ Time ____________
(name)

Comments ____

Samples from:

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

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

Page ___ of ___

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

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

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

7.3.2 Sample Transfer

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

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

Released by: ______________________________
(name)

At: ______________________________
(location)

Shipment Method ______________
Shipment Destination ______________________________

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

Released by: ______________________________
(name)

At: ______________________________
(location)

Comments ______________________________

____

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

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

Sample IDs:

Laboratory Chain of Custody

Relinquished by	Received by	Purpose	Location

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

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SECTION 8

LABORATORY PROCEDURES II — SAMPLE ANALYSES

Sample analyses may be conducted by one or more state or private contract laboratories. Because of the toxicity of dioxins/furans and the difficulty and cost of these analyses, relatively few laboratories currently have the capability of performing them. Table 8-1 lists contract laboratories experienced in dioxin/furan analyses. This list is provided for information purposes only and is not an endorsement of specific laboratories.

8.1 RECOMMENDED ANALYTES

8.1.1 Target Analytes

All recommended target analytes listed in Table 4-1 should be included in screening studies unless reliable historic tissue, sediment, or pollutant source data indicate that an analyte is not present at a level of concern for human health. Additional target analytes should be included in screening studies if states have site-specific information (e.g., historic tissue or sediment data, discharge monitoring reports from municipal and industrial sources) that these contaminants may be present at levels of concern for human health.

Intensive studies should include only those target analytes found to exceed screening values in screening studies (see Section 5.2).

8.1.2 Lipid

A lipid analysis should also be performed and reported (as percent lipid by wet weight) for each composite tissue sample in both screening and intensive studies. This measurement is necessary to ensure that gel permeation chromatography columns are not overloaded when used to clean up tissue extracts prior to analysis of organic target analytes. In addition, because bioconcentration of nonpolar organic compounds is dependent upon lipid content (i.e., the higher the lipid content of the individual organism, the higher the residue in the organism), lipid analysis is often considered essential by users of fish and shellfish monitoring data. Consequently, it is important that lipid data are obtained for eventual inclusion in a national database of fish and shellfish contaminant data.

Table 8-1. Contract Laboratories Conducting Dioxin/Furan Analyses in Fish and Shellfish Tissues[a]

Alta Analytical Laboratory[b]
5070 Robert J. Matthews Parkway, Suite 2
Eldorado Hills, CA 95762
916/933-1640
FAX: 916/933-0940
Bill Luksemburg

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

Midwest Research Institute[b]
425 Volker Boulevard
Kansas City, MO 64110
816/753-7600, ext. 1160/1557
FAX: 816/753-8240
John Stanley/Tom Sack
e-mail: JStanley@mriresearch.org
tsack@mriresearch.org

New York State Department of Health[b]
Wadsworth Center
Empire State Plaza
P.O. Box 509
Albany, NY 12201-0509
518/473-3378
FAX: 518/473-2895
Patrick O'Keefe

Pacific Analytical, Inc.[b]
6349 Paseo Del Argo
Carlsbad, CA 92009
760/438-3100
FAX: 760/931-9479
Bruce Colby

Axys Analytical Services[b]
P.O. Box 2219
2045 Mills Road
Sidney, BC V8L 3
Canada
250/656-0881; Toll Free 1-888-373-0881
Coreen Hamilton/Dale Hover/Laurie Phillips

Pace Analytical Services[b]
7726 Moller Road
Indianapolis, IN 46268
317/875-5894
FAX: 317/872-6189
Mick Mayse

Triangle Laboratories[b]
Alston Technical Park
801 Capitola Drive
Durham, NC 27713
919/544-5729
FAX: 919/544-5491
Phil Albro

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

Wright State University[b]
175 Brehm Laboratory
3640 Colonel Glen Highway
Dayton, OH 45435
937/775-2202
FAX: 937/775-3122
Thomas Tiernan/Garrett Van Ness

Quanterra Environmental Services
Knoxville Laboratory
5815 Middlebrook Pike
Knoxville, TN 37921
423/588-6401
FAX: 423/584-4315
David Thal/Tom Yoder

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

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

8.2 ANALYTICAL METHODS

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

8.2.1 Lipid Method

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

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

8.2.2 Target Analyte Methods

EPA has published interim procedures for sampling and analysis of priority pollutants in fish tissue (U.S. EPA, 1981); however, official EPA-approved methods are available only for the analysis of low parts-per-billion concentrations of some metals in fish and shellfish tissues (U.S. EPA, 1991g). Because of the lack of official EPA-approved methods for all recommended target analytes, and to allow states and Regions flexibility in developing their analytical programs, specific analytical methods for recommended target analytes in fish and shellfish monitoring programs are not included in this guidance document.

Note: A performance-based analytical program is recommended for the analysis of target analytes. This recommendation is based on the assumption that the analytical results produced by different laboratories and/or different methods will be comparable if appropriate 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. This approach is intended to allow states to use cost-effective procedures and to encourage the use of new or improved analytical methods without compromising data quality. Performance-based analytical programs currently are used in several fish and shellfish monitoring programs, including the NOAA Status and Trends Program (Battelle, 1989b; Cantillo, 1991; NOAA, 1987), the EPA Environmental Monitoring and Assessment Program (EMAP) (U.S. EPA, 1991e), and the Puget Sound Estuary Program (1990d, 1990e).

Analytical methods used in fish and shellfish contaminant monitoring programs should be selected using the following criteria:

- Technical merit—Methods should be technically sound; they should be specific for the target analytes of concern and based on current, validated analytical techniques that are widely accepted by the scientific community.

- Sensitivity—Method detection and quantitation limits should be sufficiently low to allow reliable quantitation of the target analytes of concern at or below selected screening values. Ideally, the method detection limit (in tissue) should be at least five times lower than the selected SV for a given target analyte (Puget Sound Estuary Program, 1990e).

- Data quality—The accuracy and precision should be adequate to ensure that analytical data are of acceptable quality for program objectives.

- Cost-efficiency—Resource requirements should not be unreasonably high.

A review of current EPA guidance for chemical contaminant monitoring programs and of analytical methods currently used or recommended in several of these programs (as shown in Table 8-2) indicates that a limited number of analytical

Table 8-2. Current References for Analytical Methods for Contaminants in Fish and Shellfish Tissues

- Analytical Chemistry of PCBs (Erickson, 1991)
- Analytical Methods for Pesticides and Plant Growth Regulators, Vol. 11 (Zweig and Sherma, 1980)
- 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 Xenobiotic Chemical Contaminants in Fish (U.S. EPA, 1989c)
- Analytical Procedures and Quality Assurance Plan for the Determination of PCDD/PCDF in Fish (U.S. EPA, 1989b)
- Arsenic Speciation by Coupling High-Performance Liquid Chromatography with Inductively Coupled Plasma Mass Spectrometry (Demesmay et al., 1994)
- Assessment and Control of Bioconcentratable Contaminants in Surface Water (U.S. EPA, 1991a).
- 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)
- Determination of Arsenic Species by High-Performance Liquid Chromatography - Inductively Coupled Plasma Mass Spectrometry (Beauchemin et al., 1989)
- Determination of Arsenic Species in Fish by Directly Coupled High-Performance Liquid Chromatography-Inductively Coupled Plasma Mass Spectrometry (Branch et al., 1994)
- The quantitation of butyltin and cyclohexyltin compounds in the marine environment of British Columbia. *Appl. Organometal. Chem.* 4:581-590 (Cullen et al., 1990)
- Determination of Butyltin, Methyltin and Tetraalkyltin in Marine Food Products with Gas Chromatography-Atomic Absorption Spectrometry (Forsyth and Cleroux, 1991)
- Determination of Tributyltin Contamination in Tissues by Capillary Column Gas Chromatography-Flame Photometric Detection with Confirmation by Gas Chromatography-Mass Spectroscopy (Wade et al., 1988)
- Determination of Tributyltin in Tissues and Sediments by Graphite Furnace Atomic Absorption Spectrometry (Stephenson and Smith, 1988)
- Environmental Monitoring and Assessment Program Near Coastal Virginian Province Quality Assurance Project Plan (Draft) (U.S. EPA, 1991e)
- Guidelines for Studies of Contaminants in Biological Tissues for the National Water-Quality Assessment Program (Crawford and Luoma, 1993)
- Interim Methods for the Sampling and Analysis of Priority Pollutants in Sediments and Fish Tissue (U.S. EPA, 1981)
- Laboratory Quality Assurance Program Plan (California Department of Fish and Game, 1990)
- Methods for Organic Analysis of Municipal and Industrial Wastewater (40 CFR 136, Appendix A).
- Methods for the Chemical Analysis of Water and Wastes (U.S. EPA, 1979b)
- Methods for the Determination of Metals in Environmental Samples (U.S. EPA, 1991g)
- Official Methods of Analysis of the Association of Official Analytical Chemists (Williams, 1984)
- Pesticide Analytical Manual (PAM Vols. I and II) (U.S. FDA, 1990)
- Puget Sound Estuary Program Plan (1990d, 1990e)
- Quality Assurance/Quality Control (QA/QC) for 301(h) Monitoring Programs: Guidance on Field and Laboratory Methods (U.S. EPA, 1987e)

(continued)

Table 8-2 (continued)

- Sampling and Analytical Methods of the National Status and Trends Program National Benthic Surveillance and Mussel Watch Projects 1984-92. Volume II. Comprehensive Descriptions of Complementary Measurements (NOAA, 1993a)
- Sampling and Analytical Methods of the National Status and Trends Program National Benthic Surveillance and Mussel Watch Projects 1984-92. Volume III. Comprehensive Descriptions of Elemental Analytical Methods (NOAA, 1993b)
- Sampling and Analytical Methods of the National Status and Trends Program National Benthic Surveillance and Mussel Watch Projects 1984-92. Volume IV. Comprehensive Descriptions of Trace Organic Analytical Methods (NOAA, 1993c)
- Separation of Seven Arsenic Compounds by High-performance Liquid Chromatography with On-line Detection by Hydrogen-Argon Flame Atomic Absorption Spectrometry and Inductively Coupled Plasma Mass Spectrometry (Hansen et al., 1992)
- Speciation of Selenium and Arsenic in Natural Waters and Sediments by Hydride Generation Followed by Atomic Absorption Spectroscopy (Crecelius et al., 1986)
- Standard Analytical Procedures of the NOAA National Analytical Facility (Krahn et al., 1988; MacLeod et al., 1985)
- Standard Methods for the Examination of Water and Wastewater (Greenburg et al., 1992)
- Test Methods for the Chemical Analysis of Municipal and Industrial Wastewater (U.S. EPA, 1982)
- Test Methods for the Evaluation of Solid Waste, Physical/Chemical Methods (SW-846) (U.S. EPA, 1986d)
- U.S. EPA Contract Laboratory Program Statement of Work for Inorganic Analysis (U.S. EPA, 1991b)
- U.S. EPA Contract Laboratory Program Statement of Work for Organic Analysis (U.S. EPA, 1991c)
- U.S. EPA Method 1613B: Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS (U.S. EPA, 1995b)
- U.S. EPA Method 1625: Semivolatile Organic Compounds by Isotope Dilution GC/MS (40 CFR 136, Appendix A)
- U.S. EPA Method 1631: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry (U.S. EPA, 1995c)
- U.S. EPA Method 1632: Determination of Inorganic Arsenic in Water by Hydride Generation Flame Atomic Absorption (U.S. EPA, 1995d)
- U.S. EPA Method 1637: Determination of Trace Elements in Ambient Waters by Chelation Preconcentration with Graphite Furnace Atomic Absorption (U.S. EPA, 1995e)
- U.S. EPA Method 1638: Determination of Trace Elements in Ambient Waters by Inductively Coupled Plasma-Mass Spectrometry (U.S. EPA, 1995f)
- U.S. EPA Method 1639: Determination of Trace Elements in Ambient Waters by Stabilized Temperature Graphite Furnace Atomic Absorption (U.S. EPA, 1995g)
- U.S. EPA Method 625: Base/Neutrals and Acids by GC/MS (40 CFR 136, Appendix A).
- U.S. EPA Method 8290: Polychlorinated Dibenzodioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) by High Resolution Gas Chromatography/High Resolution Mass Spectrometry (HRGC/HRMS) (U.S. EPA, 1990b)
- U.S. EPA Method 1668: Draft Method 1668 Toxic Polychlorinated Biphenols by Isotope Dilution High Gas Chromatography/High Resolution Mass Spectrometry (U.S. EPA, 1997a)

techniques are most commonly used for the determination of the recommended target analytes. These techniques are listed in Table 8-3. As shown in Table 8-4, analytical methods employing these techniques have typically achievable detection and/or quantitation limits that are well below the recommended SVs for most target analytes, with the possible exception of dieldrin, heptachlor epoxide, toxaphene, PCBs, and dioxins/furans. Recommended procedures for determining method detection and quantitation limits are given in Section 8.3.3.3.

