EPA 823-R-95-007

GUIDANCE FOR ASSESSING CHEMICAL CONTAMINANT DATA FOR USE IN FISH ADVISORIES

VOLUME 1: FISH SAMPLING AND ANALYSIS

SECOND EDITION

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

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LIST OF ACRONYMS

AFS	American Fisheries Society
ANOVA	Analysis of Variance
ATSDR	Agency for Toxic Substances and Disease Registry
BCF	bioconcentration factor
BW	body weight
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
COC	chain-of-custody
CR	consumption rate
CRADAs	Cooperative Research and Development Agreements
CSOs	combined sewer overflows
DOT	U.S. Department of Transportation
EPA	U.S. Environmental Protection Agency
FDA	U.S. Food and Drug Administration
FWS	U.S. Fish and Wildlife Service
γ-BHC	benzene hexachloride
γ-HCH	hexachlorocyclohexane
GC/ECD	gas chromatography/electron capture detection
GC/MS	gas chromatography/mass spectrometry
GPS	Global Positioning System
HRGC/MRMS	high-resolution gas chromatography/high-resolution mass spectrometry
IRIS	Integrated Risk Information System
MDL	method detection limit
MQL	method quantitation limit
NAS	National Academy of Sciences
NCBP	National Contaminant Biomonitoring Program

NCR	no-carbon-required
NFTDR	National Fish Tissue Data Repository
NIST	National Institute of Standards and Technology
NOAA	National Oceanic and Atmospheric Administration
OAPCA	Organotin Antifouling Paint Control Act
OAQPS	Office of Air Quality Planning and Standards
ODES	Ocean Discharge Evaluation System
ODW	Office of Drinking Water
OHEA	Office of Health and Environmental Assessment
OPPs	Office of Pesticide Programs
ORSANCO	Ohio River Valley Water Sanitation Commission
PAHs	polycyclic aromatic hydrocarbons
PCBs	polychlorinated biphenyls
PCDDs	polychlorinated dibenzo-p-dioxins
PCDFs	polychlorinated dibenzofurans
PEC	potency equivalency concentration
PNAs	polynuclear aromatic hydrocarbons
PTFE	polytetrafluoroethylene
QA	quality assurance
QC	quality control
RCRA	Resource Conservation and Recovery Act
RfD	reference dose
RPs	relative potencies
SF	slope factor
SOPs	standard operating procedures
SVs	screening values
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
2,3,7,8-TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
2,3,7,8-TCDF	2,3,7,8-tetrachlorodibenzofuran
2,4,5-TCP	2,4,5-trichlorophenol
TECs	toxicity equivalent concentrations
TVA	Tennessee Valley Authority

USDA	U.S. Department of Agriculture
USGS	United States Geological Survey
WHO	World Health Organization

EXECUTIVE SUMMARY

State, local, and Federal agencies currently use various methods to sample and analyze chemical contaminants in fish and shellfish in order to develop fish consumption advisories. A 1988 survey, funded by the U.S. Environmental Protection Agency (EPA) and conducted by the American Fisheries Society, identified the need for standardizing the approaches to evaluating risks and developing fish consumption advisories that are comparable across different jurisdictions (Cunningham et al., 1990; Cunningham et al., 1994). Four major components were identified as critical to the development of a consistent risk-based approach: standardized practices for sampling and analyzing fish, standardized risk assessment methods, standardized procedures for making risk management decisions, and standardized approaches for communicating risk to the general public (Cunningham et al., 1990).

To address concerns raised by the survey respondents, EPA is developing a series of four documents designed to provide guidance to State, local, regional, and tribal environmental health officials responsible for designing contaminant monitoring programs and issuing fish and shellfish consumption advisories. It is essential that all four documents be used together, since no single volume addresses all of the topics involved in the development of risk-based fish consumption advisories. The documents are meant to provide guidance only and do not constitute a regulatory requirement. This document series includes:

Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories

Volume I: Fish Sampling and Analysis
Volume II: Risk Assessment and Fish Consumption Limits
Volume III: Risk Management
Volume IV: Risk Communication.

Volume I was first released in September 1993 and this current revision to the Volume I guidance provides the latest information on sampling and analysis procedures based on new information provided by the Environmental Protection Agency. The major objective of Volume I is to provide information on sampling strategies for a contaminant monitoring program. In addition, information is provided on selection of target species; selection of chemicals as target analytes; development of human health screening values; sample collection procedures including sample processing, sample preservation, and shipping; sample analysis; and data reporting and analysis.

Volume II was released in June 1994 and provides guidance on the development of risk-based meal consumption limits for the high-priority chemical fish contaminants (target analytes). In addition to the presentation of consumption limits, Volume II contains a discussion of risk assessment methods used to derive the consumption limits as well as a discussion of methods to modify these limits to reflect local conditions.

Volume III will be released in FY 1996 and provides guidance on risk management procedures. This volume provides information regarding the selection and implementation of various options for reducing health risks associated with the consumption of chemically contaminated fish and shellfish. Using a human health risk-based approach, States can determine the level of the advisory and the most appropriate type of advisory to issue. Methods to evaluate population risks for specific groups, waterbodies, and geographic areas are also presented.

Volume IV was released in March 1995 and provides guidance on risk communication as a process for sharing information with the public on the health risks of consuming chemically contaminated fish and shellfish. This volume provides guidance on problem analysis and program objectives, audience identification and needs assessments, communication strategy design, implementation and evaluation, and responding to public inquiries.

The EPA welcomes your suggestions and comments. A major goal of this guidance document series is to provide a clear and usable summary of critical information necessary to make informed decisions regarding the development of fish consumption advisories. We encourage comments, and hope this document will be a useful adjunct to the resources used by the States, local governments, and Tribal bodies in making decisions regarding the development of fish advisories within their various jurisdictions.

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 20 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, ground water recharge, and atmospheric deposition).

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). 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.

As a result of these NAS concerns and State concerns expressed in the AFS survey, the EPA Office of Water established a Fish Contaminant Workgroup. It is 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 are recommended as standard procedures for use by the States and are described in this manual.

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 States 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 the EPA Office of Water 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.

In order to enhance the use of this guidance as a working document, the 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 the guidance will be issued to include the addition of major new areas of guidance such as using frogs and waterfowl as target species for assessment of human health risks or when major changes are made to the Agency's risk assessment procedures.

The EPA 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.

These tradeoffs will limit 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 the 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, the lower (more conservative) of the two calculated SVs is 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.

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 the EPA 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 I: Fish Sampling and Analysis (EPA 823-R-93-00), published August 1993
- Volume II: Risk Assessment and Fish Consumption Limits (EPA 823-B-94-004), published June 1994
- Volume III: Risk Management, to be published in FY 1996
- Volume IV: 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, and QA and QC considerations for fish and shellfish contaminant monitoring programs. Key references are provided that detail various aspects of these topics.

In addition, interested individuals may obtain a software program (on five 3.5-inch diskettes) of all fish consumption advisories for the 50 States and U.S. Territory waters entitled *The National Listing of Fish Consumption Advisories* (EPA-823-C-95-001) by contacting:

U.S. Environmental Protection Agency
National Center for Environmental Publications and Information
11029 Kenwood Road
Cincinnati, OH 45242
(513) 489-8190

In October 1995, EPA also will make this database available for downloading from the Internet. Point your World Wide Web browser to the following URL:

http://www.epa.gov/water

1.5 ORGANIZATION OF THIS MANUAL

This manual provides specific guidance on sampling, chemical analysis, and data reporting and analysis procedures for State fish and shellfish contaminant monitoring programs. Appropriate QA and QC considerations are integral parts of each of the recommended procedures.

Monitoring Strategy: 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 and (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.

Target Species: 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.

Target Analytes: Section 4 presents a list of recommended target analytes to be considered for inclusion in screening studies and discusses criteria used in selecting these analytes.

Screening Values: Section 5 describes the EPA risk-based procedure for calculating screening values for target analytes.

Field Procedures: 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.

Laboratory Procedures: 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 and Federal agencies currently conducting interlaboratory comparison programs.

Data Analysis and Reporting: Section 9 includes procedures for data analysis to determine the need for additional monitoring and risk assessment and for data reporting. This section also describes the National Fish Tissue Data Repository (NFTDR), a national database of fish and shellfish contaminant monitoring data.

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

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

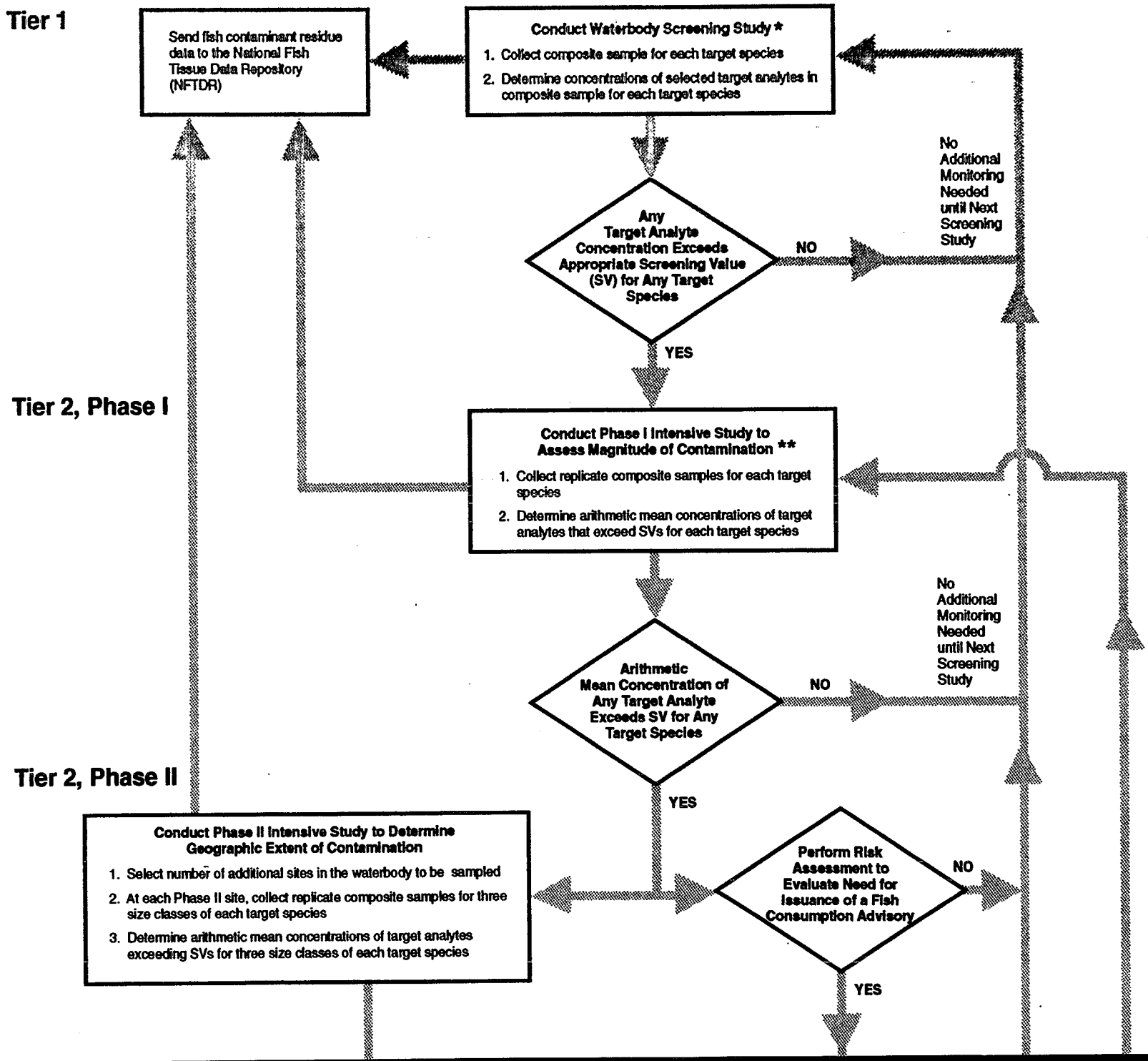
- **Tier 1—Screening studies** of a large number of sites for chemical contamination where sport, subsistence, and/or commercial fishing is conducted. This screening will help States identify those sites where concentrations of chemical contaminants in edible portions of commonly consumed fish and shellfish 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**).

This basic approach of using relatively low-cost, nonintensive screening studies to identify areas for more intensive followup sampling is used in a variety of water quality programs involving public health protection (California Environmental Protection Agency, 1991; Oregon Department of Environmental Quality, 1990; TVA, 1991; U.S. EPA, 1989d).

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.


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

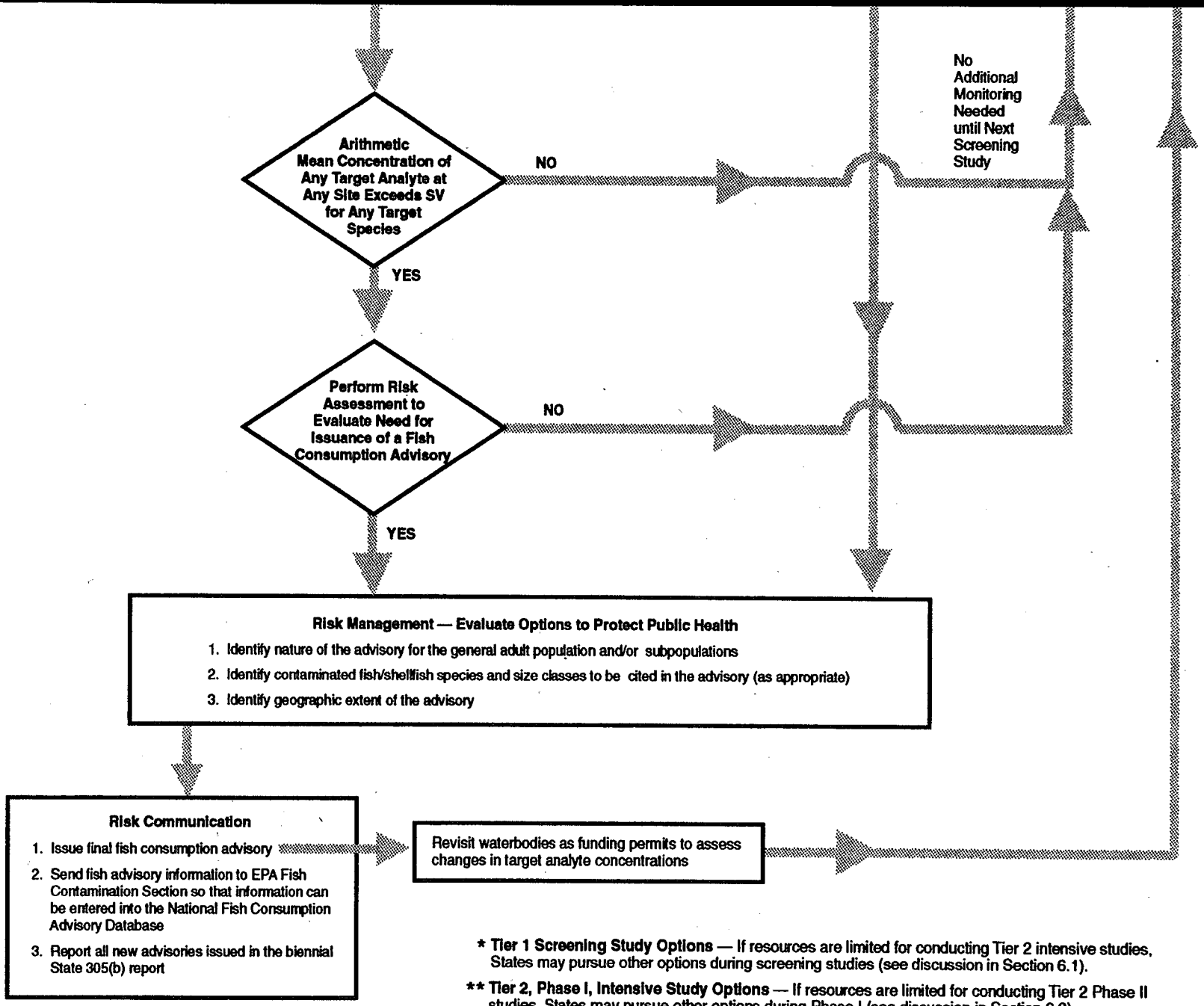


Figure 2-1. Recommended strategy for State fish and shellfish contaminant monitoring programs.

- 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).

- 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. **Note:** If consumers remove the skin and fatty areas from

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 three size classes of the target species found to be contaminated 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 species and size classes (continued)	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.
Target analytes (see Section 4)	Consider all target analytes listed in Table 4-1 for analysis as resources allow. Include additional site-specific target analytes as appropriate based on 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.

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)
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 6.5 g/d to 140 g/d or more of fish and shellfish (based on site-specific dietary data). **Note:** In this guidance document, EPA's Office of Water used a 6.5-g/d consumption rate, 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 as resources allow.	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 three 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)

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.
	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.	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.

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)
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.
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.
	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, waterbody 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

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)
Data analysis and reporting (continued)	• 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
	• 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
	• 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.

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)
Data analysis and reporting (continued)	• 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
	- 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.

a fish before preparing it for eating, exposures to some contaminants can be reduced (Armbruster et al., 1987, 1989; Cichy, Zabik, and Weaver, 1979; Foran, Cox, and Croxton, 1989; Gall and Voiland, 1990; Reinert, Stewart, and Seagram, 1972; Sanders and Haynes, 1988; Skea et al., 1979; Smith, Funk, and Zabik, 1973; Voiland et al., 1991; Wanderstock et al., 1971; Zabik, Hoojjat, and Weaver, 1979).

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, the Agency 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:

- Use of commonly consumed target species that are dominant in the catch and have high bioaccumulation potential
- 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
- 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 6.5 g/d for the general population, and a 70-yr lifetime exposure period to calculate SVs for carcinogens. **Note:** The EPA is currently reviewing the 6.5-g/d consumption rate for the general population.
- Use of a human body weight of 70 kg (average adult) and a consumption rate of 6.5 g/d for the general population to calculate SVs for noncarcinogens.

- 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 subpopulations 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 subpopulations 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 (i.e., 30 g/d for sport fisherman or 140 g/d for subsistence fishermen 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:** The EPA is currently reviewing the consumption rate for sport and subsistence fishermen.

- Use of body weights less than 70 kg for women and children and site-specific consumption rates (i.e., 30 g/d for sport fishermen or 140 g/d for subsistence fishermen or other consumption rates based on dietary studies of local fish-consuming populations) to calculate SVs for noncarcinogens.

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 A.

States should consider the potential effects of these study design features when evaluating screening study results.

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 the public's 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 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 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 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.

The EPA Fish Contaminant Workgroup believes the most important criterion for selecting target fish, shellfish, and turtle species for State contaminant monitoring programs assessing human consumption concerns is that the species are commonly consumed in the study area and are 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 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

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.*

programs to assess the 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 by the EPA Fish Contaminant Workgroup 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 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[a] and NCBP[b]	U.S. EPA NSCRF[c]	USGS NAWQA[d]
BOTTOM FEEDERS				
Family *Cyprinidae*				
Carp *(Cyprinus carpio)*	●	●	●	●
Family *Ictaluridae*				
Channel catfish *(Ictalurus punctatus)*	●	● Or other ictalurid	●	●
Family *Catostomidae*				
White sucker *(Catastomus commersoni)*	●	● Or other catostomid	●	●
Longnose sucker *(C. catostomus)*				●
Largescale sucker *(C. macrocheilus)*				●
Spotted sucker *(Minytrema melanops)*			●	
Redhorse sucker *(Moxostoma sp.)*			●	
included variety of species:				
Silver redhorse *(M. anisurum)*				
Grey redhorse *(M. congestum)*				
Black redhorse *(M. duquesnei)*				
Golden redhorse *(M. erythrurum)*				
Shorthead redhorse *(M. macrolepidotum)*				
Blacktail redhorse *(M. poecilurum)*				
PREDATORS				
Family *Salmonidae*				
Rainbow trout *(Oncorhynchus mykiss)* [formerly *Salmo gairdneri*]	●	●		
Brown trout *(Salmo tru*	●	●	●	●
Brook trout *(Salvelinus fontinalis)*	●	●		●
Lake trout *(Salmo namaycush)*	●	●		
Family *Percidae*				
Walleye *(Stizostedion vitreum)*	● Or other percid	● Or other percid	●	
Sauger *(Stizostedion canadense)*	○	○		
Yellow perch *(Perca flavescens)*	○	○		
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

[a]National Pesticide Monitoring Program
[b]National Contaminant Biomonitoring Program
[c]National Study of Chemical Residues in Fish
[d]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 B lists the freshwater fish species cited in consumption advisories for each State.

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*

Table 3-5. Average Fish Tissue Concentrations 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 (ppm)	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	Pentachloro-anisole	Pentachloro-benzene	DDE	Total Chlordane	Total Nonachlor	123 TCB	124 TCB	135 TCB	1234 TECB	Trifluralin	Hexachloro-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	18.42	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 Cat	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	ND	ND	ND	ND
Bluefish	0.05	ND	29.13	7.74	7.56	6.25	4.66	0.57	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. Values below detection have been replaced by one-half detection limit for the given sample. Asterisk indicates all values below detection. Units = ppt (parts per trillion).

Source: U.S. EPA, 1991h.

Table 3-6. Average Fish Tissue Concentrations 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	TEC
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 Catfish	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. Individual values below detection were set at zero. Units = ppb, unless noted. ND = not detected.

Source: U.S. EPA, 1991h.

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

[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.*

Source: RTI, 1993.

(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) have been used in all four of the national monitoring programs reviewed (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 EPA Workgroup reviewed average tissue concentrations of xenobiotic contaminants for major 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 current fish consumption advisories for these predator target species are further justification for their recommended use. As was shown for the bottom-feeder target species, States are already sampling the recommended predator target species listed in Table 3-7. The largest number of States issuing advisories 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).

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.

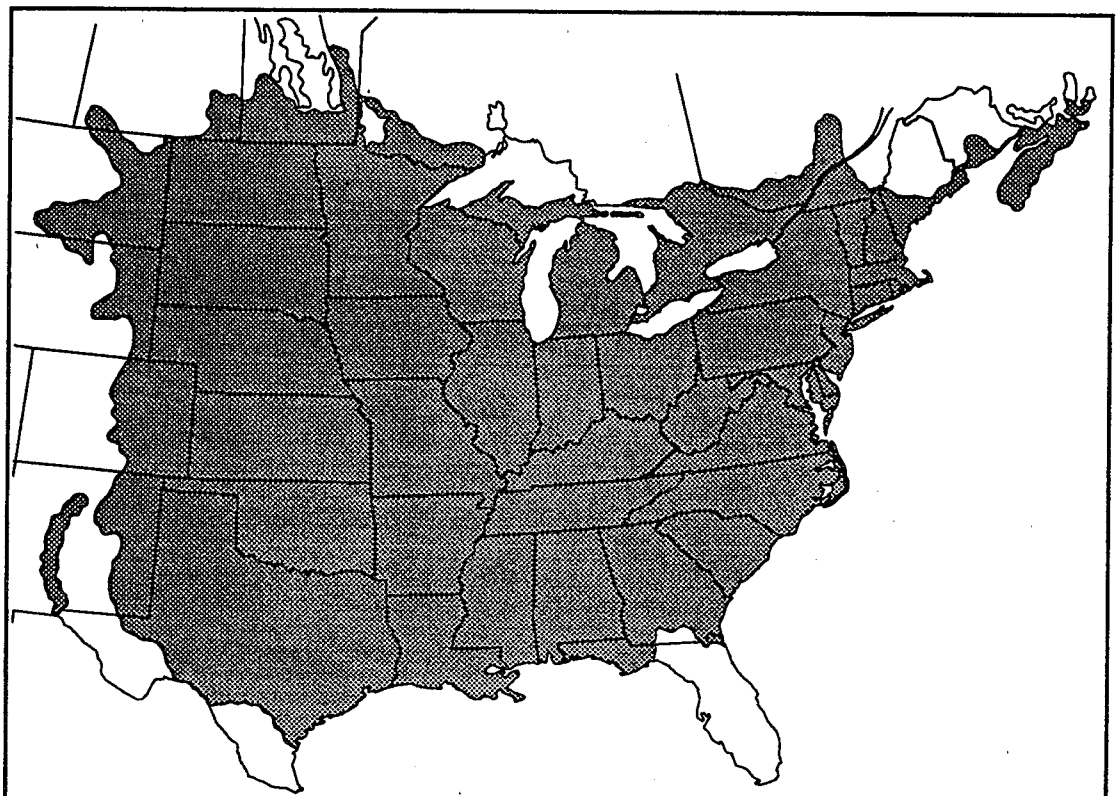
State personnel, with their knowledge of site-specific fisheries and human consumption patterns, must be the ultimate judge of the species selected for use in freshwater fish contaminant monitoring programs within their jurisdiction.

3.3.2 Target Turtle Species

EPA recommends that, in States where freshwater turtles are consumed by recreational, subsistence, or ethnic populations, States consider monitoring turtles to assess the level of environmental contamination and whether it poses 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 EPA Workgroup reviewed turtle species cited in State consumption advisories and those species identified 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 (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 species that should be considered for use as target species are also listed in Table 3-4.

Four States (Arizona, Massachusetts, Minnesota, and New York) currently have consumption advisories in force for various turtle species (New York State Department of Health, 1994; RTI, 1993). 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 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 have been shown to accumulate extremely high concentrations of a variety of environmental contaminants in comparison to muscle tissue (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.



Source: Conant and Collins, 1991.

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

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, mercury	AZ

PCB = Polychlorinated biphenyls.
DDT = 1,1,1-trichloro-2,2 bis(p-chlorophenyl)ethane.
[a]Source: RTI, 1993.
[b]Source: New York State Department of Health, 1994.

To identify those freshwater turtle species with a known ability to bioaccumulate chemical contaminants in their tissues, the EPA Workgroup reviewed several studies that identified freshwater turtle species as useful biomonitors of PCBs (Bryan et al., 1987; Hebert et al., 1993; Helwig and Hora, 1983; Olafsson et al., 1983; 1987; Safe, 1987; and Stone et al., 1980), dioxins and dibenzofurans (Rappe et al., 1981; Ryan et al., 1986), organochlorine pesticides (Hebert et al., 1993; Stone et al., 1980), heavy metals (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.

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

Table 3-9. Summary of Recent 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 and furans	Ryan et al., 1986
Snapping turtle (*Chelydra serpentina*)	PCBs Mercury Cadmium	Helwig and Hora, 1983
Snapping turtle (*Chelydra serpentina*)	PCDFs	Rappe et al., 1981
Snapping turtle (*Chelydra serpentina*)	Organochlorine pesticides DDE Dieldrin Hexachlorobenzene Heptachlor epoxide Mirex PCBs Cadmium Mercury	Stone et al., 1980
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.
PCDFs = Polychlorinated dibenzofurans.
DDE = 1,1-Dichloro-2,2-bis(p-chlorophenyl)-ethylene.

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 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.

The seven separate regional lists of target species recommended by the EPA Workgroup for estuarine/marine ecosystems were developed because of differences in species' geographic distribution and abundance and the nature of the

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*
	Hard clam	*Mercenaria mercenaria*
	Ocean quahog	*Arctica islandica*
	Surf clam	*Spisula solidissima*
	Blue mussel	*Mytilus edulis*
Crustaceans	American lobster	*Homarus americanus*
	Eastern rock crab	*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	*Ictalurus punctatus*
	White catfish	*Ictalurus catus*
Percichthyidae	White perch	*Morone americana*
	Striped bass	*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 Indies 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*

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 recent 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 EPA Workgroup also reviewed fish and shellfish species cited in State consumption advisories for estuarine/marine waters (Appendix B). 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 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 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. 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 B), seven coastal States issued advisories for unspecified bivalves or shellfish species that may have included

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.E. 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.
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.
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.
	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.

Table 3-18. Estuarine/Marie 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,c]
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)

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,c]
SHELLFISH				
Bivalves				
Hard clam *(Mercenaria mercenaria)*			●	
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)*			●	

[a]Only freshwater finfish were identified as target species; bivalves were identified as estuarin e/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.
[c]National Study of Chemical Residues in Fish.

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 comparisons within a State or between States sharing coastal waters (Figure 3-2). Because these three species meet all of the selection criteria, they are recommended as target species for use in geographic areas in which they occur.

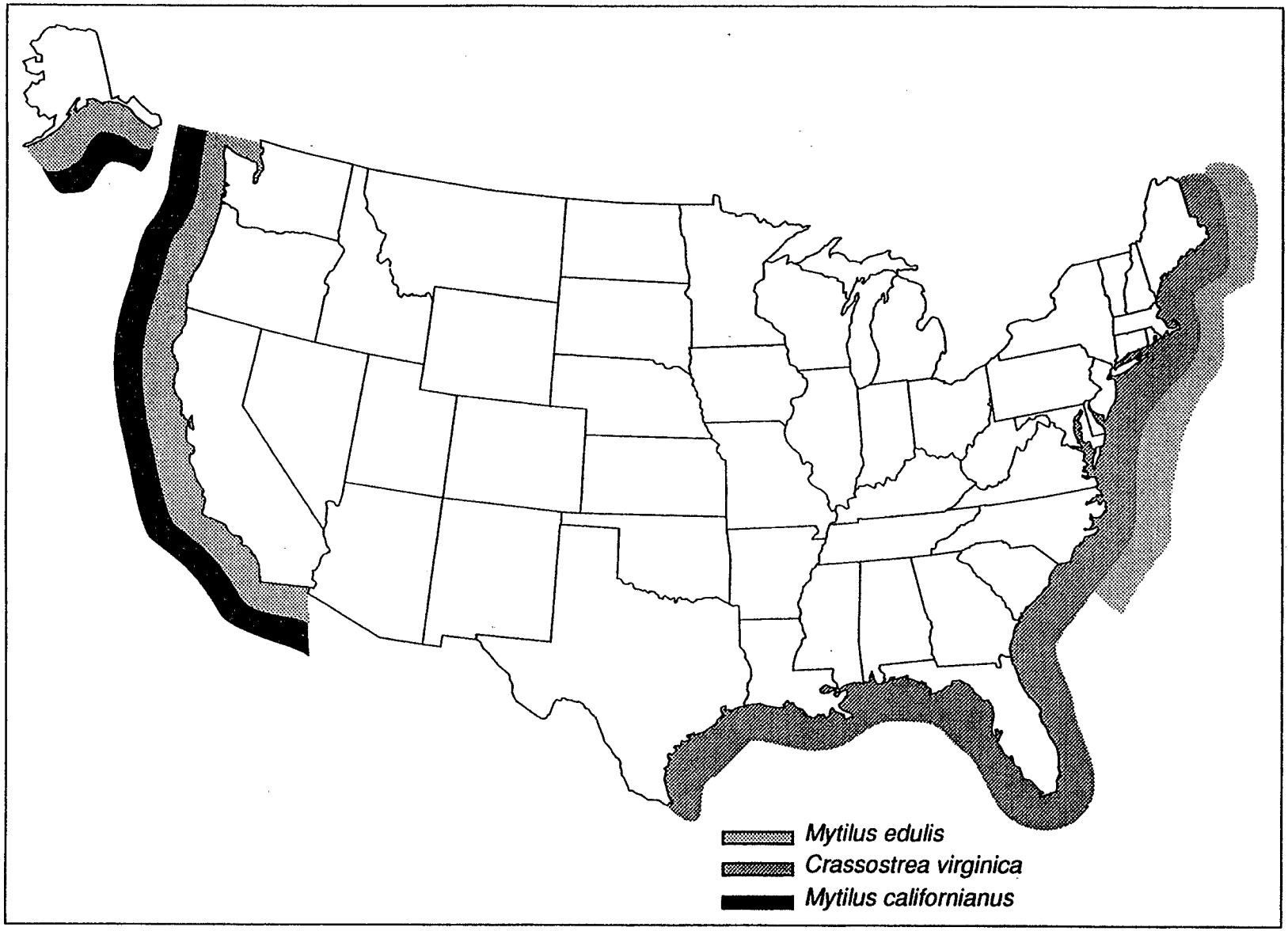
In addition, several species of edible clams were added to the various estuarine/marine target species lists based on recommendations received from specific State and regional fisheries experts.

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 have been recommended in one national monitoring program, and the Dungeness crab has been 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, 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 are 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



Source: Abbott, 1974.

Figure 3-2. Geographic distributions of three bivalve species used extensively in national contaminant 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
Finfish			
Percichthyidae	**Striped bass**	***Morone saxatilis***	**5**
	White perch	***Morone americana***	**3**
Ictaluridae	**White catfish**	***Ictalurus catus***	**4**
	Channel catfish	***Ictalurus punctatus***	**5**
Anguillidae	**American eel**	***Anguilla rostrata***	**6**
Pomatomidae	**Bluefish**	***Pomatomus saltatrix***	**4**
Belonidae	Atlantic needlefish	*Strongylura marina*	1
Serranidae	**Kelp bass**	***Paralabrax clathratus***	**1**
Sciaenidae	Black croaker	*Cheilotrema saturnum*	1
	White croaker	***Genyonemus lineatus***	**1**
	Queenfish	*Seriphus politus*	1
	Corbina	***Menticirrhus undulatus***	**1**
Shellfish			
Crustaceans[c]	**American lobster**	***Homarus americanus***	**1**
	Blue crab	***Callinectes sapidus***	**3**

[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] Seven coastal States (American Samoa, California, Louisiana, Massachusetts, New Jersey, South Carolina, and Texas) report advisories for unspecified shellfish or bivalve species.

Source: RTI, 1993.

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.

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 EPA Workgroup consulted with regional and State fisheries experts and reviewed the list of current 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 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 B); 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).

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 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
   - FDA recommendations
   - 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 C. Over 200 potential contaminants are listed, including metals, pesticides, base/neutral organic compounds, dioxins, dibenzofurans, acidic organic compounds, and volatile organic compounds.

2. **Pesticides with active registrations**—The EPA Office of Pesticide Programs (OPPs) 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

Table 4-1. Recommended Target Analytes[a]

Metals
- Arsenic (inorganic)
- Cadmium
- Mercury
- Selenium
- Tributyltin

Organochlorine Pesticides
- Chlordane, total (cis- and trans-chlordane, cis- and trans-nonachlor, oxychlordane)
- DDT, total (2,4'-DDD, 4,4'-DDD, 2,4'-DDE, 4,4'-DDE, 2,4'-DDT, 4,4'-DDT)
- Dicofol
- Dieldrin
- Endosulfan (I and II)
- Endrin
- Heptachlor epoxide[b]
- Hexachlorobenzene
- Lindane (γ-hexachlorocyclohexane; γ-HCH)[c]
- Mirex[d]
- Toxaphene

Organophosphate Pesticides[e]
- Chlorpyrifos
- Diazinon
- Disulfoton
- Ethion
- Terbufos

Chlorophenoxy Herbicides
- Oxyfluorfen

PAHs[f]

PCBs
- Total Aroclors[g]

Dioxins/furans[h,i]

PAHs = Polycyclic aromatic hydrocarbons.
PCBs = Polychlorinated biphenyls.

[a] States should include all recommended target analytes in screening studies, if resources allow, unless historic tissue or sediment 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, pesticide use application information) that these chemicals may be present at levels of concern for human health.

[b] Heptachlor epoxide is not a pesticide but is a metabolite of the pesticide heptachlor.

[c] Also known as γ-benzene hexachloride (γ-BHC).

[d] 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.

[e] The reader should note that carbophenothion was included on the original list of target analytes. Because the registrant did not support reregistration of this chemical, it will no longer be used. For this reason and because of its use profile, carbophenothion was removed from the recommended list of target analytes.

[f] It is recommended that, in both screening and intensive studies, tissue samples be analyzed for benzo[*a*]pyrene, benz[*a*]-anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, chrysene, dibenz[*a,h*]anthracene, and indeno[*1,2,3-cd*]pyrene, and that the order-of-magnitude relative potencies given for these PAHs in the EPA provisional guidance for quantitative risk assessment of PAHs (U.S. EPA, 1993c) be used to calculate a potency equivalency concentration (PEC) for each sample for comparison with the recommended SV for benzo[*a*]pyrene (see Section 5.3.2.3). At this time, EPA's recommendation for risk assessment of PAHs (U.S. EPA, 1993c) is considered provisional because quantitative risk assessment data are not available for all PAHs. This approach is under Agency review and over the next year will be evaluated as new health effects benchmark values are developed. Therefore, the method provided in this guidance document is subject to change pending results of the Agency's reevaluation.