If lower SVs are used in a study (e.g., for susceptible populations), it is the responsibility of program managers 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 SVs. If analytical methodology is not sensitive enough to reliably quantitate target analytes at or below selected SVs (e.g., dieldrin, heptachlor epoxide, toxaphene, PCBs, dioxins/furans), program managers must determine appropriate fish consumption guidance based on lowest detectable concentrations or provide justification for adjusting SVs to values at or above achievable method detection limits. It should be emphasized that when SVs are below detection limits, the failure to detect a target analyte cannot be assumed to mean that there is no cause for concern for human health effects.

The analytical techniques identified in Table 8-3 are recommended for use in state fish and shellfish contaminant monitoring programs. However, alternative techniques may be used if acceptable detection limits, accuracy, and precision can be demonstrated. **Note:** Neither rotenone, the most widely used piscicide in the United States, nor its biotransformation products (e.g., rotenolone, 6',7'-dihydro-6',7'-dihydroxyrotenone, 6',7'-dihydro-6',7'-dihydroxyrotenolone) would be expected to interfere with the analyses of organic target analytes using the recommended gas chromatographic methods of analysis. Furthermore, rotenone has a relatively short half-life in water (3.7, 1.3, and 5.2 days for spring, summer, and fall treatments, respectively) (Dawson et al., 1991) and does not bioaccumulate significantly in fish (bioconcentration factor= 26 in fish carcass) (Gingerich and Rach, 1985), so that tissue residues should not be significant.

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

The references in Table 8-2 should be consulted in selecting appropriate analytical methods. **Note:** Because many laboratories may have limited experience in determining inorganic arsenic, a widely accepted method for this analysis is included in Appendix H.

Table 8-3. Recommended Analytical Techniques for Target Analytes

Target analyte	Analytical technique
Metals	
Arsenic (inorganic)	HAA, or HPLC with ICP-MS
Cadmium	GFAA or ICP[a]
Mercury	CVAA
Selenium	GFAA, ICP, or HAA[a,b]
Tributyltin	GFAA or GC/FPD[c]
Organics	
PAHs	GC/MS or HRGC/HRMS[d]
PCBs	
Total Aroclors	GC/ECD[e,f,g,h]
Non-ortho coplanar PCBs	HRGC/HRMS[i]
Other cogeners/homologs	HRGC/LRMS
Organochlorine pesticides	GC/ECD[f,g]
Organophosphate pesticides	GC/MS, GC/FPD, or GC/NPD[j]
Chlorophenoxy herbicides	GC/ECD[f,g]
Dioxins/dibenzofurans	HRGC/HRMS[k,l]

CVAA = Cold vapor atomic absorption spectrophotometry.
GC/ECD = Gas chromatography/electron capture detection.
GC/FPD = Gas chromatography/flame photometric detection.
GC/MS = Gas chromatography/mass spectrometry.
GC/NPD = Gas chromatography/nitrogen-phosphorus detection.
GFAA = Graphite furnace atomic absorption spectrophotometry.
HAA = Hydride generation atomic absorption spectrophotometry.
HPLC = High-performance liquid chromatography.
HRGC/HRMS = High-resolution gas chromatography/high-resolution mass spectrometry.
ICP = Inductively coupled plasma emission spectrometry.
ICP-MS = Inductively coupled plasma mass spectrometry.
LRMS = Low resolution mass spectrometry.
PAHs = Polycyclic aromatic hydrocarbons.
PCBs = Polychlorinated biphenyls.

[a] Atomic absorption methods require a separate determination for each element, which increases the time and cost relative to the broad-scan ICP method. However, GFAA detection limits are typically more than an order of magnitude lower than those achieved with ICP.

[b] Use of HAA can lower detection limits for selenium by a factor of 10-100 (Crecelius, 1978; Skoog, 1985).

[c] GC/FDP is specific for tributyltin and the most widely accepted analytical method. GFAA is less expensive (see Table 8-5) but is not specific for tributyltin. Depending on the extraction scheme, mono-, di-, and tetrabutyltin and other alkyltins may be included in the analysis. Contamination of samples with tin may also be a potential problem, resulting in false positives (E. Crecelius, Battelle Pacific Northwest Laboratories, Marine Sciences Laboratory, Sequim, WA, personal communication, 1999).

[d] GC/MS is also recommended for base/neutral organic target analytes (except organochlorine pesticides and PCBs) that may be included in a study. Detection limits of less than 1 ppb can be achieved for PAHs using HRGC/HRMS. It is recommended that, in both screening and intensive studies, tissue samples be analyzed for benzo[*a*]pyrene and 14 other PAHs and that the relative potencies given for these PAHs (Nesbit and LaGoy, 1992; U.S. EPA, 1993c) be used to calculate PEC for each sample for comparison with the recommended SV for benzo[*a*]pyrene (see Section 5.3.2.4).

[e] Analysis of total PCBs, as the sum of PCB congeners or sum of Aroclors, is recommended for conducting human health risk assessments for PCBs. A standard method for Aroclor analyses is available (EPA Method 608). EPA is currently testing a draft method (1668) for PCB congener analysis; however, it has not been finalized.

(continued)

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Table 8-3 (continued)

[f] GC/ECD does not provide definitive compound identification, and false positives due to interferences are commonly reported. Confirmation by an alternative GC column phase (with ECD), or by GC/MS with selected ion monitoring, is required for positive identification of PCBs, organochlorine pesticides, and chlorophenoxy herbicides.

[g] GC/MS with selected ion monitoring may be used for quantitative analyses of these compounds if acceptable detection limits can be achieved.

[h] PCB congener analysis using capillary GC columns is recommended (NOAA, 1989b; Dunn et al., 1984; Schwartz et al., 1984; Mullin et al., 1984; Stalling et al., 1987). An enrichment step, employing an activated carbon column, may also be required to separate and quantify coeluting congeners or congeners present at very low concentrations (Smith, 1981; Schwartz et al., 1993).

[i] Includes PCBs -77, -81, -126 and -169.

[j] Some of the chlorinated organophosphate pesticides (e.g., chlorpyrifos) may be analyzed by GC/ECD (USGS, 1987).

[k] The analysis of the 17 2,3,7,8-substituted congeners of tetra- through octa-chlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) using isotope dilution is recommended.

[l] Because of the toxicity of dioxins/furans and the difficulty and cost of these analyses, relatively few laboratories currently have the capability of performing these analyses. Contract laboratories experienced in conducting dioxin/furan analyses are listed in Table 8-1.

Table 8-4. Range of Detection and Quantitation Limits of Current Analytical Methods for Recommended Target Analytes[a]

Target analyte	Recreational SV	Subsistence SV[b]	Range of detection limits	Range of quantitation limits
Metals				
Arsenic (inorganic)	26 ppb	3.27 ppb	5-50 ppb[c]; 50-100 ppb[d]	5-25 ppb
Cadmium	4,000 ppb	491 ppb	5-46 ppb[e], 400 ppb[f]	5-500 ppb
Mercury	400 ppb	49 ppb	1.3-100 ppb[g]	2-10 ppb
Selenium	20,000 ppb	2,457 ppb	17-150 ppb[c]; 20 ppb[h], 600 ppb[f]	20-600 ppb
Tributyltin	1,200 ppb	147 ppb	2.5 ppb[e]; 2-5 ppb[i]	2-10 ppb
Organochlorine Pesticides[i]				
Chlordane (total)	—	—		
cis-Chlordane	114 ppb	14 ppb	1-5 ppb	2-20 ppb[j,k]
trans-Chlordane			1-5 ppb	2-15 ppb
cis-Nonachlor			1-5 ppb	2-15 ppb
trans-Nonachlor			1-7 ppb	2-15 ppb
Oxychlordane			1-5 ppb	2-15 ppb
DDT (total)	117 ppb	14.4 ppb		
4,4′-DDT			0.1-13 ppb	2-20 ppb
2,4′-DDT			0.1-10 ppb	2-15 ppb
4,4′-DDD			0.1-10 ppb	2-15 ppb
2,4′-DDD			0.1-10 ppb	2-15 ppb
4,4′-DDE			0.1-38 ppb	2-15 ppb[j,k]
2,4′-DDE			0.1-10 ppb	2-15 ppb
Dicofol	1,600 ppb	196 ppb	1-5 ppb	2-10 ppb
Dieldrin	2.50 ppb	0.307 ppb	0.1-5 ppb	2-15 ppb
Endosulfan (total)	24,000 ppb	2,949 ppb	5-70 ppb	10-70 ppb
Endosulfan I			5-10 ppb	2-15 ppb
Endosulfan II			5-70 ppb	10-70 ppb
Endrin	1,200 ppb	147 ppb	0.1-15 ppb	2-15 ppb[j,k]
Heptachlor epoxide	4.39 ppb	0.54 ppb	0.1-5 ppb	2-15 ppb[j,k]
Hexachlorobenzene	25 ppb	3.07 ppb	0.1-2 ppb	2-15 ppb[j,k]
Lindane	30 ppb	3.78 ppb	0.1-5 ppb	2-15 ppb[j,k]
Mirex	800 ppb	98 ppb	0.1-5 ppb	2-15 ppb[j,k]
Toxaphene	36 ppb	4.46 ppb	3-100 ppb	60-153 ppb
Organophosphate Pesticides[i]				
Chlorpyrifos	18,000 ppb	147 ppb	2-5 ppb	2-15 ppb[k]
Diazinon	4,200 ppb	344 ppb	2-5 ppb	2-15 ppb
Disulfoton	240 ppb	19 ppb	2-5 ppb	2-15 ppb
Ethion	3,000 ppb	245 ppb	2-5 ppb	2-15 ppb
Terbufos	120 ppb	9 ppb	2-5 ppb	2-15 ppb
Chlorophenoxy Herbicides				
Oxyfluorfen	546 ppb	679 ppb	10-20 ppb	20-200 ppb
PAHs[i]	5.47 ppb	0.67 ppb	1-10 ppt	2-20 ppt
PCBs total	20 ppb	2.45 ppb	—	—
(sum of Aroclors)[j]			(20-62 ppb)[m]	(110-170 ppb)[m]
Non-ortho coplanar PCBs[k]			2-5 ppt	2-10 ppt
Other congeners/ homologues[n]			2-5 ppb	10 ppb
Dioxins/furans[k] (total)	0.256 ppt	0.031 ppt	1.0 ppt	5-10 ppt
TCDD/TCDF			0.1 ppt	0.5 ppt
PeCDD/PeCDF			0.5 ppt	2.5 ppt
HxCDD/HxCDF			0.5 ppt	2.5 ppt
HpCDD/HpCDF			0.5 ppt	2.5 ppt
OCDD/OCDF			1.0 ppt	5 ppt

PAHs = Polycyclic aromatic hydrocarbons. PCBs = Polychlorinated biphenyls. SV = Screening value (wet weight).