[g] Analysis of total PCBs, as the sum of Aroclor equivalents, is recommended in both screening and intensive studies because of the lack of adequate toxicologic data to develop screening values (SVs) for individual PCB congeners (see Section 4.3.5). However, because of the wide range of toxicities among different PCB congeners and the effects of metabolism and degradation on Aroclor composition in the environment, congener analysis is deemed to be a more scientifically sound and accurate method for determining total PCB concentrations. Consequently, States that currently do congener-specific PCB analyses should continue to do so. Other States are encouraged to develop the capability to conduct PCB congener analysis.

[h] Note: The EPA Office of Research and Development is currently reassessing the human health effects of dioxins/furans.

[i] Dioxins/furans should be considered for analysis primarily at sites of pulp and paper mills using a chlorine bleaching process and at industrial sites where the following organic compounds are formulated: herbicides (containing 2,4,5-trichlorophenoxy acids and 2,4,5-trichlorophenol), hexachlorophene, pentachlorophenol, and PCBs (U.S. EPA, 1987d). It is recommended that the 2,3,7,8-substituted tetra- through octa-chlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) be determined and a toxicity-weighted total concentration calculated for each sample (Barnes and Bellin, 1989; U.S. EPA, 1987d) (see Section 5.3.2.4). If resources are limited, 2,3,7,8-TCDD and 2,3,7,8-TCDF should be determined at a minimum.

- 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 have triggered advisories in the largest number of States: polychlorinated biphenyls (PCBs), mercury, chlordane, and dioxins/furans.

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 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, and on their origins, chemistry, potential to bioaccumulate, estimated human health risk, and feasibility of analysis. Primary consideration was given to the recommendations of the Committee on Evaluation of the Safety of Fishery Products, published in *Seafood Safety* (NAS, 1991), and to the recommendations of the EPA Fish Contaminant Workgroup.

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

Contaminant	Number of States issuing advisories
Metals	
Arsenic (total)	1
Cadmium	2
Chromium	1
Copper	1
Lead	4
Mercury	27
Selenium	5
Zinc	1
Organometallics	1
Unidentified metals	3
Pesticides	
Chlordane	24
DDT and metabolites	9
Dieldrin	3
Heptachlor epoxide	1
Hexachlorobenzene	2
Kepone	1
Mirex	3
Photomirex	1
Toxaphene	2
Unidentified pesticides	2
Polycyclic aromatic hydrocarbons (PAHs)	3
Polychlorinated biphenyls (PCBs)	31
Dioxins/furans	22
Other chlorinated organics	
Dichlorobenzene	1
Hexachlorobutadiene	1
Pentachlorobenzene	1
Pentachlorophenol	1
Tetrachlorobenzene	2
Tetrachloroethane	1
Others	
Creosote	2
Gasoline	1
Multiple pollutants	2
Phthalate esters	1
Polybrominated biphenyls (PBBs)	1
Unspecified pollutants	3

Source: RTI, 1993.

4.2 SELECTION OF TARGET ANALYTES

States should include all recommended target analytes (Table 4-1) in screening studies, if resources allow, unless historic tissue or sediment or pollutant source data indicate that an analyte is not present at a level of concern (see Section 5). 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 have been used historically or are currently used and are likely to be present in aquatic systems as a result of agricultural runoff or drift.

It is important to note that pesticide uses and labels may change over time. 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 locations, 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.

Additional target analytes should be included in screening programs if States have site-specific chemical information (e.g., historic tissue or sediment data, discharge monitoring reports from municipal and industrial sources, or pesticide use data) that these contaminants may be present at levels of concern for human health. Compounds that are currently under review by the EPA Office of Water for inclusion as recommended target analytes are discussed in Section 4.4. Specific factors that were considered in the selection of currently recommended target analytes are summarized in the following sections.

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 six major fish contaminant monitoring programs (see Appendix C). It should be noted, however, that with respect to arsenic, all monitoring programs measured total arsenic rather than inorganic arsenic. Selenium has been monitored in five national monitoring programs. Tributyltin has been recommended for analysis in the FDA monitoring program. Consumption advisories are currently in effect for arsenic, cadmium, mercury, selenium, and tributyltin in one, two, twenty-seven, five, and one States respectively (Table 4-2). 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 (Eisler, 1988). 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 that 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; Jongen 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, 1989). 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 (*Farm Chemicals Handbook*, 1989). 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) (IRIS, 1995) and long-term effects include dermal hyperkeratosis, dermal melanosis and carcinoma, hepatomegaly, and peripheral neuropathy (NAS, 1991) (Appendix D).

Total arsenic (inclusive of both inorganic and organic forms) has been included in six national monitoring programs (Appendix C); however, no national program is currently monitoring total inorganic arsenic in fish or shellfish tissues. 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, 1995). A BCF value of 350 was reported for the American oyster (*Crassostrea virginica*) exposed to trivalent arsenic (Zaroogian and Hoffman, 1982). Only one State (Oregon) currently has an advisory in effect for arsenic contamination (RTI, 1993).

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).

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. Total inorganic arsenic 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 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 (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 as a probable human carcinogen by inhalation (B1) by EPA (IRIS, 1992).

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 the National Contaminant Biomonitoring Program (NCBP), 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). Two States (New York and Ohio) have issued advisories for cadmium contamination (RTI, 1993).

Cadmium should be considered for inclusion in all State fish and shellfish contaminant monitoring programs.

4.3.1.3 Mercury—

The 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 are industrial discharges and wastes (e.g., the chlorine-alkali industry) and atmospheric deposition resulting from combustion of coal and municipal refuse incinerators (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 and in chlor-alkali production facilities (*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 are released into the environment from fossil fuel burning (Klaassen et al., 1986). There is also increasing evidence of elevated mercury concentrations in areas where acid rain is believed to be a factor, although the extent of this problem has not been documented with certainty (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, which is toxic to humans (NAS, 1991; Tollefson, 1989).

The EPA has determined that the evidence of carcinogenicity of mercury in both animals and humans is inadequate and has assigned this metal a D carcinogenicity classification (IRIS, 1992). 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). 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, 1981a). 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. 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).

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 contrast to cadmium and selenium, concentrations of mercury in freshwater fish tissue did not change between 1976 and 1984 (Schmitt and Brumbaugh, 1990). Mercury, the only metal analyzed in the National Study of Chemical Residues in Fish, was detected at 92.2 percent of 374 sites surveyed. Maximum, arithmetic mean, and median concentrations in fish tissue were 1.80, 0.26, and 0.17 ppm, respectively (U.S. EPA, 1991h, 1992c, 1992d). Fish consumption advisories have been issued in 27 States as a result of mercury contamination (see Figure 4-1). In particular, mercury is responsible for a large number of the fish advisories currently in effect for lakes in Wisconsin, Michigan, and Minnesota and for rivers and lakes in Florida (RTI, 1993).

Mercury should be considered for inclusion in all State fish and shellfish contaminant monitoring programs. Only two national programs (301(h) and the FDA) currently analyze specifically for methylmercury; however, six programs analyze for total mercury (Appendix C). Because of the higher cost of methylmercury 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 in order 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 and has been shown to act as a mutagen in animals (NAS, 1991). Long-term adverse effects from ingestion by humans have not been studied thoroughly. The 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. However, selenium sulfide has been classified as a probable human carcinogen (B2) (IRIS, 1992).

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, 1987, 1989a) and in freshwater fish (Schmitt and Brumbaugh, 1990) from several areas nationwide. Selenium has been monitored in five national fish contaminant

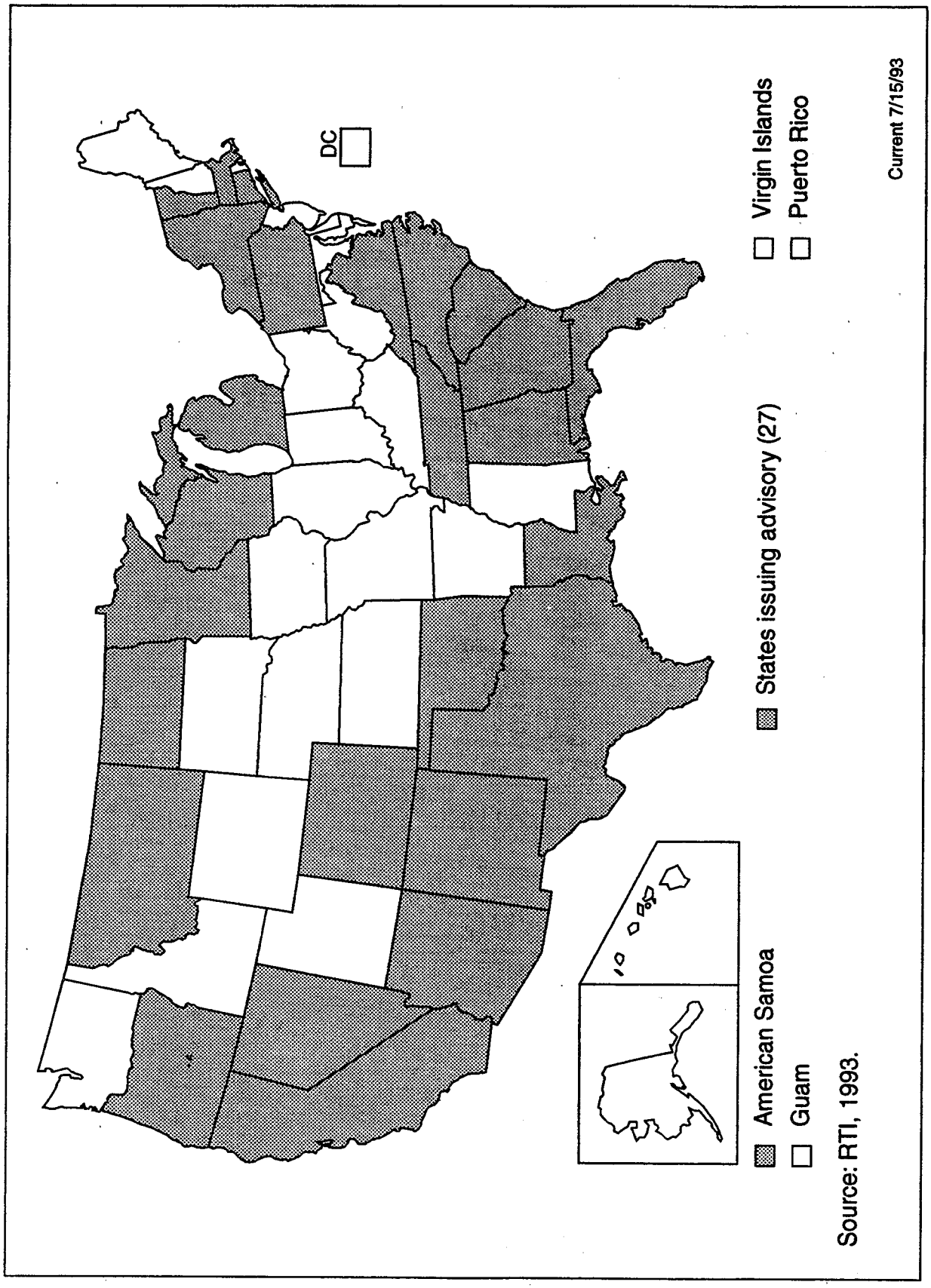


Figure 4-1. States issuing fish and shellfish advisories for mercury.

monitoring programs (Appendix C). Definitive information concerning the chemical forms of selenium found in fish and shellfish is not available (NAS, 1976, 1991). Five States (California, Colorado, North Carolina, Texas, and Utah) have issued advisories for selenium contamination in fish (RTI, 1993).

Selenium should be considered for inclusion in all State fish and shellfish monitoring programs.

4.3.1.5 Tributyltin

Tributyltin belongs 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. 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 are 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). The Agency 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 µg organotin/ cm^2/day 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 compounds are highly toxic to mammals (i.e., LD_{50} values ranged from 0.04 to 60 mg/kg) based on animal testing (Eisler, 1989; IRIS, 1995). Immunotoxicity and neurotoxicity are the critical effects produced by tributyltin. Insufficient data are available to evaluate the carcinogenicity of tributyltin compounds (IRIS, 1995) (Appendix D).

Tributyltins have been found to bioaccumulate in fish, bivalve mollusks, and crustaceans. Bioconcentration factors (BCF) ranging from 200 to 4,300 for finfish, from 2,000 to 6,000 for bivalves, and of 4,400 have been 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 is recommended for monitoring by the FDA but has not been monitored in any other national fish contaminant monitoring program (Appendix C). Only one State, Oregon, currently has an advisory in effect for tributyltin contamination in shellfish (RTI, 1993).

Tributyltin 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 are reported to be highest in areas of heavy boating and shipping activities including shipyards 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'-isomers of DDT, DDD, and DDE), dicofol, dieldrin, endosulfan I and II, endrin, heptachlor epoxide, hexachlorobenzene, lindane (γ-hexachlorocyclohexane), mirex, and toxaphene (see Appendix D). 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, 1992; 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) has been included in at least five major fish contaminant monitoring programs (Appendix C), and seven of the compounds have triggered at least one State fish consumption advisory (Table 4-2). Although use of some of these pesticides has been terminated or suspended within the United States for as long as 20 years (Appendix D), these compounds still require long-term monitoring. Many of the organochlorine pesticides were used in large quantities for over a decade and are present in sediments at high concentrations. 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 two of the organochlorine pesticides have active registrations: endosulfan and dicofol. 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 D). 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 (IRIS, 1992; 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 eight national fish contaminant programs (Appendix C) 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. The cis- and trans-isomers of chlordane and nonachlor, which are primary constituents of technical-grade chlordane, and oxychlordane, the major metabolite of chlordane, were monitored as part of the National Study of Chemical Residues in Fish. 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). Chlordane's presence in fish tissue has resulted in consumption advisories in 24 States (see Figure 4-2).

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).

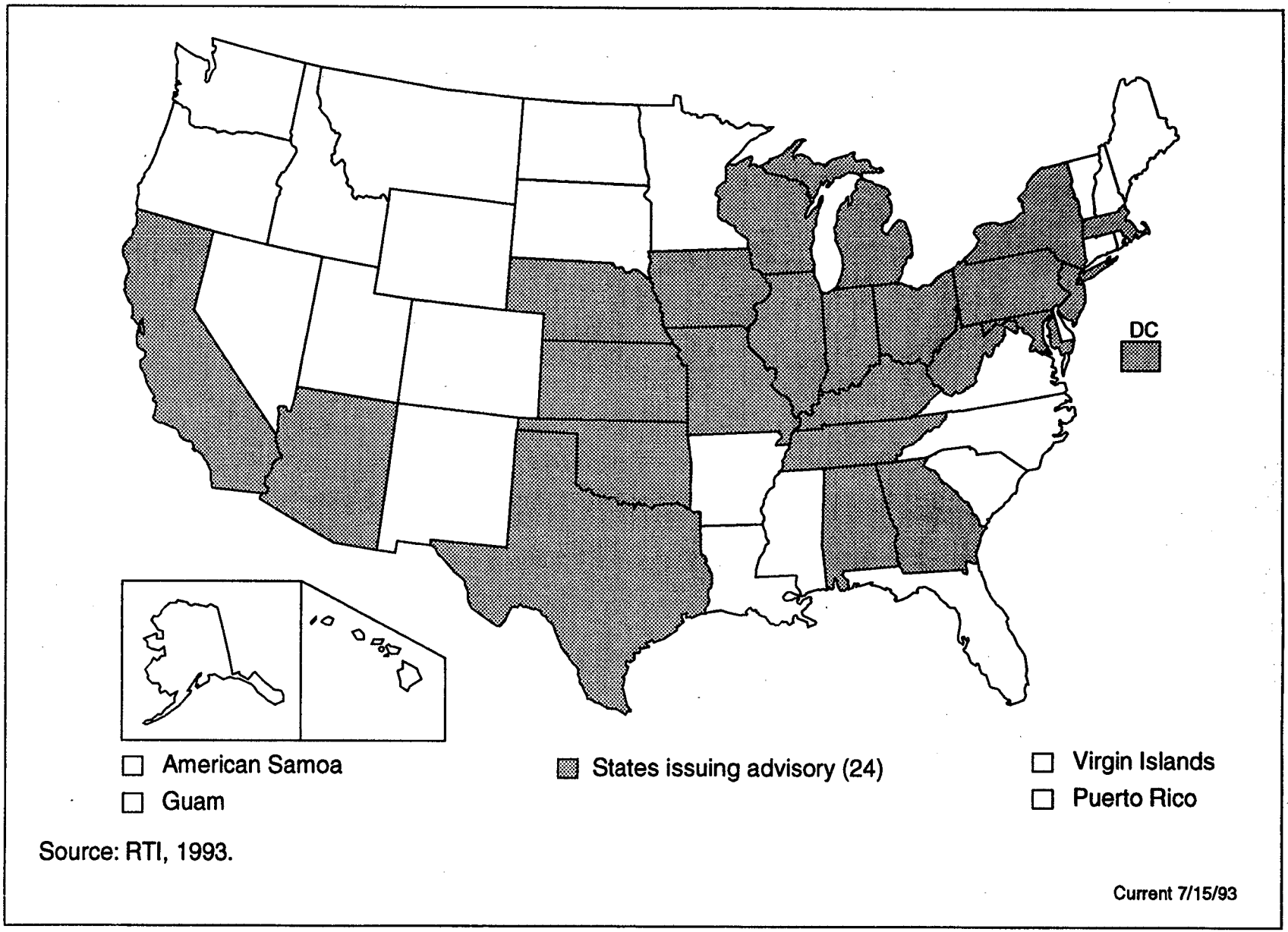


Figure 4-2. States issuing fish and shellfish advisories for chlordane.

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) (IRIS, 1992).

DDT or its metabolites have been included as target analytes in eight major fish and shellfish monitoring programs (Appendix C) and contamination has been found to be widespread (NOAA, 1987, 1989a; Schmitt et al., 1990). DDE, the only DDT metabolite surveyed in fish tissue in the National Study of Chemical Residues in Fish, was detected at more sites than any other single pollutant (99 percent of the 362 sites sampled) (U.S. EPA, 1992c, 1992d). Nine States (Alabama, American Samoa, Arizona, California, Delaware, Massachusetts, Nebraska, New York, and Texas) currently have fish consumption advisories in effect for DDT or its metabolites (RTI, 1993).

Total DDT (i.e., sum of the 4,4'- and 2,4'- isomers of DDT and of its metabolites, DDE and DDD) should be considered for inclusion in all State fish and shellfish contaminant monitoring programs.

4.3.2.3 Dicofol—

This chlorinated hydrocarbon acaricide was first registered in 1957 and is structurally similar to DDT (U.S. EPA, 1992c, 1992d). Technical-grade dicofol may contain impurities of the p,p' and o,p' isomers of DDT, DDE, DDD, and a compound known as extra-chlorine DDT (Cl-DDT) that are inherent as a result of the manufacturing process (U.S. EPA, 1983b). 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 Toxicity Class III based on oral exposure studies (Appendix D). Technical-grade dicofol induced hepatocellular (liver) carcinomas in male mice; however, results were negative in female mice and in rats (NCI, 1978). EPA has classified dicofol as a possible human carcinogen (C) (U.S. EPA, 1992a). Because of concern that dicofol would have the same effect as DDT on thinning of egg shells, the FDA required all dicofol products to contain less than 0.1 percent DDT and related contaminants after June 1, 1989 (51 FR 19508).

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 national monitoring programs (see Appendix C). In the National Study of Chemical Residues in Fish, dicofol was detected at 16 percent of the sites monitored (U.S. EPA, 1992c, 1992d).

Dicofol concentrations were greater than the quantification limit (2.5 ppb) in samples from 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 is used. Dicofol residues in fish could be much higher if sampling were targeted for pesticide runoff. Experimental evidence indicates this compound bioaccumulates in Bluegill sunfish (BCF from 6,600 to 17,000) (U.S. EPA, 1993a); however, no consumption advisories are currently in effect for dicofol (RTI, 1993).

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.

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 D).

Dieldrin has been classified by EPA as a probable human carcinogen (B2) (IRIS, 1992) and has been identified as a human neurotoxin (ATSDR, 1987a). Dieldrin has been included in eight national minitoring programs (Appendix C) and is still detected nationwide in freshwater finfish (Schmitt et al., 1990) and estuarine/marine finfish and shellfish (NOAA, 1987, 1989a). Dieldrin was detected in fish tissue at 60 percent of the 362 sites surveyed as part of the National Survey of Chemical Residues in Fish (U.S. EPA, 1992c, 1992d). 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). Three States (Arizona, Illinois, and Nebraska) have issued advisories for dieldrin contamination in fish (RTI, 1993).

Dieldrin should be considered for inclusion in all State fish and shellfish contaminant monitoring programs.

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 D). Two stereoisomers (I and II) exist and exhibit approximately equal effectiveness and toxicity (Worthing, 1991).

Endosulfan is highly toxic to humans and has been assigned to Toxicity Class I. To date, no studies have been found concerning carcinogenicity in humans after oral exposure to endosulfan (ATSDR, 1990). EPA has given endosulfan the carcinogenicity classification E, indicating there is no evidence of carcinogenicity for humans (U.S. EPA, 1992a).

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 currently insufficient to assess nationwide endosulfan contamination (NAS, 1991). Endosulfan was recommended for monitoring by the FDA and has been included in one national fish contaminant monitoring program (U.S. EPA 301(h) Program) evaluated by the EPA Workgroup (Appendix C). No consumption advisories are currently in effect for endosulfan I or II (RTI, 1993).

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 State agencies to obtain information on the historic and current uses of this pesticide.

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 (U.S. EPA, 1980a) and 1979 (44 FR 43632) and to final cancellation of its registration in 1984 (U.S. EPA, 1984a) (Appendix D).

Endrin is highly toxic to humans (Toxicity Class I), 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 (IRIS, 1992).

Although endrin has been included in six national fish contaminant monitoring programs (Appendix C), it has not been found widely throughout the United States. Endrin was 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) and was found in only 29 percent of 112 stations sampled in the NCBP (Schmitt et al., 1990). No States have issued fish consumption advisories for endrin (RTI, 1993).

Endrin should be considered for inclusion in all State fish and shellfish contaminant monitoring programs.

4.3.2.7 Heptachlor Epoxide—

Heptachlor epoxide is not a formulated pesticide, but is a metabolic degradation product of the pesticide heptachlor. It is also found as a contaminant in heptachlor and chlordane formulations (Appendix D). 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, 1987b). 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, 1986e). In animals, liver, kidney, and blood disorders can occur (IRIS, 1989). Exposure to this compound produced an increased incidence of liver carcinomas in rats and mice and hepatomas in female rats (IRIS, 1989). Heptachlor epoxide has been classified by EPA as a probable human carcinogen (B2) (IRIS, 1992).

Heptachlor epoxide has been included in seven national fish monitoring programs (Appendix C) and has been detected widely in freshwater finfish (Schmitt et al., 1990) but infrequently in bivalves and marine fish (NOAA, 1987, 1989a). Heptachlor epoxide was detected in fish tissue at 16 percent of the 362 sites where it was surveyed in the National Study of Chemical Residues in Fish (U.S. EPA, 1992c, 1992d). One State (Nebraska) currently has fish advisories for heptachlor epoxide contamination (RTI, 1993).

Heptachlor epoxide should be considered for inclusion in all State fish and shellfish monitoring programs.

4.3.2.8 Hexachlorobenzene—

Hexachlorobenzene is a fungicide that was widely used as a seed protectant in the United States until 1985 (Appendix D). 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 a toxicity classification of IV (i.e., oral LD_{50} greater than 5,000 ppm in laboratory animals (*Farm Chemicals Handbook*, 1989). However, nursing infants are particularly susceptible to hexachlorobenzene poisoning as lactational transfer can increase infant tissue levels to levels two to five times maternal tissue levels (ATSDR, 1989b). Hexachlorobenzene is a known animal carcinogen (ATSDR, 1989b) and has been classified by EPA as a probable human carcinogen (B2) (IRIS, 1992) (Appendix D).

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 (Appendix C). Hexachlorobenzene was detected

in fish tissue at 46 percent of the 362 sites where it was surveyed in the National Study of Chemical Residues in Fish (U.S. EPA, 1992c, 1992d). Two States (Louisiana and Ohio) have issued advisories for hexachlorobenzene contamination in fish and shellfish (RTI, 1993).

Hexachlorobenzene should be considered for inclusion in all State fish and shellfish monitoring programs.

4.3.2.9 Lindane—

Lindane is a mixture of isomers 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. Since 1985, many uses of lindane have been banned or restricted (see Appendix D). At present, its application is permitted only under supervision of a certified applicator (U.S. EPA, 1985c).

Lindane is a neurotoxin (assigned to Toxicity Class II) 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). Available data for this pesticide need to be reviewed, but at a minimum the carcinogenicity classification will be C (U.S. EPA, 1992a).

Lindane has been included in eight major fish contaminant monitoring programs (Appendix C). This pesticide has been detected in freshwater fish (Schmitt et al., 1990) and in marine fish and bivalves (NOAA, 1987, 1989a) nationwide. Lindane was detected in fish tissue at 42 percent of 362 sites surveyed in the National Study of Chemical Residues in Fish (U.S. EPA, 1992c, 1992d). Although lindane has been widely monitored and widely detected, no consumption advisories are currently in effect for lindane (RTI, 1993).

Lindane should be considered for inclusion in all State fish and shellfish monitoring programs.

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, more widely, under the name Dechlorane as a fire retardant and polymerizing agent in plastics (Kaiser, 1978) (Appendix D).

Mirex has been assigned to Toxicity Class II and has been classified as a probable human carcinogen by the International Agency for Research on Cancer (IARC, 1987); however, the carcinogenicity data are currently under review by EPA (IRIS, 1992). EPA instituted restrictions on the use of mirex in 1975, and,

shortly thereafter, the U.S. Department of Agriculture (USDA) suspended the fire ant control program (Hodges, 1977).

Mirex has been included in eight major fish contaminant monitoring programs (Appendix C). It has been found primarily in the Southeast and the Great Lakes regions (NAS, 1991; Schmitt et al., 1990). Mirex was detected in fish tissue at 36 percent of 362 sites surveyed in the National Study of Chemical Residues in Fish (U.S. EPA, 1992c, 1992d). Three States (New York, Ohio, and Pennsylvania) currently have fish consumption advisories for mirex (RTI, 1993).

Mirex should be considered for inclusion in all State fish and shellfish monitoring programs.

4.3.2.11 Toxaphene—

Toxaphene is a mixture of chlorinated camphenes. Historically, it was used in the United States as an insecticide primarily on cotton (Hodges, 1977). 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).

Like many of the other organochlorine pesticides, toxaphene has been assigned to Toxicity Class II (Appendix D). Unlike the other organochlorine pesticides, toxaphene is fairly easily metabolized by mammals and is not stored in the fatty tissue to any great extent. Toxaphene has been classified by EPA as a probable human carcinogen (B2) (IRIS, 1992).

Toxaphene has been included in five major fish contaminant monitoring programs (Appendix C). It has been detected frequently in both fresh (Schmitt et al., 1990) and estuarine (NOAA, 1989a) waters but is only consistently found in Georgia, Texas, and California (NAS, 1991). **Note:** A toxaphene-like compound that is a byproduct of the paper industry has been identified in the Great Lakes Region (J. Hesse, Michigan Department of Public Health, personal communication, 1993). Two States (Arizona and Texas) currently have fish advisories in effect for toxaphene (RTI, 1993).

Toxaphene should be considered for inclusion in all State fish and shellfish monitoring programs.

4.3.3 Organophosphate Pesticides

The following organophosphate pesticides are recommended as target analytes in screening studies: chlorpyrifos, diazinon, disulfoton, ethion, and terbufos (Appendix D). Most of these organophosphate pesticides share two distinct features. Organophosphate pesticides are generally more 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 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 Workgroup and none of these pesticides (including chlorpyrifos) has triggered State fish consumption advisories. All of the compounds have active pesticide registrations and have been recommended for monitoring because they have a Toxicity Classification of I or II (Appendix D), have BCFs > 300, 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 reader should note that all of the organophosphate pesticides recommended as target analytes have active registrations. States should contact their appropriate State agencies to obtain information on both the historic and current uses of these pesticides. In addition, if a State determines that use of these pesticides may be occurring in its waters, sampling 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 by fish species. Additional discussion of appropriate sampling times for fish contaminant monitoring programs is provided in Section 6.1.1.5.

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, 1986e) and has been used for a broad range of insecticide applications (Appendix D). Chlorpyrifos is used primarily to control soil and foliar insects on cotton, peanuts, and sorghum (Worthing, 1991; U.S. EPA, 1986e). 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, 57 percent of all chlorpyrifos manufactured in the United States is used on corn, while 22 percent is used for pest control and lawn and garden services (U.S. EPA, 1993a).

Chlorpyrifos has a moderate mammalian toxicity and has been assigned to Toxicity Class II based on oral feeding studies (*Farm Chemicals Handbook*, 1989). No teratogenic or fetotoxic effects were found in mice or rats (IRIS, 1989). No carcinogenicity was found in chronic feeding studies with rats, mice and dogs (U.S. EPA, 1983a). EPA has assigned chlorpyrifos a carcinogenicity classification of D—not classifiable based on inadequate evidence of carcino-

genicity or lack of data in at least two animal studies or in both epidemiologic and animal studies (U.S. EPA, 1992a).

Chlorpyrifos was recommended for monitoring by the FDA and has been included in one national monitoring program, the National Study of Chemical Residues in Fish (see Appendix C). In this latter 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 (2.5 to 344 ppb) were scattered throughout the East, Midwest, and in California; the highest concentrations detected (60 to 344 ppb) were found either in agricultural areas or in urban areas with a variety of nearby industrial sources. 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. Experimental evidence indicates this compound bioaccumulates in rainbow trout (BCF from 1,280 to 3,903) (U.S. EPA, 1993a); however, no consumption advisories are currently in effect for chlorpyrifos (RTI, 1993).

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 State agencies to obtain information on the historic and current uses of this pesticide.

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, 1986f).

Diazinon is moderately toxic to mammals and has been assigned to Toxicity Class II based on oral toxicity tests (Appendix D). EPA has assigned diazinon to carcinogenicity classification D—not classifiable based on a lack of data or inadequate evidence of carcinogenicity in at least two animal tests or in both epidemiologic and animal studies (U.S. EPA, 1992a). This compound is also highly toxic to birds, fish, and other aquatic invertebrates (U.S. EPA, 1986f).

Diazinon has not been included in any national fish contaminant monitoring program evaluated by the EPA Workgroup (Appendix C). Experimental evidence indicates this compound accumulates in trout (BCF of 542) (U.S. EPA, 1993a); however, no consumption advisories are currently in effect for diazinon (RTI, 1993).

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 State agencies to obtain information on the historic and current uses of this pesticide.

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 D).

Disulfoton is highly toxic to all mammalian systems and has been assigned to Toxicity Class I on the basis of all routes of exposure (*Farm Chemicals Handbook*, 1989). All labeling precautions and use restrictions are based on human health risk. Disulfoton and its major metabolites are potent cholinesterase inhibitors primarily attacking acetylcholinesterase. Contradictory evidence is available on the mutagenicity of this compound and the EPA has concluded that the mutagenic potential is not adequately defined (U.S. EPA, 1984d). EPA has assigned disulfoton to carcinogenicity classification D—not classifiable based on a lack of data or inadequate evidence of carcinogenicity in at least two animal tests or in both epidemiologic and animal studies (U.S. EPA, 1992a).

Disulfoton has not been included in any national fish contaminant monitoring program evaluated by the EPA Workgroup (Appendix C). Experimental evidence indicates this compound accumulates in fish (BCF from 460 to 700) (U.S. EPA, 1993a); however, no consumption advisories are currently in effect for disulfoton (RTI, 1993).

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 State agencies to obtain information on the historic and current uses of this pesticide.

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 D). 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 (Toxicity Class II) (*Farm Chemicals Handbook*, 1989). In a chronic rat toxicity study, a decrease in serum cholinesterase was observed in both males and females. Ethion was not found to be carcinogenic in rats and mice (U.S. EPA, 1989e). EPA has assigned ethion to carcinogenicity classification D—not classifiable based on a lack of data or inadequate evidence

of carcinogenicity in at least two animal tests or in both epidemiologic and animal studies (U.S. EPA, 1992a).

Ethion has not been included in any national fish contaminant monitoring program evaluated by the EPA Workgroup (Appendix C). Experimental evidence indicates this compound accumulates in Bluegill sunfish (BCF from 880 to 2,400) (U.S. EPA, 1993a); however, no consumption advisories are currently in effect for ethion (RTI, 1993).

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 State agencies to obtain information on the historic and current uses of this pesticide.

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).

Terbufos is highly toxic to humans and has been assigned to Toxicity Class I (Appendix D). Symptoms of acute cholinesterase inhibition have been reported in all acute studies, and cholinesterase inhibition was reported in several chronic mammalian feeding studies (U.S. EPA, 1985d). EPA has assigned terbufos to carcinogenicity classification D—not classifiable based on a lack of data or inadequate evidence of carcinogenicity in at least two animal tests or in both epidemiologic and animal studies (U.S. EPA, 1992a). Terbufos is also highly toxic to birds, fish, and other aquatic invertebrates (U.S. EPA, 1985d).

Terbufos has not been included in any national fish contaminant monitoring program evaluated by the EPA Workgroup (Appendix C). Experimental evidence indicates this compound accumulates in fish (BCF from 320 to 1,400) (U.S. EPA, 1993a); however no consumption advisories are currently in effect for terbufos (RTI, 1993).

Terbufos 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.

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 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 D).

Evidence suggests that oxyfluorfen is moderately toxic to mammals and has been assigned to Toxicity Class II based on a chronic mouse feeding study (*Farm Chemicals Handbook*, 1989; IRIS, 1993). 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) (U.S. EPA, 1992c).

Although oxyfluorfen has an active registration, it has not been included in any national fish contaminant monitoring program evaluated by the EPA Workgroup (Appendix C). Experimental evidence indicates this herbicide accumulates in Bluegill sunfish (BCF from 640 to 1,800) (U.S. EPA, 1993a); however, no consumption advisories are currently in effect for oxyfluorfen (RTI, 1993).

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 State agencies to obtain information on the historic and current uses of this pesticide.

4.3.5 Polycyclic Aromatic Hydrocarbons (PAHs)—

Polycyclic aromatic hydrocarbons 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 include those listed as priority pollutants (U.S. EPA, 1995a)

- Acenaphthene
- Acenaphthylene
- Anthracene
- Benz[*a*]anthracene
- Benzo[*a*]pyrene
- Benzo[*b*]fluoranthene
- Benzo[*k*]fluoranthene
- Benzo[*g,h,i*]perylene
- Chlorinated naphthalenes
- Chrysene
- Dibenz[*a,h*]anthracene
- Fluoranthene
- Fluorene
- Indeno[*1,2,3-cd*]pyrene
- Naphthalene
- 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) have been shown in laboratory test systems to be carcinogens, cocarcinogens, teratogens, and/or mutagens (Moore and Ramamoorthy, 1984; U.S. DHHS, 1990). Benzo[*a*]pyrene, one of the most widely occurring and potent PAHs, and several other PAHs (e.g., benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, chrysene, cyclopenta[*cd*]pyrene, dibenz[*a,h*]anthracene, dibenzo[*a,e*]fluoranthene, dibenzo[*a,e*]pyrene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,l*]pyrene, indeno[*1,2,3-cd*]pyrene) have been classified by EPA as probable human carcinogens (B2) (IRIS, 1992). 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 (U.S. DHHS, 1990).

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 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 (Neff, 1985). Historic coal gasification sites have also been identified as significant sources of PAH contamination (J. Hesse, Michigan Department of Public Health, personal communication, March 1991).

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 (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 and crustaceans have frequently been reported to be in the range of 100 to 2,000 (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 oxidose system required for biotransformation of PAHs, some molluscs lack this system and are unable to metabolize PAHs efficiently (Varanasi et al., 1985). Thus, bivalves are good bioaccumulators of some PAHs. NAS (1991) reported that PAH contamination in bivalves has been found in all areas of the United States. 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). If PAHs are included as target analytes at a site, preference should be given to selection of a bivalve mollusc as one of the target species (if available) and a finfish as the other target species.

Three States (Massachusetts, Michigan, and Ohio) have issued advisories for PAH contamination in finfish (RTI, 1993).

Although several PAHs have been classified as probable human carcinogens (Group B2), benzo[*a*]pyrene is the only PAH for which an oral cancer slope factor (SF) is currently available in IRIS (1995). It is recommended that, in both screening and intensive studies, tissue samples be analyzed for benzo[*a*]pyrene, benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, chrysene, dibenz[*a,h*]anthracene, and indeno*[1,2,3-cd]*pyrene, and that the relative potencies given for these PAHs in the EPA provisional guidance for quantitative risk assessment of PAHs (U.S. EPA, 1993c) be used to calculate a potency equivalency concentration (PEC) for each sample for comparison with the recommended SV for benzo[*a*]pyrene (see Section 5.3.2.3). At this time, EPA's recommendation for risk assessment of PAHs (U.S. EPA, 1993c) is considered provisional because quantitative risk assessment data are not available for all PAHs. This approach is under Agency review and over the next year will be

evaluated as new health effects benchmark values are developed. Therefore, the method provided in this guidance document is subject to change pending results of the Agency's reevaluation.

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 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, 1992). However, individual PCB congeners have widely varying potencies for producing a variety of adverse biological effects including hepatotoxicity, 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). 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. However, 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. PCB mixtures have been classified as probable human carcinogens (Group B2) (IRIS, 1992; U.S. EPA, 1988a).

Of particular concern are several studies that have suggested that exposure to PCBs may be damaging to the health of fetuses and children (Fein et al., 1984; Jacobson et al., 1985, 1990). However, these studies are inconclusive due to a failure to assess confounding variables (J. Hesse, Michigan Department of Public Health, personal communication, 1992). In a more recent study of prenatal exposure to PCBs and reproductive outcome, birth size was found to be associated positively with PCB exposure, contrary to expectations (Dar et al., 1992). The results of these investigations clearly indicate the need for further study. Nevertheless, it may be appropriate for States in which PCBs are found to be a problem contaminant in fish or shellfish tissue to assess the need to issue consumption advisories, particularly for pregnant women, nursing mothers, and children.

PCBs have been included in eight major fish contaminant monitoring programs (Appendix C). A recent summary of the National Contaminant Biomonitoring Program data from 1976 through 1984 indicated a significant downward trend in total PCBs, although PCB residues in fish tissue remained widespread (Schmitt et al., 1990). Total PCBs were detected at 91 percent of 374 sites surveyed in the National Study of Chemical Residues in Fish (U.S. EPA, 1992c, 1992d). Currently, PCB contamination in fish and shellfish has resulted in the issuance of consumption advisories in 31 States (Figure 4-3) (RTI, 1993).

PCBs may be analyzed quantitatively as Aroclor equivalents 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) 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;

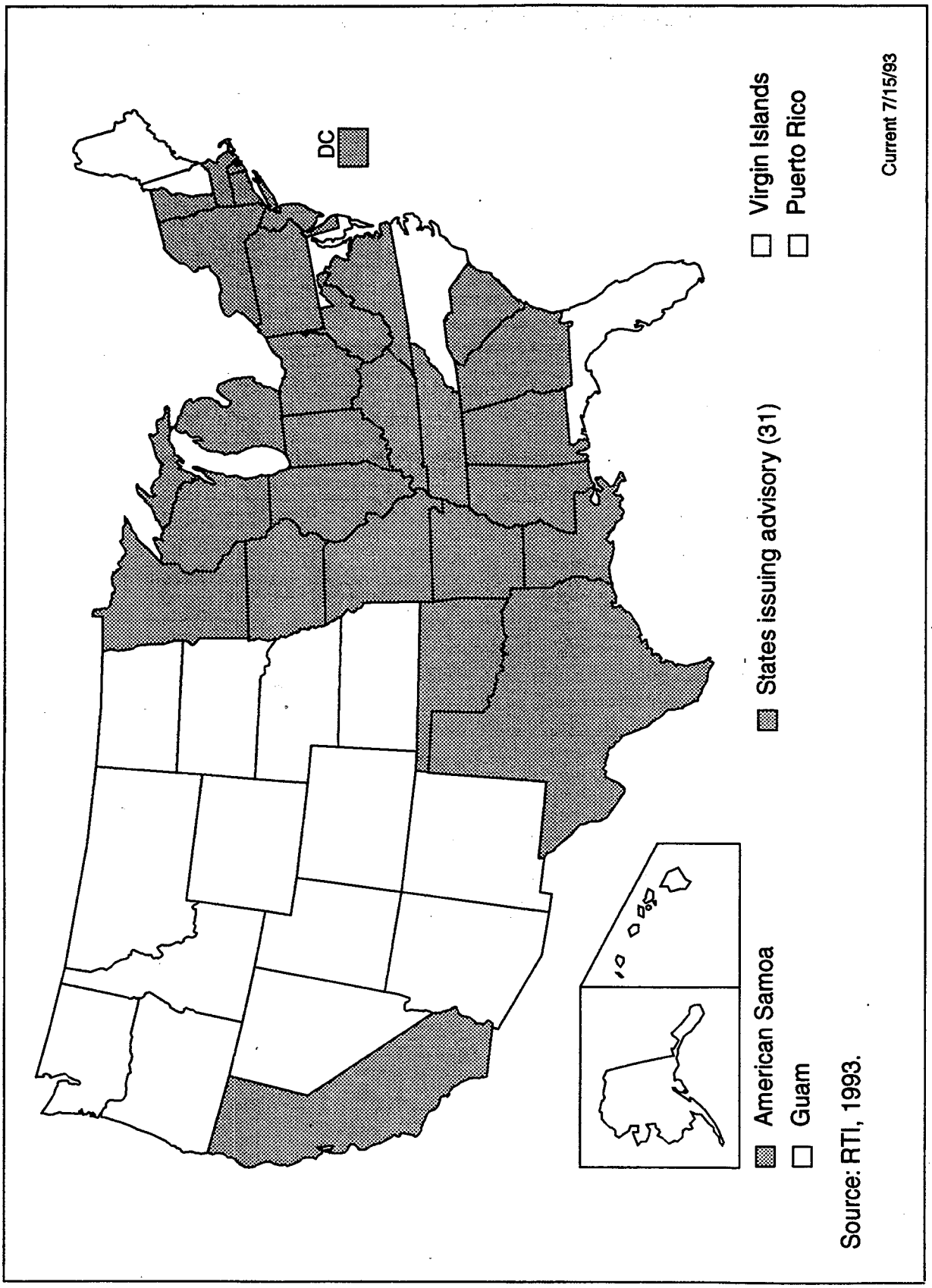


Figure 4-3. States issuing fish and shellfish advisories for PCBs.

Oliver and Niimi, 1988; Smith et al., 1990). Recent aquatic environmental studies indicate that many of the most potent, dioxin-like PCB congeners are preferentially accumulated in higher organisms (Bryan et al., 1987; Kubiak et al., 1989; Oliver and Niimi, 1988). This preferential accumulation probably results in a significant increase in the total toxic potency of PCB residues as they move up the food chain. Consequently, the congener-specific analysis of PCBs is required for more accurate determination of total PCB concentrations and for more rigorous assessment of the toxicologic effects of PCBs.

Even though the large number of congeners of PCBs and their similar chemical and physical properties present serious analytical difficulties, analytical methods for the determination of PCB congeners have been improved in recent years so that it is now possible to determine essentially all PCB congeners in mixtures (Huckins et al., 1988; Kannan et al., 1989; MacLeod et al., 1985; Maack and Sonzogni, 1988; Mes and Weber, 1989; NOAA, 1989b; Smith et al., 1990; Tanabe et al., 1987). Both NOAA (MacLeod et al., 1985; NOAA, 1989b) and the EPA Narragansett Research Laboratory conduct PCB congener analyses and have adopted the same 18 PCB congeners for monitoring fish contamination. However, quantitation of individual PCB congeners is relatively time-consuming and expensive and many laboratories do not have the capability or expertise to perform such analyses. Some States currently conduct both congener and Aroclor analysis; however, most States routinely perform only Aroclor analysis.

For the purposes of screening tissue residues against potential levels of public health concern in fish and shellfish contaminant monitoring programs, the issue of whether to determine PCB concentrations as Aroclor equivalents or as individual congeners cannot be resolved entirely satisfactorily at this time, primarily because of a lack of toxicologic data for individual congeners.

Ideally, congener analysis should be conducted. However, at present, only an Aroclor-based quantitative risk estimate of carcinogenicity is available (IRIS, 1993) for developing SVs and risk assessment. Consequently, until adequate congener-specific toxicologic data are available to develop quantitative risk estimates for a variety of toxicologic endpoints, the EPA Office of Water recommends, as an interim measure, that PCBs be analyzed as Aroclor equivalents, with total PCB concentrations reported as the sum of Aroclors.

States are encouraged to develop the capability to perform PCB congener analysis. When congener analysis is conducted, the 18 congeners recommended by NOAA (shown in Table 4-3) 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) are listed in Table 4-3.

This interim recommendation is intended to (1) allow States flexibility in PCB analysis until reliable congener-specific quantitative risk estimates are available, and (2) encourage the continued development of a reliable database of PCB

Table 4-3. Polychlorinated Biphenyl (PCB) Congeners Recommended for Quantitation as Potential Target Analytes

PCB Congener[a,b]	Recommended by NOAA[c]	Recommended by McFarland and Clarke (1989)	
		Highest Priority[d]	Second Priority[e]
2,4' diCB	8		
2,2',5 triCB	18		18
2,4,4' triCB	28		
3,4,4' triCB			37
2,2'3,5' tetraCB	44		44
2,2'4,5' tetraCB			99
2,2',5,5' tetraCB	52		52
2,3',4,4' tetraCB	66		
2,3',4',5 tetraCB			70
2,4,4',5 tetraCB			74
3,3',4,4' tetraCB	77	77	
3,4,4',5 tetraCB			81
2,2',3,4,5' pentaCB		87	
2,2',3,4',5 pentaCB		49	
2,2',4,5,5' pentaCB	101	101	
2,3,3',4,4' pentaCB	105	105	
2,3,4,4',5 pentaCB			114
2,3',4,4',5 pentaCB	118	118	
2,3',4,4',6 pentaCB			119
2',3,4,4',5 pentaCB			123
3,3',4,4',5 pentaCB	126	126	
2',3,3',4,4' hexaCB	128	128	
2,2',3,4,4',5' hexaCB	138	138	
2,2',3,5,5',6 hexaCB			151
2,2',4,4',5,5' hexaCB	153	153	
2,3,3',4,4',5 hexaCB		156	
2,3,3',4,4',5 hexaCB			157
2,3,3',4,4',6 hexaCB			158
2,3',4,4',5,5' hexaCB			167
2,3',4,4',5',6 hexaCB			168
3,3',4,4',5,5' hexaCB	169	169	

(continued)

Table 4-3 (continued)

PCB Congener[a,b]	Recommended by NOAA[c]	Recommended by McFarland and Clarke (1989)	
		Highest Priority[d]	Second Priority[e]
2,2',3,3',4,4',5 heptaCB	170	170	
2,2',3,4,4',5,5' heptaCB	180	180	
2,2',3,4,4',5',6 heptaCB		183	
2,2',3,4,4',6,6' heptaCB		184	
2,2',3,4',5,5',6 heptaCB	187		187
2,3,3',4,4',5,5' heptaCB			189
2,2',3,3',4,4',5,6 octaCB		195	
2,2',3,3',4,5,5',6' octaCB			201
2,2',3,3',4,4',5,5',6 nonaCB		206	
2,2',3,3',4,4',5,5',6,6' decaCB		209	

[a] PCB 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 (McFarland and Clarke, 1989).

[e] PCB 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 (McFarland and Clarke, 1989).

congener 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 5.3.2.3.

4.3.7 Dioxins and Dibenzofurans

Note: At this time, the EPA Office of Research and Development is reevaluating the potency of dioxins and dibenzofurans. Information provided below as well as information in Section 5.3.2.4 related to calculating toxicity equivalent concentrations (TECs) and SVs for dioxins/furans 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 isomer 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) (IRIS, 1992). 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).

Because dioxin/furan contamination is found almost exclusively 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) (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 industrial sites 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.

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 seven national monitoring programs (Appendix C). Six dioxin congeners and nine dibenzofuran congeners were measured in fish tissue and shellfish samples in the National Study of Chemical Residues in Fish. The various dioxin congeners were detected at from 32 to 89 percent of the 388 sites surveyed, while the furan congeners were detected at from 1 to 89 percent of the 388 sites surveyed (U.S. EPA, 1992c, 1992d). The dioxin/furan congeners detected at more than 50 percent of the sites are listed below:

- 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)
- 1,2,3,7,8 PeCDD (54 percent).

Currently, 22 States have issued fish consumption advisories for dioxins/furans (Figure 4-4) (RTI, 1993).

Dioxins/furans should be considered for analysis primarily 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 formulated: herbicides (containing 2,4,5-trichlorophenoxy acids and 2,4,5-trichlorophenol), hexachlorophene, pentachlorophenol, and PCBs (U.S. EPA, 1987d). If resources permit, it is recommended that the 17 2,3,7,8-substituted tetra- through octa-chlorinated dioxin and dibenzofuran congeners shown in Table 4-4 be included as target analytes. At a minimum, 2,3,7,8-TCDD and 2,3,7,8-tetra-chlorodibenzofuran (2,3,7,8-TCDF) should be determined.

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.

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

2,3,7,8-TCDD	1,2,3,7,8-PeCDF
	2,3,4,7,8-PeCDF
1,2,3,7,8-PeCDD	
	1,2,3,4,7,8-HxCDF
1,2,3,4,7,8-HxCDD	1,2,3,6,7,8-HxCDF
1,2,3,6,7,8-HxCDD	1,2,3,7,8,9-HxCDF
1,2,3,7,8,9-HxCDD	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
2,3,7,8-TCDF	

Source: Barnes and Bellin, 1989.

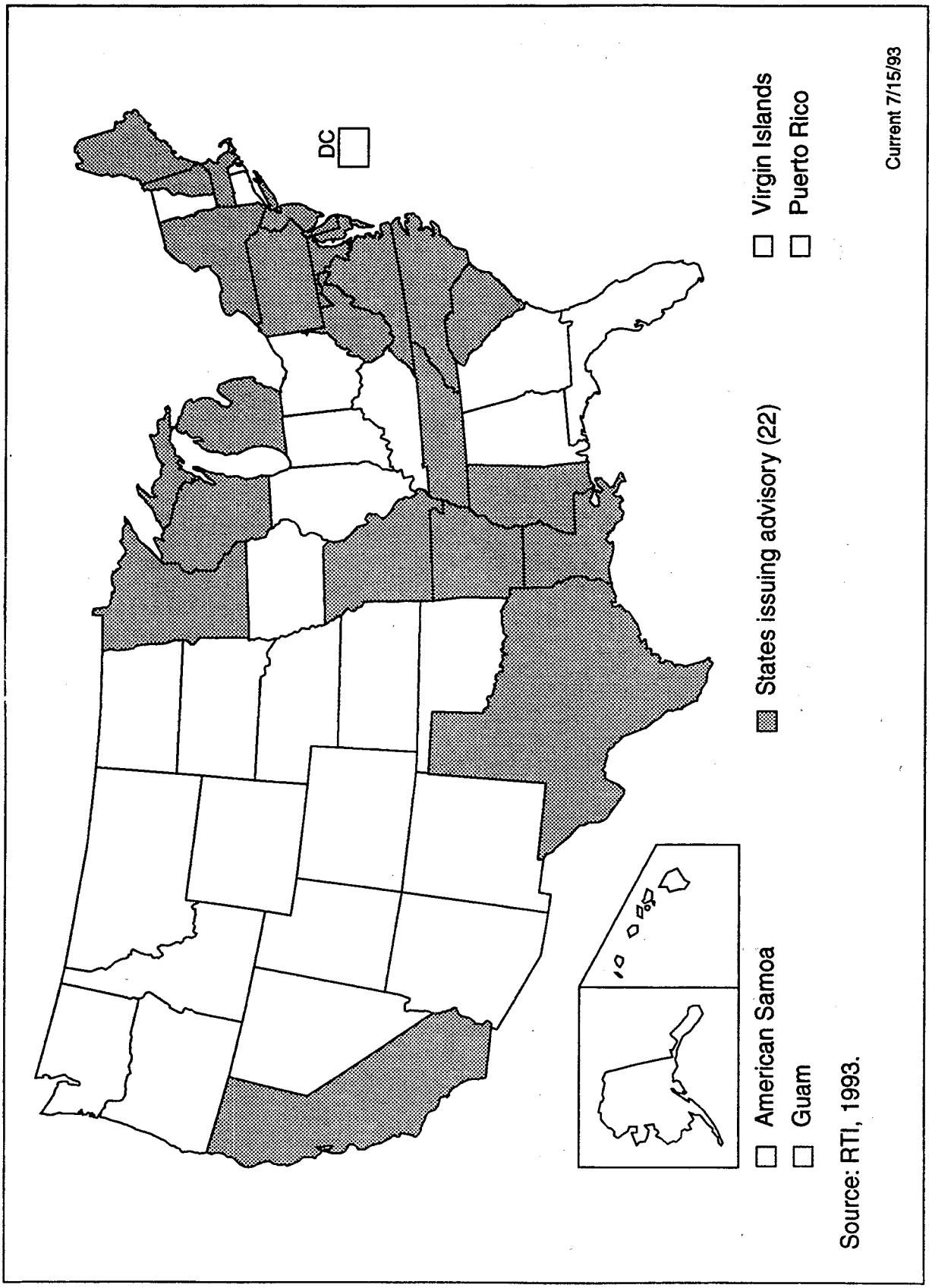


Figure 4-4. States issuing fish and shellfish advisories for dioxin/furans.

Note: Any time a State independently deems that the 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. As recently as the mid-1980s, 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. At present, lead is used primarily in batteries, electric cable coverings, some exterior paints, ammunition, and sound barriers. Currently, the major points of entry of lead into the environment are from mining and smelting operations, from fly ash resulting from coal combustion, and from the leachates of landfills (May and McKinney, 1981).

Lead has been included in six national monitoring programs (Appendix C). 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). 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). Currently three States (Massachusetts, Missouri, and Tennessee) and American Samoa have issued fish advisories for lead contamination (RTI, 1993).

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, 1988). A great deal of information on the health effects of lead has been obtained through decades of medical observation and scientific research. This information has been assessed in the development of air and water quality criteria by the Agency's Office of Health and Environmental Assessment (OHEA) in support of regulatory decisionmaking by the Office of Air Quality Planning and Standards (OAQPS) and by the Office of Drinking Water (ODW). 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. The Agency'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, 1993). Lead and its inorganic compounds have been classified as probable human carcinogens (B2) by EPA (IRIS, 1992). However, at this time, a quantitative estimate of carcinogenic risk from oral exposure is not available (IRIS, 1993).

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 tissue. Additional information is provided on this issue in Volume II—Risk Assessment and Fish Consumption Limits—in this guidance series (U.S. EPA, 1994).

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 standards 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 subpopulations such as sport and subsistence fishermen 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 and was the approach used in the National Study of Chemical Residues in Fish (U.S. EPA, 1992c, 1992d). EPA recognizes that there are many other approaches and models currently in use. 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) will be discussed in greater detail in the second guidance document in this series.

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 E. 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 (RfD).** 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., LOAEL if the 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 **slope factor (SF)**, 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, 1989d).

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

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

where

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

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

5.1.3 Recommended Values for Variables in Screening Value Equations

The recommended values in this section for variables used in Equations (5-4) and (5-5) to calculate SVs are based upon assumptions for the general adult population. For risk management purposes (e.g., to direct limited resources toward protection of sensitive subpopulations), States may choose to use values for consumption rate (CR), body weight (BW), and risk level (RL) different from those recommended in this section.

5.1.3.1 Dose-Response Variables—

EPA has developed oral RfDs and/or SFs for all of the recommended target analytes in Section 4 (see Appendix E). These are maintained in the EPA Integrated Risk Information System (IRIS, 1992), an electronic database containing health risk and EPA regulatory information on approximately 400 different chemicals. The IRIS RfDs and SFs 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 SFs 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 SFs be used to calculate SVs for target analytes, unless otherwise recommended.

A summary description of IRIS and instructions for accessing information in IRIS are found in U.S. EPA (1989d). Additional information can be obtained from IRIS User Support (Tel: 513-569-7254). IRIS is also available on the National Institutes of Health (NIH) National Library of Medicine TOXNET system (Tel: 301-496-6531).

In cases where IRIS values for oral RFDs or SFs are not available for calculating SVs for target analytes, estimates of these variables should 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 most current Reference Dose List (U.S. EPA, 1993b) and the Classification List of Chemicals Evaluated for Carcinogenicity Potential (U.S. EPA 1992a) from the Office of Pesticide Programs Health Effects Division.

5.1.3.2 Body Weight (BW) and Consumption Rate (CR)—

Values for the variables BW and CR in Equations (5-4) and (5-5) are given in Table 5-1 for the general adult population and various subpopulations. In this document, the EPA Office of Water used a BW = 70 kg and a CR = 6.5 g/d to calculate SVs for the general adult population. **Note:** The 6.5-g/d CR value that is used to establish water quality criteria is currently under review by the EPA Office of Water. This CR, which represents a consumption rate for the average fish consumer in the general adult population (45 FR 231, Part V), may not be appropriate for sport and subsistence fishermen who generally consume larger quantities of fish and shellfish (U.S. EPA, 1990a).

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 only involves calculating additional SVs and does not add to sampling or analytical costs.

The EPA has published detailed guidance on exposure factors (U.S. EPA, 1990a). In addition, 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). States should consult these documents to ensure that appropriate values are selected to calculate SVs for site-specific exposure scenarios.

5.1.3.3 Risk Level (RL)—

The EPA Office of Water recommends that an RL of 10^{-5} be used to calculate screening values for the general adult population. However, States may 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 RECOMMENDED SCREENING VALUES FOR TARGET ANALYTES

Recommended target analyte SVs, and the dose-response variables used to calculate them, are given in Table 5-2. 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 SFs (IRIS, 1992) unless otherwise noted:

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, 1990a)
	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]	6.5 g/d (0.0065 kg/d)	Estimate of the average consumption of fish and shellfish from estuarine and fresh waters by the **general U.S. population** (45 FR 231, Part V)
	14 g/d (0.014 kg/d)	Estimate of the average consumption of fish and shellfish from marine, estuarine, and fresh waters by the **general U.S. population** (45 FR 231, Part V)
	15 g/d (0.015 kg/d)	Estimate of the average consumption of fish from the Great Lakes by the **95th percentile of the regional population** (fishermen and nonfishermen) (U.S. EPA, 1992e)
	30 g/d (0.030 kg/d)	Estimate of the average consumption of fish and shellfish from marine, estuarine, and fresh waters by the **50th percentile of recreational fishermen** (U.S. EPA, 1990a)
	140 g/d (0.140 kg/d)	Estimate of the average consumption of fish and shellfish from marine, estuarine, and fresh waters by the **90th percentile of recreational fishermen** (i.e., subsistence fishermen) (U.S. EPA, 1990a)

[a] These are recommended consumption rates only. **Note:** EPA is currently evaluating the use of 6.5 g/d, 30 g/d, and 140 g/d as estimates of consumption rates for the general population, the 50th percentile of recreational fishermen, and subsistence fishermen, respectively. When local consumption rate data are available for these populations, they should be used to calculate SVs for noncarcinogens and carcinogens, as described in Sections 5.1.1 and 5.1.2, respectively.

- **For noncarcinogens:**

  BW = 70 kg, average adult body weight

  CR = 6.5 g/d (0.0065 kg/d), estimate of average consumption of fish and shellfish from estuarine and fresh waters by the general adult population (45 FR 231, Part V).

- **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.

Where both oral RfD and SF values are available for a given target analyte SVs for, both noncarcinogenic and carcinogenic effects are listed in Table 5-2. Unless otherwise indicated, the lower of the two SVs should be used. EPA recommends that the SVs in the shaded boxes (Table 5-2) 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 subpopulations (e.g., pregnant women, children, and recreational or subsistence fishermen). EPA recognizes that States may use higher CRs that are more appropriate for recreational and subsistence fishermen in calculating SVs for use in their jurisdictions rather than the 6.5-g/d CR for the general adult population used to calculate the SVs shown in Table 5-2.

Note: States should use the same SV (i.e., either for the general adult population or adjusted for other subpopulations) for a given target analyte for both screening and intensive studies. Therefore, it is critical that States clearly define their program objectives and accurately characterize the population or subpopulation(s) of concern in order to ensure that appropriate SVs are selected. If 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 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.

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 subpopulations of concern and for RL values ranging from 10^{-4} to 10^{-7} are given in Table 5-3.

Table 5-2. Dose-Response Variables and Recommended Screening Values (SVs) for Target Analytes

Target analyte	Noncarcinogens	Carcinogens	SV[a] (ppm)	
	RfD[b] (mg/kg/d)	SF[b] $(mg/kg/d)^{-1}$	Noncarcinogens	Carcinogens ($RL=10^{-5}$)
Metals				
Arsenic (inorganic)[c]	3×10^{-4} [d]	NA[e]	3	—
Cadmium	1×10^{-3}	NA	10	—
Mercury[f]				
Developmental	6×10^{-5} [g]	NA	0.6[d]	—
Chronic systemic	3×10^{-4} [h]	NA	3[h]	—
Selenium[i]	5×10^{-3}	NA	50	—
Tributyltin	3×10^{-5} [d]	NA	0.3	—
Organochlorine Pesticides				
Total chlordane (sum of cis- and trans-chlordane, cis- and trans-nonachlor, and oxychlordane)[j]	6×10^{-5}	1.3	0.6	0.08
Total DDT (sum of 4,4'- and 2,4'-isomers of DDT, DDE, and DDD)[k]	5×10^{-4}	0.34	5	0.3
Dicofol	1×10^{-3} [l]	NA	10	—
Dieldrin	5×10^{-5}	16	0.6	7×10^{-3}
Endosulfan (I and II)	6×10^{-3} [m]	NA	60	—
Endrin	3×10^{-4}	NA	3	—
Heptachlor epoxide	1.3×10^{-5}	9.1	0.1	0.01
Hexachlorobenzene	8×10^{-4}	1.6	9	0.07

See notes at end of table

(continued)

Table 5-2 (continued)

Target analyte	Noncarcinogens RfD[b] (mg/kg/d)	Carcinogens SF[b] (mg/kg/d)$^{-1}$	SV[a] (ppm) Noncarcinogens	SV[a] (ppm) Carcinogens (RL=10^{-5})
Metals				
Lindane (γ-hexachlorocyclohexane; γ-HCH)	3×10^{-4}	1.3[n]	3	0.08
Mirex	2×10^{-4}	NA[o]	2	—
Toxaphene	2.5×10^{-4} [l,p]	1.1	3	0.1
Organophosphate Pesticides				
Chlorpyrifos	3×10^{-3}	NA	30	—
Diazinon	9×10^{-5} [l]	NA	0.9	—
Disulfoton	4×10^{-5}	NA	0.5	—
Ethion	5×10^{-4}	NA	5	—
Terbufos	1.3×10^{-4} [l]	NA	1	—
Chlorophenoxy Herbicides				
Oxyfluorfen	3×10^{-3}	1.3×10^{-1}	30	0.8
PAHs	NA	7.3[d,q]	—	0.01
PCBs				
Total PCBs (sum of Aroclors)	2×10^{-5} [d,r]	7.7[s]	0.2	0.01
Dioxins/furans[t]	NA	1.56×10^{5}	—	7×10^{-7}

(continued)

Table 5-2 (continued)

NA = Not available in EPA's Integrated Risk Information System (IRIS, 1992).
PAH = Polycyclic aromatic hydrocarbon.
PCB = Polychlorinated biphenyl.
RfD = Oral reference dose (mg/kg/d).
RL = Risk level (dimensionless).
SF = Oral slope factor $(mg/kg/d)^{-1}$.

[a] Except for mercury, screening values (SVs) are target analyte concentrations in fish tissue that equal exposure levels at either the RfD for noncarcinogens or the SF and an RL=10^{-5} for carcinogens, given average consumption rates (CRs) and body weights (BWs) of 6.5 g/d and 70 kg, respectively, for the general adult population (U.S. EPA, 1989d). **Note:** These values have been determined by rounding the final calculated value to one significant figure. EPA believes that using more than one significant figure would imply a degree of precision that is not warranted given the large uncertainty factors generally used in deriving SVs. For target analytes with both carcinogenic and noncarcinogenic effects, the lower (more conservative) of the calculated SVs should be used. **Note:** Values in the shaded boxes are SVs recommended for use in State fish/shellfish consumption advisory programs for the general adult population. States may choose to use other SVs based on different CRs, BWs, and/or an RL ranging from 10^{-4} to 10^{-7}.

[b] Unless otherwise noted, values listed are the most current oral RfDs and SFs in EPA's IRIS (IRIS, 1992).

[c] Total inorganic arsenic should be determined for comparison with the recommended SV.

[d] From IRIS (1995).

[e] The SF for inorganic arsenic is currently under review by the Agency. At this time, EPA does not have a cancer SF for inorganic arsenic to recommend for use in conducting fish consumption risk assessments.

[f] Because most mercury in fish and shellfish tissue is present 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.

(continued)

[g] **Note:** The EPA has recently reevaluated the RfD for methylmercury, primarily because of concern about evidence that the fetus is at increased risk of adverse neurological effects from exposure to methylmercury (Marsh et al., 1987; Piotrowski and Inskip, 1981; NAS, 1991; WHO, 1976, 1990). On May 1, 1995, IRIS was updated to include an oral RfD of $1x10^{-4}$ mg/kg/d based on developmental neurological effects in human infants. An oral RfD of $3x10^{-4}$ mg/kg/d for chronic systemic effects of methylmercury among the general adult population was available in IRIS until May 1, 1995; however, it was not listed in the IRIS update on that date. For the purposes of calculating an SV for methylmercury that is protective of fetuses and nursing infants, the EPA Office of Water has chosen to continue to use the general adult population RfD of $3x10^{-4}$ mg/kg/d for chronic systemic effects of methylmercury until a value is relisted in IRIS, and to reduce this value by a factor of 5 to derive an RfD of $6x10^{-5}$ mg/kg/d for developmental effects among infants. This factor is based on experimental results that suggest a possible fivefold increase in fetal sensitivity to methylmercury exposure. This more protective approach recommended by the EPA Office of Water was deemed to be most prudent at this time. This approach should be considered interim until such time as the Agency has reviewed new studies on the chronic and developmental effects of methylmercury.

[h] This RfD is used in risk assessment calculations for the general adult population (see Volume II of this guidance document series [U.S. EPA, 1994]). It is not recommended that this SV be used in screening programs because it may not be protective of women of reproductive age and children.

[i] The RfD for selenium is the IRIS (1992) value for selenious acid. The evidence of carcinogenicity for various selenium compounds in animal and mutagenicity studies is conflicting and difficult to interpret. However, evidence for selenium sulfide is sufficient for a B2 classification (IRIS, 1992).

[j] The RfD and SF values listed are derived from studies using technical-grade chlordane (purity ~95%) or a 90:10 mixture of chlordane:heptachlor or analytical-grade chlordane (IRIS, 1992). No RfD or SF values are given in IRIS (1992) 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 the total concentration of cis- and trans-chlordane, cis- and trans-nonachlor, and oxychlordane be determined for comparison with the recommended SV.

[k] The RfD value listed is for DDT. The SF value is for DDT or DDE; the SF value for DDD is 0.24. The U.S. EPA Carcinogenicity Assessment Group recommended the use of SF = 0.34 for any combination of DDT, DDE, DDD, and dicofol (Holder, 1986). It is recommended that the total concentration of the 2,4′- and 4,4′-isomers of DDT and its metabolites, DDE and DDD, be determined for comparison with the recommended SV.

[l] The RfD value listed is from the Office of Pesticide Program's Reference Dose Tracking Report (U.S. EPA, 1993b).

[m] The RfD value listed is from the Office of Pesticide Program's Reference Dose Tracking Report (U.S. EPA, 1995j).

(continued)

Table 5-2 (continued)

[n] IRIS (1992) has not provided an SF for lindane. The SF value listed for lindane was calculated from the water quality criteria (0.063 µg/L) (U.S. EPA, 1992e).

[o] The National Study of Chemical Residues in Fish (U.S. EPA, 1992c, 1992d) used a value of SF = 1.8 for mirex from HEAST (1989).

[p] The RfD value is the Office of Pesticide Programs value; this value was never submitted for verification.

[q] The SF value listed is for benzo[*a*]pyrene. Values for other PAHs are not currently available in IRIS (1995). It is recommended that, in both screening and intensive studies, tissue samples be analyzed for benzo[*a*]pyrene, benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, chrysene, dibenz[*a,h*]anthracene, and indeno*[1,2,3-cd]*pyrene, and that the order-of-magnitude relative potencies given for these PAHs in the EPA provisional guidance for quantitative risk assessment of PAHs (U.S. EPA, 1993c) be used to calculate a potency equivalency concentration (PEC) for each sample for comparison with the recommended SV for benzo[*a*]pyrene (see Section 5.3.2.3). At this time, EPA's recommendation for risk assessment of PAHs (U.S. EPA 1993c) is considered provisional because quantitative risk assessment data are not available for all PAHs. This approach is under Agency review and over the next year will be evaluated as new health effects benchmark values are developed. Therefore, the method provided in this guidance document is subject to change pending results of the Agency's reevaluation.

[r] The RfD for PCBs is based on the chronic toxicity of Aroclor 1254 (IRIS, 1995). This RfD is lower than the RfD that is available in IRIS (1995) for the developmental toxicity of Aroclor 1016 ($7x10^{-5}$) and, therefore, is protective against both chronic systemic toxicity and developmental toxicity. See Volume II (Section 5.6.19) of this guidance document series (U.S. EPA, 1994b) for a more detailed discussion of toxicity data for PCBs and their use in conducting quantitative risk assessments and determination of consumption limits.

[s] The SF is based on a carcinogenicity assessment of Aroclor 1260. The SF of Aroclor 1260 is intended to represent the upper bound risk for all PCB mixtures (IRIS, 1992).

[t] The SF value listed is for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (U.S. EPA, 1986c). The National Study of Chemical Residues in Fish used a value of RfD = $1x10^{-9}$ for 2,3,7,8-TCDD from ATSDR (1987d). It is recommended that, in both screening and intensive studies, the 17 2,3,7,8-substituted tetra- through octa-chlorinated dibenzo-p-dioxins and dibenzofurans be determined and a toxicity-weighted total concentration be calculated for each sample for comparison with the recommended SV, using the revised interim method for estimating Toxicity Equivalency Concentrations (TECs) (Barnes and Bellin, 1989; U.S. EPA, 1991h). If resources are limited, the 2,3,7,8-TCDD and 2,3,7,8-TCDF congeners should be determined at a minimum.

Table 5-3. Example Screening Values (SVs) for Various Subpopulations and Risk Levels (RLs)[a]

Chemical	Subpopulation[b]	CR[c]	BW	RfD	SF	RL	SV (ppm)
Noncarcinogens							
Chlorpyrifos	Standard adults	6.5	70	3×10^{-3}	—	—	30
	Children	6.5	36[d]	3×10^{-3}	—	—	20
	Subsistence fishermen	140	70	3×10^{-3}	—	—	2
Cadmium	Standard adults	6.5	70	1×10^{-3}	—	—	10
	Children	6.5	36[d]	1×10^{-3}	—	—	6
	Subsistence fishermen	140	70	1×10^{-3}	—	—	0.5
Carcinogens							
Lindane	Standard adults	6.5	70	—	1.3	10^{-4}	8×10^{-1}
					1.3	10^{-5}	8×10^{-2}
					1.3	10^{-6}	8×10^{-3}
					1.3	10^{-7}	8×10^{-4}
	Children	6.5	36[d]	—	1.3	10^{-4}	4×10^{-1}
					1.3	10^{-5}	4×10^{-2}
					1.3	10^{-6}	4×10^{-3}
					1.3	10^{-7}	4×10^{-4}
	Subsistence fishermen	140	70	—	1.3	10^{-4}	4×10^{-2}
					1.3	10^{-5}	4×10^{-3}
					1.3	10^{-6}	4×10^{-4}
					1.3	10^{-7}	4×10^{-5}
Toxaphene	Standard adults	6.5	70	—	1.1	10^{-4}	10×10^{-1}
					1.1	10^{-5}	10×10^{-2}
					1.1	10^{-6}	10×10^{-3}
					1.1	10^{-7}	10×10^{-4}
	Children	6.5	36[d]	—	1.1	10^{-4}	5×10^{-1}
					1.1	10^{-5}	5×10^{-2}
					1.1	10^{-6}	5×10^{-3}
					1.1	10^{-7}	5×10^{-4}
	Subsistence fishermen	140	70	—	1.1	10^{-4}	5×10^{-2}
					1.1	10^{-5}	5×10^{-3}
					1.1	10^{-6}	5×10^{-4}
					1.1	10^{-7}	5×10^{-5}

CR = Mean daily fish or shellfish consumption rate, 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).
SF = Oral slope factor for carcinogens $(mg/kg/d)^{-1}$.
RL = Maximum acceptable risk level for carcinogens (dimensionless).
[a] See Equations (5-4) and (5-5).
[b] See Table 5-2 for definitions of subpopulations.
[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 9 to <12 yr (see Table 5-2).