(continued)

Table 8-4. (continued)

[a] Target analyte concentrations are given based on wet weight of fish tissue.
[b] From Tables 5-2 and 5-3. SVs shown here are for fish consumers using RfDs or CSFs available in the EPA IRIS (1999) database and assuming a consumption rate (CR) for recreational fishers of 12 g/d and for subsistence fishers of 124 g/d , average adult body weight (BW) = 72 kg, lifetime (70-yr) exposure, and, for carcinogens, a risk level (RL) = 10^{-5}. **Note:** Increasing CR, decreasing BW, and/or using an RL <10^{-5} will decrease the SV. Program managers must ensure that detection and quantitation limits of analytical methods are sufficient to allow reliable quantitation of target analytes at or below selected SVs. If analytical methodology is not sensitive enough to reliably quantitate target analytes at or below selected SVs (e.g., inorganic arsenic, dieldrin, heptachlor epoxide, toxaphene, PCBs, dioxins/furans), the program managers must determine appropriate fish consumption guidance based on lowest detectable concentrations or provide justification for adjusting SVs to values at or above achievable method detection or quantitation limits. It should be emphasized that when SVs are below method detection limits, the failure to detect a target analyte cannot be assumed to indicate that there is no cause for concern for human health effects.
[c] Analysis by hydride generation atomic absorption spectrophotometry (HAA) with preconcentration (E. Crecelius, Battelle Pacific Northwest Laboratories, Marine Sciences Laboratory, Sequim, WA, personal communication, July 1999).
[d] Analysis by high-performance liquid chromatography/mass spectrometry (HPLC/MS) (E. Crecelius, Battelle Pacific Northwest Laboratories, Marine Sciences Laboratory, Sequim, WA, personal communication, July 1999).
[e] Analysis by graphite furnace atomic absorption spectrophotometry (GFAA). **Note:** This method is not specific for tributyltin. Depending on the extraction procedure, mono-, di-, and tetrabutyltin may also be included in the analysis. Also, this method does not distinguish between butyltins and other alkyltins (E. Crecelius, Battelle Pacific Northwest Laboratories, Marine Sciences Laboratory, Sequim, WA, personal communication, July 1999).
[f] Analysis by inductively coupled plasma atomic emission spectrophotometry (ICP).
[g] Analysis by cold vapor atomic absorption spectrophotometry (CVAA).
[h] Analysis by HAA.
[i] Analysis by gas chromatography/flame photometric detection (GC/FPD) (E. Crecelius, Battelle Pacific Northwest Laboratories, Marine Sciences Laboratory, Sequim, WA, personal communication, July 1999).
[j] Analysis by gas chromatography/electron capture detection (GC/ECD), except where otherwise noted.
[k] Analysis by high-resolution GC/high-resolution mass spectrometry (HRGC/HRMS).
[l] Analysis by gas chromatography/mass spectrometry. Detection limits of less than 1 ppb can be achieved using high-resolution gas chromatography/mass spectrometry (HRGC/HRMS).
[m] Values in parentheses represent ranges for individual Aroclors.
[n] Analysis by high-resolution GC/low resolution mass spectrometry (HRGC/LRMS).

An additional resource for method selection is the EPA Environmental Monitoring Methods Index System (EMMI), an automated inventory of information on environmentally significant analytes and methods for their analysis (U.S. EPA, 1991f). The EMMI database includes information on more than 4,000 analytes from over 80 regulatory and nonregulatory lists and more than 900 analytical methods in a variety of matrices, including tissue. This searchable database provides a comprehensive cross-reference between analytes and analytical methods with detailed information on each analytical method, including sponsoring organization, sample matrix, and estimates of detection limits, accuracy, and precision.

EMMI is available from the EPA Sample Control Center for all EPA personnel and from National Technical Information Service (NTIS) for all other parties. EMMI is also available through the EPA Local Area Network (LAN).

The private sector may purchase EMMI Version 2.0 through the:

National Technical Information Service (NTIS)
5285 Port Royal Road
Springfield, VA 22161
USA
Phone: (703) 605-6000
Fax: (703) 605-6900
Rush Orders: (800) 553-NTIS
Online Orders: http:\\www.ntis.gov

The order number is PB97-502637INC for a single user, PB97-502645INC for a five-user LAN package, and PB97-502652INC for an unlimited user LAN package. Further information may be obtained by contacting:

EMMI User Support
Tech Calls
EPA Assistant Administrator for Water
Office of Science and Technology
(703) 461-2104
Alexandria, VA 22313

Because chemical analysis is frequently one of the most expensive components of a sampling and analysis program, the selection of an analytical method often will be influenced by its cost. In general, analytical costs increase with increased sensitivity (i.e., lower detection limits) and reliability (i.e., accuracy and precision). Analytical costs will also be dependent on the number of samples to be analyzed, the requested turnaround time, the number and type of analytes requested, the level of QC effort, and the amount of support documentation requested (Puget Sound Estuary Program, 1990d). However, differences in protocols, laboratory experience, and pricing policies of laboratories often introduce large variation into analytical costs. Approximate costs per sample for the analysis of target analytes by the recommended analytical techniques are provided in Table 8-5.

8.3 QUALITY ASSURANCE AND QUALITY CONTROL CONSIDERATIONS

Quality assurance and quality control must be integral parts of each chemical analysis program. The QA process consists of management review and oversight at the planning, implementation, and completion stages of the analytical data collection activity to ensure that data provided are of the quality required. The QC process includes those activities required during data collection to produce the data quality desired and to document the quality of the collected data.

During the planning of a chemical analysis program, QA activities focus on defining data quality criteria and designing a QC system to measure the quality of data being generated. During the implementation of the data collection effort, QA activities ensure that the QC system is functioning effectively and that the

Table 8-5. Approximate Range of Costs per Sample for Analysis of Recommended Target Analytes[a]

Target analyte	Approximate cost range (1999 $)
Metals[b]	
Arsenic (inorganic)[c]	200 - 400
Cadmium	55 - 60
Mercury (total)	45 - 60
Selenium	35 - 60
Tributyltin[d]	200 - 400
Organochlorine pesticides[e,f]	285 - 500
Organophosphate pesticides[g]	250 - 500
Chlorophenoxy herbicides[h]	250 - 500
PAHs[i]	250 - 525
PCBs	
Total Aroclors[e]	210 - 500
Non-ortho coplanar PCBs[j]	1,000 - 2,000
Other cogeners/homologs[k]	800 - 1,000
Dioxins/furans[j]	
TCDD/TCDF only	600 - 1,000
TCDD/TCDF through OCDD/OCDF isomers	800 - 1,600
2,3,7,8-substituted dioxins/furans	1,000 - 2,000
Lipid	30 - 40

OCDD = Octachlorodibenzo-*p*-dioxin.
OCDF = Octachlorodibenzofuran.
PAHs = Polycyclic aromatic hydrocarbons.
PCBs = Polychlorinated biphenyls.
TCDD = 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin.
TCDF = 2,3,7,8-Tetrachlorodibenzofuran.

[a] These costs include sample digestion or extraction and cleanup, but not sample preparation (i.e., resection, grinding, homogenization, compositing). Estimated cost of sample preparation for a composite homogenate of five fish is $200 to $500.

[b] Analysis of inorganic arsenic by hydride generation atomic absorption spectroscopy (HAA) or high-performance liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICP/MS). Analysis of cadmium by graphite furnace atomic absorption spectrophotometry (GFAA). Analysis of selenium by GFAA or HAA. Analysis of mercury by cold vapor atomic absorption spectrophotometry (CVAA). Analysis of tributyltin by GFAA or gas chromatography/flame photometric detection (GC/FPD).

[c] Estimated costs are for total inorganic arsenic. Estimated cost of analysis by HAA is $200. Estimated cost of analysis by HPLC-ICP/MS is $400.

[d] Estimated cost of analysis by GFAA is $200. Estimated cost of analysis by GC/FPD is $400. **Note:** Analysis by GFAA is not specific for tributyltin. Depending on the extraction procedure, other butyl- and alkyltin species may be detected.

[e] Analysis by gas chromatography/electron capture detection (GC/ECD).

[f] Estimated costs are for analysis of all recommended target analyte organochlorine pesticides (see Table 4-1).

[g] Analysis by GC/FPD or gas chromatography/nitrogen-phosphorus detection (GC/NPD). Some of the chlorinated organophosphate pesticides (e.g., chlorpyrifos) may be analyzed as organochlorine pesticides by GC/ECD (USGS, 1987).

[h] Analysis by GC/ECD.

[i] Costs are for analysis by gas chromatography/mass spectrometry (GC/MS) or gas chromatography/flame ionization detection (GC/FID). Cost for analysis by high-resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) is approximately $1,000 per sample.

[j] Analysis by HRGC/HRMS.

[k] Analysis by HRGC/low resolution mass spectrometry (LRMS).

deficiencies uncovered by the QC system are corrected. After the analytical data are collected, QA activities focus on assessing the quality of data obtained to determine its suitability to support decisions for further monitoring, risk assessments, or issuance of advisories.

The purpose of this section is to describe the general QA and QC requirements for chemical analysis programs.

8.3.1 QA Plans

Each laboratory performing chemical analyses in fish and shellfish contaminant monitoring programs must have an adequate QA program (U.S. EPA, 1984b). The QA program should be documented fully in a QA plan or in a combined Work/QA Project Plan (U.S. EPA, 1980b). (See Appendix I.) Each QA and QC requirement or procedure should be described clearly. Documentation should clearly demonstrate that the QA program meets overall program objectives and data quality requirements. The QA guidelines in the Puget Sound Estuary Program (1990d, 1990e), the NOAA Status and Trends Program (Battelle, 1989b; Cantillo, 1991; NOAA, 1987), the EPA 301(h) Monitoring Programs (U.S. EPA, 1987e), the EPA EMAP Near Coastal (EMAP-NC) Program (U.S. EPA, 1991e), and the EPA Contract Laboratory (CLP) Program (U.S. EPA, 1991b, 1991c) are recommended as a basis for developing program-specific QA programs. Additional method-specific QC guidance is given in references in Table 8-2.

8.3.2 Method Documentation

Methods used routinely for the analyses of contaminants in fish and shellfish tissues must be documented thoroughly, preferably as formal standard operating procedures (U.S. EPA, 1984b). Recommended contents of an analytical SOP are shown in Figure 8-1. Analytical SOPs must be followed exactly as written. A published method may serve as an analytical SOP only if the analysis is performed exactly as described. Any significant deviations from analytical SOPs must 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 an SOP deviation did not adversely affect method performance (i.e., detection or quantitation limits, accuracy, precision). Otherwise, the effect of the deviation on data quality must be assessed and documented and all suspect data must be identified.

8.3.3 Minimum QA and QC Requirements for Sample Analyses

The guidance provided in this section is derived primarily from the protocols developed for the Puget Sound Estuary Program (1990d, 1990e). These protocols have also provided the basis for the EPA EMAP-NC QA and QC requirements (U.S. EPA, 1991e). QA and QC recommendations specified in this document are intended to provide a uniform performance standard for all analytical protocols used in state fish and shellfish contaminant monitoring

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

Figure 8-1. Recommended contents of analytical standard operating procedures (SOPs).

programs and to enable an assessment of the comparability of results generated by different laboratories and different analytical procedures. These recommendations are intended to represent minimum QA and QC procedures for any given analytical method. Additional method-specific QC procedures should always be followed to ensure overall data quality.

For sample analyses, minimum QA and QC requirements consist of (1) initial demonstration of laboratory capability and (2) routine analyses of appropriate QA and QC samples to demonstrate continued acceptable performance and to document data quality.

Initial demonstration of laboratory capability (prior to analysis of field samples) should include

- Instrument calibration
- Documentation of detection and quantitation limits
- Documentation of accuracy and precision
- Analysis of an accuracy-based performance evaluation sample provided by an external QA program.

Ongoing demonstration of acceptable laboratory performance and documentation of data quality should include

- Routine calibration and calibration checks
- Routine assessment of accuracy and precision
- Routine monitoring of interferences and contamination
- Regular assessment of performance through participation in external QA interlaboratory comparison exercises, when available.

The QA and QC requirements for the analyses of target analytes in tissues should be based on specific performance criteria (i.e., warning or control limits) for data quality indicators such as accuracy and precision. **Warning limits** are numerical criteria that serve to alert data reviewers and data users that data quality may be questionable. A laboratory is not required to terminate analyses when a warning limit is exceeded, but the reported data may be qualified during subsequent QA review. **Control limits** are numerical data criteria that, when exceeded, require suspension of analyses and specific corrective action by the laboratory before the analyses may resume.

Typically, warning and control limits for accuracy are based on the historical mean recovery plus or minus two or three standard deviation units, respectively. Warning and control limits for precision are typically based on the historical standard deviation or coefficient of variation (or mean relative percent difference for duplicate samples) plus two or three standard deviation units, respectively. Procedures incorporating control charts (ASTM, 1976; Taylor, 1985) and/or tabular presentations of historical data should be in place for routine monitoring of analytical performance. Procedures for corrective action in the event of excursion outside warning and control limits should also be in place.

The results for the various QC samples analyzed with each batch of samples should be reviewed by qualified laboratory personnel immediately following the analysis of each sample batch to determine when warning or control limits have been exceeded. When established control limits are exceeded, appropriate corrective action should be taken and, if possible, all suspect samples reanalyzed before resuming routine analyses. If reanalyses cannot be performed, all suspect data should be identified clearly. **Note:** For the purposes of this guidance manual, a batch is defined as any group of samples from the same source that is processed at the same time and analyzed during the same analytical run.

Recommended QA and QC samples (with definitions and specifications), frequencies of analyses, control limits, and corrective actions are summarized in Table 8-6.