The need to accurately characterize the subpopulation of interest in order to establish sufficiently protective SVs cannot be overemphasized. For example, the recommended consumption rate of 140 g/d for subsistence fishermen may be an underestimate of consumption rate for some subsistence populations. In a recent study of Alaskan subsistence fishing economies (Wolf and Walker, 1987), daily consumption rates for subsistence fishermen were found to range from 6 to 1,536 g/d, with an average daily consumption rate of 304 g/d. 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),

$$SV = (0.001 \text{ mg/kg/d} \cdot 70 \text{ kg}) / (0.304 \text{ kg/d}) = 0.2 \text{ mg/kg (ppm)} \quad . \quad (5\text{-}7)$$

This value is significantly lower than the SV of 0.5 ppm for cadmium based on the recommended consumption rate of 140 g/d for subsistence fishermen, as shown in Table 5-3.

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; Jongen et al., 1985; Kaise et al. 1985; Luten et al., 1982; Sabbioni et al., 1991; Siewicki, 1981; Tam et al., 1982; Vahter et al., 1983; Yamauchi et al., 1986). Inorganic arsenic, which is of concern for human health effects (ATSDR, 1993; WHO, 1989), is generally found in seafood at concentrations 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 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 SV. For mercury, the SV that is calculated from the RfD for developmental effects of methylmercury (see Table 5-2) should be used because it is most protective.

The determination of methylmercury is not recommended even though methylmercury is the compound of greatest concern for human health (NAS, 1991; Tollefson, 1989) and the recommended SV is for methylmercury (see Table 5-2). Because most mercury in fish and shellfish tissue is present as methylmercury (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. This approach is deemed to be most protective of human health and most cost-effective.

Note: The EPA has recently reevaluated the RfD for methylmercury, primarily because of concern about evidence that the fetus is at increased risk of adverse neurological effects from exposure to methylmercury (Marsh et al., 1987; Piotrowski and Inskip, 1981; NAS, 1991; WHO, 1976, 1990). On May 1, 1995, IRIS was updated to include an oral RfD of $1x10^{-4}$ mg/kg/d based on developmental neurological effects in human infants. An oral RfD of $3x10^{-4}$ mg/kg/d for chronic systemic effects of methylmercury among the general adult population was available in IRIS until May 1, 1995; however, it was not listed in the IRIS update on that date. For the purposes of calculating an SV for methylmercury that is protective of fetuses and nursing infants, the EPA Office of Water has chosen to continue to use the general adult population RfD of $3x10^{-4}$ mg/kg/d for chronic systemic effects of methylmercury until a value is relisted in IRIS, and to reduce this value by a factor of 5 to derive an RfD of $6x10^{-5}$ mg/kg/d for developmental effects among infants. This factor is based on experimental results that suggest a possible fivefold increase in fetal sensitivity to methylmercury exposure. This more protective approach recommended by the EPA Office of Water was deemed to be most prudent at this time. This approach should be considered interim until such time as the Agency has reviewed new studies on the chronic and developmental effects of methylmercury.

5.3.1.3 Tributyltin—

Tissue samples should be analyzed specifically for tributyltin for comparison with the recommended SV for this compound.

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), or that represent classes of compounds (i.e., PAHs, PCBs, dioxins/furans), 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 SV for total chlordane is derived from technical-grade chlordane. Oral slope factors are not available in IRIS (1992) 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 cis- and trans-chlordane, cis- and trans-nonachlor, and oxychlordane be determined and summed to give a total chlordane concentration for comparison with the recommended SV for total chlordane (see Table 5-2).

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 DDE and DDD metabolites be determined and a total DDT concentration be calculated for comparison with the recommended SV for total DDT (see Table 5-2).

5.3.2.3 PAHs—

Although several PAHs have been classified as B2 carcinogens (probable human carcinogens), benzo[*a*]pyrene is the only PAH for which an SF is currently available in IRIS (1995). 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 recently issued provisional guidance for quantitative risk assessment of PAHs (U.S. EPA, 1993c) in which an estimated order of potential potency for six Group B2 PAHs relative to benzo[*a*]pyrene is recommended, as shown in Table 5-4. Based on this guidance, it is recommended that, in both screening and intensive studies, tissue samples be analyzed for the seven PAHs shown in Table 5-4 and that a potency-weighted total concentration be calculated for each sample for comparison with the recommended SV for benzo[*a*]pyrene. This potency equivalency concentration (PEC) 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 5-4)
C_i = Concentration of the ith PAH.

At this time, EPA's recommendation for risk assessment of PAHs (U.S. EPA, 1993c) is considered provisional because quantitative risk assessment data are not available for all PAHs. This approach is under Agency review and over the next year will be evaluated as new health effects benchmark values are developed. Therefore, the method provided in this guidance document is subject to change pending results of the Agency's reevaluation.

5.3.2.4 PCBs—

Using the interim approach for PCB analysis recommended by the EPA Office of Water (see Section 4.3.5), total PCB concentrations should be determined, in both screening and intensive studies, as the sum of Aroclor equivalents. The total PCB concentration should be compared with the recommended SV for PCBs (see Table 5-2). Because this SV is based on the SF for Aroclor 1260, the recommendation to use this SV for comparison with total Aroclor concentration requires the assumption that Aroclor 1260 is representative of

Table 5-4. Estimated Order of Potential Potencies of Selected PAHs

Compound	Relative Potency[a,b]	Reference
Benzo[*a*]pyrene	1.0	
Benz[*a*]anthracene	0.1	Bingham and Falk, 1969
Benzo[*b*]fluoranthene	0.1	Habs et al., 1980
Benzo[*k*]fluoranthene	0.01	Habs et al., 1980
Chrysene	0.001	Wynder and Hoffmann, 1959
Dibenz[*a,h*]anthracene	1.0	Wynder and Hoffmann, 1959
Indeno[1,2,3-*cd*]pyrene	0.1	Habs et al., 1980; Hoffmann and Wynder, 1966

[a] Model was $P(d)=1-\exp[-a(1+bd)^2]$ for all but indeno[1,2,3-*cd*]pyrene.
[b] Values listed are order-of-magnitude potencies based on the following scheme for rounding experimental values: 0.51–5.0=1.0; 0.051–0.50=0.1; 0.0051–0.050=0.01.

Source: Modified from U.S. EPA, 1993c.

other PCB mixtures, i.e., that the SF for Aroclor 1260 is an upper limit risk estimate for all other PCB mixtures as well (IRIS, 1992; U.S. EPA, 1988a). The EPA Office of Water recognizes that this assumption has significant uncertainties.

The comparison of total PCB concentrations (determined as the sum of Aroclor equivalents) with the Aroclor 1260-based SV may be overly conservative. The EPA Carcinogen Assessment Group has reported a much lower SF for Aroclor 1254 (SF = 2.6) and data from studies of Aroclor 1242 (Schaeffer et al., 1984) indicate that there are no statistically significant increases in liver tumors compared to controls. A recent reassessment of the results of five PCB studies in rats found significant differences between Aroclor 1260 and other Aroclors in the types and incidence of pathological effects on rats (IEHR, 1991). On the other hand, Aroclor 1260 may not represent an upper bound risk estimate because the PCB congener distribution in fish and shellfish tissue is usually markedly altered from, and may be more potent than, the parent Aroclor mixture (Bryan et al., 1987; Kubiak et al., 1989; Norstrom, 1988; Oliver and Niimi, 1988; Smith et al., 1990). This underscores the need to move toward congener-specific analysis based on (1) pharmacokinetics and (2) relative potency at specific site(s) of action (NAS, 1991).

EPA also recognizes that the current recommended SV of 10 ppb for total PCBs will result in widespread exceedance in waterbodies throughout the country and will drive virtually all fish and shellfish contaminant monitoring programs into the risk assessment phase for PCBs. The decision on whether to issue a consumption advisory for PCBs at this level is one that must be made by risk managers in each State.

EPA is currently giving high priority to addressing the unresolved issues related to PCB analysis and risk assessment. A work group has been convened to examine the feasibility of TEFs for PCB congeners similar to those developed for PCDDs and PCDFs (U.S. EPA, 1991j) and two EPA-sponsored national workshops have been held recently to identify problematic issues and areas for future research (U.S. EPA, 1993d; U.S. EPA, 1993e). Additional guidance on PCB analyses will be provided in addenda to this document and in subsequent documents in this series.

5.3.2.4 Dioxins and Dibenzofurans—

Note: At this time, the EPA Office of Research and Development is reevaluating the potency of dioxins/furans. Consequently, the following recommendation is subject to 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 be determined and that a toxicity-weighted total concentration be calculated for each sample for comparison with the recommended SV for 2,3,7,8-TCDD (see Table 5-2).

The revised interim method for estimating toxicity equivalency concentrations (Barnes and Bellin, 1989) should be used to estimate TCDD equivalent concentrations according to the following equation:

$$TEC = \sum_{i} (TEF_i \cdot C_i) \qquad (5\text{-}9)$$

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 are shown in Table 5-5.

If resources are limited, the 2,3,7,8-TCDD and 2,3,7,8-TCDF congeners should be determined and the calculated TEC compared with the recommended SV for 2,3,7,8-TCDD (see Table 5-2).

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

Analyte	TEF[a]
2,3,7,8-TCDD	1.00
1,2,3,7,8-PeCDD	0.50
1,2,3,4,7,8-HxCDD	0.10
1,2,3,6,7,8-HxCDD	0.10
1,2,3,7,8,9-HxCDD	0.10
1,2,3,4,6,7,8-HpCDD	0.01
OCDD	0.001
2,3,7,8-TCDF	0.10
1,2,3,7,8-PeCDF	0.05
2,3,4,7,8-PeCDF	0.50
1,2,3,4,7,8-HxCDF	0.10
1,2,3,6,7,8-HxCDF	0.10
1,2,3,7,8,9-HxCDF	0.10
2,3,4,6,7,8-HxCDF	0.10
1,2,3,4,6,7,8-HpCDF	0.01
1,2,3,4,7,8,9-HpCDF	0.01
OCDF	0.001

Source: Barnes and Bellin, 1989.

[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.

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 F). 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 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 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 dischargers
  - — 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)

Sample Request Form

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

INSTRUCTIONS TO SAMPLE COLLECTION TEAM

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

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

Target Species:	Alternate Species: *(in order of preference)*
☐ Freshwater _____	_____
☐ Estuarine _____	_____
_____	_____

Proposed Sampling Dates: _____

Proposed Sampling Method: _____

☐ Electrofishing	☐ Mechanical grab or tongs
☐ Seining	☐ Biological dredge
☐ Trawling	☐ Hand collection

☐ Other *(Specify _____)*

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

☐ _____ field replicates

(Specify number for each target species)

Number of Individuals per Composite:

_____ Fish per composite

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

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

- 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

- Unpolluted areas that can serve as reference sites for subsequent intensive studies. For example, 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 fishermen 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).

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 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 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 Table 5-2 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 in addition

to those recommended in Table 5-2 are included in a screening study, these calculation procedures should be used to estimate SVs based on typical exposure assumptions for the general population 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 Section 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 can not 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 fishermen). 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 System (1990).

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, a composite sample should consist of that portion of the individual organism that is commonly consumed by the population at risk. Skin-on fillets (with the belly flap included) are recommended for most scaled finfish (see Sections 7.2.2.6 and 7.2.2.7). Other sample types (e.g., skinless fillets) may be more appropriate for some target species (e.g., catfish and other scaleless finfish species). For shellfish, the tissue considered to be edible will vary by target species (see Section 7.2.4.4) based on local food preferences. A precise description of the sample type (including the number and

size of the individuals in the composite) should be documented in the program records for each target species. **Note:** For freshwater turtles, the tissues considered to be edible vary based on the dietary and culinary practices of local populations (see Section 7.2.3.3). The EPA recommends use of individual turtle samples rather than composite samples for evaluating turtle tissue contamination.

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:** Under no circumstance should indivi-

duals from different species be used in a 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, the individuals within each target species composite should be of similar size within a target size range. For persistent chlorinated organic compounds (e.g., DDT, PCBs, and toxaphene) and organic mercury compounds, 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 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 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.

As alluded to above, one limitation of using composite samples is that information on extreme levels of 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 A.

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 optional 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 human 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 contaminated sediments accumulate and the bioaccumulation potential might be enhanced (U.S. EPA, 1986f). 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.

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 required 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—

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 an 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 A.

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). In order 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.

The remainder of 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.

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

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

where

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

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

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

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

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

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

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 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 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 σ^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:

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

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

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

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

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

Two assumptions are made to examine the statistical power of the test of the null hypothesis of interest. First, it is assumed that the true mean of the site-specific composite target analyte concentrations (μ) is either 10 percent 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 to 100 percent. Four 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 two of the four scenarios are shown in Table 6-2.

Power estimates for the two 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 125 of the resulting 140 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 variance to the SV increases (i.e., σ/SV), the statistical power decreases.

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 variance to the SV presented in Section A of Table 6-2 is unrealistic for some fish and shellfish populations. Data in Section B, which reflect more realistic assumptions concerning the estimated population variance, show that States will be able to detect only large differences between the site-specific mean target analyte concentrations and the SV. Specifically, 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

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	6	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
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 = 1.0 and μ = 1.5 x SV:										
3	-	-	-	-	-	-	-	-	5	6
4	-	-	-	5	6	6	7	7	8	8
5	-	5	6	7	8	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
10	8	8	9	9	9	9	9	9	9	9
15	9	9	9	9	9	9	9	9	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 above 90 percent.

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.

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 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.

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.

6.2 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 6-3 for fish and in Table 6-4 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 fishermen.

A basic checklist of field sampling equipment and supplies is shown in Table 6-5. Safety considerations associated with the use of a boat in sample collection activities are summarized in Table 6-6.

Table 6-3. Summary of Fish Sampling Equipment

Device	Use	Advantages	Disadvantages
ACTIVE METHODS			
Electrofishing	Shallow rivers, lakes, and streams.	Most efficient nonselective method. Minimal damage to fish. Adaptable to a number of sampling conditions (e.g., boat, wading, shorelines). Particularly useful at sites where other active methods cannot be used (e.g., around snags and irregular bottom contours).	Nonselective—stuns or kills most fish. Cannot be used in brackish, salt, or extremely soft water. Requires extensive operator training. DANGEROUS when not used properly.
Seines	Shallow rivers, lakes, and streams. Shoreline areas of estuaries.	Relatively inexpensive and easily operated. Mesh size selection available for target species.	Cannot be used in deep water or over substrates with an irregular contour. Not completely efficient as fish can 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 fishermen	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 fishermen and should remove the required samples from the collection device. This will ensure the proper handling of the specimens and accurate recording of the collection time and sampling location.
PASSIVE METHODS			
Gill nets	Lakes, rivers, and estuaries. Where fish movement can be expected or anticipated.	Effective for collecting pelagic fish species. 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.

(continued)

TABLE 6-3. (continued)

Device	Use	Advantages	Disadvantages
PASSIVE METHODS			
Hoop, Fyke and Pound Nets	Shallow rivers, lakes, and estuaries where currents are present or when movements of fish are predictable. Frequently used in commercial operations.	Unattended operation. Very efficient in regard to long-term return and expended effort. Particularly useful in areas where active methods are impractical.	Inefficient for short term. Difficult to set up and maintain.
D-Traps	Used for long-term capture of slow-moving fish, particularly bottom species. Can be used in all environments.	Easy to operate and set. Unattended operation. Particularly useful for capturing bottom dwelling organisms in deep waters or other types of inaccessible areas. Relatively inexpensive—often can be hand made.	Efficiency is highly variable. Not effective for pelagic fish or fish that are visually oriented. Less efficient for all species when water is clear rather than turbid. Not a good choice for a primary sampling technique, but valuable as backup for other methods.

Source: Versar, 1982.

Table 6-4. 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 efficient means of sampling bivalves (e.g., clams and oysters) that are located on or buried in bottom sediments.	At depths greater than 6 m, the pole-operated devices become difficult to operate manually.
Tongs or double-handled grab sampler	Most useful in shallow water, lakes, rivers, and estuaries. Generally used from a boat.	Very efficient means of sampling oysters, clams, and scallops. Collection of surrounding or overlying sediments is not required and the jaws are generally open baskets. This reduces the weight of the device and allows the washing of collected specimens to remove sediments.	At depths greater than 6 m, the pole-operated devices become difficult to operate manually.
Line or Cable-Operated Grab Buckets:			
Ekman grab	Used from boat or pier to sample soft to semisoft substrates.	Can be used in water of varying depths in lakes, rivers, and estuaries.	Possible incomplete closure of jaws can result in sample loss. Must be repeatedly retrieved and deployed. Grab is small and is not particularly effective in collecting large bivalves (clams and oysters).
Petersen grab	Deep lakes, rivers, and estuaries for sampling most substrates.	Large sample is obtained; grab can penetrate most substrates.	Grab is heavy, may require winch for deployment. Possible incomplete closure of jaws can result in sample loss. Must be repeatedly retrieved and deployed.
Ponar grab	Deep lakes, rivers, and estuaries for sampling sand, silt or clay substrates.	Most universal grab sampler. Adequate on most substrates. Large sample is obtained intact.	Possible incomplete closure of jaws can result in sample loss. Must be repeatedly retrieved and deployed.
Orange peel grab	Deep lakes, rivers, and estuaries for sampling most substrates.	Designed for sampling hard substrates.	Grab is heavy, may require winch for deployment. Possible incomplete closure of jaws can result in sample loss. Must be repeatedly retrieved and deployed. Grab is small and not particularly effective in collecting large bivalves (clams and oysters).

(continued)

TABLE 6-4. (continued)

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

Source: Versar, 1982.

Table 6-5. 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 6-6. 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.

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, 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).

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 fishermen using active collection devices is acceptable; however, field sampling staff should accompany the commercial fishermen 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 (1970); Gunderson and Ellis (1986); Hayes (1983); Mearns and Allen (1978); Pitt, Wells, and McKone (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.

Purchasing fish and shellfish from commercial fishermen using passive collection methods is acceptable; however, field sampling staff should accompany the fishermen 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 been obviously contaminated by oils, grease, diesel fuel, or gasoline should not be used. All utensils or equipment that will be used directly in handling fish or shellfish (e.g., fish measuring board or calipers) should be cleaned in the laboratory prior to each sampling trip, rinsed in acetone and pesticide-grade hexane, and stored in aluminum foil until use (Versar, 1982). Between sampling 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 6-2 and 6-3) and **Tier 2** intensive studies as appropriate (Figures 6-4 and 6-5):

- Project number
- Sampling date and time (specify convention used, e.g., day/month/year and 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
- Collection method
- Collectors' names and signatures
- Agency (including telephone number and address)

Field Record for Fish Contaminant Monitoring Program — Screening Study

Project Number: _____ Sampling Date and Time: _____

SITE LOCATION

Site Name/Number: _____

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

Waterbody Name/Segment Number: _____

Waterbody Type: ☐ RIVER ☐ LAKE ☐ ESTUARY

Site Description: _____

_____

Collection Method: _____

Collector Name: _____
(print and sign)

Agency: _____ Phone: (____) _____

Address: _____

FISH COLLECTED

Bottom Feeder—Species Name: _____

Composite Sample #: _____ Number of Individuals: _____

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

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

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

Predator—Species Name: _____

Composite Sample #: _____ Number of Individuals: _____

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

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

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

Figure 6-2. Example of a field record for fish contaminant monitoring program—screening study.

Field Record for Shellfish Contaminant Monitoring Program — Screening Study

Project Number: _____ Sampling Date and Time: _____

SITE LOCATION

Site Name/Number: _____

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

Waterbody Name/Segment Number: _____

Waterbody Type: ☐ RIVER ☐ LAKE ☐ ESTUARY

Site Description: _____

_____

Collection Method: _____

Collector Name: _____
(print and sign)

Agency: _____ Phone: (____) _____

Address: _____

_____

SHELLFISH COLLECTED

Bivalve Species Name: _____

Composite Sample #: _____ Number of Individuals: _____

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

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

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

_____

Figure 6-3. 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 6-4. 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 6-4 (continued)

Field Record for Shellfish Contaminant Monitoring Program — Intensive Study

Project Number: _____ Sampling Date and Time: _____

SITE LOCATION

Site Name/Number: _____

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

Waterbody Name/Segment Number: _____

Waterbody Type: ☐ RIVER ☐ LAKE ☐ ESTUARY

Site Description: _____

_____

Collection Method: _____

Collector Name: _____
(print and sign)

Agency: _____ Phone: (____) _____

Address: _____

_____

SHELLFISH COLLECTED

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

Composite Sample #: _____ Number of Individuals: _____

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

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

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

_____

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

- Species collected (including species 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 6-6). 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)

- Sampling site—waterbody name and/or identification number

- Sampling date/time (specify convention, e.g., day/month/year and 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 6-7.

Species Name or Code	Sample Type
Total Length or Size (cm)	Sampling Site (name/number)
Specimen Number ☐☐☐☐☐–☐☐☐	Sampling Date (d/mo/yr) Time (24-h clock)

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

Project Number	Collecting Agency (name, address, phone)	
Sampling Site (name and/or ID number)	Sampler (name and signature)	
Composite Number/Specimen Number(s) Sampling Date (d/m/yr)/Time (24-hr clock)	Chemical Analyses ☐ All target analytes ☐ Others (specify) _____	Study Type: Screening; Intensive: Phase I ☐, Phase II ☐
Species Name or Code	Processing: Whole Body / Resection	Type of Ice: Wet / Dry
Comments		

Figure 6-7. 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 6-8).

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 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.

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 6-8) 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

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 6-8. Example of a chain-of-custody record form.

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 6-2 through 6-5).

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 fishermen 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 A 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 6-9. 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).

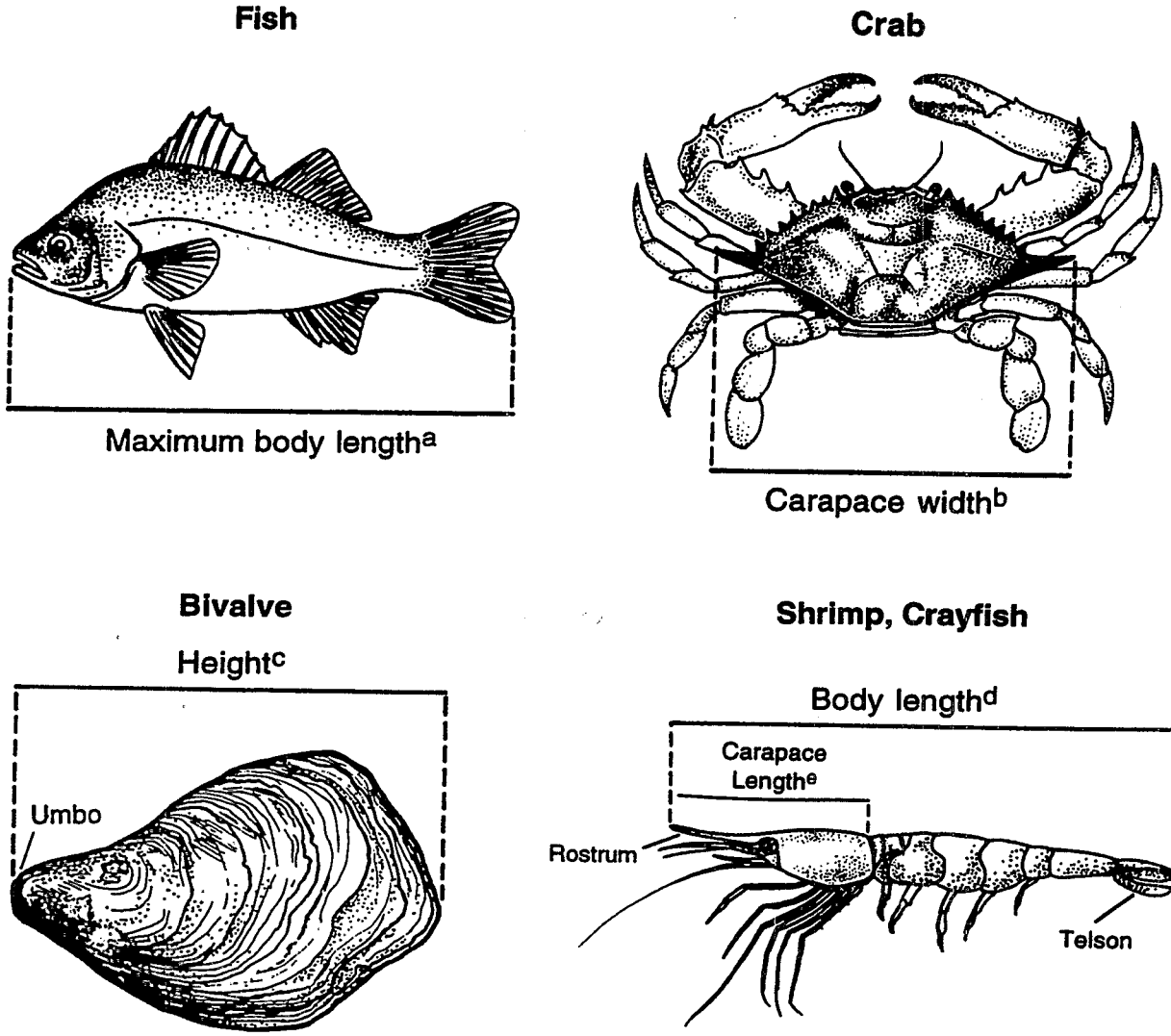
Each turtle within the selected target species should be measured to determine total carapace length (mm). To be consistent with the convention used by most herpetologists in the United States, carapace length should be measured as shown in Figure 6-9. The maximum carapace length is defined as the straight 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.).



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

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

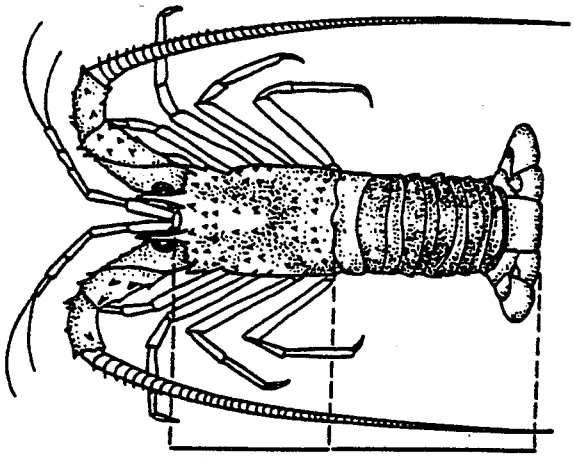
[c] Height is the distance from the umbo to the anterior (ventral) shell margin (Galtsoff, 1964).

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

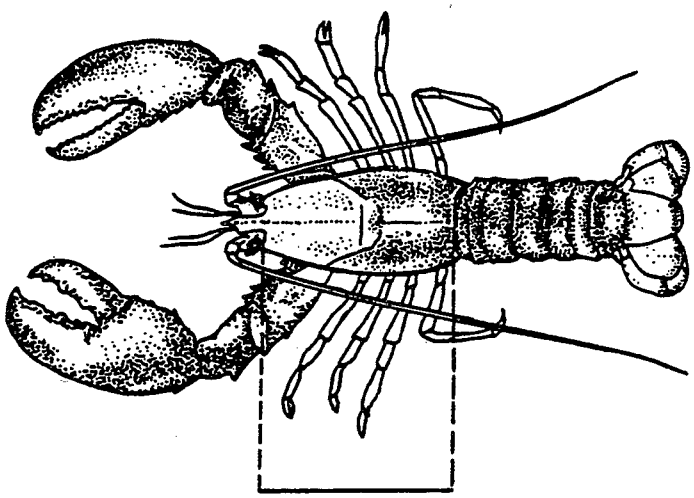
[e] Carapace length is distance from top of rostrum to the posterior margin of the carapace.

Figure 6-9. Recommended measurements of body length and size for fish, shellfish, and turtles.

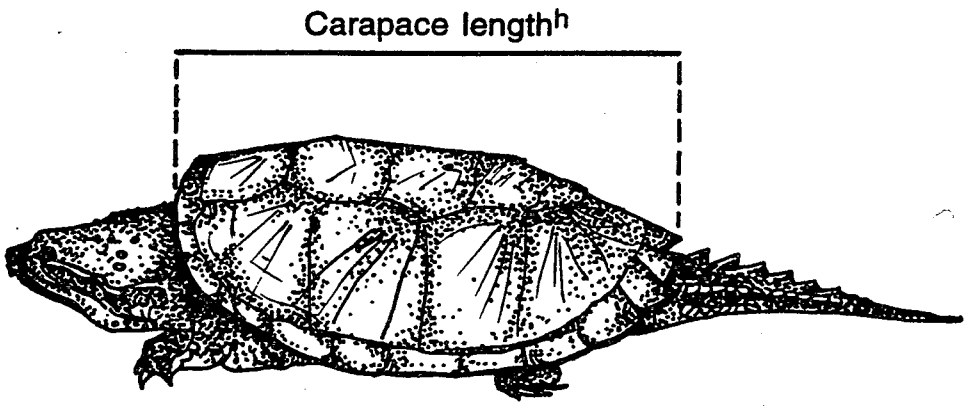
Spiny Lobster



Clawed Lobster



Turtle



[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 the 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 6-9 (continued)

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 6-6 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 6-6) 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/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 6-7).

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 6-6) 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.
- 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 6-7).

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 6-7).

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

Table 6-7. Recommendations for Preservation of Fish, Shellfish, and Turtle Samples from Time of Collection to Delivery at the Processing Laboratory

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

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

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 fieldsurveys. 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 6-8).

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

SECTION 7

LABORATORY PROCEDURES I — SAMPLE HANDLING

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

Laboratory procedures should be documented in a Work/QA Project Plan (U.S. EPA, 1980b) as described in Appendix F. 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 6-8), and the field records (Figures 6-2 through 6-5). 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 (specify convention, e.g., day/month/year)
  - — Sampling date (specify convention, e.g., day/month/year)
  - — 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, 1995k). 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 G 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 (1995b).

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

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

PTFE = Polytetrafluoroethylene (Teflon).

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

[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. The EPA (1995c) recommends a maximum holding time of 1 year at ≤-10 °C for dioxins/furans.

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

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

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

7.2.1.1 Samples for Organics Analysis—

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

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

or acetone followed by a rinse with contaminant-free distilled water between each fish sample (Stober, 1991).

7.2.1.2 Samples for Metals Analysis—

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

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

7.2.1.3 Samples for Both Organics and Metals Analyses—

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

Quartz, ceramic, borosilicate glass, and PTFE are recommended materials for sample processing equipment. If chromium and nickel are not of concern, high-quality, corrosion-resistant stainless steel utensils may be used. Knives with titanium blades and PTFE handles are recommended for performing tissue resections (Lowenstein and Young, 1986). Borosilicate glass bench liners are recommended. Filleting should be done on glass or PTFE cutting boards that are cleaned properly between fish or on cutting boards covered with heavy duty

aluminum foil that is changed after each filleting. Fillets or tissue homogenates should be stored in clean borosilicate glass, quartz, or PTFE containers with PTFE-lined lids.

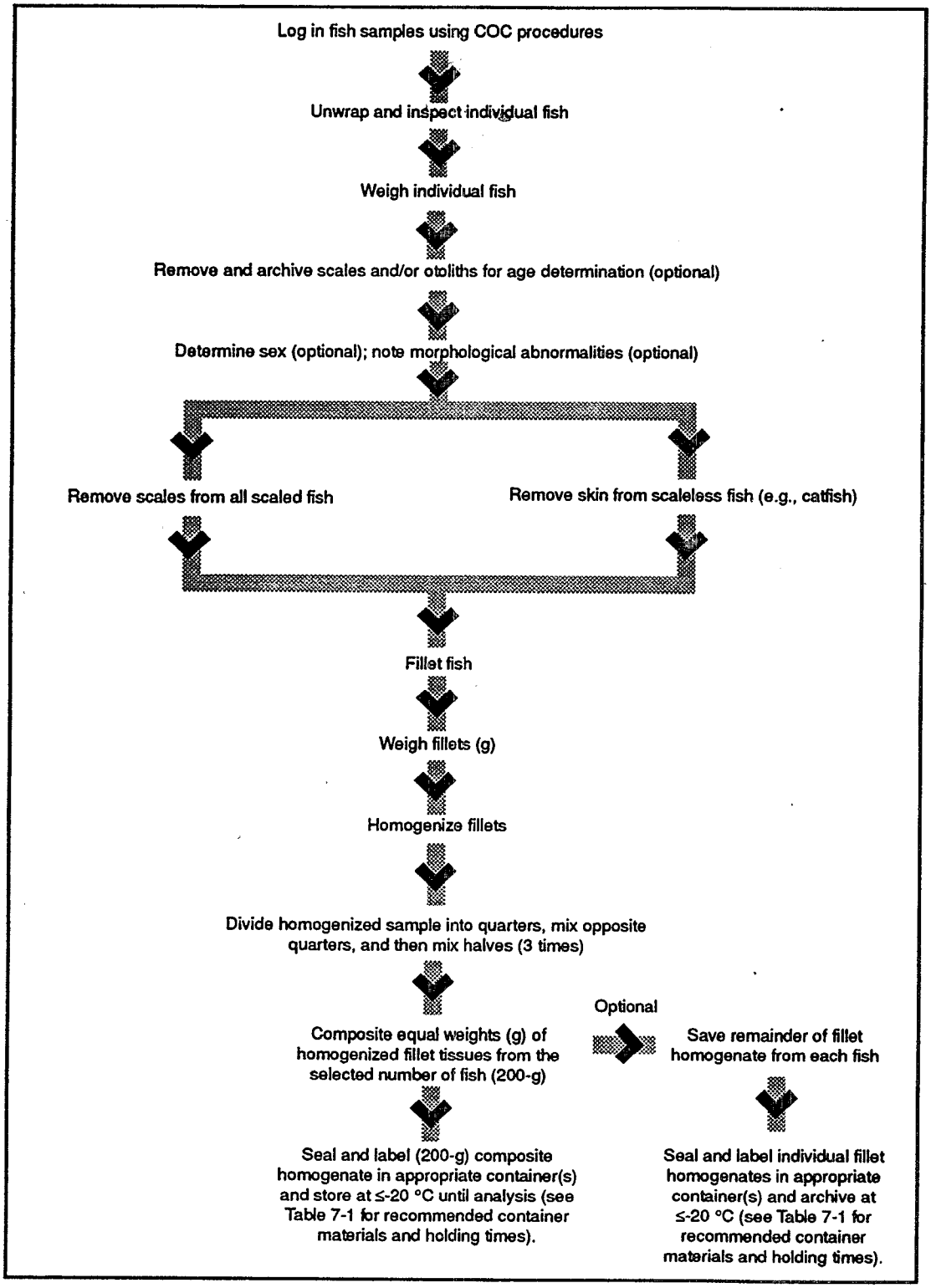
Prior to preparing each composite sample, utensils and containers should be cleaned thoroughly with a detergent solution, rinsed with tap water, soaked in 50% HNO_3, for 12 to 24 hours at room temperature, and then rinsed with organics- and metal-free water. **Note:** Chromic acid should not be used 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.



COC = Chain of custody.

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

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 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. An example 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.

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. Example of a sample processing record for fish contaminant monitoring program—fish fillet composites.