Note: EPA recognizes that resource limitations may prevent some states from fully implementing all recommended QA and QC procedures. Therefore, as additional guidance, the minimum numbers of QA and QC samples recommended for routine analyses of target analytes are summarized in Table 8-7. It is the responsibility of each program manager to ensure that the analytical QC program is adequate to meet program data quality objectives for method detection limits, accuracy, precision, and comparability.

Recommended QA and QC procedures and the use of appropriate QA and QC samples are discussed in Sections 8.3.3.2 through 8.3.3.8. Recommended procedures for documenting and reporting analytical and QA and QC data are given in Section 8.4. Because of their importance in assessing data quality and interlaboratory comparability, reference materials are discussed separately in the following section.

Table 8-6. Recommended Quality Assurance and Quality Control Samples

Sample type (definition; specifications)	Objective	Recommended frequency of analysis[a]	Recommended control limits[b]	Recommended corrective action
External Calibration				
Calibration standards (3-5 standards over the expected range of sample target analyte concentrations, with the lowest concentration standard at or near the MDL; see Section 8.3.3.2.1)	Full calibration: Establish relationship between instrument response and target analyte concentration. Used for organics analysis by GC/ECD and for metals analysis.	Instrument/method-dependent; follow manufacturer's recommendations or procedures in specific analytical protocols. At a minimum, perform a 3-point calibration each time an instrument is set up for analysis, after each major equipment change or disruption, and when routine calibration check exceeds specific control limits.	*Organics*: RSD of RFs of calibration standards ≤20%. *Metals*: %R of all calibration standards = 95-105.	Determine cause of problem (e.g., instrument instability or malfunction, contamination, inaccurate preparation of calibration standards) and take appropriate corrective action. Recalibrate and reanalyze all suspect samples or flag all suspect data.
Internal Standard Calibration				
Instrument internal standards (e.g., 2,2'-difluorobiphenyl) (see Section 8.3.3.2.1 for definition)	Full calibration: Determine RRFs of organic target analytes for quantitative analysis. Required for internal calibration of GC/MS systems. Optional calibration technique for GC/ECD.	In every calibration standard, sample, and blank analyzed; added to final sample extract. Internal standard calibration performed at same frequency recommended for external calibration.	RSD of RRFs of calibration standards ≤30%.	Determine cause of problem (e.g., instrument instability or malfunction, contamination, inaccurate preparation of internal standards or calibration standards) and take appropriate corrective action. Recalibrate and reanalyze all suspect samples or flag all suspect data.
Calibration Verification				
Calibration check standards (minimum of one mid-range standard prepared independently from initial calibration standards; an instrument internal standard must be added to each calibration check standard when internal standard calibration is being used; see Section 8.3.3.2.1)	Verify calibration.	*Organics (GC/MS)*: After initial calibration or recalibration. At beginning and end of each work shift, and once every 12 h (or every 10-12 analyses, whichever is more frequent). *Organics (GC/ECD)*: After initial calibration or recalibration. At beginning and end of each work shift, and once every 6 h (or every 6 samples, whichever is less frequent). *Metals*: After initial calibration or recalibration. Every 10 samples or every 2 h, whichever is more frequent.	*Organics*: Percent difference between the average RF (or RRF) from initial calibration and the RF (or RRF) from the calibration check ≤25%. *Mercury*: %R = 80-120. *Other Metals*: %R = 90-110.	Determine cause of problem (e.g., instrument instability or malfunction, contamination, inaccurate preparation of calibration standards) and take appropriate corrective action. Recalibrate and reanalyze all suspect samples or flag all suspect data.

(continued)

Table 8-6. (continued)

Sample type (definition; specifications)	Objective	Recommended frequency of analysis[a]	Recommended control limits[b]	Recommended corrective action
Method Detection Limit Determination				
Spiked matrix samples (analyte-free tissue samples to which known amounts of target analytes have been added; one spike for each target analyte at 3-5 times the estimated MDL; see Section 8.3.3.3.1)	Establish or confirm MDL for analyte of interest (Keith, 1991a; Keith et al., 1983).	Seven replicate analyses prior to use of method for routine analyses, and after any significant change to a method currently in use. Reevaluation of MDL annually.	Determined by program manager.	Redetermine MDL.
Accuracy and Precision Assessment				
Reference materials[c] (see Section 8.3.3.1 for definitions) (SRMs or CRMs, prepared from actual contaminated fish or shellfish tissue if possible, covering the range of expected target analyte concentrations.	Assess method performance (initial method validation and routine accuracy 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:* Measured value <95% confidence intervals, if certified. Otherwise, %R = 70-130.[d] *Metals:* %R = 85-115.[d]	NA
		Routine accuracy assessment: one (preferably blind) per 20 samples or one per batch, whichever is more frequent.	*Organics:* Measured value <95% confidence intervals, if certified. Otherwise, %R = 70-130.[d] *Metals:* %R = 85-115.[d]	Determine cause of problem (e.g., inaccurate calibration, contamination), take appropriate corrective action, and reanalyze all suspect samples or flag all suspect data.
Laboratory control samples (Accuracy-based samples consisting of fish or shellfish tissue homogenates spiked with target analytes of interest; may be SRMs or CRMs; sometimes referred to as QC samples. When available, CRMs are recommended for routine use as laboratory control samples; see Appendix M)	Assess method performance (initial method validation and routine accuracy assessment). Used for initial accuracy assessment only if reference materials prepared from actual contaminated fish or shellfish are not available.	*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).	Determined by program manager.	NA
		Routine accuracy assessment. One per 20 samples or one per batch, whichever is more frequent.	*Organics*: %R = 70-130.[d] *Metals*: %R = 85-115.[d]	Determine cause of problem (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 all suspect data.

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Table 8-6. (continued)

Sample type (definition; specifications)	Objective	Recommended frequency of analysis[a]	Recommended control limits[b]	Recommended corrective action
Matrix spikes (composite tissue homogenates of field samples to which known amounts of target analytes have been added; 0.5 to 5 times the concentration of the analyte of interest or 5 times the MQL)	Assess matrix effects and accuracy (%R) routinely.	One per 20 samples or one per batch, whichever is more frequent.	*Organics:* %R ≥50 with good precision. *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.
Matrix spike replicates (replicate aliquots of matrix spike samples; 0.5 to 5 times the concentration of the analyte of interest or 5 times the MQL)	Assess method precision routinely.	One duplicate 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: IRPDI ≤20 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.
Laboratory replicates[e] (replicate aliquots of composite tissue homogenates of field samples)	Assess method precision routinely.	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:* IRPDI ≤20 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 (replicate aliquots of final sample extract or digestate)	Assess analytical precision.	Duplicate injections for all metal analyses.[f]	Determined by program manager.[g]	Determine cause of problem (e.g., instrument instability or malfunction), take appropriate corrective action, and reanalyze sample.

(continued)

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Table 8-6. (continued)

Sample type (definition; specifications)	Objective	Recommended frequency of analysis[a]	Recommended control limits[b]	Recommended corrective action
Field replicates (replicate composite tissue samples)	Assess total variability (i.e., population variability, field or sampling variability, and analytical method variability).	*Screening studies*: **OPTIONAL**; if program resources allow, a minimum of one blind replicate (i.e., duplicate) for each primary target species at 10 percent of screening sites.[9]	Determined by program manager.[9]	Determined by program manager.
		Intensive studies: Blind replicate samples for each target species (and size, age or sex class, if appropriate) at each sampling site. Number of replicates determined by program manager (see Section 6.1.2.7).	Determined by program manager.[9]	Determined by program manager.
Contamination Assessment				
Blanks (field, method, processing, bottle, reagent) (see Section 8.3.3.6 for definitions)	Assess contamination from equipment, reagents, etc.	One field blank per sampling site. One method 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 batch of samples, whichever is more frequent. One reagent blank prior to use of a new batch of reagent and whenever method blank exceeds control limits.	Concentration of any analyte <MDL or MQL, as determined by program manager.	Determine cause of problem (e.g., contaminated reagents, equipment), remove sources of contamination, and reanalyze all suspect samples or flag all suspect data.
Routine Monitoring of Method Performance for Organic Analyses				
Surrogate spikes (see Section 8.3.3.7.1 for definition)				
Prepared from isotopically labeled target analytes	Assess method performance and estimate recovery of organic target analytes analyzed by GC/MS. Determine RRFs of organic target analytes quantitated by isotope dilution techniques.	In every calibration standard, sample, and blank analyzed for organics by isotope dilution GC/MS; added to samples prior to extraction.	Determined by program manager.	Determine cause of problem (e.g., incomplete extraction or digestion, contamination, inaccurate preparation of internal standard), take appropriate corrective action, and reanalyze all suspect samples or flag all suspect data.

(continued)

Table 8-6. (continued)

Sample type (definition; specifications)	Objective	Recommended frequency of analysis[a]	Recommended control limits[b]	Recommended corrective action
Prepared from other surrogate compounds	Assess method performance and estimate the recovery of organic target analytes analyzed by GC/MS or GC/ECD.	In every calibration standard, sample, and blank analyzed for organics, unless isotope dilution technique is used: *Semivolatiles*: 3 for neutral fraction 2 for acid fraction *Volatiles*: 3 *Pesticides/PCBs*: 1 Added to samples prior to extraction.	Determined by program manager according to most recent EPA CLP guidelines.[h]	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.
External QA Assessment				
Accuracy-based performance evaluation samples (QA samples from NOAA interlaboratory comparison program; see Section 8.3.3.8.1)	Initial demonstration of laboratory capability.	Once prior to routine analysis of field samples (blind).	*Organics*: %R=70-130.[d] *Metals*: %R=85-115.[d]	Determine cause of problem and reanalyze sample. Do not begin analysis of field samples until performance evaluation sample results are acceptable.
	Ongoing demonstration of laboratory capability.	One exercise (four to six samples) per year (blind).	Determined by NOAA. Based on consensus value of all participating laboratories.	Determine cause of problem. Do not continue analysis of field samples until laboratory capability is clearly demonstrated.

(continued)

Table 8-6. (continued)

Sample type (definition; specifications)	Objective	Recommended frequency of analysis[a]	Recommended control limits[b]	Recommended corrective action
Split samples (laboratory replicates analyzed by different laboratories; see Section 8.3.3.8.2)	Assess interlaboratory comparability.	5-10% of composite homogenates split between states and/or Regions that routinely share monitoring results, or as determined by program managers.[g]	Determined by program managers.	Review sampling and analytical methods. Identify sources of noncomparability. Standardize and validate methods to document comparability.

CLP = Contract laboratory program.
CRM = Certified reference material (see Section 8.3.3.1).
GC/ECD = Gas chromatography/electron capture detection.
GC/MS = Gas chromatography/mass spectrometry.
MDL = Method detection limit (see Section 8.3.3.3.1).
MQL = Method quantitation limit (see Section 8.3.3.3.2).
NA = Not applicable.
NOAA = National Oceanic and Atmospheric Administration.
PCBs = Polychlorinated biphenyls.
QA = Quality assurance.
%R = Percent recovery (see Sections 8.3.3.4 and 8.3.3.7.1).
RF = Response factor (see Section 8.3.3.2.1).
RPD = Relative percent difference (see Section 8.3.3.5).
RRF = Relative response factor (see Section 8.3.3.2.1).
RSD = Relative standard deviation (see Section 8.3.3.5).
SRM = Standard reference material (see Section 8.3.3.1).

[a] Recommended frequencies are based primarily on recommendations in U.S. EPA (1986d, 1987e, 1989c, 1991b, 1991c), Puget Sound Estuary Program (1990d, 1990e), and Battelle (1989b).
[b] From Puget Sound Estuary Program (1990d, 1990e) action limits, except where otherwise noted. **Note:** Individual programs may require more stringent 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.
[c] As available (see Table 8-8 and Appendix M).
[d] From U.S.EPA (1991e).
[e] Sometimes referred to as analytical replicates (e.g., in Puget Sound Estuary Program, 1990d).
[f] From U.S. EPA (1987e).
[g] Recommended by EPA for this guidance document.
[h] From U.S. EPA (1991b, 1991c).

(continued)

Table 8-7. Minimum Recommended QA and QC Samples for Routine Analysis of Target Analytes[a]

Sample Type	Target analyte: Metals	Target analyte: Organics
Accuracy-based performance evaluation sample[b]	Once prior to routine analysis of field samples, plus one exercise (four to six samples) per year.	Once prior to routine analysis of field samples, plus one exercise (four to six samples) per year.
Method blank	1	1
Laboratory duplicate	1	1
Matrix spike/matrix spike replicate	1	1
Laboratory control sample (SRM or CRM, if available)	1	1
Calibration check standard	2[c]	2[c]
Surrogate spike (isotopically labeled target analyte or other surrogate compound added prior to extraction)	NA	Each sample
Instrument (injection) internal standard; added prior to injection	NA	Each calibration or calibration check standard and each sample or blank analyzed by GC/MS[d]

CRM = Certified reference material (see Section 8.3.3.1).
GC/MS = Gas chromatography/mass spectroscopy.
NA = Not applicable.
QA = Quality assurance.
QC = Quality control.
SRM = Standard reference material (see Section 8.3.3.1).

[a] Unless otherwise specified, the number given is the recommended number of QC samples per 20 samples or per batch, whichever is more frequent. Additional method-specific QC requirements should always be followed provided these minimum requirements have been met.

[b] QA samples from National Oceanic and Atmospheric Administration interlaboratory comparison program (see Section 8.3.3.8.1).

[c] One every 10 samples (plus one at beginning and end of each analytical run).

[d] Optional for analyses by GC/electron capture detection (ECD), GC/flame ionization detection (FID), or GC with other nonspecific detectors.

8.3.3.1 Reference Materials—

The appropriate use of reference materials is an essential part of good QA and QC practices for analytical chemistry. The following definitions of reference materials (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 has (have) been certified by a variety of technically valid procedures. CRMs are accompanied by or traceable to a certificate or other documentation that is issued by the certifying organization (e.g., U.S. EPA, 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 and QC procedures is given in Taylor (1985). The following general guidelines should be followed to ensure proper use of reference materials (NOAA, 1992):

- 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. **Note:** Because of a lack of low- and high-concentration reference materials for most analytes in fish and shellfish tissue matrices, potential problems at low or high concentrations often cannot be documented.

- Reference materials should be analyzed prior to beginning the analyses of field samples to assess laboratory capability and regularly thereafter to detect and document any changes in laboratory performance over time. Appropriate corrective action should be taken whenever changes are observed outside specified performance limits (e.g., accuracy, precision).

- 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. However, because of the limited number of certified fish and shellfish 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.

- Results of reference material analyses are essential to assess interlaboratory or intermethod comparability. 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 reference materials for the analysis of priority pollutants and selected related compounds in fish and shellfish tissues are given in Appendix M. Available marine or estuarine tissue reference materials that may be appropriate for use by analytical laboratories in fish and shellfish contaminant monitoring programs are given in Table 8-8.

8.3.3.2 Calibration and Calibration Checks—

General guidelines for initial calibration and routine calibration checks are provided in this section. Method-specific calibration procedures are included in the references in Table 8-2. It is the responsibility of each program manager to ensure that proper calibration procedures are developed and followed for each analytical method to ensure the accuracy of the measurement data.

All analytical instruments and equipment should be maintained and calibrated properly 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. Calibration 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.

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, NIST-SRMs or other certified reference standards should be used for calibration standards (see Section 8.3.3.4 and Appendix M). 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 8-8. Fish and Shellfish Tissue Reference Materials

Identification code	Analytes	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
GBW-08571	Elements	NRCCRM	Mussel tissue (freeze-dried)
GBW-08572	Elements	NRCCRM	Prawn tissue
MA-A-1/OC	Organic compounds	IAEA	Copepod homogenate (freeze-dried)
MA-A-3/OC	Organic compounds	IAEA	Shrimp homogenate (freeze-dried)
MA-B-3/OC	Organic compounds	IAEA	Fish tissue (freeze-dried)
MA-M-2/OC	Organic compounds	IAEA	Mussel tissue
MA-A-1/TM	Elements	IAEA	Copepod homogenate (freeze-dried)
MA-A-2/TM	Elements	IAEA	Fish flesh homogenate
MA-B-3/TM	Elements	IAEA	Fish tissue (freeze-dried)
MA-B-3/RN	Isotopes	IAEA	Fish tissue (freeze-dried)
IAEA-350	Elements	IAEA	Tuna homogenate (freeze-dried)
IAEA-351	Organic compounds	IAEA	Tuna homogenate (freeze-dried)
IAEA-352	Isotopes	IAEA	Tuna homogenate (freeze-dried)
CRM-278	Elements	BCR	Mussel tissue (freeze-dried)
CRM-422	Elements	BCR	Cod muscle (freeze-dried)
EPA-FISH	Pesticides	EPA1	Fish tissue
EPA-SRS903	Chlordane	EPA2	Fish tissue
EPA-0952	Mercury	EPA1	Fish tissue
EPA-2165	Mercury	EPA1	Fish tissue
RM-50	Elements	NIST	Albacore tuna (freeze-dried)
SRM-1566a	Elements	NIST	Oyster tissue (freeze-dried)
SRM-1974	Organic compounds	NIST	Mussel tissue (frozen)
SRM-2974	Organic compounds	NIST	Mussel tissue (freeze-dried)
NIES-6	Elements	NIES	Mussel tissue

Sources:

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, OH, 45268, USA. (EPA1: Material available from Supelco, Inc., Supelco Park, Bellefonte, PA, 16823-0048, USA. EPA2: Material available from Fisher Scientific, 711 Forbes Ave., Pittsburgh, PA 15219.)

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

NRCCRM = National Research Center for CRMs, Office of CRMs, No. 7, District 11, Hepingjie, Chaoyangqu, Beijing, 100013, China.

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.

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

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

8.3.3.2.1 Initial and routine calibration

Prior to beginning routine analyses of samples, a minimum of three (and preferably five) calibration standards should be used to construct a calibration curve for each target analyte, covering the normal working range of the instrument or the expected target analyte concentration range of the samples to be analyzed. The lowest-concentration calibration standard should be at or near the estimated method detection limit (see Section 8.3.3.3.1). Calibration standards should be prepared in the same matrix (i.e., solvent) as the final sample extract or digestate. Criteria for acceptable calibration (e.g., acceptable limits for r^2, slope, intercept, percent recovery, response factors) should be established for each analytical method. If these control limits are exceeded, the source of the problem (e.g., inaccurate standards, instrument instability or malfunction) should be identified and appropriate corrective action taken. No analyses should be performed until acceptable calibration has been achieved and documented.

In addition to the initial calibration, 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, after any major maintenance, and whenever a calibration check exceeds the recommended control limits (see Table 8-6).

Two types of calibration procedures are used in the analytical methods recommended for the quantitation of target analytes: external calibration and internal standard calibration.

External calibration

In external calibration, calibration standards with known concentrations of target analytes are analyzed, independent of samples, to establish the relationship between instrument response and target analyte concentration. External calibration is used for the analyses of metals and, at the option of the program manager, for the analyses of organics by gas chromatography/electron capture detection (GC/ECD), gas chromatography/flame ionization detection (GC/FID), or GC methods using other nonspecific detectors.

External calibration for metals analysis is considered acceptable if the percent recovery of all calibration standards is between 95 and 105 percent; external calibration for organic analyses is considered acceptable if the relative standard deviation (RSD) of the response factors (RFs) is $\leq$20 percent (see Table 8-6). If these limits are exceeded, the initial calibration should be repeated.

Internal standard calibration

Calibration of GC/mass spectrometry (MS) systems used for the analysis of organic target analytes requires the addition of an **internal standard** to each calibration standard and determination of the response of the target analyte of interest relative to that of the internal standard. Internal standard calibration may also be used with nonspecific detector GC methods such as GC/ECD and GC/FID. Internal standards used to determine the relative response factors (RRFs) are termed instrument or injection internal standards (Puget Sound Estuary Program, 1990d; U.S. EPA, 1991e). The addition of instrument internal standards to both calibration standards and sample extracts ensures rigorous quantitation, particularly accounting for shifts in retention times of target analytes in complex sample extracts relative to calibration standards. Recommended instrument internal standards for semivolatile organic compounds are included in analytical methods for these compounds (see references in Table 8-2).

The RRF for each target analyte is calculated for each calibration standard as follows:

$$RRF_t = (A_t)\,(C_{is}) \,/\, (A_{is})\,(C_t) \qquad (8\text{-}1)$$

where

A_t = Measured response (integrated peak area) for the target analyte
C_{is} = Concentration of the instrument internal standard in the calibration standard
A_{is} = Measured response (integrated peak area) for the instrument internal standard
C_t = Concentration of the target analyte in the calibration standard.

If the RSD of the average RRF_t for all calibration standards ($\overline{RRF_t}$) is ≤30 percent, RRF_t can be assumed to be constant across the working calibration range and $\overline{RRF_t}$ can be used to quantitate target analyte concentrations in the samples as follows:

$$C_t \text{ (ppm or ppb, wet weight)} = (A_t)\,(C_{is})\,(V_e) \,/\, (A_{is})\,(\overline{RRF_t})\,(W) \qquad (8\text{-}2)$$

where

C_t = Concentration of the target analyte in the sample
C_{is} = Concentration of the instrument internal standard in the sample extract
V_e = Volume of the final sample extract (mL)
W = Weight of sample extracted (g)

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and A_t, A_{is}, and $\overline{RRF}_t$ are defined as in Equation (8-1).

If the RSD of $\overline{RRF}_t$ for all calibration standards is >30 percent, the initial calibration should be repeated (see Table 8-6).

8.3.3.2.2 Routine calibration checks

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 calibration check standard. A **calibration check standard** is a mid-range calibration standard that has been prepared independently (i.e., using a different stock) from the initial calibration standards. When internal standard calibration is being used, an instrument internal standard must be added to each calibration check standard.

Routine calibration checks should be conducted often enough throughout each analysis run to ensure adequate maintenance of instrument calibration (see Table 8-6). A calibration check should always be performed after analyzing the last sample in a batch and at the end of each analysis run.

If a calibration check does not fall within specified calibration control limits, the source of the problem should be determined and appropriate corrective action taken (see Table 8-6). After acceptable calibration has been reestablished, all suspect analyses should be repeated. If resources permit, it is recommended that all samples after the last acceptable calibration check be reanalyzed. Otherwise, the last sample analyzed before the unacceptable calibration check should be reanalyzed first and reanalysis of samples should continue in reverse order until the difference between the reanalysis and initial results is within the control limits specified in Table 8-6. If reanalysis is not possible, all suspect data (i.e., since the last acceptable calibration check) should be identified clearly in the laboratory records and the data report.

8.3.3.2.3 Calibration range and data reporting

As noted in Section 8.3.2.1, the lowest-concentration calibration standard should be at or near the method detection limit. The highest-concentration calibration standard should be selected to cover the full range of expected concentrations of the target analyte in fish and shellfish tissue samples. If a sample concentration occurs outside the calibration range, the sample should be diluted or concentrated as appropriate and reanalyzed or the calibration range should 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 or modification of the extraction procedure may be required.

All reported concentrations must be within the upper limit of the demonstrated working calibration range. Procedures for reporting data, with appropriate qualifications for data below method detection and quantitation limits, are given in Section 8.3.3.3.3.

8.3.3.3 Assessment of Detection and Quantitation Limits—

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. When available scientific literature demonstrates that the selected SVs are analytically attainable, the laboratory is responsible for ensuring that these limits are sufficiently low to allow reliable quantitation of the analyte at or below the selected SVs (see Section 5.2). Detection and quantitation limits must be determined prior to the use of any method for routine analyses and after any significant changes are made to a method during routine analyses. 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. The limits of detection given in Table 8-4 are considered to be representative of typically attainable values. Depending upon individual laboratory capabilities and fish tissue matrix properties, it should be noted that SVs for some recommended target analytes (e.g., inorganic arsenic, dieldrin, heptachlor epoxide, toxaphene, PCBs, and dioxins/ furans) may not always be analytically attainable quantitation limits. In these instances, all historic and current data on contaminant sources and on water, sediment, and fish and shellfish contaminant tissue data should be reviewed to provide additional information that could aid in the risk assessment process and in making risk management decisions.

The EPA has previously issued guidance on detection limits for trace metal and organic compounds for analytical methods used in chemical contaminant monitoring programs (U.S. EPA, 1985a). However, 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, detection and quantitation limits reported in the literature are seldom clearly defined. 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).

8.3.3.3.1 Detection limits

The EPA recommends that the method detection limit (MDL) defined below and determined according to 40 CFR 136, Appendix B, be used to establish the limits of detection for the analytical methods used for analyses of all target analytes:

- **Method Detection Limit**: The minimum concentration of an analyte in a given matrix (i.e., fish or shellfish tissue homogenates for the purposes of this guidance) 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 three to five times the estimated MDL (Glaser et al., 1981; 40 CFR 136, Appendix B):

$$MDL = (t_{0.99})\ (S). \tag{8-3}$$

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.