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 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—

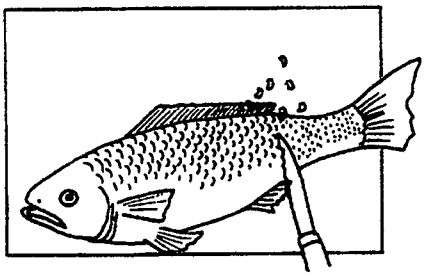
Filleting should be conducted only by or under the supervision of an experienced fisheries biologist. If gloves are worn, they should be talc- or dust-free, and of non- contaminating materials. Prior to filleting, hands should be washed with Ivory soap and rinsed thoroughly in tap water, followed by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

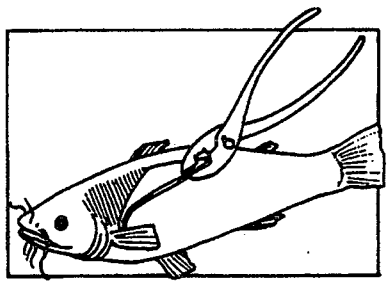
1 **Scaled Fish**

After removing the scales (by scraping with the edge of a knife) and rinsing the fish:



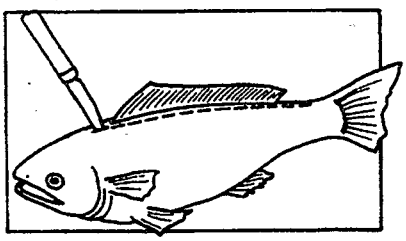
1b **Scaleless Fish**

Grasp the skin at the base of the head (preferably with pliers) and pull toward the tail.



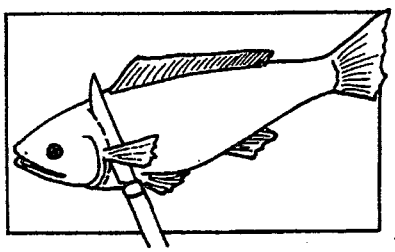
Note: This step applies only for catfish and other scaleless species.

2



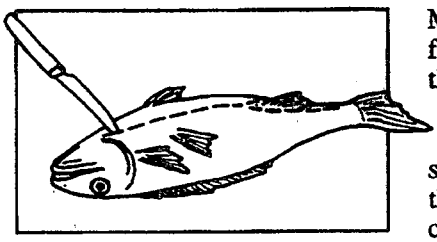
Make a shallow cut through the skin (on either side of the dorsal fin) from the top of the head to the base of the tail.

3



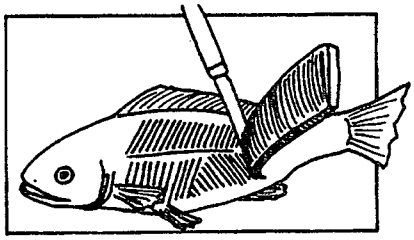
Make a cut behind the entire length of the gill cover, cutting through the skin and flesh to the bone.

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.

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.

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 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.

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).

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.

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. An example 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.

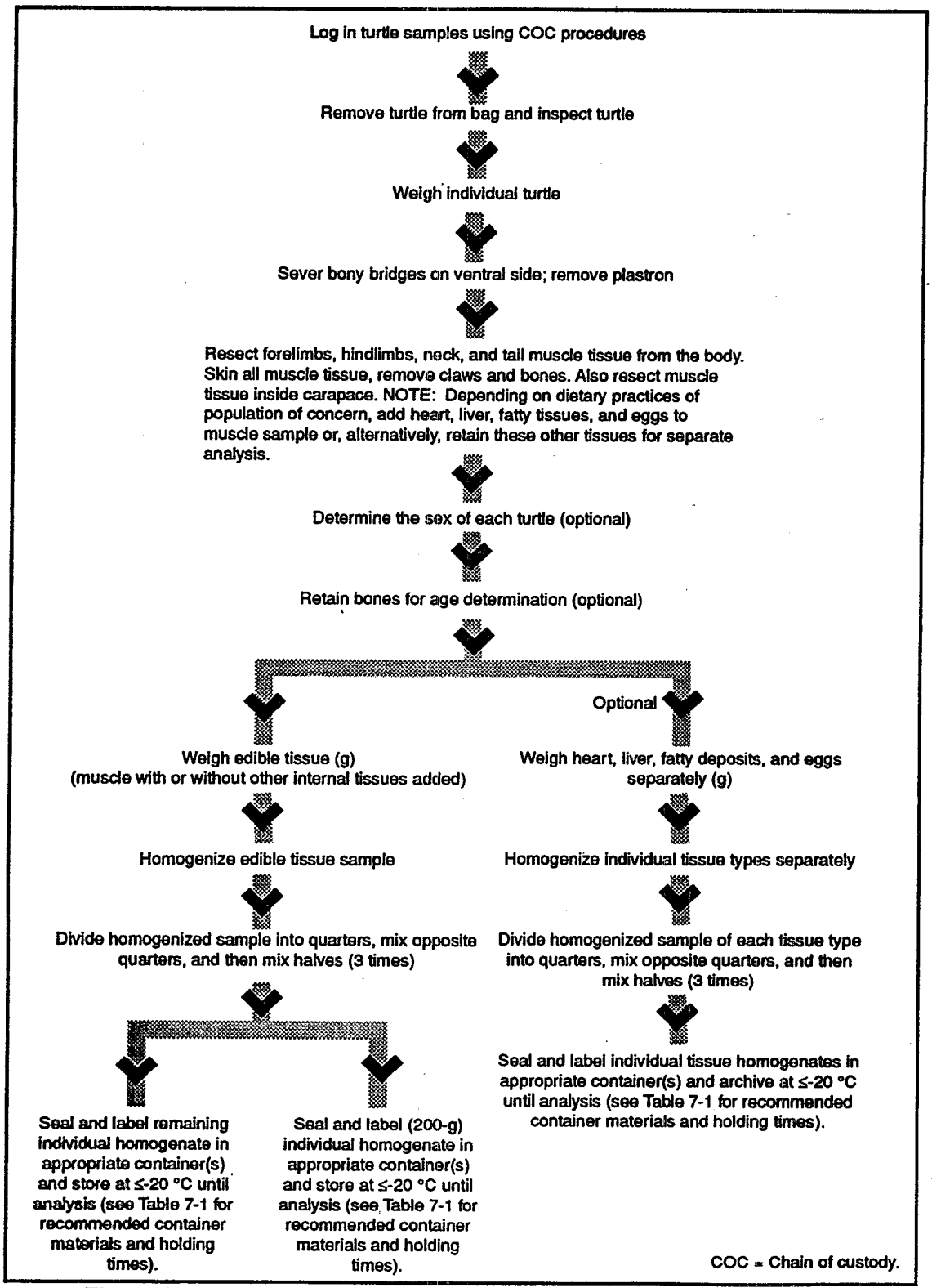


Figure 7-4. Preparation of individual turtle homogenate samples.

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. Example of a sample processing record for a contaminant monitoring program—individual turtle samples.

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.

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 I.

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 fore 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.

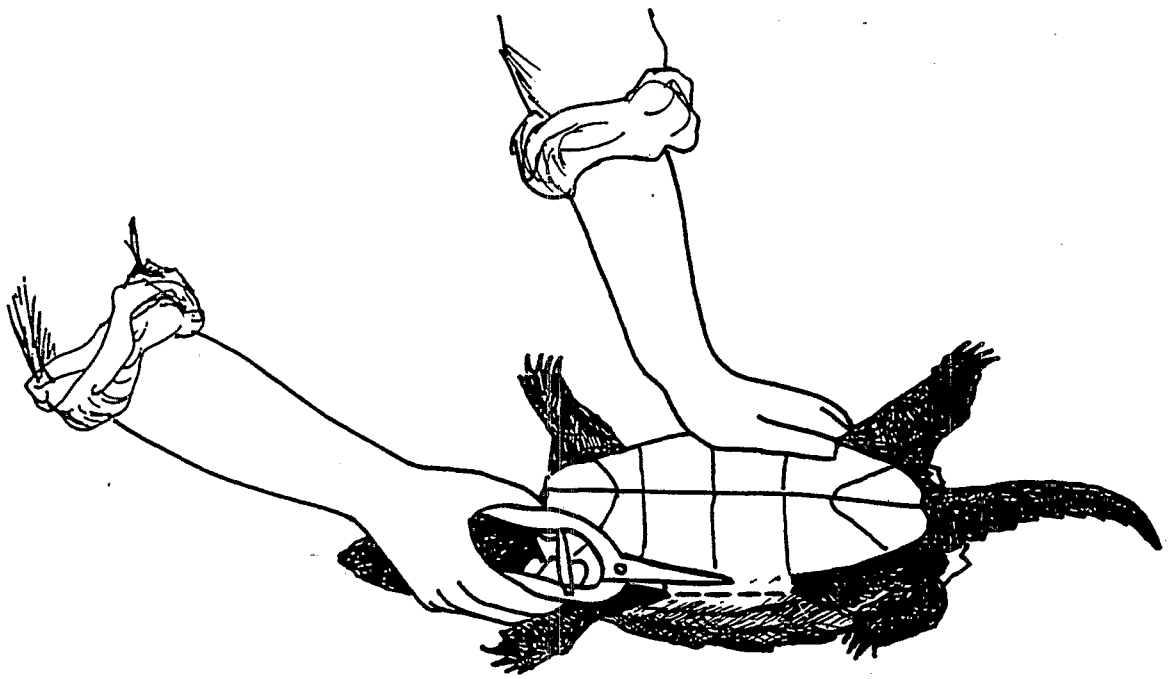
Skin on the forelimbs, hindlimbs, neck, and tail should be removed. Claws should be removed from the fore and hindlimbs. Bones still present in the muscle tissue after resection should be removed carefully (U.S. EPA, 1991d) and may be used in age determination (see Section 7.2.3.5).

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 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



Source: Hamerstrom, 1989.

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

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 I). 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 may be performed by visually examining the plastron of the turtle during the resection, or the plastron may be tagged with the sample identification number of the turtle and retained 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 ≤-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."

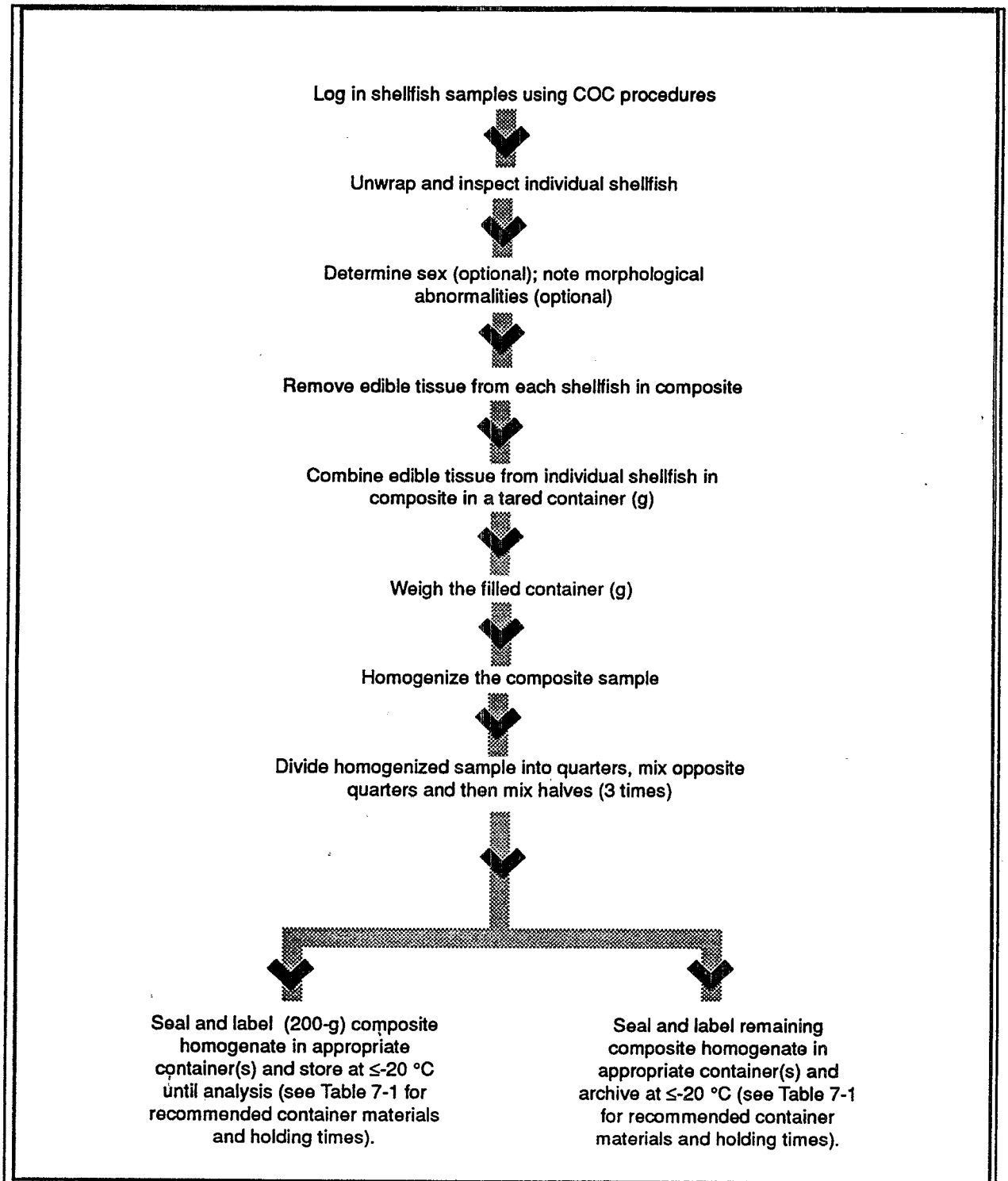
It is essential that the weight of individual homogenate samples is of adequate size to perform all necessary analyses. The recommended sample size of 200 g for screening studies is intended to provide sufficient sample material to (1) analyze for all recommended target analytes (see Table 4-1) at appropriate 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 particular, it is recommended that separate composite



COC = Chain of custody.

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

homogenates be prepared for the analysis of metals and organics if resources allow. An example 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 possesses 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

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. Example of a sample processing record for shellfish contaminant monitoring program—edible tissue composites.

Sinderman and Rosenfield (1967), Rosen (1970), and Murchelano (1982) for 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 I.

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.

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 for a routine sample that is split for analysis by a second laboratory; MS1 and MS2 for sample pairs, one of which will be prepared as a matrix spike). For example, the aliquot identification number may be of the form 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

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).

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:	

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

number that has not been used for an actual sample and to indicate clearly on the 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.

Fish and Shellfish Monitoring Program
Sample Transfer Record

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

Released by: _____
(name)

At: _____
(location)

Shipment Method _____

Shipment Destination _____

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

Received by: _____
(name)

At: _____
(location)

Comments _____

_____

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

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 95630
916/933-1640
FAX: 916/933-0940
Bill Luksemburg

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

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

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

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

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

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

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

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

Triangle Laboratories[b]
Alston Technical Park
801 Capitola Drive, Suite 10
Research Triangle Park, NC 27713
919/544-5729
Laurie White

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

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

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

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

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

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.

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, 1986b)
- 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)
- Determination of Butyltin and Cyclohexyltin Compounds in the Marine Environment by High-performance Liquid Chromatography-Graphite Furnace Atomic Absorption Spectrometry with Confirmation by Mass Spectrometry (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 (EMAP) 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, 1981b)
- 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, 1986f)
- 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, 1995c)
- 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, 1995d)
- U.S. EPA Method 1632: Determination of Inorganic Arsenic in Water by Hydride Generation Flame Atomic Absorption (U.S. EPA, 1995e)
- U.S. EPA Method 1637: Determination of Trace Elements in Ambient Waters by Chelation Preconcentration with Graphite Furnace Atomic Absorption (U.S. EPA, 1995f)
- U.S. EPA Method 1638: Determination of Trace Elements in Ambient Waters by Inductively Coupled Plasma-Mass Spectrometry (U.S. EPA, 1995g)
- U.S. EPA Method 1639: Determination of Trace Elements in Ambient Waters by Stabilized Temperature Graphite Furnace Atomic Absorption (U.S. EPA, 1995h)
- 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)

8.2.2 Target Analyte Methods

EPA has published interim procedures for sampling and analysis of priority pollutants in fish tissue (U.S. EPA, 1981b); however, at present, 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 (SVs). 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

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 and Appendix J, 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'-dihydroxyretonone, 6',7'-dihydro-6',7'-dihydroxyretonolone) 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 [BCF] = 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 K. 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

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 Arochlors)[e]	GC/ECD[f,g,h]
Organochlorine pesticides	GC/ECD[f,g]
Organophosphate pesticides	GC/MS, GC/FPD, or GC/NPD[i]
Chlorophenoxy herbicides	GC/ECD[f,g]
Dioxins/dibenzofurans	HRGC/HRMS[j,k]

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.
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, 1995).

[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, benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, chrysene, dibenz[*a,h*]anthracene, and indeno*[1,2,3-cd]*pyrene, and that the relative potencies given for these PAHs in the EPA provisional guidance for quantitative risk assessment of PAHs (U.S. EPA, 1993c) be used to calculate a potency equivalency concentration (PEC) for each sample for comparison with the recommended SV for benzo[a]pyrene (see Section 5.3.2.3). At this time, EPA's recommendation to use the PEC approach for risk assessment of PAHs (U.S. EPA 1993c) is considered provisional because quantitative risk assessment data are not available for all PAHs. This approach is under Agency review and over the next year will be evaluated as new health effects benchmark values are developed. Therefore, the method provided in this guidance document is subject to change pending results of the Agency's reevaluation.

(continued)

Table 8-3 (continued)

[e] Analysis of total PCBs, as the sum of Aroclor equivalents, is recommended in both screening and intensive studies because of the lack of adequate toxicologic data to develop screening values (SVs) for individual PCB congeners (see Section 4.3.5). However, because of the wide range of toxicities among different PCB congeners and the effects of metabolism and degradation on Aroclor composition in the environment, congener analysis is deemed to be a more scientifically sound and accurate method for determining total PCB concentrations. Consequently, States are encouraged to develop the capability to conduct PCB congener analysis.

[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] If PCB congener analysis is conducted, capillary GC columns are 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] Some of the chlorinated organophosphate pesticides (i.e., chlorpyrifos, diazinon, ethion) may be analyzed by GC/ECD (USGS, 1987).

[j] 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. Note: If resources are limited, at a minimum, 2,3,7,8-TCDD and 2,3,7,8-TCDF should be analyzed.

[k] 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.

their analysis (U.S. EPA, 1991f). At present, the EMMI database includes information on more than 2,600 analytes from over 80 regulatory and nonregulatory lists and more than 900 analytical methods in a variety of matrices, including tissue. When fully implemented, this database will provide 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. As of September 1995, a new version of EMMI will be 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) 487-4650
Fax: (703) 321-8547
Rush Orders: (800) 553-NTIS

The order number is PB95-50174B for a single user, PB95-502399B for a 5-user LAN package, and PB95-502407B for an unlimited user LAN package. Further information may be obtained by contacting:

EEMI User Support
U.S. EPA Sample Control Center
Operated by DynCorp EENSP
P.O. Box 1407
Alexandria, VA 22313
USA
Phone: (703) 519-1222
Fax: (703) 684-0610
Monday—Friday 8:00 a.m. to 5:00 p.m.

Internet: EMMIUSER@USVA5.DYNCORP.COM

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 may be expected to 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.

Table 8-4. Range of Detection and Quantitation Limits of Current Analytical Methods for Recommended Target Analytes[a]

Target analyte	SV[b]	Range of detection limits	Range of quantitation limits
Metals			
Arsenic (inorganic)	3 ppm	5-50 ppb[c]; 50-100 ppb[d]	—
Cadmium	10 ppm	0.005-0.046 ppm[e]; 0.4 ppm[f]	—
Mercury	0.6 ppm	0.0013-0.1 ppm[g]	—
Selenium	50 ppm	0.017-0.15 ppm[c]; 0.02 ppm[h]; 0.6 ppm[f]	—
Tributyltin	0.3 ppm	2.5 ppb[e]; 2-5 ppb[i]	—
Organochlorine Pesticides[j]			
Chlordane (total)	80 ppb		
cis-Chlordane		<1.5-5 ppb	2-20 ppb[j,k]
trans-Chlordane		<1.5-5 ppb	2-15 ppb
cis-Nonachlor		<1.5-5 ppb	2-15 ppb
trans-Nonachlor		<1.5-7 ppb	2-15 ppb
Oxychlordane		<1.5-5 ppb	2-15 ppb
DDT (total)	300 ppb		
4,4′-DDT		0.1-13 ppb	2-15 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	10,000 ppb	100 ppb	2.5 ppb
Dieldrin	7 ppb	0.1-5 ppb	2-15 ppb
Endosulfan (total)	60,000 ppb		
Endosulfan I		5 ppb	—
Endosulfan II		5-70 ppb	—
Endrin	3,000 ppb	<1-15 ppb	2-15 ppb[j,k]
Heptachlor epoxide	10 ppb	0.1-5 ppb	2-15 ppb[j,k]
Hexachlorobenzene	70 ppb	0.1-2 ppb	2-15 ppb[j,k]
Lindane	80 ppb	0.1-5 ppb	2-15 ppb[j,k]
Mirex	2,000 ppb	0.1-5 ppb	2-15 ppb[j,k]
Toxaphene	100 ppb	3-100 ppb	60-153 ppb
Organophosphate Pesticides[j]			
Chlorpyrifos	30,000 ppb	10 ppb	2.5 ppb[k]
Diazinon	900 ppb	50 ppb	—
Disulfoton	500 ppb	—	—
Ethion	5,000 ppb	20 ppb	—
Turbufos	10,000 ppb	—	—
Chlorophenoxy Herbicides			
Oxyfluorfen	800 ppb	—	—
PAHs[l]	10 ppb	10-100 ppb	330 ppb
PCBs[j] (total Aroclors)	10 ppb	50 ppb (20-62 ppb)[m]	— (110-170 ppb)[m]
Dioxins/furans[k] (total)	0.7 ppt		
TCDD/TCDF		1 ppt	—
PeCDD/PeCDF		2 ppt	—
HxCDD/HxCDF		4 ppt	—
HpCDD/HpCDF		10 ppt	—

PAHs = Polycyclic aromatic hydrocarbons. PCBs = Polychlorinated biphenyls. SV = Screening value (wet weight).

(continued)

Table 8-4 (continued)

[a] Wet weight. Summarized from Appendix H.

[b] From Table 5-2. Except for mercury, SVs are for general adult population using RfDs or oral slope factors available in the EPA IRIS database and assuming a consumption rate (CR) = 6.5 g/d, average body weight (BW) = 70 kg, lifetime (70-yr) exposure, and, for carcinogens, a risk level (RL) = 10^{-5}. The RfD of $3x10^{-4}$ mg/kg/d for chronic systemic effects of methylmercury that was listed in IRIS through April 1995 was lowered by a factor of 5 to calculate the recommended SV of 0.6 ppm in order to account for a possible fivefold increase in fetal sensitivity to methylmercury exposure (WHO, 1990). This approach is consistent with, but somewhat more protective than, use of the current IRIS (1995) RfD of $1x10^{-5}$ mg/kg/d for the developmental effects of methylmercury (see Section 5.3.1.2). This approach should be considered interim until such time as the Agency has reviewed new studies on the chronic and developmental effects of methylmercury. **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., 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, June 1995).. .

[d] Analysis by high-performance liquid chromatography/mass spectrometry (HPLC/MS) (E. Crecelius, Battelle Pacific Northwest Laboratories, Marine Sciences Laboratory, Sequim, WA, personal communication, June 1995).

[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, June 1995).

[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, June 1995).

[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.

Table 8-5. Approximate Range of Costs per Sample for Analysis of Recommended Target Analytes[a]

Target analyte	Approximate cost range (1992 $)
Metals[b]	
Arsenic (inorganic)[c]	150 - 300
Cadmium	25 - 50
Mercury	35 - 50
Selenium	25 - 50
Tributyltin[d]	150 - 350
Organochlorine pesticides[e,f]	285 - 500
Organophosphate pesticides[g]	250 - 500
Chlorophenoxy herbicides[h]	250 - 500
PAHs[i]	250 - 525
PCBs[e]	
Total Aroclors	210 - 500
Dioxins/furans[j]	
TCDD/TCDF only	200 - 1,000
TCDD/TCDF through OCDD/OCDF isomers	450 - 1,600
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 $150 to $200. Estimated cost of analysis by HPLC-ICP/MS is $250 to $300.

[d] Estimated cost of analysis by GFAA is $150 to $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 (i.e., chlorpyrifos, diazinon, ethion) 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 $800 per sample.

[j] Analysis by HRGC/HRMS.

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 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 E.) 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.

- 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).

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

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.

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.

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.

(continued)

Table 8-6 (continued)

Sample type (definition; specifications)	Objective	Recommended frequency of analysis[a]	Recommended control limits[b]	Recommended corrective action
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.
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.

(continued)

Table 8-6 (continued)

Sample type (definition; specifications)	Objective	Recommended frequency of analysis[a]	Recommended control limits[b]	Recommended corrective action
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.

(continued)

Table 8-6 (continued)

Sample type (definition; specifications)	Objective	Recommended frequency of analysis[a]	Recommended control limits[b]	Recommended corrective action
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, EPA-CRMs are recommended for routine use as laboratory control samples; see Appendix I)	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.
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.

(continued)

Table 8-6 (continued)

Sample type (definition; specifications)	Objective	Recommended frequency of analysis[a]	Recommended control limits[b]	Recommended corrective action
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*: |RPD| ≤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*: |RPD| ≤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.

(continued)

Table 8-6 (continued)

Sample type (definition; specifications)	Objective	Recommended frequency of analysis[a]	Recommended control limits[b]	Recommended corrective action
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.
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.[g]	Determined by program manager.[g]	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.[g]	Determined by program manager.

(continued)

Table 8-6 (continued)

Sample type (definition; specifications)	Objective	Recommended frequency of analysis[a]	Recommended control limits[b]	Recommended corrective action
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 labelled 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 (1986f, 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 I).

[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).

Table 8-7. Minimum Recommended QA and QC Samples for Routine Analysis of Target Analytes[a]

Sample Type	Target analyte	
	Metals	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.

- 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 EPA-certified and other recommended reference materials for the analysis of priority pollutants and selected related compounds in fish and shellfish tissues are given in Appendix L. Currently 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 EPA-certified standards should be used for calibration standards (see Section 8.3.3.4 and Appendix I). 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.

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

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-1974a[a]	Organic compounds	NIST	Mussel tissue (frozen)
SRM-2974[a]	Organic compounds	NIST	Mussel tissue (freeze-dried)
NIES-6	Elements	NIES	Mussel tissue

[a] Certification in progress as of June 1995. SRM-1974a is a renewal of SRM-1974, which was issued in 1990.

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, Chacyangqu, 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.

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 ≤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 relative standard deviation (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)

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 and Appendix H 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., 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. Appendix H clearly illustrates the widespread inconsistency in defining and reporting limits of detection and quantitation. 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 (MDL)**: 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):

$$MDL = (t_{0.99})\ (S). \qquad (8\text{-}3)$$

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:

$$MDL_{tissue}\ (\text{ppm or ppb}) = (MDL_{extract} \bullet V)\ /W \qquad (8\text{-}4)$$

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 GG/MS methods used to analyze organic pollutants in bioaccumulation monitoring programs (U.S. EPA, 1986b). 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.

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:

$$\text{PQL } (\mu g/g;\text{ppm}) = \frac{C\,(\mu g/mL) \cdot V\,(mL)}{W\,(g)} \quad . \qquad (8\text{-}5)$$

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 and Appendix I), **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 and Appendix I). 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:

$$\% \text{ Recovery} = 100 \, (M/T) \qquad (8\text{-}6)$$

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:

$$\% \text{ Recovery} = [(M_s - M_u)/T_s] \times 100 \qquad (8\text{-}7)$$

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 which 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 (RSD) or coefficient of variation (CV) for multiple samples, and the relative percent 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

$$RPD = 100 \{(x_1 - x_2)/[(x_1 + x_2)/2]\} \quad . \qquad (8\text{-}9)$$

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 8-7). 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 (see next page) 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., ≤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.

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_a = 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:

$$N = 16\,(RT/W)^2 \qquad (8\text{-}11)$$

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 the 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. In 1993, the estimated cost of participation in the NIST Intercomparison Exercise Program for Organic Contaminants in the Marine Environment will be $2,000 and $2,300 for private laboratories within and outside the United States, respectively. This cost covers samples for one exercise per year. Samples may be obtained directly from NIST by contacting Ms. Reenie Parris, NIST, Chemistry B158, Gaithersburg, MD 20899; Tel:301-975-3103, FAX:301-926-8671. At present, the cost of participation in trace inorganic exercises by private laboratories has not been established. Once this cost has been set, trace inorganic samples will be available directly from NRCC.

To obtain additional information about participation in the NS&T QA program, contact Dr. Adriana Cantillo, QA Manager, NOAA/National Status and Trends Program, N/ORCA21, Rockville, MD 20852, Tel: 301-443-8655.

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, 1995i) 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 and (2) data reporting requirements for a national database (National Fish Tissue Data Repository) for fish and shellfish contaminant monitoring programs.

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 E). 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 (SV) 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 (MDL), including a datum reported as not detected (i.e., ND, no observed response) should be assigned a value of one-half the MDL.

- A datum reported between the MDL and the method quantitation limit (MQL) 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 (SVs); 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 M.

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.

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 9-2.

9.2.2 Reports to the National Fish Tissue Data Repository

The EPA Office of Science and Technology within the Office of Water has established a NFTDR. The NFTDR is a collection of fish and shellfish contaminant monitoring data gathered by various Federal, State, and local agencies. The objectives of the NFTDR are to:

- Facilitate the exchange of fish and shellfish contaminant monitoring data nationally by improving the comparability and integrity of the data
- Encourage greater cooperation among regional and State fish advisory programs
- Assist States in their data collection efforts by providing ongoing technical assistance.

The NFTDR is currently part of the EPA's Ocean Discharge Evaluation System (ODES) database, a primary source for maintaining, retrieving, and analyzing freshwater, estuarine, and marine data. The EPA Office of Water selected the ODES database to serve as a national repository for fish and shellfish contaminant monitoring data for both inland and coastal waters. Unfortunately,

- Study identification (e.g., project number, title, and study type)
- Program manager
- Sampling site name
- Latitude (in degrees, minutes, and seconds)
- Longitude (in degrees, minutes, and seconds)
- Type of waterbody (lake, river, estuary, etc.)
- Name of waterbody
- Sampling date (e.g., DD, MM, YY)
- Sampling time (e.g., HH, MM in a 24-h format)
- Sampling gear type used (e.g., dredge, seine, trawl)
- Sampling depth
- Scientific name of target species
- Common name of target species
- 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 or size (mm)
  - Description of edible portion (tissue type)

(continued)

Figure 9-1. Recommended data reporting requirements for screening and intensive studies.

- Analytical methods used (including method for lipid analysis)
- Method detection and quantitation limits for each target analyte
- 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
  - 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 and indication of whether SV was exceeded (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 and indication of whether SV was exceeded.

Figure 9-1 (continued)

ODES has not evolved into a widely used database and there is relatively little fish and shellfish contaminant monitoring data currently stored in the NFTDR. To make this database more accessible, EPA intends to modify the existing NFTDR and incorporate it as a major prototype during the modernization (Phase III) of the STORET database. During prototype development, EPA will use actual fish contaminant monitoring data in ODES to identify needed data fields, to test the data structure, and to develop the necessary data analysis programs in the STORET database. During 1996, EPA intends to completely convert the NFTDR to a STORET-based fish contaminant monitoring database. The primary benefit of including the NFTDR as a subset of STORET is that one platform will be able to store both water quality data and biological data, such as fish and shellfish contaminant monitoring data. Existing data sets would be able to easily migrate to the new STORET system when it is completed in 1997.

State, regional, and local agency staff may obtain more information by writing to

National Fish Tissue Data Repository
U.S. Environmental Protection Agency
401 M Street, SW
Washington, DC 20460

SECTION 10

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APPENDIX A

USE OF INDIVIDUAL SAMPLES IN FISH CONTAMINANT MONITORING PROGRAMS

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USE OF INDIVIDUAL SAMPLES IN FISH CONTAMINANT MONITORING PROGRAMS

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 the mean contaminant concentration compared to the contaminant screening values. 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.

Analyzing individual fish incurs additional expenses, particularly when one considers that a number of individual analyses are required to achieve measurements of a reasonable statistical power. However, the recommendation that States archive the individual fish homogenates from which composite samples are prepared for both screening and intensive studies (see Section 6.1.1.6) would make it possible to perform individual analyses where needed without incurring additional sampling costs.

Individual analysis is especially well-suited for intensive studies, in which results from multiple stations and time periods are to be compared. The remainder of this appendix discusses how the sampling design might be affected by analyzing individual rather than composite samples and how contaminant data from individuals versus composites might be used in risk assessments.

A.1 SAMPLING DESIGN

There are seven major components of the sampling design for a fish or shellfish monitoring program: site selection, target species, target analytes, target analyte screening values (SVs), sampling time, sampling type and size class, and replicate samples. Of these, only the number of replicate samples and possibly the

target species would be expected to differ if individual samples were analyzed rather than composites. Target species becomes a limiting factor when individuals of the target species are not large enough to provide adequate tissue mass for all the required chemical analyses.

The five factors that determine the optimal number of fish or shellfish to analyze are presented in Section 6.1.2.7. Briefly, the five factors are:

- Cost components
- Minimum detectable difference between site-specific mean target analyte concentration and SV
- Level of significance
- Population variance
- Power of the hypothesis test

Each of these characteristics will be examined in detail for the collection and analysis of individual samples.

A.1.1 Cost Components

The cost of obtaining contaminant data from individual fish or shellfish is compared to the cost of obtaining contaminant data from composite samples in Table A-1. These costs are dependent on the separate costs of collecting, preparing, and analyzing the samples.

Typically, the cost of collecting individual samples will be less than that of collecting composite samples when the target species is scarce or difficult to capture. The cost of collecting individuals may not be a factor if the sample

Table A-1 Relative Cost of Obtaining Contaminant Data from Individual Versus Composite Samples

	Relative cost	
Cost component	**Composite samples**	**Individual samples**
Collection	Moderate to high	Low to moderate
Preparation	Very low to moderate	Very low to low
Analysis	Low to moderate	Moderate to high

collection method used typically allows for the collection of a large number of individuals in a short period of time. In some situations, seines or gill nets might have this characteristic. Also, in estuaries, coastal water, or large lakes where productivity is high, the additional cost of collecting large numbers of individuals for composite sampling may be minimal compared to the effort expended for collecting individual samples.

The cost of preparing individual samples for analysis is typically lower than either the costs of collection or analysis. Generally, the cost of preparing composite samples for analysis will be greater than that of preparing individual samples. Sample preparation procedures can range in complexity from the grinding of whole fish to delicate and time-consuming operations to resect specific tissues. Costs of composite sampling depend largely on the number of individuals required per composite sample and the number of replicate composite samples required to achieve the desired statistical power; however, these costs can be somewhat controlled (see Section 6.1.2.7).

The cost of analyzing individual samples is also typically higher than the cost of analyzing composite samples. The cost differential between the two approaches is directly correlated to the cost for the analysis of a single sample. For some intensive studies, the number of target analytes exceeding the SV is small, so few analyses are required. In these cases, the relative costs between the two approaches may not differ greatly if the number of samples analyzed using the two different approaches is similar (e.g., three to five samples). A sampling design with such a small number of individual samples would be appropriate only if the expected mean target analyte concentration was much greater than the SV.

A.1.2 Minimum Detectable Difference

The difference between the mean target analyte concentration at a site and the SV will not often be known before the screening study has been performed. The minimum detectable difference between the mean concentration and the SV will depend on the level of significance (see Section A.1.3), population variance (Section A.1.4), and the number of replicates collected. In practice, the sample size is often determined by establishing the minimum detectable difference prior to the study according to the objectives of the project. For an SV that has not been multiplied by an uncertainty factor, the cost of detecting a 10 percent difference may be warranted. The issue of minimum detectable difference is discussed in greater detail in Section A.1.5.

A.1.3 Level of Significance

The level of significance (LS) refers to the probability of incorrectly rejecting the null hypothesis, that there is no difference between the mean target analyte concentration and the SV. This probability is also called Type I error. The LS can be thought of as the chance of a "false positive" or of detecting a difference that does not exist. The LS affects the sampling design by modifying the required

power (thus impacting the sample size) of the statistical test to detect a significant difference between the mean target analyte concentration and the SV (see Section A.1.5). A typical LS used in biological sampling is 0.05. In some cases, an LS other than 0.05 could be appropriate. If the ramifications of a statistically significant difference are severe, a more conservative LS (e.g. 0.01) might be used. On the other hand, if the statistical test is being conducted to identify whether additional sampling should be performed (i.e., a screening survey), then a less conservative LS (e.g. 0.10) might be used.

A.1.4 Population Variance

The variability in target analyte concentrations within a given fish or shellfish population is a critical factor in determining how many individual samples to collect and analyze. The population variance directly affects the power of the statistical test to detect a significant difference between the mean target analyte concentration and the SV (see Section A.1.5) by impacting the sample size. The population variance may not be known prior to sampling, but it can be estimated from similar data sets from the same target species, which could in many cases be obtained by analyzing individual fish homogenates if these have been archived as recommended in Section 6.1.1.6. In using historical data to estimate population variance, it is important to consider contaminant data only from individual fish or shellfish of the same species. By its very nature, a data set consisting of replicate composite samples tends to smooth out the variability inherent in a group of individual organisms. An extreme example of this phenomenon was presented by Fabrizio et al. (1995) in a study on procedures for compositing fish samples. They used computer simulations to predict PCB concentrations in composite samples of striped bass that had previously been analyzed individually. The predicted variance in these concentrations in the composite samples was approximately 20 percent of the variance obtained from individual analyses.

A.1.5 Power of Statistical Test

Another critical factor in determining the sample size is the power of the statistical test, that is, the probability of detecting a true difference between the mean target analyte concentration and the SV. Because of its profound influence on sample size, it is the power of the test that may ultimately control whether the objectives of the survey are met. The effect of joint consideration of the desired power, the population variance, and the minimum detectable difference on the sample size is described by the following formula (Steel and Torrie, 1980):

$$n = \frac{(Z_\alpha + Z_\beta)^2 \, 2\sigma^2}{\delta^2}$$

where

n = sample size
Z_α = Z statistic for Type I error (α)
Z_β = Z statistic for Type II error (β)
σ^2 = population variance (estimated from historical data)
δ = minimum detectable difference between mean target analyte concentration and SV.

Recall that the Type I error is equal to the LS, and the value is generally between 0.01 and 0.10. Type II error is the probability of accepting the null hypothesis (that there is no difference between the mean target population concentration and the SV) when it is actually false. This type of error can be thought of as the chance of a "false negative," or not detecting a difference that does in fact exist. The complement of Type II error (1-β) is the power of the statistical test.

The above equation for determining sample size was solved for powers ranging from 0.5 to 0.9 (50 to 90 percent; Figure A-1) assuming an LS of 0.05. The values for σ (standard deviation) and δ were set relative to the SV. A similar exercise was performed in Section 6.1.2.7 and two examples were provided. In example A, both the standard deviation and minimum detectable difference were set to 0.5 SV. Example A corresponds to a ratio of 1 on the x-axis of Figure A-1. Applying example A to the collection of individual fish, the recommended sample size would range from approximately 6 individual samples for a power of 50 percent to 18 individual samples for a power of 90 percent (Figure A-1). In example B, the standard deviation was set to 1.0 SV, while the minimum detectable difference was kept at 0.5 SV. Example B corresponds to a ratio of 2 on the x-axis of Figure A-1. Applying example B to the collection of individual samples, the sample size would have to be almost 40 individual samples to achieve even a modest statistical power (i.e., 70 percent).

It is common to set the power of the statistical test to at least 80 percent (Fairweather, 1991). Figure A-1 indicates that, to achieve a statistical power of 80 percent using the variability assumptions in examples A and B, 13 and 50 fish would have to be collected, respectively. The estimated sample sizes for individual fish or shellfish is similar to those calculated for composite samples (see Section 6.1.2.7). For example A as applied to composite samples, 12 to 18 fish would have to be collected. For example B as applied to composite samples, 30 to 50 fish would have to be collected. Thus, the cost of collecting the fish to achieve a power of 80 percent would not be significantly different for composite versus individual samples (see Section A.1.1). The number of

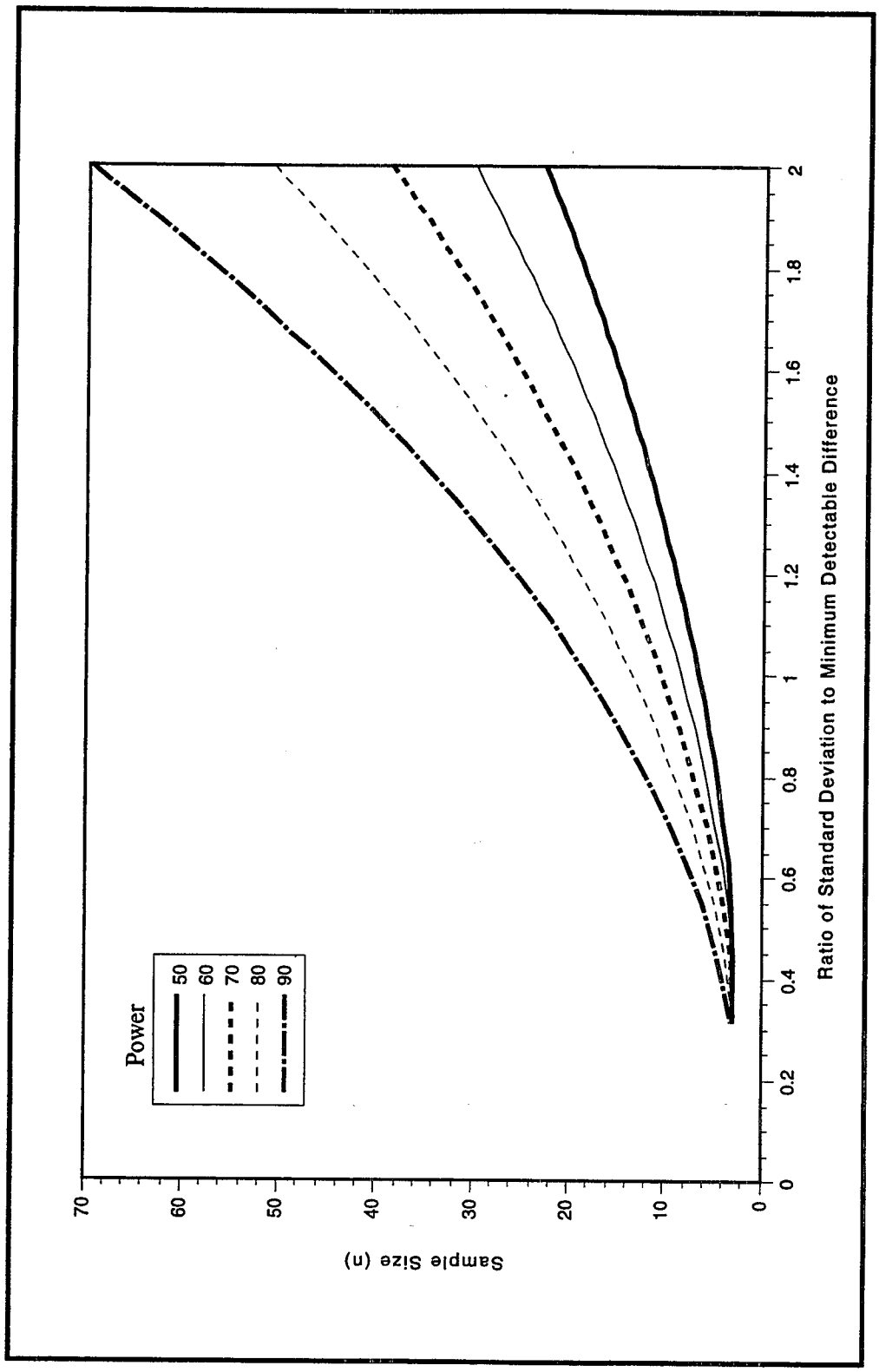

Figure A-1. Recommended sample sizes to achieve various statistical powers.

analyses, however, would be considerably less for composite samples (3 to 10 analyses of composite samples versus 13 or 50 analyses of individual samples).

Figure A-1 also indicates that 10 or fewer individual fish or shellfish should be analyzed only if the ratio of the standard deviation to the minimum detectable difference is 0.85 or less. For ratios less than 0.5, the effect of sample size on the statistical power is minor. If the expected mean target analyte concentration is many times greater than the SV, it may not be necessary to allocate resources toward the collection and analysis of more than a minimum number (e.g., three to five samples) of individual fish or shellfish.

A.2 USE OF CONTAMINANT DATA FROM INDIVIDUAL FISH/SHELLFISH IN RISK ASSESSMENTS

Target analyte concentrations in composite samples represent averages for specific target species populations. The use of these values in risk assessments is appropriate if the objective is to estimate the average concentration to which consumers of the target species might be exposed over a long period of time. The use of long exposure durations (e.g., 30 to 70 years) is typical of the assessment of carcinogenic target analytes, the health effects of which may be manifested over an entire lifetime (see Volume II of this series). Target analytes that produce noncarcinogenic effects, on the other hand, may cause acute effects to human health over a relatively short period of time on the order of hours or days. The use of average contaminant concentrations derived from the analysis of composite samples may not be protective against acute health effects because high concentrations in an individual organism may be masked by lower concentrations in other individuals in the composite sample. Contaminant data from individual samples permits the use of alternative estimates of contaminant concentration for a group of fish or shellfish (e.g., maximum). Therefore, the decision whether to collect and analyze individual fish or shellfish may depend on the target analytes included in the monitoring program.

EPA has recommended that 25 target analytes be included in screening studies (see Section 4). All of the target analytes except PCBs, PAHs, and dioxins/furans have reference doses for noncarcinogenic health effects, although the carcinogenic risk is likely to be greater than the noncarcinogenic risk for eight other target analytes (see Table 5-2). EPA's draft reassessment of the health effects of 2,3,7,8-TCDD (dioxin) indicated that this chemical may also pose a significant noncarcinogenic health risk in some cases (U.S. EPA, 1994).

A.3 EXAMPLE CASE STUDY

The presentation of a case study will illustrate some of the sample size and data interpretation issues discussed in Sections A.1 and A.2, respectively. A State has prepared a composite sample of target species A from a particular water-body of concern. This composite sample was analyzed for all 25 target analytes listed in Table 4-1. Of the 25 target analytes, only cadmium was detected at a concentration exceeding the SV (10 ppm) for cadmium listed in Table 5-2.

Cadmium was detected at 20 ppm, twice the SV calculated for cadmium. Because the SV for at least one target analyte was exceeded, an intensive study was warranted. The State decided to collect and analyze individual fish in the intensive study for the following reasons: (1) the cost of collecting individual fish is less than the cost of collecting fish for composites, (2) the analytical costs for analyzing cadmium are relatively low (<$50 sample), and (3) the cadmium concentrations in individual fish should more accurately reflect the potential acute (noncarcinogenic) health risk from cadmium than the mean cadmium concentration derived from composite samples.

The first issue the State must decide is how many individual fish to collect and analyze. The important factors in this decision are the minimum detectable difference the State wishes to test and the variability in cadmium concentrations within the target species population. The first factor can be obtained from the results of the screening survey. The State wishes to test whether the difference between the concentration detected in the single composite sample (20 ppm) and the SV (10 ppm) is significant. This assumes that the mean cadmium concentration for the individual is also 20 ppm. The expected standard deviation (8 ppm) was obtained from a previous investigation performed on individuals of the target species and was equal to 0.8 of the SV (10 ppm). Using Figure A-1, it can be seen that, for a ratio of standard deviation (0.8 x SV) to detectable difference (1.0 x SV) of 0.8, the sample size necessary to achieve a statistical power of 80 percent would be eight fish.

The State determines that the mean cadmium concentration of eight individual fish of the target species is 30 ppm and the standard deviation is equal to the predicted value of 8 ppm. The State performs a *t*-test to determine if the mean concentration is significantly greater than the SV. As described in Section 6.1.2.7, the statistic

(mean - SV)/standard deviation

has a *t*-distribution with n-1 degrees of freedom. For this example, the *t* statistic is 2.5 ([(30-10)/8] with 7 degrees of freedom. This value exceeds the critical t-statistic (1.895) for a one-tailed LS of 0.05. Therefore, the State determines that the mean cadmium concentration for these eight individual fish of the target species is significantly greater than the SV and a risk assessment is performed.

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APPENDIX B

FISH AND SHELLFISH SPECIES FOR WHICH STATE CONSUMPTION ADVISORIES HAVE BEEN ISSUED

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FISH AND SHELLFISH SPECIES FOR WHICH STATE CONSUMPTION ADVISORIES HAVE BEEN ISSUED

FRESHWATER FINFISH SPECIES FOR WHICH STATE CONSUMPTION ADVISORIES HAVE BEEN ISSUED

AL catfish (unspecified), fish species (unspecified), bigmouth buffalo, brown bullhead, channel catfish, white bass

AK no consumption advisories

AS no consumption advisories

AZ fish species (unspecified)

AR fish species (unspecified)

CA goldfish, Sacramento blackfish, brown bullhead, crappie (unspecified), hitch, common carp, largemouth bass, smallmouth bass, channel catfish, white catfish, rainbow trout, croaker (unspecified), orangemouth corvina, sargo, tilapia (unspecified), squawfish, sucker (unspecified), trout (unspecified), fish species (unspecified)

CO rainbow trout, yellow perch, northern pike, walleye, smallmouth bass, largemouth bass, black crappie, kokanee salmon, channel catfish, trout (unspecified), fish species (unspecified)

CT common carp and fish species (unspecified)

DE white catfish, channel catfish, fish species (unspecified)

DC channel catfish, common carp, American eel

FL largemouth bass, gar, bowfin, warmouth, yellow bullhead, Mayan cichlid, oscar, spotted sunfish

GA common carp, largemouth bass, catfish (unspecified), fish species (unspecified)

GU no consumption advisories

HI no consumption advisories

ID no consumption advisories

IL lake trout, coho salmon, chinook salmon, brown trout, common carp, catfish (unspecified), bigmouth buffalo, channel catfish, flathead catfish, smallmouth buffalo, shovelnose sturgeon, bluegill, crappie (unspecified), freshwater drum, largemouth bass, spotted bass, alewife

IN common carp, catfish (unspecified), coho salmon, brown trout, lake trout, chinook salmon, channel catfish, fish species (unspecified)

IA channel catfish, common carp, carpsucker (unspecified), fish species (unspecified)

KS buffalo (unspecified), catfish (unspecified), common carp, freshwater drum, sturgeon (unspecified), carpsucker (unspecified)

KY channel catfish, paddlefish, white bass, common carp, fish species (unspecified)

LA bass (unspecified), fish species (unspecified)

ME fish species (unspecified)

MD channel catfish, American eel, black crappie, common carp, bullhead (unspecified), sunfish (unspecified)

MA brown trout, yellow perch, white sucker, American eel, smallmouth bass, largemouth bass, lake trout, channel catfish, brown bullhead, common carp, white catfish, fish species (unspecified)

MI common carp, rock bass, crappie (unspecified), yellow perch, largemouth bass, smallmouth bass, walleye, northern pike, muskellunge, sauger, white bass, longnose sucker, white perch, carpsucker (unspecified), brown bullhead, bullhead (unspecified), bluegill, freshwater drum, sturgeon (unspecified), brown trout, ciscowet, lake trout, coho salmon, chinook salmon, splake, catfish (unspecified), rainbow trout, brook trout, sucker (unspecified), gizzard shad, freshwater drum, white sucker, lake whitefish

MN yellow perch, brown bullhead, black bullhead, yellow bullhead, quillback carpsucker, brown trout, brook trout, lake trout, chinook salmon, ciscowet, walleye, northern pike, brook trout, muskellunge, splake, smallmouth bass, largemouth bass, rock bass, white bass, rainbow trout, white sucker, tullibee, bluegill, black crappie, white crappie, shorthead redhorse, silver redhorse, common carp, smallmouth buffalo, redhorse sucker, sauger, bigmouth buffalo, channel catfish, lake whitefish, freshwater drum, pumpkinseed, chub bloater, lake herring, flathead catfish, bowfin

MS fish species (unspecified), catfish (unspecified), buffalo (unspecified)

MO sturgeon (unspecified), common carp, channel catfish, buffalo (unspecified), flathead catfish, sucker (unspecified), paddlefish, catfish (unspecified), redhorse, freshwater drum

MT fish species (unspecified)

NE common carp, channel catfish

NV fish species (unspecified)

NH fish species (unspecified)

NJ striped bass, American eel, white perch, white catfish, fish species (unspecified)

NM white crappie, channel catfish, common carp, brown trout, river carpsucker, kokanee salmon, largemouth bass, bluegill, white bass, walleye, white sucker, yellow perch, black bullhead, black crappie, bass (unspecified), crappie (unspecified), rainbow trout, longnose dace, walleye, northern pike, trout (unspecified), carpsucker (unspecified), bullhead (unspecified), black bass

NY common carp, lake trout, brown trout, yellow perch, smallmouth bass, splake, American eel, goldfish, striped bass, white perch, bluefish, largemouth bass, brown bullhead, white catfish, walleye, rainbow smelt, tiger muskellunge, white sucker, northern pike, chinook salmon, coho salmon, rainbow trout

NC largemouth bass, fish species (unspecified)

ND walleye, white bass, yellow perch, northern pike, bigmouth buffalo, common carp, crappie (unspecified), bullhead (unspecified), white sucker, channel catfish, goldeye, chinook salmon, sauger, carpsucker (unspecified), sunfish (unspecified), smallmouth bass

OH common carp, catfish (unspecified), white bass, sucker (unspecified), fish species (unspecified)

OK channel catfish, largemouth bass, fish species (unspecified)

OR fish species (unspecified), crayfish

PA white sucker, white perch, common carp, American eel, channel catfish, goldfish, largemouth bass, green sunfish, quillback carpsucker, white bass, lake trout, walleye, smallmouth bass, shorthead redhorse, sucker (unspecified), fish species (unspecified)

PR no fish consumption advisories

RI striped bass

SC fish and shellfish species (unspecified)

SD no fish consumption advisories

TN catfish (unspecified), largemouth bass, crappie (unspecified), common carp, rainbow trout, striped bass, sauger, white bass, smallmouth buffalo, fish species (unspecified)

TX catfish (unspecified), fish species (unspecified)

UT fish species (unspecified)

VT brown trout, lake trout, walleye

VA fish species (unspecified)

VI no fish consumption advisories

WA no fish consumption advisories

WV channel catfish, brown bullhead, common carp, sucker (unspecified), fish species (unspecified)

WI lake trout, coho salmon, chinook salmon, brown trout, common carp, catfish (unspecified), splake, rainbow trout, brook trout, lake trout, ciscowet, northern pike, white bass, white sucker, walleye, yellow perch, muskellunge, flathead catfish, freshwater drum, channel catfish, bullhead (unspecified), bluegill, black crappie, crappie (unspecified), rock bass, smallmouth bass, redhorse (unspecified), largemouth bass, lake sturgeon, buffalo (unspecified), fish species (unspecified)

WY no fish consumption advisories

Source: RTI, 1993. *National Listing of State Fish and Shellfish Consumption Advisories and Bans.* (Current as of July 22, 1993.) Research Triangle Institute, Research Triangle Park, NC.

ESTUARINE/MARINE FISH AND SHELLFISH SPECIES FOR WHICH STATE CONSUMPTION ADVISORIES HAVE BEEN ISSUED

AL	no consumption advisories
AK	no consumption advisories
AS	fish and shellfish species (unspecified)
CA	white croaker, black croaker, corbina, surfperch, queenfish, sculpin, rockfish, kelp bass, striped bass, fish and shellfish species (unspecified)
CT	striped bass, bluefish
DE	no consumption advisories
DC	channel catfish, American eel
FL	shark (unspecified)
GA	no consumption advisories
GU	no consumption advisories
HI	no consumption advisories
LA	fish and shellfish species (unspecified)
ME	no consumption advisories
MD	channel catfish, American eel
MA	American eel, flounder, American lobster, bivalves (unspecified), fish species (unspecified)
MS	no consumption advisories
NH	no consumption advisories
NJ	striped bass, bluefish, American eel, white perch, white catfish, blue crab, fish and shellfish species (unspecified)
NY	American eel, striped bass, bluefish, white perch, white catfish, rainbow smelt, Atlantic needlefish, blue crab
NC	fish species except herring, shad, striped bass, and shellfish species (unspecified)

OR	no consumption advisories
PA	white perch, channel catfish, American eel
PR	no consumption advisories
RI	striped bass, bluefish
SC	fish and shellfish species (unspecified)
TX	blue crab, catfish (unspecified), fish species (unspecified)
VA	fish species (unspecified)
VI	no consumption advisories
WA	no consumption advisories

Source: RTI, 1993. *National Listing of State Fish and Shellfish Consumption Advisories and Bans.* (Current as of July 22, 1993.) Research Triangle Institute, Research Triangle Park, NC.

APPENDIX C

TARGET ANALYTES ANALYZED IN NATIONAL OR REGIONAL MONITORING PROGRAMS

Table C-1. Target Analytes Analyzed in National or Regional Monitoring Programs

Analyte	Monitoring program								
	a	b	c	d[1]	e	f	g	h	i
Metals									
Aluminum (Al)					●				●
Antimony (Sb)	●				●				●
Arsenic (As) (total)	●	●		●	●		●		●
Barium (Ba)									●
Beryllium (Be)	●								●
Cadmium (Cd)	●	●		●	●		●		●
Chromium (Cr)	●	●		●	●				●
Copper (Cu)	●			●	●		●		●
Cyanide	●								
Iron (Fe)					●				●
Lead (Pb)	●	●		●	●		●		●
Manganese (Mn)				●	●				●
Mercury (Hg)	●		●	●	●		●		●
Methylmercury	●	●							
Molybdenum									●
Nickel (Ni)	●	●			●				●
Selenium (Se)	●	●			●		●		●
Silicon (Si)					●				
Silver (Ag)	●				●				●
Thallium (Tl)	●				●				
Tin (Sn)					●				
Tributyltin		●							
Vanadium									●
Zinc (Zn)	●				●		●		●
Pesticides									
Aldrin	●			●	●		●	●	
Butachlor									●
Chlordane (cis & trans)	●	●	●	●	●[2]		●	●	●
Chlorpyrifos		●	●						
Danitol								●	

(continued)

Table C-1 (continued)

Analyte	Monitoring program								
	a	b	c	d[1]	e	f	g	h	i
DCPA (chlorthal)		●					●		
DDT (total)				●	●		●		
2,4'-DDD (2,4'-TDE)				●	●		●		●
4,4'-DDD (4,4'-TDE)	●	●		●	●		●	●	●
2,4'-DDE				●	●		●		●
4,4'-DDE	●	●	●	●	●		●	●	●
2,4'-DDT				●	●		●		●
4,4'-DDT	●	●		●	●		●	●	●
Demeton	●								
Dicofol			●					●	●
Dieldrin	●	●	●	●	●		●	●	●
Diphenyl disulfide			●						
Endosulfan									
α-Endosulfan (endosulfan I)	●	●							
ß-Endosulfan (endosulfan II)	●	●							
Endosulfan sulfate	●	●							
Endrin	●	●	●	●			●		●
Endrin aldehyde	●								
Ethyl-p-nitrophenylphenylphosphorothioate (EPN)								●	
Fonofos		●							
Guthion	●								
Heptachlor	●		●	●	●		●	●	●
Heptachlor epoxide	●	●	●		●		●	●	●
Hexachlorocyclohexane (HCH) also known as Benzene hexachloride (BHC)									
α-Hexachlorocyclohexane	●	●	●	●			●	●	●
ß-Hexachlorocyclohexane	●			●				●	●
δ-Hexachlorocyclohexane	●			●					●
γ-Hexachlorocyclohexane (lindane)	●	●	●	●	●		●	●	●
Technical-hexachlorocyclohexane								●	
Hexachlorophene								●	
Isopropalin			●						●
Kepone		●							●
Malathion	●								

(continued)

Table C-1 (continued)

Analyte	Monitoring program								
	a	b	c	d[1]	e	f	g	h	i
Methoxychlor	●		●	●					●
Mirex	●	●	●	●	●		●	●	●
Nitrofen			●						
cis-Nonachlor		●	●				●		●
trans-Nonachlor		●	●		●		●		●
Oxychlordane		●	●				●		●
Parathion	●								
Toxaphene (mixture)	●	●		●			●	●	
Triazine herbicides		●[3]							
Trichloronate									●
Trifluralin		●	●						●
Base/Neutral Organic Compounds									
Acenaphthene	●				●				●
Acenaphthylene	●				●				●
Anthracene	●				●				●
Benzidine	●								
Benzo(a)anthracene	●				●				●
Benzo(a)pyrene	●				●				●
Benzo(e)pyrene					●				
Benzo(b)fluoranthene	●				●				●
Benzo(k)fluoranthene	●				●				●
Benzo(g,h,i)perylene	●				●				●
Benzyl butyl phthalate	●								
Biphenyl			●		●				
4-Bromophenyl ether	●								
bis(2-Chloroethoxy)methane	●								
bis(2-Chloroethyl)ether	●								
bis(2-Chloroisopropyl)ether	●								
bis(2-Ethylhexyl)phthalate (BEHP)								●	
Chlorinated benzenes		●							
2-Chloronaphthalene	●								
4-Chlorophenyl ether	●								
Chrysene	●				●				●

(continued)

Table C-1 (continued)

Analyte	Monitoring program								
	a	b	c	d[1]	e	f	g	h	i
Dibenzo(a,h)anthracene	●				●				●
Di-n-butyl phthalate	●								
1,2-Dichlorobenzene	●								
1,3-Dichlorobenzene	●								
1,4-Dichlorobenzene	●								
3,3'-Dichlorobenzidine	●							●	
Diethyl phthalate	●								
2,6-Dimethylnaphthalene					●				●
2,3,5-Trimethylnaphthalene					●				
Dimethyl phthalate	●								
2,4-Dinitrotoluene	●								
2,6-Dinitrotoluene	●								
Di-n-octyl phthalate	●								
1,2-Diphenylhydrazine	●								
bis(2-Ethylhexyl) phthalate	●								
Fluoranthene	●				●				●
Fluorene	●				●				●
Heptachlorostyrene				●					
Hexachlorostyrene				●					
Hexachlorobenzene	●		●	●	●		●	●	●
Hexachlorobutadiene	●		●						
Hexachlorocyclopentadiene	●								●
Hexachloroethane	●								
Indeno(1,2,3-cd)pyrene	●				●				
Isophorone	●								
4,4'-Methylene bis(N,N'-dimethyl)aniline								●	
1-Methylnaphthalene					●				
2-Methylnaphthalene					●				
1-Methylphenanthrene					●				
Naphthalene	●				●				●
Nitrobenzene	●								
N-Nitroso-di-n-butylamine								●	
N-Nitrosodimethylamine	●								

(continued)

Table C-1 (continued)

Analyte	Monitoring program								
	a	b	c	d[1]	e	f	g	h	i
N-Nitrosodiphenylamine	●								
N-Nitrosodipropylamine	●								
Octachlorostyrene			●	●					●
PAHs (polycyclic aromatic hydrocarbons)		●[3]							
PBBs (polybrominated biphenyls)				●					
PCBs (polychlorinated biphenyls)		●	●	●	●				●
Aroclor 1016 (mixture)	●							●	
Aroclor 1221 (mixture)	●							●	
Aroclor 1232 (mixture)	●							●	
Aroclor 1242 (mixture)	●						●	●	
Aroclor 1248 (mixture)	●						●	●	
Aroclor 1254 (mixture)	●						●	●	
Aroclor 1260 (mixture)	●						●	●	
Selected individual congeners					●				
Pentachloroanisole (PCA)			●				●		●
Pentachlorobenzene			●					●	●
Pentachloronitrobenzene (PCNB)			●						●
Pentachlorophenyl methyl ether		●							
Pentachlorophenyl methyl sulfide		●							
Pentachlorostyrene				●					
Perthane			●						●
Perylene					●				
Phenanthrene	●				●				●
Pyrene	●				●				●
Terphenyl				●					
1,2,3,4-Tetrachlorobenzene			●						●
1,2,3,5-Tetrachlorobenzene			●						●
1,2,4,5-Tetrachlorobenzene			●					●	●
1,2,3-Trichlorobenzene			●						
1,2,4-Trichlorobenzene	●		●						●
1,3,5-Trichlorobenzene			●						●
Triphenyl phosphate									●

(continued)

Table C-1 (continued)

Analyte	Monitoring program								
	a	b	c	d[1]	e	f	g	h	i
Dioxins									
1,2,3,7,8-Pentachlorodibenzodioxin (PeCDD)			●						●
2,3,7,8-Tetrachlorodibenzodioxin (TCDD)	●	●	●	●		●		●	●
1,2,3,4,6,7,8-Heptachlorodibenzodioxin (HpCDD)			●						●
1,2,3,4,7,8-Hexachlorodibenzodioxin (HxCDD)			●						●
1,2,3,6,7,8-Hexachlorodibenzodioxin (HxCDD)			●						●
1,2,3,7,8,9-Hexachlorodibenzodioxin (HxCDD)			●						●
Dibenzofurans									
1,2,3,4,6,7,8-Heptachlorodibenzofuran (HpCDF)			●						●
1,2,3,4,7,8,9-Heptachlorodibenzofuran (HpCDF)			●						●
1,2,3,4,7,8-Hexachlorodibenzofuran (HxCDF)			●						●
1,2,3,6,7,8-Hexachlorodibenzofuran (HxCDF)			●						●
1,2,3,7,8,9-Hexachlorodibenzofuran (HxCDF)			●						●
2,3,4,6,7,8-Hexachlorodibenzofuran (HxCDF)			●						●
1,2,3,7,8-Pentachlorodibenzofuran (PeCDF)			●						●
2,3,4,7,8-Pentachlorodibenzofuran (PeCDF)			●						●
2,3,7,8-Tetrachlorodibenzofuran (TCDF)			●						●
Acidic Organic Compounds									
Chlorinated phenols		●[3]							
4-Chloro-3-cresol	●								
2-Chlorophenol	●								
2,4-Dichlorophenol	●								
2,4-Dimethylphenol	●								
4,6-Dinitro-2-cresol	●								
2-4-Dinitrophenol	●								
2-Nitrophenol	●								
4-Nitrophenol	●								
Pentachlorophenol (PCP)	●								●
Phenol	●								
2,4,6-Trichlorophenol	●								
Volatile Organic Compounds									
Acrolein	●								
Acrylonitrile	●								

(continued)

Table C-1 (continued)

Analyte	Monitoring program								
	a	b	c	d[1]	e	f	g	h	i
Benzene	●								
Bromodichloromethane	●								
Bromoform	●								
Bromomethane	●								
Carbon tetrachloride	●								
Chlorobenzene	●								
Chloroethane	●								
2-Chloroethylvinyl ether	●								
Chloroform	●								
Chloromethane	●								
Dibromochloromethane	●								
1,1-Dichloroethane	●								
1,2-Dichloroethane	●								
1,1-Dichloroethene	●								
trans-1,2-Dichloroethene	●								
1,2-Dichloropropane	●								
cis-1,3-Dichloropropene	●								
trans-1,3-Dichloropropene	●								
Ethylbenzene	●								
Methylene chloride	●								
1,1,2,2-Tetrachloroethane	●								
Tetrachloroethene	●								
Toluene	●								
1,1,1-Trichloroethane	●								
1,1,2-Trichloroethane	●								
Trichloroethene	●								
Vinyl chloride	●								

[1] Contaminants listed were monitored by at least one Great Lakes State. NOTE: Contaminants monitored exclusively by the Canadian Province of Ontario were not included.

[2] Only the cis-isomer is monitored.

[3] FDA recommends method development/improvement for this analysis.

[a] 301(h) Monitoring Program. Source: U.S. EPA. 1985. *Bioaccumulation Monitoring Guidance: 1. Estimating the Potential for Bioaccumulation of Priority Pollutants and 301(h) Pesticides Discharged into Marine and Estuarine Waters.* EPA 503/3-90-001. Office of Marine and Estuarine Protection, Washington, DC.

Table C-1 (continued)

[b] Food and Drug Administration recommendations. Source: Michael Bolger, FDA, personal communication, 1990.

[c] National Study of Chemical Residues in Fish. Source: U.S. EPA. 1992. *National Study of Chemical Residues in Fish.* Volumes I and II. EPA 823/R-92-008a and 008b. Office of Science and Technology, Washington, DC.

[d] Great Lakes Sport Fish Contaminant Advisory Program. Source: Hesse, J. L. 1990. *Summary and Analyses of Existing Sportfish Consumption Advisory Programs in the Great Lakes Basin—the Great Lakes.* Fish Consumption Advisory Task Force, Michigan Department of Health, Lansing, MI.

[e] NOAA Status and Trends Program. Source: NOAA. 1989. *National Status and Trends Program for Marine Environmental Quality--Progress Report: A Summary of Selected Data on Tissue Contamination from the First Three Years (1986-1988) of the Mussel Watch Project.* NOAA Technical Memorandum NOS OMA 49. U.S. Department of Commerce, Rockville, MD.

[f] EPA National Dioxin Study. Source: U.S. EPA. 1987. *National Dioxin Study. Tiers 3, 5, 6 and 7.* EPA 440/4-87-003. Office of Water Regulations and Standards, Washington, DC.

[g] U.S. Fish and Wildlife Service National Contaminant Biomonitoring Program. Sources: C. J. Schmitt, J. L. Zajicek, and P. H. Peterman, 1990, National Contaminant Biomonitoring Program: Residues of organochlorine chemicals in U.S. freshwater fish, 1976-1984, *Arch. Environ. Contam. Toxicol.* 19:748-781; and T. P. Lowe, T. W. May, W. G. Brumbaugh, and D. A. Kane, 1985, National Contaminant Biomonitoring Program: Concentrations of seven elements in freshwater fish, 1978-1981, *Arch. Environ. Contam. Toxicol.* 14:363-388.

[h] U.S. EPA. 1991. *Assessment and Control of Bioconcentratable Contaminants in Surface Waters.* Draft. Office of Water, Office of Research and Development, Washington, DC.

[i] U.S. Geological Survey National Water-Quality Assessment Program. Source: J.K. Crawford and S.N. Luoma. 1993. Guidelines for Studies of Contaminants in Biological tissues for the National Water-Quality Assessment Program. USGS Open-File Report 92-494. U.S. Geological Survey, Lemoyne, PA.

APPENDIX D

PESTICIDES AND HERBICIDES RECOMMENDED AS TARGET ANALYTES

Table D-1. Pesticides and Herbicides Recommended as Target Analytes

Pesticide	Family	Use	Registration	Toxicity class[a]	EPA carcinogenicity classification[b]
Metal Containing Pesticides					
Arsenicals (including arsenic acid, arsenic trioxide, copper acetoarsenite, lead arsenate, calcium arsenate, sodium arsenite)	Inorganic arsenicals	A variety of inorganic arsenic compounds are used as herbicides, fungicides, insecticides and rodenticides, but registered uses of some were superseded because of their hazard to man and other nontarget species (*Farm Chemicals Handbook*, 1989)	Some inorganic arsenic compound registrations have been canceled; others are under restricted use application and others are in special review (U.S. EPA, 1993)	I	A[f]
Tributyltins	Organotins	A variety of organotin compounds are used as wood preservatives, antifoulants, biocides, and disinfectants (*Farm Chemicals Handbook*, 1989)	Some organotin compounds have been actively registered since the mid-1960s. Several registrations have been canceled or manufacturers discontinued production (U.S. EPA, 1988a)	I	NA[f]

See notes and references at end of table.

(continued)

Table D-1 (continued)

Pesticide	Family	Use	Registration	Toxicity class[a]	EPA carcinogenicity classification[b]
Organochlorines					
Chlordane	Chlorinated cyclodiene	Termite control. Historically used for control of fire ants, cutworms, grasshoppers, and on other insects on corn, grapes, strawberries, and other crops and as a dip for nonfood roots and tips of plants (Hartley and Kidd, 1987).	In March 1978, the EPA issued a cancellation proceeding on chlordane, allowing only limited use on certain crops and pests until July 1983, but no use thereafter except for underground termite control (43 FR 12372). All uses except subsurface ground insertion for termite control were canceled November 30, 1987. All chlordane/heptachlor products were voluntarily canceled by the registrant, Velsicol. All other chlordane/heptachlor products are either voluntarily canceled or suspended for failure to meet EPA data requirements. The only commercial use of chlordane/heptachlor products still permitted is for fire ant control in power transformers (U.S. EPA, 1990). The sale, distribution, and shipment of existing stocks of all canceled chlordane/heptachlor products is prohibited in the U.S. as of April 15, 1988. The use of existing stocks of termiticide products in the possession of homeowners is also permitted (53 FR 11798; 54 FR 20194).	II	B2

See notes and references at end of table.