In addition to the MDL, three other types of detection limits have been defined by the American Chemical Society Committee on Environmental Improvement (Keith, 1991a):

- **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 at a specified level of confidence. The recommended value for the LOD is three times the standard deviation of the blank in replicate analyses, corresponding to a 99 percent confidence level.

- **Reliable Detection Limit (RDL):** The concentration level of an analyte in a given matrix at which a detection decision is extremely likely. The RDL is generally set higher than the MDL. When RDL=MDL, the risk of a false positive at 3σ from zero is <1 percent, whereas the corresponding risk of a false negative is 50 percent. When RDL=2MDL, the risk of either a false positive or a false negative at 3σ from zero is <1 percent.

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 or RDL may be too stringent and result in the rejection of valid data; however, these are the only detection limit estimates that account for matrix effects and interferences and provide a high level of statistical confidence in sample results. The MDL is the recommended detection limit in the EPA EMAP-NC Program (U.S. EPA, 1991e).

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

$$MDL_{tissue}\ (\text{ppm or ppb}) = (MDL_{extract} \bullet V)\ /W \tag{8-4}$$

where

V = Final extract or digestate volume, after dilution or concentration (mL)
W = Weight of sample digested or extracted (g).

Equation 8-4 clearly illustrates that the MDL in tissue may be improved (reduced) by increasing the sample weight (W) and/or decreasing the final extract or digestate volume (V).

The initial MDL is a statistically derived empirical value that may differ in actual samples depending on several factors, including sample size, matrix effects, and percent moisture. Therefore, it is recommended that each laboratory reevaluate annually all MDLs for the analytical methods used for the sample matrices typically encountered (U.S. EPA, 1991e).

Experienced analysts may use their best professional judgment to adjust the measured MDL to a lower "typically achievable" detection limit (Puget Sound Estuary Program, 1990e; U.S. EPA, 1985a) or to derive other estimates of detection limits. For example, EPA recommends the use of lower limits of detection (LLDs) for GC/MS 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 are 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.

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.

8.3.3.3.2 Quantitation limits

In addition to the MDL, a method quantitation limit (MQL), or minimum concentration allowed to be reported at a specified level of confidence without qualifications, should be derived for each analyte. Ideally, MQLs should account for matrix effects and interferences. The MQL can be greater than or equal to the MDL. At present, there is no consistent guidance in the scientific literature for determining MQLs; therefore, it is not possible to provide specific recommendations for determining these limits at this time.

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The American Chemical Society Committee on Environmental Improvement (Keith, 1991b; 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 ±30 percent in the measured value ($10\sigma \pm 3\sigma$) at the 99 percent confidence level.

The LOQ is the recommended quantitation limit in the EPA EMAP-NC Program (U.S. EPA, 1991e). However, the LOQ does not account for matrix effects or interferences.

The U.S. EPA (1986d) 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, 1987d) used a PQL based on the lowest concentration of the initial calibration curve (C, in µg/mL), the amount of sample typically analyzed (W, in g), and the final extract volume (V, in mL) of that method:

$$\text{PQL}\ (\mu g / g[ppm]) = \frac{C\ (\mu g / mL) \bullet V(mL)}{W\ (g)} \qquad (8\text{-}5)$$

However, this PQL is also applicable only to samples without substantial matrix effects or interferences.

A reliable detection limit (RDL) equal to 2 MDL may also be used as an estimate of the MQL (see Section 8.3.3.3.1). The RDL accounts for matrix effects and provides a high level of statistical confidence in analytical results.

Analysts must use their expertise and professional judgment to determine the best estimate of the MQL for each target analyte. MQLs, 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.

8.3.3.3.3 Use of detection and quantitation limits in reporting data

The analytical laboratory does not have responsibility or authority to censor data. Therefore, all data should be reported with complete documentation of limitations and problems. Method detection and quantitation limits should be used to qualify reported data for each composite sample as follows (Keith, 1991b):

- "Zero" concentration (no observed response) should be reported as not detected (ND) with the MDL noted, e.g., "ND(MDL=X)".

- Concentrations below the MDL should be reported with the qualification that they are below the MDL.

- Concentrations between the MDL and the MQL should be reported with the qualification that they are below the quantitation limit.

- Concentrations at or above the MQL may be reported and used without qualification.

The use of laboratory data for comparing target analyte concentrations to SVs in screening and intensive studies is discussed in Sections 9.1.1 and 9.1.2.

8.3.3.4 Assessment of Method Accuracy—

The accuracy of each analytical method should be assessed and documented for each target analyte of interest, in a fish or shellfish tissue matrix, prior to beginning routine analyses and on a regular basis during routine analyses.

Method accuracy may be assessed by analysis of appropriate reference materials (i.e., SRMs or CRMs prepared from actual contaminated fish or shellfish tissue, see Table 8-8, **laboratory control samples** (i.e., accuracy-based samples consisting of fish and shellfish tissue homogenates spiked with compounds representative of the target analytes of interest), and/or **matrix spikes**. If possible, laboratory control samples should be SRMs or CRMs. **Note:** Only the analysis of fish or shellfish tissue SRMs or CRMs prepared from actual contaminated fish or shellfish tissue allows rigorous assessment of total method accuracy, including the accuracy with which an extraction or digestion procedure isolates the target analyte of interest from actual contaminated fish or shellfish. The analysis of spiked laboratory control samples or matrix spikes provides an assessment of method accuracy including sample handling and analysis procedures but does not allow rigorous assessment of the accuracy or efficiency of extraction or digestion procedures for actual contaminated fish or shellfish. Consequently, these samples should not be used for the primary assessment of total method accuracy unless SRMs or CRMs prepared from actual contaminated fish or shellfish tissue are not available.

The concentrations of target analytes in samples used to assess accuracy should fall within the range of concentrations found in the field samples; however, this may not always be possible for reference materials or laboratory control samples because of the limited number of these samples available in fish and shellfish tissue matrices (see Table 8-8). Matrix spike samples should be prepared using spike concentrations approximately equal to the concentrations found in the unspiked samples. An acceptable range of spike concentrations is 0.5 to 5 times the expected sample concentrations (U.S. EPA, 1987e). Spikes should always be added to the sample homogenates prior to digestion or extraction.

Accuracy is calculated as percent recovery from the analysis of reference materials, or laboratory control samples, as follows:

$$\% \text{ Recovery} = 100\,(M/T) \tag{8-6}$$

where

M = Measured value of the concentration of target analyte
T = "True" value of the concentration of target analyte.

Accuracy is calculated as percent recovery from the analysis of matrix spike samples as follows:

$$\% \text{ Recovery} = [(M_s - M_u)/T_s] \times 100 \tag{8-7}$$

where

M_s = Measured concentration of target analyte in the spiked sample
M_u = Measured concentration of target analyte in the unspiked sample
T_s = "True" concentration of target analyte added to the spiked sample.

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

8.3.3.4.1 Initial assessment of method accuracy

As discussed above, method accuracy should be assessed initially by analyzing appropriate SRMs or CRMs that are prepared from actual contaminated fish or shellfish tissue. The 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 with concurrence of the program manager. If such SRMs or CRMs are not available, laboratory control samples or matrix spikes may be used for initial assessment of method accuracy.

8.3.3.4.2 Routine assessment of method accuracy

Laboratory control samples and matrix spikes should be analyzed for continuous assessment of accuracy during routine analyses. It is recommended that 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, 1990e). Ideally, CRMs or SRMs should also be analyzed at this recommended frequency; however, limited availability and cost of these materials may make this impractical.

For organic compounds, isotopically labeled or surrogate recovery standards that must be added to each sample to monitor overall method performance also provide an assessment of method accuracy (see Section 8.3.3.7.1).

Percent recovery values for spiked samples must fall within established control limits (see Table 8-6). If the percent recovery falls outside the control limit, 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.

Note: Reported data should not be corrected for percent recoveries. Recovery data should be reported for each sample to facilitate proper evaluation and use of analytical results.

Poor performance on the analysis of reference materials or poor spike recovery may be caused by inadequate mixing of the composite homogenate sample before aliquotting, inconsistent digestion or extraction procedures, matrix interferences, or instrumentation problems. If replicate analyses are acceptable (see Section 8.3.3.5), 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 significantly different, the digestion technique should be modified to obtain acceptable recoveries. If there is no significant 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 additions or use of a matrix modifier is indicated (U.S. EPA, 1987e).

8.3.3.5 Assessment of Method Precision—

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

Precision is defined as the agreement among a set of replicate measurements without assumption of knowledge of the true value. Method precision (i.e., total variability due to sample preparation and analysis) is estimated by means of the analyses of duplicate or replicate tissue homogenate samples containing concentrations of the target analyte of interest above the MDL. All samples used for assessment of total method precision must be carried through the complete analytical procedure, including extraction or digestion.

The most commonly used estimates of precision are the relative standard deviation or coefficient of variation (CV) for multiple samples, and the relative percent

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difference (RPD) when only two samples are available. These are defined as follows:

$$RSD = CV = 100\ S/\bar{x}_i \qquad (8\text{-}8)$$

where

S = Standard deviation of the x_i measurements
$\bar{x}_i$ = Arithmetic mean of the x_i measurements

and

$$RPD = 100\ \{(x_1 - x_2)/[(x_1 + x_2)/2]\}\ . \qquad (8\text{-}9)$$

8.3.3.5.1 Initial assessment of method precision

Method precision should be assessed prior to routine sample analyses by analyzing replicate samples of the same reference materials, laboratory control samples, and/or matrix spikes that are used for initial assessment of method accuracy (see Section 8.3.3.4.1). The number of replicates required to be analyzed for the initial assessment of method precision should be determined by each laboratory for each analytical procedure with concurrence of the program manager. Because precision may be concentration-dependent, initial assessments of precision across the estimated working range should be obtained.

8.3.3.5.2 Routine assessment of method precision

Ongoing assessment of method precision during routine analysis should be performed by analyzing replicate aliquots of tissue homogenate samples taken prior to sample extraction or digestion (i.e., **laboratory replicates**) and **matrix spike replicates**. Matrix spike concentrations should approximate unspiked sample concentrations; an acceptable range for spike concentrations is 0.5 to 5 times the sample concentrations (U.S. EPA, 1987e).

For ongoing assessment of method precision, it is recommended that 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 over time.

Precision estimates obtained from the analysis of laboratory duplicates, matrix spike duplicates, and repeated laboratory control sample analyses must fall within specified control limits (see Table 8-6). If these values fall outside the control limits, the analyses should 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).

8.3.3.5.3 Routine assessment of analytical precision

The analysis of replicate aliquots of final sample extracts or digestates **(analytical replicates)** provides an estimate of analytical precision only; it does not provide an estimate of total method precision. For organic target analytes, analytical replicates may be included at the discretion of the program manager or laboratory supervisor. For the analysis of target metal analytes by graphite furnace atomic absorption spectrophotometry (GFAA) and cold vapor atomic absorption spectrophotometry (CVAA), it is recommended that duplicate injections of each sample be analyzed and the mean concentration be reported. The RPD should be within control limits established by the program manager or laboratory supervisor, or the sample should be reanalyzed (U.S. EPA, 1987e).

8.3.3.5.4 Assessment of overall variability

Estimates of the overall variability of target analyte concentrations in a sample fish or shellfish population and of the sampling and analysis procedures can be obtained by collecting and analyzing **field replicates**. Replicate field samples are optional in screening studies; however, if resources permit, it is recommended that duplicate samples be collected at 10 percent of the screening sites as a minimal QC check. Analysis of replicate field samples provides some degree of variability in that the samples themselves are typically collected and exposed to the same environmental conditions and contaminants. There are many points of potential dissimilarity between samples of the type described here; however, this variability is reduced when well-homogenized composite samples are analyzed. In intensive studies, replicate samples should be collected at each sampling site (see Section 6.1.2.7). Although the primary purpose of replicate field samples in intensive studies is to allow more reliable estimates of the magnitude of contamination, extreme variability in the results of these samples may also indicate that sampling and/or analysis procedures are not adequately controlled.

8.3.3.6 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 6.2.2 and 7.2 should be followed carefully to avoid contamination of samples in the field and laboratory.

Many metal contamination problems are due to airborne dust. High zinc blanks may result from airborne dust or galvanized iron, and high chromium and nickel blanks often indicate contamination from stainless steel. Mercury thermometers should not be used in the field because broken thermometers can be a source of significant mercury contamination. In the laboratory, samples to be analyzed for mercury should be isolated from materials and equipment (e.g., polarographs) that are potential sources of mercury contamination. Cigarette smoke is a source of cadmium. Consequently, care should be taken to avoid the presence of cigarette smoke during the collection, handling, processing, and analysis of samples for cadmium. In organic analyses, phthalates, methylene chloride, and toluene are common laboratory contaminants that are often detected in blanks at concentrations above the MDL (U.S. EPA, 1987e).