(continued)

Table D-1 (continued)

Pesticide	Family	Use	Registration	Toxicity class[a]	EPA carcinogenicity classification[b]
DDT	Chlorinated hydrocarbon	Insecticide	All uses in U.S. were canceled as of January 1, 1973, except for emergency public health uses. Effective December 31, 1988, all uses were canceled unless registered formulas contain less than 0.1% DDT (51 FR 19508).	III	B2
Dicofol	Chlorinated hydrocarbon	Acaricide on many fruit, vegetable, ornamental and fried crops. Historically used to control mites on cotton and citrus (60%). Other major uses included control of mites on apples (10%), ornamental plants and turf (10%) and 20% on a variety of other agricultural products (pears, apricots and cherries), seed crop soil treatment, vegetables, (e.g., beans and corn) and shade trees (51 FR 19515) (U.S. EPA, 1986b).	Active since 1957; however all uses are to be canceled after January 1989 unless registered formulas contain less than 0.1% DDT and related contaminants (51 FR 19508).[c]	III	C[d]

See notes and references at end of table.

(continued)

Table D-1 (continued)

Pesticide	Family	Use	Registration	Toxicity class[a]	EPA carcinogenicity classification[b]
Dieldrin	Chlorinated cyclodiene	Control of locusts, tropical disease carriers (e.g., mosquitoes), and termites, use as wood preservative, and moth proofing for woolen clothes and carpets (Worthing, 1991).	All uses on food products were suspended in 1974 (ATSDR, 1987a). All uses in the U.S. were banned in 1985 except for subsurface termite control, dipping of nonfood roots and tops, and moth proofing in a closed system (U.S. EPA, 1985b). These uses have been voluntarily canceled by industry (ATSDR, 1987a).	II	B2
Endosulfan (I and II)	Chlorinated bicyclid sulfite	Insecticide and acaricide on citrus, deciduous, small fruits, coffee, tea, fiber crops, forage crops, forest, grains, nuts, oil crops, tobacco, ornamentals, and vegetables.	Active since 1954. Used for control of aphids, thrips, beetles, foliar feeding larvae, mites, borers, cutworms, bollworms, bugs, whiteflies, leafhoppers and slugs on citrus, deciduous, small fruits, coffee, tea, fiber crops, forage crops, forest, grains, nuts, oil crops, ornamentals, tobacco, and vegetables.[c]	I	E[d]

See notes and references at end of table.

(continued)

Table D-1 (continued)

Pesticide	Family	Use	Registration	Toxicity class[a]	EPA carcinogenicity classification[b]
Endrin	Chlorinated cyclodiene	Historically used to control cotton bollworms, as a foliar treatment for citrus, potatoes, small grains, apple orchards, sugarcane, and as flower and bark treatment on trees. Endrin has also been used to control populations of birds and rodents (U.S. EPA, 1980).	In 1964, endrin persistence in soils led to cancellation of its use on tobacco (U.S. EPA, 1980). By 1979, specified uses on cotton, small grains, apple orchards, sugarcane and ornamentals were also restricted (44 FR 43632). In 1984, the sole producer of endrin voluntarily requested cancellation of all endrin products (U.S. EPA, 1984a).	I	D
Heptachlor epoxide	Chlorinated cyclodiene	Heptachlor epoxide is an oxidation product of heptachlor. It is a contaminant of both heptachlor and chlordane. Heptachlor was widely used as a termiticide and insecticide, primarily for ant control (Hodges, 1977).	Termide (chlordane) sales halted per Velsicol and EPA agreement pending results of specific application tests. Restrictions on heptachlor were first instituted in 1978 and heptachlor can no longer be sold in the U.S. as of August 1987 but remaining stocks can be used in some States by commercial exterminators for termite control. All uses have been banned in Minnesota, Massachusetts, and New York (ATSDR, 1987b).	NA	B2

See notes and references at end of table.

(continued)

Table D-1 (continued)

Pesticide	Family	Use	Registration	Toxicity class[a]	EPA carcinogenicity classification[b]
Hexachlorobenzene	Chlorinated benzene	Primary use prior to 1985 was as a fungicide seed protectant in small grain crops, particularly wheat.	Registration of hexachlorobenzene as a pesticide was voluntarily canceled in 1984 (Morris and Cabral, 1986).	IV	B2
Lindane (γ-hexachlorocyclohexane)	Chlorinated hydrocarbon	Seed treatments, soil treatments for tobacco transplants, foliage applications on fruit and nut trees, vegetables, and wood and timber protection.	Use of lindane in smoke fumigation devices for indoor domestic purposes was banned in 1985 (48 FR 48512, 50 FR 5424). Use in dog dips permitted only for veterinary use (U.S. EPA, 1985a). Application permitted only under supervision of certified applicator (U.S. EPA, 1985a).	II	B2/C[d,e]
Mirex	Chlorinated cyclodiene	Historically used primarily in fire ant control in southeastern States (Kutz et al., 1985) and was used industrially as a fire retardant and polymerizing agent in plastics under the name dechlorane.	All registered uses of mirex were canceled in 1977 (41 FR 56703). All existing stocks were not to be sold, distributed, or used after June 30, 1978 (NAS, 1978).	II	R
Toxaphene	Chlorinated camphene	Historically used extensively on cotton (*Farm Chemicals Handbook*, 1989). Note: A toxaphene-like compound can be a byproduct of the paper industry and has been identified in the Great Lakes region (J. Hesse, Michigan Department of Public Health, personal communication, 1992).	Registration for all uses was canceled in the U.S. in November 1982 (47 FR 53784; U.S. EPA, 1990).	II	B2

See notes and references at end of table.

(continued)

Table D-1 (continued)

Pesticide	Family	Use	Registration	Toxicity class[a]	EPA carcinogenicity classification[b]
Organophosphates					
Chlorpyrifos	Heterocyclic organophosphate	Insecticide primarily used to control soil and foliar insect pests on cotton, peanuts, and sorghum (Worthing, 1983; U.S. EPA, 1986a). In addition, it is used to control root-infesting and boring insects on a variety of fruits (e.g., citrus crops, apples, bananas, peaches, grapes, nectarines), nuts (e.g., almonds, walnuts), vegetables (e.g., beans, broccoli, brussel sprouts, cauliflower, soybeans, cabbage, peas) and field crops (e.g., alfalfa and corn) (U.S. EPA, 1984b) and to control ticks on cattle and sheep (Thomson, 1985). As a household insecticide it has been used to control ants, cockroaches, fleas, and mosquitoes (Worthing, 1983) and is registered for use in controlling subsurface termites in California (U.S. EPA, 1983).	Active since 1965 (U.S. EPA, 1984b).[c]	II	D[d]

See notes and references at end of table.

(continued)

Table D-1 (continued)

Pesticide	Family	Use	Registration	Toxicity class[a]	EPA carcinogenicity classification[b]
Diazinon	Heterocyclic organophosphate	Insecticide and nematicide for control of soil insects and pests of fruits, vegetables, tobacco, forage, field crops, range, pasture, grasslands and ornamentals. Used to control cockroaches and other household insects; grubs and nematodes in turf; as a seed treatment and for fly control (*Farm Chemicals Handbook*, 1989).	Active since 1952 (U.S. EPA, 1986c).[c]	II	D[d]
Disulfoton	Aliphatic organophosphate	Systemic insecticide and a caricide on grain, nut, cole and root crops; pome, strawberry and pineapple fruits; forage, field and vegetable crops, sugarcane, seed crops, forest plantings, ornamentals and potted plants (houseplants) (U.S. EPA, 1984b).	Active since 1958 (U.S. EPA, 1984c).[c]	I	D[d]
Ethion	Organothiophosphate	Insecticide (nonsystemic) for control of leaf-feeding insects, mites, and scale insects. Citrus accounts for 86%-89% of total pounds of ethion used in the U.S. with the remaining 11%-14% applied to cotton, a variety of fruit trees, nut trees, and vegetables (U.S. EPA, 1989).	Active since 1965 (U.S. EPA, 1989).[c]	II	D[d]

See notes and references at end of table.

(continued)

Table D-1 (continued)

Pesticide	Family	Use	Registration	Toxicity class[a]	EPA carcinogenicity classification[b]
Terbufos	Organophosphate	Systemic insecticide and nematicide on corn, sugar beets, and grain sorghum (U.S. EPA, 1985c).	Active since 1974; however, granular end-use products containing 15% or more terbufos were classified as "Restricted Use" after September 1985 (U.S. EPA, 1985c).[c]	I	D[d]
Chlorophenoxy Herbicides					
Oxyfluorfen	Diphenyl ether	Pre- and postemergence herbicide for a wide spectrum of annual broadleaf weeds and grasses in apples, artichokes, corn, cotton, tree fruit, grapes, nuts, spearmint, peppermint, certain topical plantation, and ornamental crops (*Farm Chemicals Handbook*, 1989)	Active since 1979.[c]	II	C[d]

[a] Designations are from *Farm Chemicals Handbook* (1989):
I = Oral LD_{50} up to and including 50 mg/kg in laboratory animals.
II = Oral LD_{50} from 50 through 500 mg/kg in laboratory animals.
III = Oral LD_{50} from 500 through 5,000 mg/kg in laboratory animals.
IV = Oral LD_{50} greater than 5,000 mg/kg in laboratory animals.
NA = No value available.

[b] Designations are from IRIS (1992) unless otherwise noted: NA = not evaluated; A = human carcinogen; B1, B2 = probable human carcinogen; C = possible human carcinogen; D = inadequate evidence of animal carcinogenicity; E = no evidence of carcinogenicity for humans; R = under review by EPA.

(continued)

Table D-1 (continued)

[c] This pesticide has an active registration for agricultural use. The EPA Office of Pesticide Programs is responsible for registration and reregistration of pesticides. The 1988 Amendment of FIFRA requires EPA to reregister each "registered pesticide containing any active ingredient contained in any pesticide first registered before November 1, 1984, except for any pesticide as to which the Administration has determined, after November 1, 1984 .... that—(1) there are no outstanding data requirements; and (2) the requirements of section 3(c)(5) have been satisfied" (U.S. EPA, 1988). The Agency will review all relevant data submitted by the registrant for each pesticide reregistration and will use the data to conduct a risk assessment. Any subsequent regulatory action will be based on the results of the risk assessment. If the data submitted are incomplete at the predetermined review time, the pesticide may be suspended.

[d] EPA carcinogenicity classification based on Classification List of Chemicals Evaluated for Carcinogenicity Potential (U.S. EPA, 1992).

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APPENDIX E

TARGET ANALYTE DOSE-RESPONSE VARIABLES AND ASSOCIATED INFORMATION

Table E-1. Target Analyte Dose-Response Variables and Associated Information

Target analyte	Noncarcinogens: RfD[a] (degree of confidence; uncertainty factor)	Noncarcinogens: Critical toxic effect	Carcinogens: SF[b] (discussion of confidence)	Carcinogens: Critical carcinogenic effect[c]	Carcinogens: EPA carcinogenicity classification[d]
Metals					
Arsenic (inorganic)	3×10^{-4} [e] (medium; 3)	Hyperpigmentation, keratosis and possible vascular complications in humans	NA[f]	—	A[e]
Cadmium	1×10^{-3} (high; 10)	Significant proteinurea in humans	NA	—	B1[g]
Mercury (as methylmercury)	3×10^{-4} [h] (medium; 10)	Central nervous system effects (e.g., ataxia, parathesia) in humans	NA	—	C
	1×10^{-4} [e,h] (medium; 10)	Developmental neurological abnormalities in human infants.			
Selenium[i]	5×10^{-3} (high; 3)	Selenosis in humans	NA	—	D
Tributyltin	3×10^{-5} [e] (low; 1000)	Immunotoxicity in rats	NA	—	NA[e]

See notes and references at end of table.

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Table E-1 (continued)

Target analyte	Noncarcinogens: RfD[a] (degree of confidence; uncertainty factor)	Noncarcinogens: Critical toxic effect	Carcinogens: SF[b] (discussion of confidence)	Carcinogens: Critical carcinogenic effect[c]	Carcinogens: EPA carcinogenicity classification[d]
Organochlorine Pesticides					
Chlordane (sum of cis- and trans-chlordane, cis- and trans-nonachlor, and oxychlordane)	6×10^{-5} (low; 1000)	Regional liver lesions (hypertrophy) in one strain of female rats	1.3 (Adequate number of animals observed. SF is the geometric mean of SFs for four data sets from two studies. This SF is consistent with SF = 1.1 derived from less sensitive rat species.)	Hepatocellular carcinomas in two strains of mice (male and female)	B2
DDT (sum of 4,4'- and 2,4'- isomers of DDT, DDE, and DDD)	5×10^{-4} (medium; 100)	Liver lesions in rats	3.4×10^{-1} (SF is geometric mean of SFs from 10 data sets. SF from mouse data only = 4.8×10^{-1}; SF from rat data only = 1.5×10^{-1}.)	DDT: Liver tumors in six studies in two mouse strains and two studies in two rat strains	B2

See notes and references at end of table.

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Table E-1 (continued)

Target analyte	Noncarcinogens: RfD[a] (degree of confidence; uncertainty factor)	Noncarcinogens: Critical toxic effect	Carcinogens: SF[b] (discussion of confidence)	Carcinogens: Critical carcinogenic effect[c]	Carcinogens: EPA carcinogenicity classification[d]
			3.4×10^{-1} (Adequate number of animals observed. SF for mouse studies alone is within a factor of 2 for mouse and hamster data combined.)	DDE: Liver tumors (including carcinomas) in two strains of mice and in hamsters	B2
			2.4×10^{-1} (Adequate number of animals observed. SF calculated using tumor incidence data from only one dose.)	DDD: Liver tumors in one strain of mice (males only)	B2
Dicofol	1×10^{-3}[j] (NA, 1000)	Increase liver to body weight ratios observed in 2-yr rat feeding study.[j]	NA	—	C[k]
Dieldrin	5×10^{-5} (medium; 100)	Liver lesions (focal proliferation and focal hyperplasia) in one strain of female rats	16 (SF is the geometric mean of SFs from 13 data sets. Individual SFs ranged within a factor of 8.)	Liver carcinomas in five strains of mice (male and female)	B2
Endosulfan (sum of endosulfan I and II)	6×10^{-3}[l] (medium; 100)	Decreased body weight gain and progressive glomerulonephrosis and blood vessel aneurysmsin one strain of male rats[l]	NA	—	E[k]

See notes and references at end of table.

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Table E-1 (continued)

Target analyte	Noncarcinogens: RfD[a] (degree of confidence; uncertainty factor)	Noncarcinogens: Critical toxic effect	Carcinogens: SF[b] (discussion of confidence)	Carcinogens: Critical carcinogenic effect[c]	Carcinogens: EPA carcinogenicity classification[d]
Endrin	3×10^{-4} (medium; 100)	Mild histological lesions in livers in dogs (both sexes)	NA	—	D
Heptachlor epoxide	1.3×10^{-5} (low; 1000)	Increased liver-to-body weight ratios in male and female dogs	9.1 (Adequate number of animals observed in both studies, but survival in one study was low. This SF is consistent with SF = 5.8 for one strain of seven rats.)	Hepatocellular carcinomas in two strains of mice (male and female)	B2
Hexachlorobenzene	8×10^{-4} (medium; 100)	Liver effects (hepatic centrilobular basophilic chromogenesis) in one strain of rats (both sexes)	1.6 (Significant increases in malignant tumors observed among an adequate number of animals observed for their lifetime.)	Hepatocellular carcinomas in one strain of rats (females only)	B2
Lindane (γ-BHC)	3×10^{-4} (medium; 1000)	Liver and kidney toxicity (liver hypertrophy, kidney tubular degeneration, hyaline droplets, tubular distension, interstitial nephritis, and basiophilic tubules) in both sexes of one strain of rats	1.3[m]	—	B2/C[k,n]

See notes and references at end of table.

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Table E-1 (continued)

Target analyte	Noncarcinogens: RfD[a] (degree of confidence; uncertainty factor)	Noncarcinogens: Critical toxic effect	Carcinogens: SF[b] (discussion of confidence)	Carcinogens: Critical carcinogenic effect[c]	Carcinogens: EPA carcinogenicity classification[d]
Mirex	2×10^{-4} (high; 300)	Liver cytomegaly, fatty metamorphosis, angiectasis and thyroid cystic follicles in one strain of rats.	NA[o]	—	R
Toxaphene	2.5×10^{-4} [j,p] (NA, 1000)	Slight liver degeneration—granularity and vacuolization of hepatocytes.[j]	1.1 (Adequate number of animals observed. A dose-response effect was seen in a study with three non-zero dose levels.)	Hepatocellular carcinomas and neoplastic nodules in one strain of mice (males only)	B2
Organophosphate Pesticides					
Chlorpyrifos	3×10^{-3} (medium, 10)	Decreased plasma ChE activity observed in 20-day human feeding study.	NA	—	D[k]
Diazinon	9×10^{-5} [j] (NA, 100)	Inhibition of plasma ChE observed in 90-day rat feeding study.[j]	NA	—	D[k]
Disulfoton	4×10^{-5} (medium, 1000)	ChE inhibition and degeneration of the optic nerve observed in 2-yr dog feeding study.	NA	—	D[k]

See notes and references at end of table.

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Table E-1 (continued)

Target analyte	Noncarcinogens: RfD[a] (degree of confidence; uncertainty factor)	Noncarcinogens: Critical toxic effect	Carcinogens: SF[b] (discussion of confidence)	Carcinogens: Critical carcinogenic effect[c]	Carcinogens: EPA carcinogenicity classification[d]
Ethion	5×10^{-4} (medium, 100)	Plasma ChE inhibition and inhibition of brain ChE observed in 21-day human feeding study.	NA	—	D[k]
Terbufos	1.3×10^{-4} [j] (NA, 10)	Inhibition of plasma ChE observed in 28-day dog feeding study.[j]	NA	—	D[k]
Chlorophenoxy Herbicides					
Oxyfluorfen	3×10^{-3} (high, 100)	Increased absolute liver weight and nonneoplastic lesions observed in 20-month mouse feeding study.	1.3×10^{-1} [k]	Evidence of carcinogenicity (liver tumors) in mice.	C[k]
PAHs[q]					
Benzo[*a*]pyrene	NA	—	7.3[e] (Data less than optimal, but acceptable. Four data sets used from two different studies using two different species (rats and mice; both sexes) to derive geometric mean of four calculated slope factors.)	Squamous cell carcinoma of the forestomach in one strain of mice (both sexes). Forestomach, larynx, and esophagus papillomas and carcinomas in one strain of rats (both sexes)	B2

See notes and references at end of table.

(continued)

Table E-1 (continued)

Target analyte	Noncarcinogens: RfD[a] (degree of confidence; uncertainty factor)	Noncarcinogens: Critical toxic effect	Carcinogens: SF[b] (discussion of confidence)	Carcinogens: Critical carcinogenic effect[c]	Carcinogens: EPA carcinogenicity classification[d]
PCBs					
Total PCBs (sum of Aroclors)	2×10^{-5} [e,r] (medium; 300)	Ocular exudate, inflamed, prominent Meibomian glands, distorted growth of finger and toe nails, decreased antibody response to sheep erythrocytes in monkey clinical and immunologic studies	7.7[s] (Adequate number of animals observed for their normal lifespan. Only one non-zero test dose used.)	Trabecular carcinomas/adenocarcinomas, neoplastic nodules in one strain of rats (females only)	B2
	7×10^{-5} [e,t] (medium; 100)	Reduced birth weights in monkeys			
Dioxins/furans	NA	—	1.56×10^{5} [u]	NA	B2

NA = Not available in IRIS (1992).
PAHs = Polycyclic aromatic hydrocarbons.
PCBs = Polychlorinated biphenyls.

[a] RfD = Oral reference dose (mg/kg/day); from IRIS (1992) unless otherwise noted (see Section 5.1.1).

[b] SF = Oral slope factor $(mg/kg/day)^{-1}$; from IRIS (1992) unless otherwise noted (see Section 5.1.2).

[c] The critical effect is the effect observed in oral dose response studies used to determine the SF.

[d] Except where noted, all EPA carcinogenicity classifications are taken from IRIS (1992):
- A = Human carcinogen based on sufficient evidence from epidemiologic studies.
- B1 = Probable human carcinogen based on at least limited evidence of carcinogenicity to humans.
- B2 = Probable human carcinogen based on a combination of sufficient evidence in animals and inadequate data in humans.
- C = Possible human carcinogen based on limited evidence of carcinogenicity in animals in the absence of human data.
- D = Not classifiable based on lack of data or inadequate evidence of carcinogenicity from animal data.

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E = No evidence of carcinogenicity for humans (no evidence of carcinogenicity in at least two adequate animal tests in different species or in both epidemiologic and animal studies).
R = Currently under review by EPA.

[e] From IRIS (1995).

[f] The SF for inorganic arsenic is currently under review by the Agency. At this time, EPA does not have a cancer SF to recommend for use in conducting fish consumption risk assessments.

[g] Based on limited evidence from human occupational epidemiologic studies where the primary route of exposure was by inhalation, and on sufficient evidence from studies in which rats and mice were exposed by inhalation and intramuscular and subcutaneous injection. However, data are inadequate to conclude that cadmium is carcinogenic via ingestion. The EPA Office of Drinking Water classifies cadmium as a Group D carcinogen in the health advisory for cadmium (U.S. EPA, 1987).

[h] The EPA has recently reevaluated the RfD for methylmercury, primarily because of concern about evidence that the fetus is at increased risk of adverse neurological effects from exposure to methylmercury (Marsh et al., 1987; Piotrowski and Inskip, 1981; Seafood Safety, 1991; WHO, 1976, 1990). On May 1, 1995, IRIS was updated to include an oral RfD of $1x10^{-4}$ mg/kg/d based on developmental neurological effects in human infants. An oral RfD of $3x10^{-4}$ mg/kg/d for chronic systemic effects of methylmercury among the general adult population was available in IRIS until May 1, 1995; however, it was not listed in the IRIS update on that date. For the purposes of calculating an SV for methylmercury that is protective of developing fetuses and nursing infants, the EPA Office of Water has chosen to continue to use the general adult population RfD of $3x10^{-4}$ mg/kg/d for chronic systemic effects of methylmercury until a value is relisted in IRIS, and to reduce this value by a factor of 5 to derive an RfD of $6x10^{-5}$ mg/kg/d for developmental effects among fetuses and nursing infants. The protective factor of 5 is based on experimental results that suggest a possible fivefold increase in fetal sensitivity to methylmercury exposure. This more protective approach recommended by the EPA Office of Water was deemed to be most prudent at this time. This approach should be considered interim until such time as the Agency has reviewed new studies on the chronic and developmental effects of methylmercury.

[i] The oral RfD is for selenious acid (IRIS, 1992). The evidence of carcinogenicity for various selenium compounds in animals and mutagenicity studies is conflicting and difficult to interpret. However, evidence for selenium sulfides is sufficient for a B2 classification (IRIS, 1992).

[j] Reference dose information is taken from the Office of Pesticide Programs Reference Dose Tracking Report (U.S. EPA, 1993).

[k] EPA carcinogenicity classification taken from Classification List of Chemicals Evaluated for Carcinogenicity Potential (U.S. EPA, 1992a).

(continued)

[l] Reference dose information is taken from the Office of Pesticide Programs Reference Dose Tracking Report (U.S. EPA, 1995).

[m] IRIS (1992) has not provided an SF for lindane. The SF value listed for lindane was calculated from the water quality criteria (0.063 µg/L) (U.S. EPA, 1992d) and is comparable to the SF of 1.33 mg/kg/d^{-1} from the Public Health Risk Evaluation Database (U.S. EPA, 1988b).

[n] Previously classified by EPA as B2 (IRIS, 1989). Available data need to be reviewed further, but at a minimum lindane will be classified as a C carcinogen (U.S. EPA, 1992a).

[o] The National Study of Chemical Residues in Fish (U.S. EPA, 1992b, 1992c) used a value of SF = 1.8 for mirex from HEAST (1989).

[p] The RfD value is the Office of Pesticide Programs value; this value was never submitted for verification.

[q] This RfD is for benzo[*a*]pyrene (IRIS, 1995). There are no other RfDs or SFs listed for PAHs in IRIS (1995). It is recommended that, in both screening and intensive studies, tissue samples be analyzed for benzo[*a*]pyrene, benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, chrysene, dibenz[*a,h*]anthracene, and indeno*[1,2,3-cd]*pyrene, and that the relative potencies given for these PAHs in the EPA provisional guidance for quantitative risk assessment of PAHs (U.S. EPA, 1993c) be used to calculate a potency equivalency concentration (PEC) for each sample for comparison with the recommended SV for benzo[*a*]pyrene (see Section 5.3.2.3). At this time, EPA's recommendation for risk assessment of PAHs (U.S. EPA, 1993c) is considered provisional because quantitative risk assessment data are not available for all PAHs. This approach is under Agency review and over the next year will be evaluated as new health effects benchmark values are developed. Therefore, the method provided in this guidance document is subject to change pending results of the Agency's reevaluation.

[r] This RfD for PCBs is based on the chronic toxicity of Aroclor 1254 (IRIS, 1995).

[s] This SF is based on a carcinogenicity assessment of Aroclor 1260. The SF of Aroclor 1260 is intended to represent the upper bound risk for all PCB mixtures (IRIS, 1992). **Note:** EPA is currently reevaluating the SF for PCBs and a revised value may be available for comment in the Fall of 1995.

[t] This RfD for PCBs is based on the developmental toxicity of Aroclor 1016 (IRIS, 1995).

[u] The SF value listed is for 2,3,7,8-tetrachlorodibenzo-p-dioxin 2,3,7,8-TCDD (U.S. EPA, 1986). The National Study of Chemical Residues in Fish used a value of RfD = 1x10^{-9} for 2,3,7,8-TCDD from ATSDR (1987). It is recommended that, in both screening and intensive studies, the tetra- through octa-chlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) be determined and a toxicity-weighted total concentration be calculated for each sample for comparison with the recommended SV, using the revised interim method for estimating Toxicity Equivalency Concentration (TECs) (Barnes and Bellin, 1989; U.S. EPA, 1991). If resources are limited, the 2,3,7,8-TCDD and 2,3,7,8-TCDF congeners should be determined, at a minimum.

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APPENDIX F

QUALITY ASSURANCE AND QUALITY CONTROL GUIDANCE

APPENDIX F

QUALITY ASSURANCE(QA) AND QUALITY CONTROL (QC) GUIDANCE

F.1 GENERAL QA AND QC CONSIDERATIONS

The primary objective of the specific QA and QC guidance provided in this document is to ensure that

- Appropriate data quality objectives or requirements are established **prior** to sample collection and analysis
- Samples are collected, processed, and analyzed according to scientifically valid, cost-effective, standardized procedures
- The integrity and security of samples and data are maintained at all times
- Recordkeeping and documentation procedures are adequate to ensure the traceability of all samples and data from initial sample collection through final reporting and archiving, and to ensure the verifiability and defensibility of reported results
- Data quality is assessed, documented, and reported properly
- Reported results are complete, accurate, and comparable with those from other similar monitoring programs.

F.2 QA PLAN REQUIREMENTS

To ensure the quality, defensibility, and comparability of the data used to determine exposure assessments and fish consumption advisories, it is essential that an effective QA program be developed as part of the overall design for each monitoring program. The QA program should be documented in a written QA plan or in a combined Work/QA Project Plan and should be implemented strictly throughout all phases of the monitoring program. The QA plan should include the following information either in full or by reference to appropriate standard operating procedures (SOPs):

1. A clear statement of program objectives

2. A description of the program organization and personnel roles and responsibilities, including responsibility for ensuring adherence to the QA plan

3. Specification of data quality objectives in terms of accuracy, precision, representativeness, and completeness, for data generated from each type of measurement system

4. Detailed descriptions of field sample collection and handling procedures, including documentation of

   - Target species and size (age) class
   - Sampling site locations
   - Target contaminants
   - Sampling times/schedules
   - Numbers of samples and sample replication strategy
   - Sample collection procedures
   - Sample processing procedures, including sample identification, labeling, preservation, and storage conditions
   - Sample shipping procedures

5. A detailed description of chain-of-custody procedures, including specification of standard chain-of-custody forms and clear assignment of field and laboratory personnel responsibilities for sample custody

6. Detailed descriptions of laboratory procedures for sample receipt, storage, and preparation, including specification of the kinds of samples to be prepared for analyses (e.g., composite vs. individual, whole body vs. fillet, replicates)

7. Detailed descriptions of the analytical methods used for quantitation of target contaminants, and percent lipid determination including

   - Specification and definition of method detection limits
   - Method validation procedures for verification of specifications for method accuracy, precision, and detection limits prior to analysis of field samples

8. Detailed descriptions of methods routinely used to assess data accuracy, precision, and completeness, including

- Internal QC checks using field, reagent, or method blanks; spiked samples; split samples; QC samples prepared from standard reference materials; and replicate analyses
- Calibration checks
- Data quality assessments

9. Detailed descriptions of calibration procedures for all measurement instruments, including specification of reference materials used for calibration standards and calibration schedules

10. Detailed descriptions of preventive maintenance procedures for sampling and analysis equipment

11. Detailed description of health and safety procedures

12. Detailed descriptions of recordkeeping and documentation procedures, including requirements for

    - Maintaining field and laboratory logs and notebooks
    - Use of standard data collection and reporting forms
    - Making changes to original records
    - Number of significant figures to be recorded for each type of data
    - Units of reporting
    - Routine procedures to assess the accuracy and completeness of records

13. Detailed descriptions of data analysis procedures, including

    - Statistical treatment of data
    - Data summary formats (e.g., plots, tables)

14. Detailed descriptions of data management and reporting procedures, including requirements for

    - Technical reports
    - QA and QC reports
    - Data coding procedures
    - Database specifications
    - QA review of reported data
    - Data storage and archiving procedures

15. Detailed descriptions of procedures for internal QC performance and/or systems audits for sampling and analysis programs.

16. Detailed descriptions of procedures for external QA performance and/or systems audits for sampling and analysis programs, including participation in certified QA proficiency testing or interlaboratory comparison programs.

17. Detailed descriptions of corrective action procedures in both sampling and analysis programs, including

    - Criteria and responsibility for determining the need for corrective action
    - Procedures for ensuring that effective corrective action has been taken
    - Procedures for documenting and reporting corrective actions

18. A description of procedures for documenting deviations from standard procedures, including deviations from QA or QC requirements

19. A description of the procedure for obtaining approval for substantive changes in the monitoring program.

Guidance for addressing each of the QA or QC elements outlined above, including a list of recommended standard reference materials and external QA or interlaboratory comparison programs for the analyses of target analytes, is incorporated in the appropriate sections of this guidance document.

APPENDIX G

RECOMMENDED PROCEDURES FOR PREPARING WHOLE FISH COMPOSITE HOMOGENATE SAMPLES

APPENDIX G

RECOMMENDED PROCEDURES FOR PREPARING WHOLE FISH COMPOSITE HOMOGENATE SAMPLES

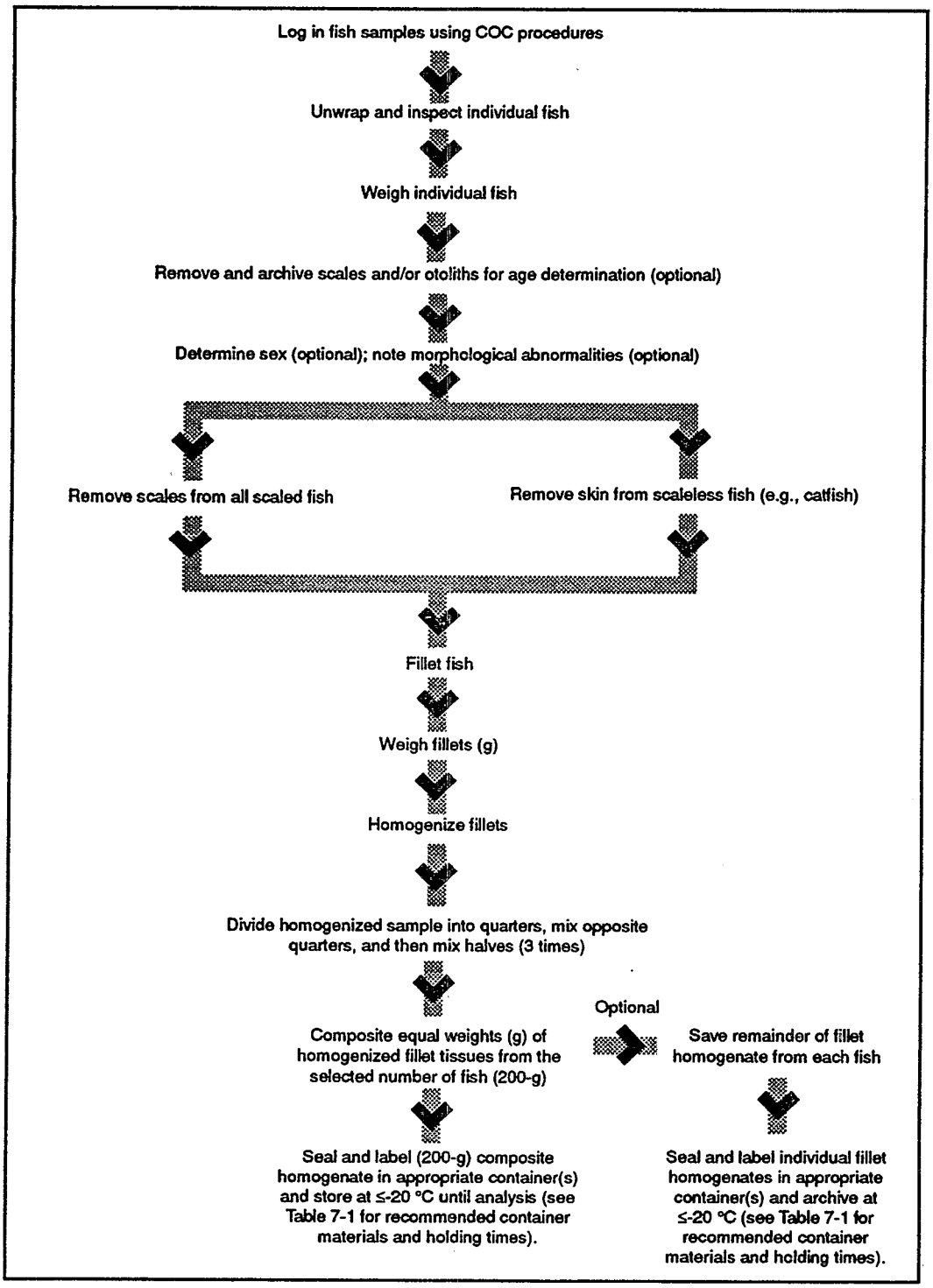
G.1 GENERAL GUIDELINES

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

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

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

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



COC = Chain of custody.

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

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

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

PTFE = polytetrafluoroethylene; Teflon.

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

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

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

The thawed or partially thawed whole fish should then be homogenized individually, and equal weights of each homogenate should be combined to form the composite sample. Individual homogenates and/or composite homogenates may be frozen; however, frozen individual homogenates must be rehomogenized before compositing, and frozen composite homogenates must be rehomogenized before aliquotting for analysis. The maximum holding time from sample collection to analysis for mercury is 28 days at ≤-20 °C; for all other analytes, the holding time is 1 year at ≤-20 °C (Stober, 1991). Recommended container materials, preservation temperatures, and holding times are given in Table G-1. **Note:** Holding times in Table G-1 are maximum times recommended for holding samples from the time they are received at the laboratory until they are anaylzed. These holding times are based on guidance that is sometimes administrative rather than technical in nature; there are no promulgated holding time criteria for tissues (U.S. EPA, 1995b). If States choose to use longer holding times, they must demonstrate and document the stability of the target analyte residues over the extended holding times.