Cross-contamination between samples should be avoided during all steps of analysis of organic contaminants by GC-based methods. 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 may be required following the analysis of a high-level sample to assess carryover (U.S. EPA, 1987e).

To monitor for interferences and contamination, the following blank samples should be analyzed prior to beginning sample collection and analyses and on a routine basis throughout each study (U.S. EPA, 1987e):

- **Field blanks** are rinsates of empty field sample containers (i.e., aluminum foil packets and plastic bags) that are prepared, shipped, and stored as actual field samples. Field blanks 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 analytes of interest and the total amount of target analyte 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** are rinsates of utensils and equipment used for dissecting and homogenizing fish and shellfish. Processing blanks 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 study and preferably once with each batch of 20 or fewer samples.

- **Bottle blanks** are rinsates of empty bottles used to store and ship sample homogenates. Bottle blanks should be collected after the bottles are cleaned prior to use for storage or shipment of homogenates. They should be analyzed, using the procedure described above for field blanks, to evaluate their potential as sources of contamination. It is recommended that one bottle blank be analyzed for each lot of bottles or with each batch of 20 or fewer samples, whichever is more frequent.

- **Method blanks** are samples of extraction or digestion solvents that are carried through the complete analytical procedure, including extraction or digestion; they are also referred to as **procedural blanks.** Method blanks should be analyzed to evaluate contaminants resulting from the total analytical method (e.g., contaminated glassware, reagents, solvents, column packing materials, processing equipment). It is recommended that one method blank be analyzed with every 20 samples or with each batch of samples, whichever is more frequent.

- **Reagent blanks** are samples of reagents used in the analytical procedure. It is recommended that each lot of analytical reagents be analyzed for target analytes 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 the absence of interfering impurities should be verified prior to use (U.S. EPA, 1987e).

Because the contamination in a blank sample may not always translate into contamination of the tissue samples, analysts and program managers must use their best professional judgment when interpreting blank analysis data. Ideally, there should be no detectable concentration of any target analyte in any blank sample (i.e., the concentration of target analytes in all blanks should be less than the MDL). However, program managers may set higher control limits (e.g., $\leq$MQL) depending on overall data quality requirements of the monitoring program. If the concentration of a target analyte in any blank is greater than the established control limit, all steps in the relevant sample handling, processing, and analysis procedures should be reviewed to identify the source of contamination and appropriate corrective action should be taken. If there is sufficient sample material, all samples associated with the unacceptable blank should be reanalyzed. If reanalysis is not possible, all suspect data should be identified clearly.

Note: Analytical data should not be corrected for blank contamination by the reporting laboratory; however, blank concentrations should always be reported with each associated sample value.

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8.3.3.7 Special QA and QC Procedures for the Analysis of Organic Target Analytes—

8.3.3.7.1 Routine monitoring of method performance

To account for losses during sample preparation (i.e., extraction, cleanup) and to monitor overall method performance, a standard compound that has chemical and physical properties as similar as possible to those of the target analyte of interest should be added to each sample prior to extraction and to each calibration standard. Such compounds may be termed **surrogate recovery standards.** A stable, **isotopically labeled analog of the target analyte** is an ideal surrogate recovery standard for GC/MS analysis.

If resources permit, an isotope dilution GC/MS technique such as EPA Method 1625 (40 CFR 136, Appendix A) is recommended for the analysis of organic target analytes for which isotopically labeled analogs are available. In this technique, RRFs used for quantitation may be calculated from measured isotope ratios in calibration standards and not from instrument internal standards. However, an instrument internal standard still must be added to the final sample extract prior to analysis to determine the percent recoveries of isotopically labeled recovery standards added prior to extraction. Thus, in isotope dilution methods, instrument internal standards may be used only for QC purposes (i.e., to assess the quality of data) and not to quantify analytes. Control limits for the percent recovery of each isotopically labeled recovery standard should be established by the program manager, consistent with program data quality requirements. Control limits for percent recovery and recommended corrective actions given in EPA Method 1625 (40 CFR 136, Appendix A) should be used as guidance.

If isotopically labeled analogs of target analytes are not available or if the isotope dilution technique cannot be used (e.g., for chlorinated pesticides and PCBs analyzed by GC/ECD), other surrogate compounds should be added as recovery standards to each sample prior to extraction and to each calibration standard. These surrogate recovery standards should have chemical and physical properties similar to the target analytes of interest and should not be expected to be present in the original samples. Recommended surrogate recovery standards are included in the methods referenced in Table 8-2 and in EMMI (U.S. EPA, 1991f).

Samples to which surrogate recovery standards have been added are termed **surrogate spikes**. The percent recovery of each surrogate spike ($\% R_s$) should be determined for all samples as follows:

$$\% R_s = 100 \, (C_m/C_a) \qquad (8\text{-}10)$$

where

$\% R_s$ = Surrogate spike percent recovery
C_m = Measured concentration of surrogate recovery standard

C_s = Actual concentration of surrogate recovery standard added to the sample.

Control limits for the percent recovery of each surrogate spike should be established by the program manager consistent with program data quality requirements. The control limits in the most recent EPA CLP methods (U.S. EPA, 1991c) are recommended for evaluating surrogate recoveries.

Note: Reported data should not be corrected for percent recoveries of surrogate recovery standards. Recovery data should be reported for each sample to facilitate proper evaluation and use of the analytical results.

8.3.3.7.2 Other performance evaluation procedures

The following additional procedures are required to evaluate the performance of GC-based analytical systems prior to the routine analysis of field samples (U.S. EPA, 1989c; U.S. EPA, 1991c). It is the responsibility of each program manager to determine specific evaluation procedures and control limits appropriate for their data quality requirements.

Evaluation of the GC system

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

Column Resolution: 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 20 percent during an analysis session. The equation for N is given as follows:

$$N = 16\,(RT/W)^2 \tag{8-11}$$

where

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

Relative Retention Time: 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 control limits, 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 should 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 a 50-ng injection of DFTPP should meet the following criteria (U.S. EPA, 1991c):

m/z	Criteria
51	30-80% mass 198
68	<2% mass 69
69	present
70	<2% mass 69
127	25-75% mass 198
197	<1% mass 198
198	base peak, 100% relative abundance
199	5-9% mass 198
275	10-30% mass 198
365	>0.75% mass 198
441	present and <mass 443
442	40-110% mass 198
443	15-24% mass 442

If the control limits for 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 analytes are consistently collected in the proper fraction. 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 8.3.3.6). Silica gel columns should be evaluated by their ability to resolve cholesterol from a selected target analyte.

8.3.3.8 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 strongly recommended for several reasons:

- To demonstrate laboratory capability prior to conducting routine analyses of field samples
- To provide an independent ongoing assessment of each laboratory's capability to perform the required analyses
- To enhance the comparability of data between states and Regions.

Two types of external QA programs are recommended: **round-robin interlaboratory comparisons** (often referred to as **interlaboratory calibration programs**) and **split-sample interlaboratory comparisons.**

8.3.3.8.1 Round-robin analysis interlaboratory comparison program

At present, the only external round-robin QA program available for analytical laboratories conducting fish and shellfish tissue analyses for environmental pollutants is administered by NOAA in conjunction with its National Status and Trends (NS&T) Program (Cantillo, 1991). This QA program has been designed to ensure proper documentation of sampling and analysis procedures and to evaluate both the individual and collective performance of participating laboratories. Recently, NOAA and EPA have agreed to conduct the NS&T Program and the EMAP-NC Program as a coordinated effort. As a result, EMAP-NC now cosponsors and cooperatively funds the NS&T QA Program, and the interlaboratory comparison exercises include all EMAP-NC laboratories (U.S. EPA, 1991e).

Note: Participation in the NS&T QA program by all laboratories performing chemical analyses for state fish and shellfish contaminant monitoring programs is recommended to enhance the credibility and comparability of analytical data among the various laboratories and programs.

Each laboratory participating in the NS&T QA program is required to demonstrate its analytic capability prior to the analysis of field samples by the blind analysis of a fish and shellfish tissue sample that is uncompromised, homogeneous, and contains the target analytes of interest at concentrations of interest. A laboratory's performance generally will be considered acceptable if its reported results are within ±30 percent (for organics) and ±15 percent (for metals) of the actual or certified concentration of each target analyte in the sample (U.S. EPA, 1991e). If any of the results exceed these control limits, the laboratory will be required to repeat the analysis until all reported results are within the control limits. Routine analysis of field samples will not be allowed until initial demonstration of laboratory capability is acceptable.

Following the initial demonstration of laboratory capability, each participating laboratory is required to participate in one intercomparison exercise per year as a continuing check on performance. This intercomparison exercise includes both organic and 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. Sample types and matrices vary yearly. Performance evaluation samples used in the past have included accuracy-based solutions, sample extracts, and representative matrices (e.g., tissue or sediment samples). Laboratories are required to analyze the performance evaluation samples blind and to submit their results to NIST or NRCC, as instructed. Individual laboratory performance is evaluated against the consensus values (i.e., grand means) of the results reported by all participating laboratories. Laboratories that fail to achieve acceptable performance must take appropriate corrective action. NIST and NRCC will provide technical assistance to participating laboratories that have problems with the intercomparison analyses. At the end of each calendar year, the results of the intercomparison exercises are reviewed at a workshop sponsored by NIST and NRCC. Representatives from each laboratory are encouraged to participate in these workshops, which provide an opportunity for discussion of analytical problems encountered in the intercomparison exercises.

Note: Nonprofit laboratories (e.g., EPA and other federal laboratories, state, municipal, and nonprofit university laboratories) may participate in the NS&T QA program at no cost on a space-available basis. The cost of participation in the NIST Intercomparison Exercise Program for Organic Contaminants in the Marine Environment is $2,500 for private laboratories within and outside the United States. This cost covers samples for one exercise per year. Samples may be obtained directly from NIST by contacting Ms. Michele Shantz, NIST, 100 Bureau Drive, Stop 8392, Gaithersburg, MD 20899-8392; Tel: 301-975-3106, FAX: 301-997-0685. Trace inorganic samples are available directly from NRCC by contacting Mr. Scott Willis, NRCC, Ottawa, Ontario, Canada K1A029, e-mail: scott.willie@NRC.CA, Tel: 613-993-4969.

To obtain additional information about participation in the NS&T QA program, contact Dr. Adriana Cantillo, QA Manager, NOAA/National Status and Trends Program, NYSCI1, 1305 East West Highway, Silver Spring, MD 20910; Tel: 301-713-3028, ext. 147, FAX: 301-713-4388.

8.3.3.8.2 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 samples analyzed by each state or Region are divided and distributed for analyses among laboratories from other states or Regions. Because actual 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 results from different states or Regions.

The NS&T QA program does not include an interlaboratory split-sample analysis program. However, it is recommended that split-sample analysis programs be established by states and/or Regions that routinely share results.

8.4 Documentation and Reporting of Data

The results of all chemical analyses must be documented adequately and reported properly to ensure the correct evaluation and interpretation of the data.

8.4.1 Analytical Data Reports

The documentation of analytical data for each sample should include, at a minimum, the following information:

- Study identification (e.g., project number, title, phase)
- Description of the procedure used, including documentation and justification of any deviations from the standard procedure
- Method detection and quantitation limits for each target analyte
- Method accuracy and precision for each target analyte
- Discussion of any analytical problems and corrective action taken
- Sample identification number
- Sample weight (wet weight)
- Final dilution volume/extract volume
- 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 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 GC/FID
- Raw data quantitation reports for each sample

- Description of all QC samples associated with each sample (e.g., reference materials, field blanks, rinsate blanks, method blanks, duplicate or replicate samples, spiked samples, laboratory control samples) and results of all QC analyses. 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 and matrix 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 ppm or ppb wet weight, to two significant figures unless otherwise justified). **Note:** Reported data should not be recovery- or blank-corrected.

- Lipid content (as percent wet weight)

- 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 of reported data, 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, 1991b, 1991c) may serve as useful examples for analytical laboratories.

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 data quality specifications (e.g., detection and quantitation limits, precision, accuracy) and other performance criteria established in the Work/QA Project Plan. In many instances, it may be necessary to qualify reported data values; qualifiers should always be defined clearly in the data report. Recent guidance on the documentation and evaluation of trace metals data collected for Clean Water Act compliance monitoring (U.S. EPA, 1995h) provides additional useful information on data review procedures.

8.4.2 Summary Reports

Summaries of study data should be prepared for each target species at each sampling site. Specific recommendations for reporting data for screening and intensive studies are given in Section 9.2.

SECTION 9

DATA ANALYSIS AND REPORTING

This section provides guidance on (1) analysis of laboratory data for both screening and intensive studies that should be included in state data reports, (2) data reporting requirements for both state-conducted screening and intensive studies, and (3) data reporting requirements for a national data repository for state-collected fish tissue data housed within the National Listing of Fish and Wildlife Advisories (NLFWA) database.

All data analysis and reporting procedures should be documented fully as part of the Work/QA Project Plan for each study, prior to initiating the study (see Appendix I). All routine data analysis and reporting procedures should be described in standard operating procedures. In particular, the procedures to be used to determine if the concentration of a target analyte in fish or shellfish tissue differs significantly from the selected screening value must be clearly documented.

9.1 DATA ANALYSIS

9.1.1 Screening Studies

The primary objective of **Tier 1** screening studies is to assist states in identifying potentially contaminated harvest areas where further investigation of fish and shellfish contamination may be warranted. The criteria used to determine whether the measured target analyte concentration in a fish or shellfish tissue composite sample is different from the SV (greater than or less than) should be clearly documented. If a reported target analyte concentration exceeds the SV in the screening study, a state should initiate a **Tier 2**, **Phase I**, intensive study (see Section 6.1.2.1) to verify the level of contamination in the target species. Because of resource limitations, some states may choose to conduct a risk assessment using screening study data; however, this approach is not recommended because a valid statistical analysis cannot be performed on a single composite sample. If a reported analyte concentration is close to the SV but does not exceed the SV, the state should reexamine historic data on water, sediment, and fish tissue contamination at the site and evaluate data on laboratory performance. If these data indicate that further examination of the site is warranted, the state should initiate a **Tier 2**, **Phase I**, intensive study to verify the magnitude of the contamination.

Because replicate composite samples are not required as part of a screening study, estimating the variability of the composite target analyte concentration at any site is precluded. The following procedure is recommended for use by states for analysis of the individual target analyte concentration for each composite sample from reported laboratory data (see Section 8.3.3.3)

- A datum reported below the method detection limit, including a datum reported as not detected (i.e., ND, no observed response) should be assigned a value of one-half the MDL or zero.
- A datum reported between the MDL and the method quantitation limit should be assigned a value of the MDL plus one-half the difference between the MQL and the MDL.
- A datum reported at or above the MQL should be used as reported.

This approach is similar to that published in 40 CFR Parts 122, 123, 131, and 132—Proposed Water Quality Guidance for the Great Lakes System.

If resources permit and replicate composite samples are collected at a suspected site of contamination, then a state may conduct a statistical analysis of differences between the mean target analyte concentration and the SV, as described in Section 9.1.2.

9.1.2 Intensive Studies

The primary objectives of **Tier 2** intensive studies are to confirm the findings of the screening study by assessing the magnitude and geographic extent of the contamination in various size classes of selected target species. The EPA Office of Water recommends that states collect replicate composite samples of three size classes of each target species in the study area to verify whether the mean target analyte concentration of replicate composite samples for any size class exceeds the SV for any target analyte identified in the screening study. The statistical approach for this comparison is described in Section 6.1.2.7.

The following procedure is recommended for use by states in calculating the mean arithmetic target analyte concentration from reported laboratory data (see Section 8.3.3.3.3).

- Data reported below the MDL, including data reported as not detected (i.e., ND, no observed response) should be assigned a value of one-half the MDL.
- Data reported between the MDL and the MQL should be assigned a value of the MDL plus one-half the difference between the MQL and the MDL.
- Data reported at or above the MQL should be used as reported.

This approach is similar to that published in 40 CFR Parts 122, 123, 131, and 132—Proposed Water Quality Guidance for the Great Lakes System.

Secondary objectives that may be assessed as part of **Tier 2** intensive studies can include defining the geographical region where fish contaminant concentrations exceed screening values; identifying geographical distribution of contaminant concentrations; and, in conjunction with historical data or future data collection, assessing changes in fish contaminant concentrations over time. The statistical considerations involved in comparing fish contaminant levels measured at different locations or times are discussed in Appendix N.

State staff should consult a statistician in interpreting intensive study tissue residue results to determine the need for additional monitoring, risk assessment, and issuance of a fish or shellfish consumption advisory. Additional information on risk assessment, risk management, and risk communication procedures will be provided in later volumes in this guidance series (see Section 1.4).

9.2 DATA REPORTING

9.2.1 State Data Reports

State data reports should be prepared by the fish contaminant monitoring program manager responsible for designing the screening and intensive studies. Summaries of **Tier 1** screening study data should be prepared for each target species sampled at each screening site. For **Tier 2** intensive studies (**Phase I** and **Phase II**), data reports should be prepared for each target species (by size class, as appropriate) at each sampling site within the waterbody under investigation (see Section 6.1.2). Screening and intensive study data reports should include, at a minimum, the information shown in Figure 9-1.

9.2.2 Reports to the National Fish Tissue Residue Data Repository (NFTRDR)

The EPA Office of Science and Technology within the Office of Water has established the NFTRDR, which is housed within the NLFWA database. This repository is a collection of fish and shellfish contaminant monitoring data gathered by various state, federal, and local agencies for advisory purposes. The objectives of the repository are to:

- Facilitate the exchange of fish and shellfish contaminant monitoring data nationally by improving the comparability and integrity of state data
- Encourage greater cooperation among regional and state fish advisory programs
- Assist states in their fish tissue data collection efforts by providing ongoing technical assistance.

The NLFWA database now contains a facility for storing fish tissue residue data as well as for documenting and mapping active and rescinded fish consumption advisories. Since 1996, a stand-alone version of the NFLWA database has been available for Internet downloads. Internet WEB-based tools have recently been developed to support queries and interactive mapping of both the general advisory information as well as fish tissue residue data. Internet-based tools are also being

- Study identification (e.g., project number, title, and study type)
- Program manager
- Sampling site name
- Latitude (decimal degrees preferred)
- Longitude (decimal degrees preferred)
- Type of waterbody (lake, river, estuary, etc.)
- Name of waterbody
- Sampling date (e.g., YYYYMMDD, Year 2000 compliant format)
- Sampling time (e.g., HH, MM in a 24-h format)
- Sampling gear type used (e.g., dredge, seine, trawl, gill net)
- Sampling depth (feet or meters)
- Standard common name of target species (preferably name sanctional by American Fisheries Society for inland waters or by NOAA for coastal waters)
- Composite sample numbers
- Number of individuals in each composite sample
- Number of replicate composite samples
- Predominant characteristics of specimens used in each composite sample
  - Predominant life stage of individuals in composite
  - Predominant sex of individuals in composite (if applicable)
  - Average age of individuals in composite (if applicable)
  - Average body length (cm)
  - Description of edible portion (tissue type)
- Analytical methods used (including method for lipid analysis)
- Method detection and quantitation limits for each target analyte analyzed
- Sample cleanup procedures (e.g., additional steps taken to further purify the sample extracts or digestates)
- Data qualifiers (e.g., additional qualifying information about the measurement)
- Percent lipid (wet weight basis) in each composite sample
- For each target analyte in each composite sample:
  - Total wet weight of composite sample (g) used in analysis
  - Measured concentration (wet weight basis) as reported by the laboratory (see Section 8.3.3.3.3)
  - Units of measurement for target analyte concentration (e.g., ppm)
  - Evaluation of laboratory performance (i.e., description of all QA and QC samples associated with the sample(s) and results of all QA and QC analyses)
- In screening studies with only one composite sample for each target species, the state should provide for each target analyte a comparison of reported concentration with selected SV (see Section 4 tables) and indication of whether SV was exceeded for recreational or subsistence fishers (see Section 9.1.1).
- In intensive studies, for each target analyte in each set of replicate composite samples, the state should provide
  - Range of target analyte concentrations for each set of replicate composite samples
  - Mean (arithmetic) target analyte concentration for each set of replicate composite samples (see Section 9.1.2)
  - Standard deviation of mean target analyte concentration
  - Comparison of target analyte arithmetic mean concentration with selected SV (see Section 5) and indication of whether SV was exceeded for recreational or subsistence fishers

Figure 9-1. Recommended data reporting requirements for screening and intensive studies.

developed as a way for state agencies to add fish advisory and contaminant monitoring data to the NLFWA database and may be developed to perform some types of standard data analysis on the fish tissue residue data.

EPA has recently developed an Internet-based data entry facility for the NLFWA using some of the data elements included in Figure 9-1. This Internet-based data entry facility is housed within the EPA's NLFWA database and allows states to archive fish advisory information as well as fish tissue residue data generated through their fish contaminant monitoring programs. States may prepare their own data tables and arrange to transfer these to EPA to be formatted and reviewed before entry into the repository. The information in the NFTRDR can be organized into three different tables (STATIONS, SAMPLES, and RESULTS tables) using such readily available PC relational database packages as ACCESS (Figure 9-2). If states submit their monitoring data in other file formats (e.g., spreadsheet files or ASCII files exported from other in-house database systems), a short data dictionary (metadata) file should be included (ASCII, Wordperfect, or WORD format) clearly documenting the meaning of all data fields and any codes, abbreviations, or measurement units used in the files.

State, regional, and local agency staff may obtain further information on the new Internet WEB-based database EPA now has available by contacting:

U.S. Environmental Protection Agency
Office of Science and Technology
National Fish and Wildlife Contamination Program-4305
1200 Pennsylvania Avenue, NW
Washington, DC 20460
PHONE: 202-260-7301
FAX: 202-260-9830

Jeffrey D. Bigler
U.S. Environmental Protection Agency-4305
1200 Pennsylvania Avenue, NW
Washington, DC 20460
PHONE: 202-260-1305
E-MAIL: bigler.jeff@epa.gov

Fish Tissue Chemical Residue Data Tables: STATIONS, SAMPLES and RESULTS

The **STATIONS** table includes basic locational data.

Field name	Field description
STATION_ID	Waterbody, Station or Monitoring Site Identifier. This field becomes a database key field. Each record must have a unique STATION_ID.
STATE	State 2-character postal code abbreviation.
WATERBODY (or SITENAME)	A short caption to identify the waterbody or sampling station.
LOCATION	Additional descriptive information on the waterbody or station location.
ADVNUM	If the waterbody or site is associated with an advisory (active or rescinded), include the number assigned to this advisory in the current National Listing of Fish and Wildlife Advisories (NLFWA) database.
COUNTY	County name.
LAT	Station latitude. A format in decimal degrees is preferred.
LNG	Station longitude. A format in decimal degrees is preferred.

The **SAMPLES** table includes data on the type of tissue sample collected.

Field name	Field description
SAMPLE_ID	An identifier to each specific fish tissue sample from a waterbody or station. This is used as a database key, so each record must have a unique SAMPLE_ID
STATION_ID	Waterbody, Station or Monitoring Site Identifier as defined in the STATIONS table.
SAMPLE_DATE	The date the sample was collected in the field. Give date in a Year 2000 compliant format (YYYYMMDD).
FISH_SPECIES	Fish species names. Standard English common names as established by the American Fisheries Society for inland waters or NOAA for coastal water are preferred.
SAMPLE_TYPE	How the sample was prepared (e.g., fillet with skin-on or skin-off, whole fish). In the NUMBER_OF_FISH field below, multiple fish in a sample indicate a composite sample.
LENGTH	The length of the sample fish. For composites, an average length should be given.
LENGTH_UNIT	Length units of fish (cm or inches)
WEIGHT	Specimen or composite weight used for residue analysis.
WEIGHT_UNIT	Weight units (usually in grams).
LIPID	Percent extractable lipids.
NUMBER_OF_FISH	Number of fish (specimens) in sample. Number greater than a value of 1 indicates a composite sample.

The **RESULTS** table includes chemical-specific tissue sample concentrations.

Field name	Field description
SAMPLE_ID	An identifier to each specific fish tissue sample from a waterbody or station. This is used as a database key, so each record must have a unique SAMPLE_ID
PARAMETER	Chemical name. File should specify all acronyms or abbreviations used.
DETECTION_INFO	A caption to document detection limit information (e.g., "less than detection limit").
RESULT	A number representing the concentration of a chemical (or the detection limit).
RESULT_UNIT	Units associated with concentration (e.g., "ppm").

Figure 9-2. Key information fields for the National Fish Tissue Residue Data Repository.