G.2 SAMPLE PROCESSING PROCEDURES

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

G.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.

G.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.

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

Project No. _____ Sampling Date and Time: _____

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

SITE LOCATION

Site Name/Number: _____

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

State Waterbody Segment Number: _____ Waterbody Type: _____

Bottom Feeder – Species Name: _____

Composite Sample #: _____ Number of Individuals: _____

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

Total Composite Homogenate Weight _____

Predator – Species Name: _____

Composite Sample #: _____ Number of Individuals: _____

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

Total Composite Homogenate Weight _____

Notes: _____

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

Fish shipped on wet or blue ice should be weighed directly on a foil-lined balance tray. To prevent cross contamination between individual fish, the foil lining should be replaced after each weighing. Frozen fish (i.e., those shipped on dry ice) should be weighed in clean, tared, noncontaminating containers if they will thaw before the weighing can be completed. Liquid from the thawed sample must be kept in the container as part of the sample because it will contain lipid material that has separated from the tissue (Stober, 1991).

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

G.2.3 Age Determination

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

G.2.4 Sex Determination (Optional)

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

G.2.5 Assessment of Morphological Abnormalities (Optional)

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

G.2.6 Preparation of Individual Homogenates

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

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

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

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

G.2.7 Preparation of Composite Homogenates

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

Each composite homogenate should be blended by dividing it into quarters, mixing opposite quarters together by hand, and mixing the two halves together. The quartering and mixing should be repeated at least two more times. If the sample is to be analyzed only for metals, the composite homogenate may be mixed by hand in a polyethylene bag (Stober, 1991). At this time, the composite homogenate may be processed for analysis or frozen and stored at ≤-20 °C (see Table G-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 G-2. The total composite weight required for intensive studies may be less than in screening studies if the number of target analytes is reduced significantly.

The recommended sample size of 200 g for screening studies is intended to provide sufficient sample material to (1) analyze for all recommended target analytes (see Table 4-1) at appropriate detection limits, (2) meet minimum QA and QC requirements for the analyses of replicate, matrix spike, and duplicate matrix spike samples (see Section 8.3.3.4), and (3) allow for reanalysis if the QA and QC control limits are not met or if the sample is lost. However, sample size requirements may vary among laboratories and the analytical methods used.

Table G-2. Weights (g) of Individual Homogenates Required for Screening Study Composite Homogenate Sample[a]

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.

Therefore, it is the responsibility of each program manager to consult with the analytical laboratory supervisor to determine the actual weights of composite homogenates required to analyze for all selected target analytes at appropriate detection limits.

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APPENDIX H

GENERAL PROCEDURES FOR REMOVING EDIBLE TISSUES FROM FRESHWATER TURTLES

APPENDIX H

GENERAL PROCEDURES FOR REMOVING EDIBLE TISSUES FROM FRESHWATER TURTLES

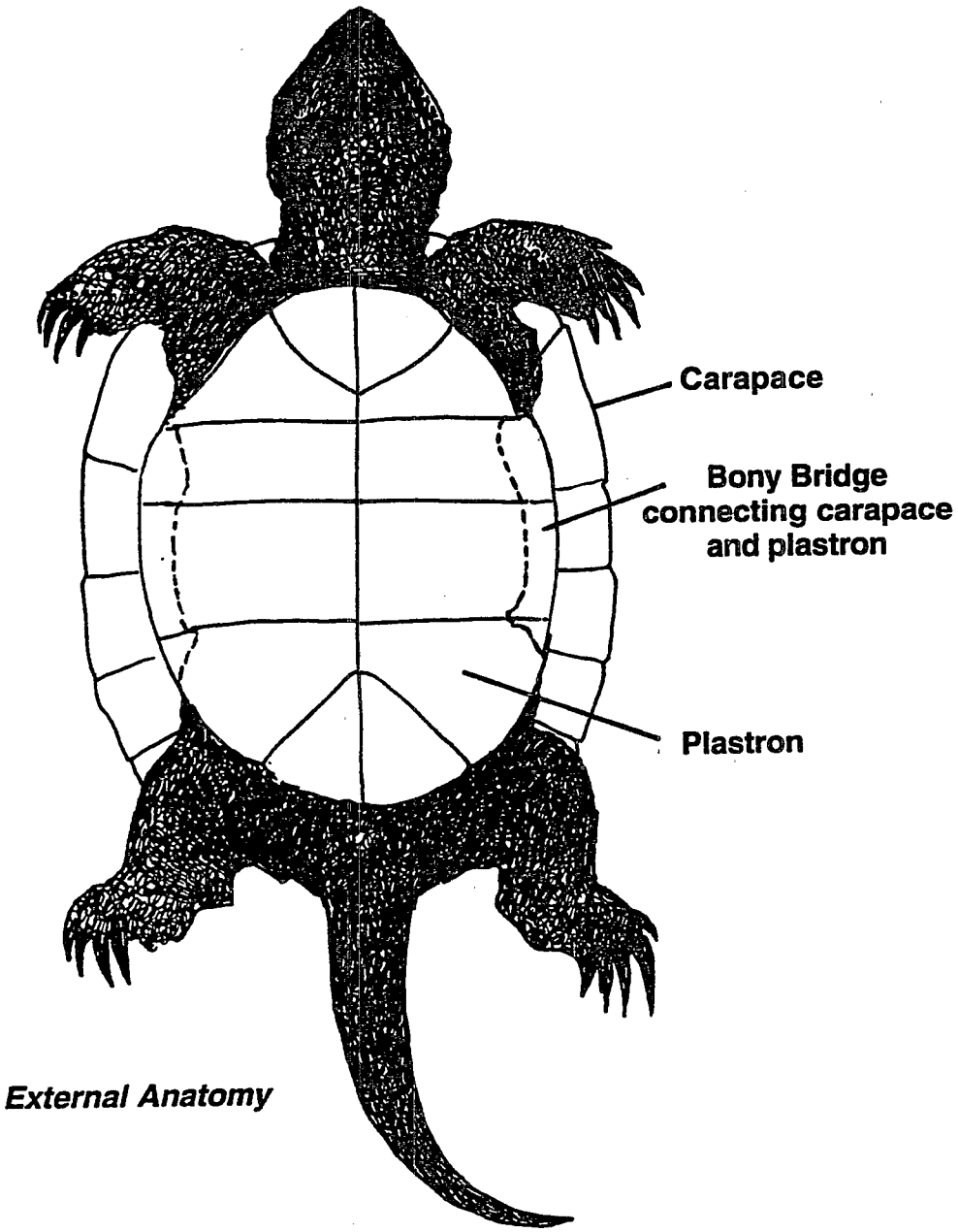
1. Turtles brought to the processing laboratory on wet, blue, or dry ice should be placed in a freezer for a minimum of 48 hours prior to resection. Profound hypothermia can be employed to induce death (Frye, 1994) Decapitation of alert animals is not recommended because there is evidence that decapitation does not produce instantaneous loss of consciousness (Frye, 1994).

2. The turtle should be placed on its back with the plastron (ventral plate) facing upwards. The carapace and plastron are joined by a bony bridge on each side of the body extending between the fore and hindlimbs (Figure H-1). Using a bone shears, pliers, or sharp knife, break away the two sides of the carapace from the plastron between the fore and hind legs on each side of the body.

3. Remove the plastron to view the interior of the body cavity. At this point, muscle tissue from the forelimbs, hindlimbs, tail (posterior to the anus), and neck can be resected from the body. The muscle tissue should be skinned and the bones should be removed prior to homogenization of the muscle tissue. Typically, the muscle tissue is the primary tissue consumed and turtle meat sold in local markets usually contains lean meat and bones only (Liner, 1978).

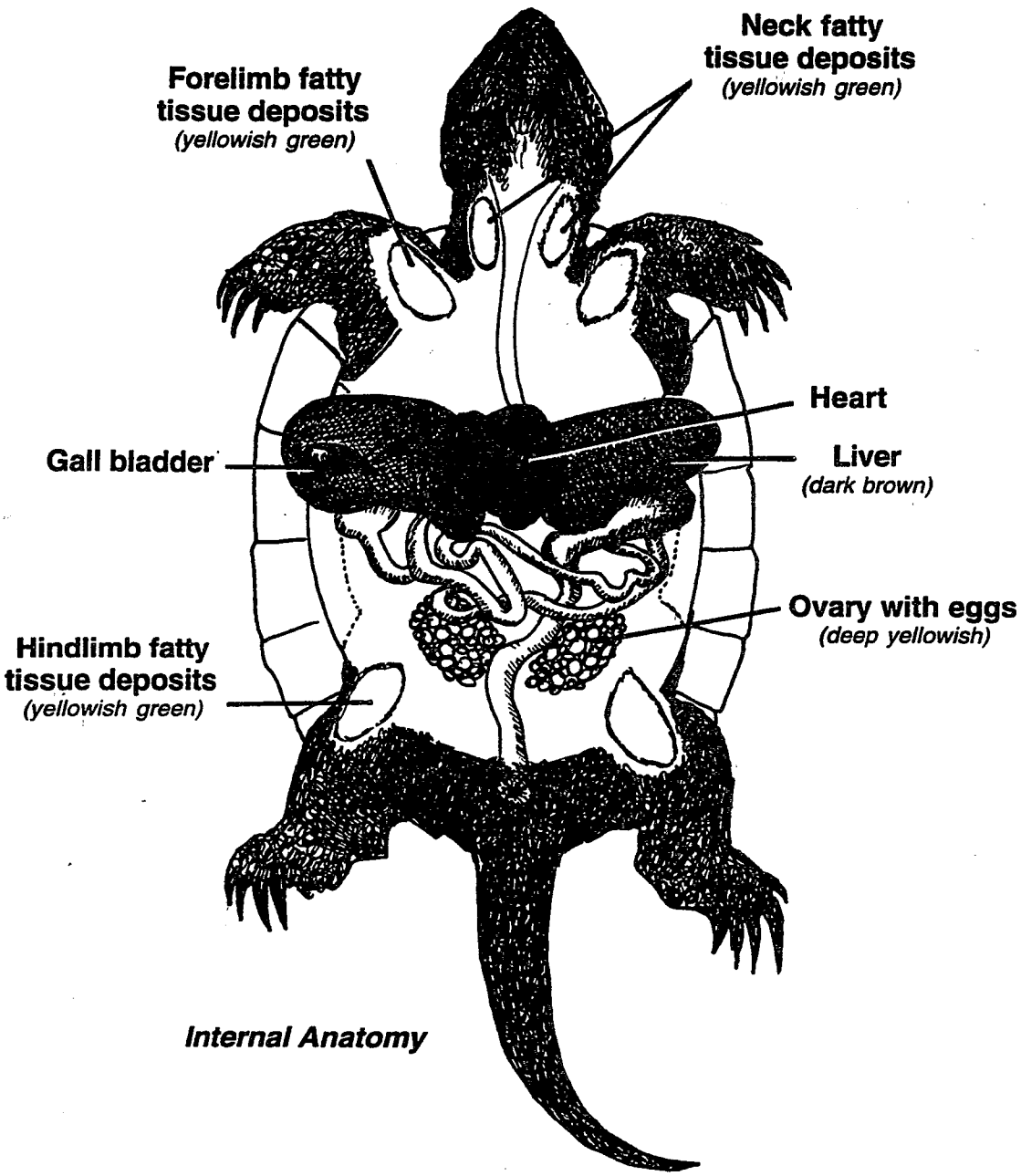
   Dietary and culinary habits with regard to which turtle tissues are edible, however, differ greatly among various populations. In some populations, the liver, heart, eggs, fatty deposits, and skin are also used (Liner, 1978). Therefore only general information on the types of turtle tissues most frequently considered edible can be presented here. State staff familiar with the dietary and culinary habits of the turtle-consuming populations within their jurisdictions are the best judge of which edible tissues should be included as part of the tissue samples used to assess the health risks to the turtle-consuming public.

4. Several of the tissue types that are considered edible include the fatty deposits found in various parts of the body, the heart, liver (usually with the gall bladder removed), and the eggs (if the specimen is a female). These edible tissues are shown in Figure H-2.



Source: Ashley, 1962.

Figure H-1.



Source: Ashley, 1962.

Figure H-2.

- Masses of yellowish-green fatty deposits may be removed from above the forelimbs and from above and in front of the hindlimbs. Fatty deposits can also be found at the base of the neck near the point where the neck enters the body cavity.

- The centrally located heart is positioned anterior to the liver.

- The large brownish liver is the predominant tissue in the body cavity and is an edible tissue eaten by some populations. Note: The small greenish-colored gall bladder lies on the dorsal side of the right lobe of the liver (not visible unless the liver is lifted upward and turned over). The gall bladder is usually removed and discarded by consumers because of its acrid taste (Liner, 1978).

- If the turtle specimen is a female, ovaries containing bright yellow-colored spherical eggs of varying sizes are located posterior to the liver and lie against the dorsal body wall.

Note: The fatty deposits, liver tissue, and eggs are highly lipophilic tissues and have been shown to accumulate chemical contaminants at concentrations 10 to more than 100 times the concentrations reported from muscle tissue (Bryan et al., 1987; Hebert et al., 1993; Olafsson et al., 1983, 1987; Ryan et al., 1986; Stone et al., 1980). States may wish to resect the fatty tissues, liver, heart, and eggs for inclusion in the turtle muscle tissue sample to obtain a conservative estimate of the concentration to which the turtle-consuming public would be exposed. Alternatively, States may want to retain these tissues for individual analysis. Some States already advise their residents who consume turtles to remove all fatty tissues (Minnesota Department of Health, 1994; New York State Department of Health, 1994) and not to consume the liver and eggs (New York State Department of Health, 1994). These cleaning procedures are recommended as a risk-reducing strategy.

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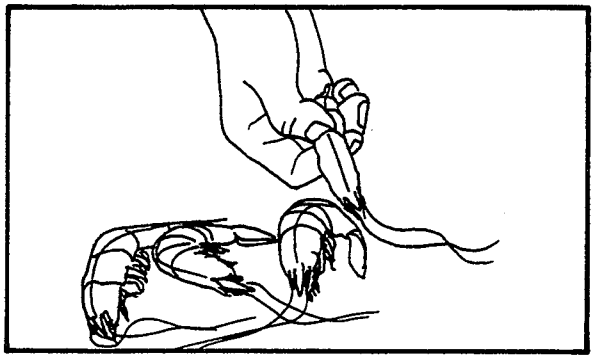
APPENDIX I

GENERAL PROCEDURES FOR REMOVING EDIBLE TISSUES FROM SHELLFISH

Heading, peeling and deveining shrimp

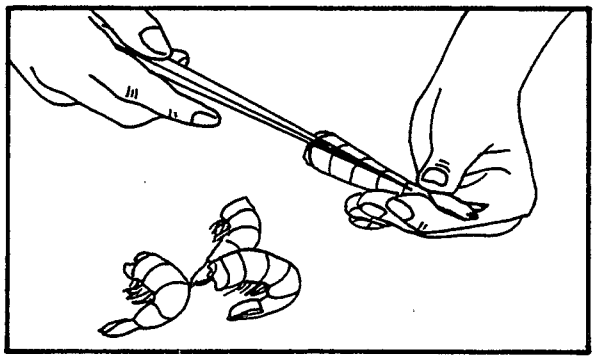
1

To head a shrimp, hold it in one hand. With your thumb behind shrimp head, push head off. Be sure to push just the head off so that you do not lose any meat.



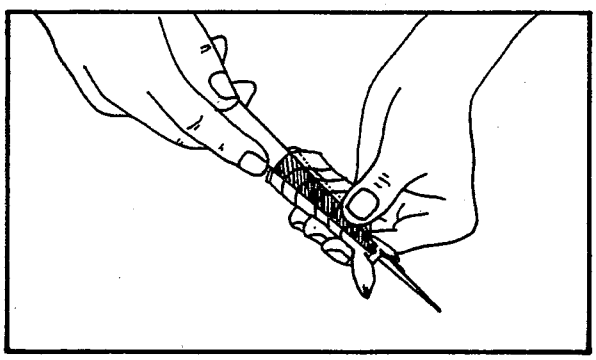
2

If using a deveiner, insert it at head end, just above the vein.



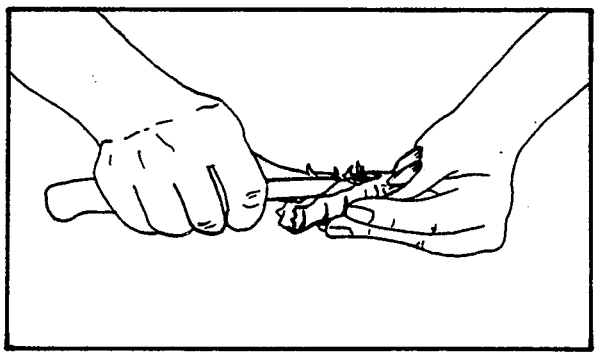
3

Push through shrimp to the tail and split and remove shell. This removes vein at the same time.



4

If you prefer to use a paring knife, shell shrimp with your fingers or knife. Then use knife to gently remove vein.

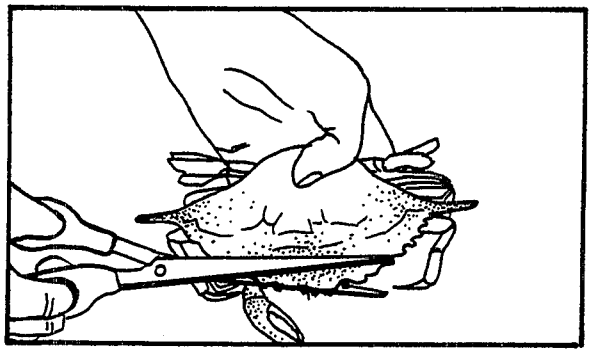


Source: UNC Sea Grant Publication UNC-SG-88-02

Cleaning soft-shell crabs

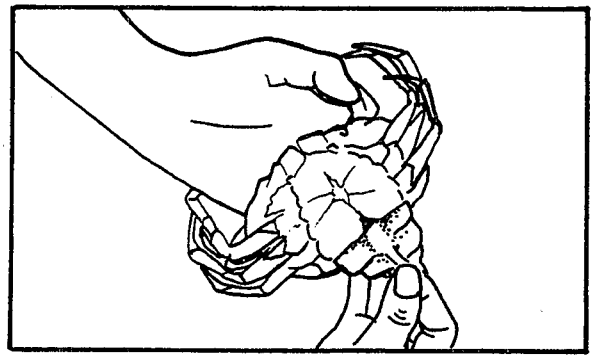
1

Hold crab in one hand and cut across body just behind eyes to remove eyes and mouth.



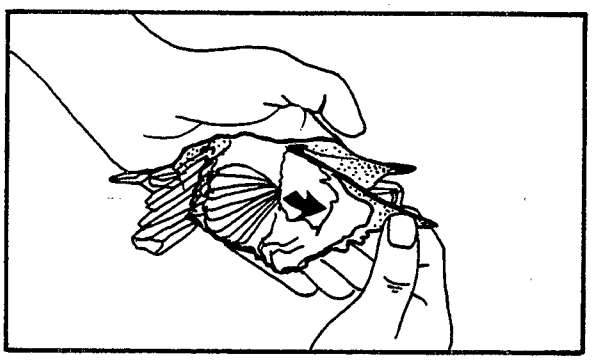
2

Turn crab on its back. Lift and remove apron and vein attached to it.



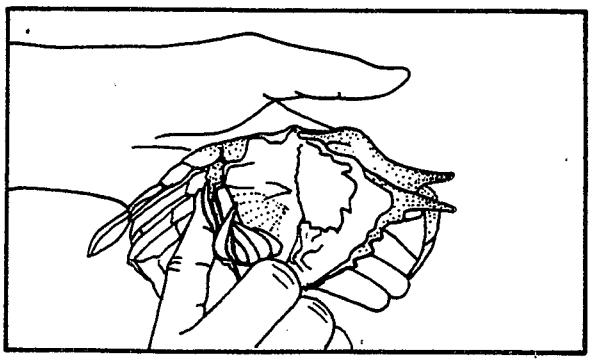
3

Turn crab over and lift one side of top shell.



4

With a small knife, scrape off grayish-feathery gills. Repeat procedure on other side.

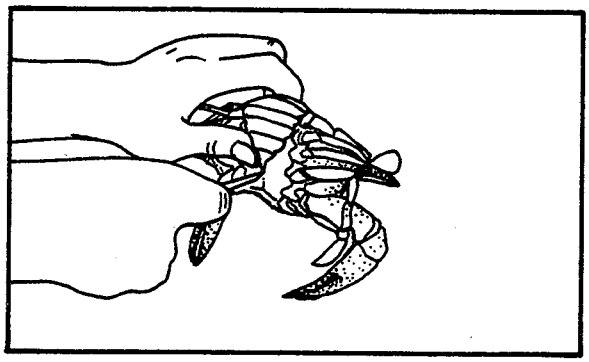


Source: UNC Sea Grant Publication UNC-SG-88-02

Cleaning hard-shell crabs

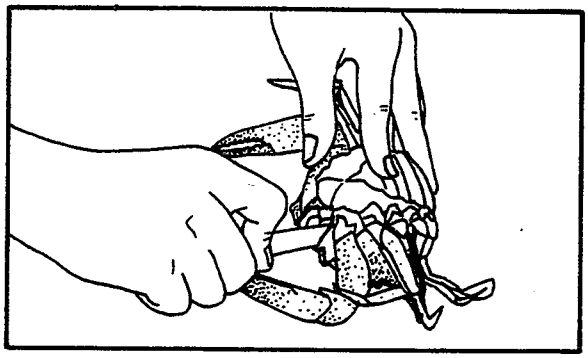
1

Hold crab in one hand. Turn crab over and stab straight down at point of apron with a knife.



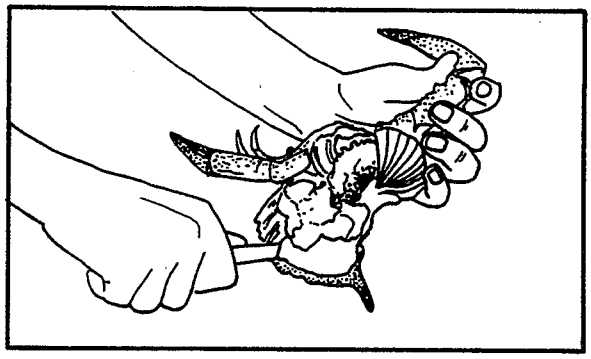
2

Make two cuts from this point to form a V-pattern that will remove mouth.



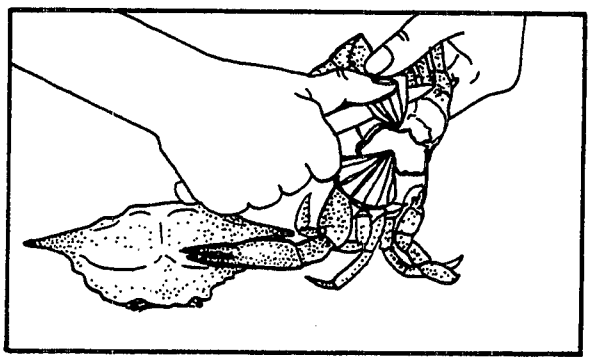
3

Do not remove knife after making second cut. Firmly press crab shell to cutting surface without breaking back shell. With other hand, grasp crab by legs and claws on the side where you are holding knife, and pull up. This should pull crab body free from back shell.



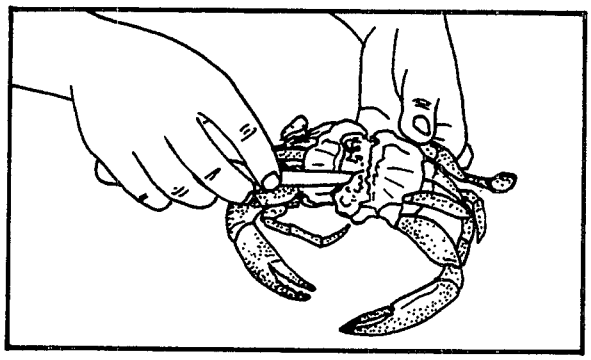
4

Remove gray, feathery gills, which are attached just above legs. Cut and scrape upward to remove gills.



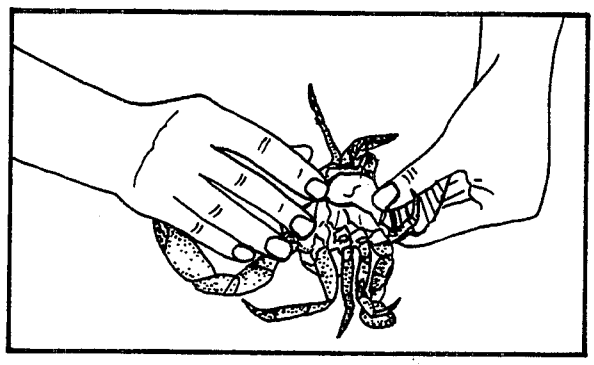
5

Remove all loose material—viscera and eggs—from body cavity.



6

If apron did not come loose with shell, remove it.

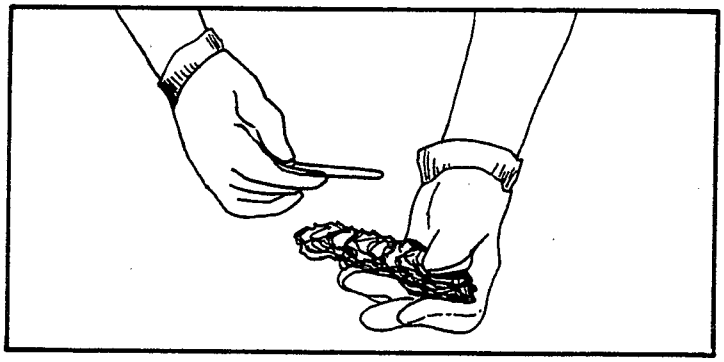


Source: UNC Sea Grant Publication UNC-SG-88-02

Shucking oysters

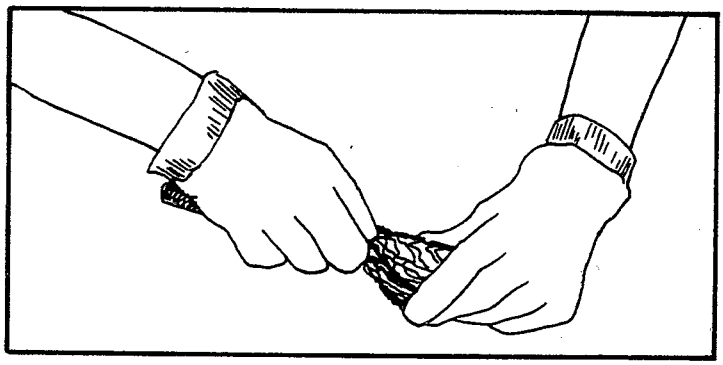
1

Oyster shells are especially sharp; be sure to wear gloves to protect your hands. Chip off a small piece of shell from the thin lip of the oyster until there is a small opening.



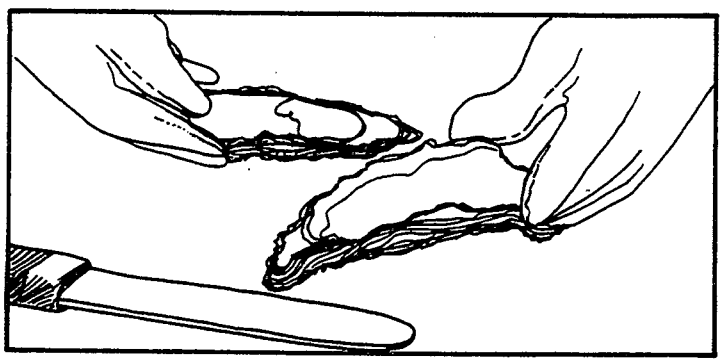
2

Insert knife blade into the opening and cut muscle free from top and bottom shells.



3

Remove oyster meat from the shell.

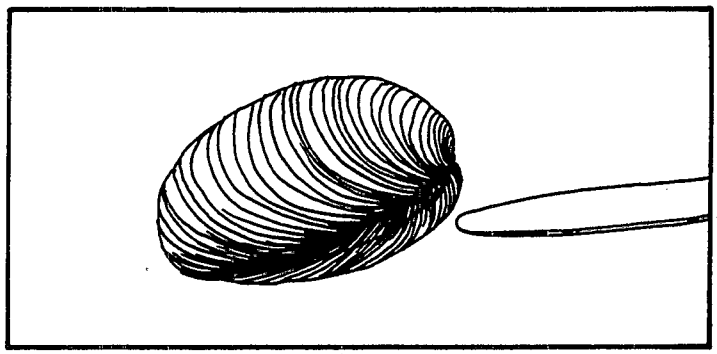


Source: UNC Sea Grant Publication UNC-SG-88-02

Shucking clams

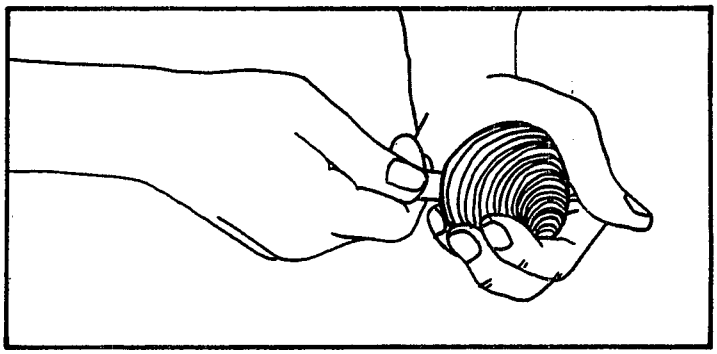
1

In the back of clam near the hinge is a black ligament. Toward the front where ligament ends is a weak spot. Insert your knife at this spot.



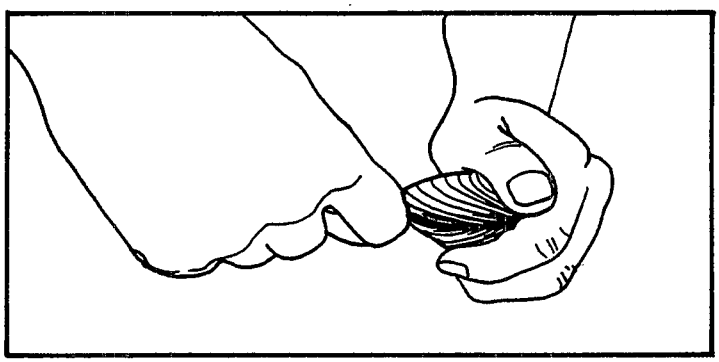
2

Inside are two muscles. Run the knife around the shell to sever both muscles.



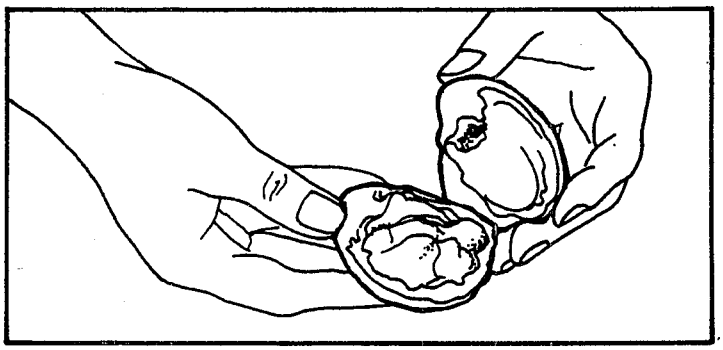
3

Now insert the knife blade into the front of the shell and separate the two shells.



4

Scrape the meat free from the top and bottom shell.



Source: UNC Sea Grant Publication UNC-SG-88-02

APPENDIX J

COMPARISON OF TARGET ANALYTE SCREENING VALUES (SVs) WITH DETECTION AND QUANTITATION LIMITS OF CURRENT ANALYTICAL METHODS

Table J-1. Comparison of Target Analyte Screening Values (SVs) with Reported Detection and Quantitation Limits of Current Analytical Methods[a]

		Methods									
		Puget Sound Protocols[c]		National Study of Chemical Residues in Fish[d]		EMSL[e]	National Contaminant Biomonitoring Program[f]		California OEHHA[g]	State of California, Dept. of Fish and Game Environmental Services Division[h]	EPA 301(h) Monitoring Program[i]
Target Analyte	SV[b]	LOD[j]	PQL[k]	MLD[l]	TQL[m]	MDL[n]	LOD[o]	LOQ[p]	MDL[q]	LOD[r]	Detection Limits[s]
Metals											
Arsenic (inorganic)[t]	3 ppm	N/I	N/I	N/I	N/I	N/I	N/I	N/I	N/I	N/I	N/I
Cadmium	10 ppm	0.01 ppm	N/R	N/I	N/I	0.02 ppm	0.005-0.046 ppm	N/R	N/I	0.01-0.1 ppm	0.01 ppm (GFAA); 0.4 ppm (ICP)
Mercury	0.6 ppm	0.01 ppm	N/R	1.3 ppb (LOD)[u]	N/R	0.1 ppm	0.01-0.05 ppm	N/R	0.050 ppm	0.02 ppm	0.01 ppm (CVAA)
Selenium	50 ppm	N/I	N/I	N/I	N/I	0.6 ppm	0.017-0.15 ppm	N/R	N/I	0.05 ppm	0.02 ppm (GFAA)
Tributyltin	0.3 ppm	N/I	N/I	N/I	N/I	N/I	N/I	N/I	2.5 ppb[v]	0.05 ppm (dry weight)[v]	N/I
Organochlorine Pesticides						N/I					0.1-5 ppb[w]
Chlordane (total)	80 ppb										
cis-Chlordane		1-5 ppb	20 ppb	N/R	2.5 ppb		<1.5 ppb	2-15 ppb	3-5 ppb	5 ppb	
trans-Chlordane		N/I	N/I	N/R	2.5 ppb		<1.5 ppb	2-15 ppb	2-5 ppb	5 ppb	
cis-Nonachlor		N/I	N/I	N/R	2.5 ppb		<1.5 ppb	2-15 ppb	N/I	5 ppb	
trans-Nonachlor		N/I	N/I	N/R	2.5 ppb		<1.5 ppb	2-15 ppb	4-7 ppb	5 ppb	
Oxychlordane		N/I	N/I	N/R	2.5 ppb		<1.5 ppb	2-15 ppb	N/I	5 ppb	
DDT (total)	300 ppb								38 ppb		
4,4´-DDT		0.1-2 ppb	4 ppb	N/I	N/I		<1.5 ppb	2-15 ppb	7-13 ppb	10 ppb	
2,4´-DDT		0.1-2 ppb	4 ppb	N/I	N/I		<1.5 ppb	2-15 ppb	5-6 ppb	10 ppb	
4,4´-DDD		0.1-2 ppb	4 ppb	N/I	N/I		<1.5 ppb	2-15 ppb	5-6 ppb	10 ppb	
2,4´-DDD		0.1-2 ppb	4 ppb	N/I	N/I		<1.5 ppb	2-15 ppb	3-5 ppb	10 ppb	
4,4´-DDE		0.1-2 ppb	4 ppb	N/R	2.5 ppb		<1.5 ppb	2-15 ppb	15-38 ppb	5 ppb	
2,4´-DDE		0.1-2 ppb	4 ppb	N/I	N/I		<1.5 ppb	2-15 ppb	6-10 ppb	10 ppb	
Dicofol	10,000 ppb	N/I	N/I	N/R	2.5 ppb		N/I	N/I	N/I	100 ppb	
Dieldrin	7 ppb	0.1-2 ppb	4 ppb	N/R	2.5 ppb		<1.5 ppb	2-15 ppb	N/I	5 ppb	
Endosulfan (total)	60,000 ppb										
Endosulfan I		N/I	N/I	N/I	N/I		N/I	N/I	N/I	5 ppb	
Endosulfan II		N/I	N/I	N/I	N/I		N/I	N/I	N/I	70 ppb	
Endrin	3,000 ppb	N/I	N/I	N/R	2.5 ppb		<1-5 ppb	2-15 ppb	N/I	15 ppb	

See notes and references at end of table.

(continued)

Table J-1 (continued)

		Methods									
		Puget Sound Protocols[e]		National Study of Chemical Residues in Fish[d]		EMSL[o]	National Contaminant Biomonitoring Program[f]		California OEHHA[g]	State of California, Dept. of Fish and Game Environmental Services Division[h]	EPA 301(h) Monitoring Program[i]
Target Analyte	SV[b]	LOD[j]	PQL[k]	MLD[j]	TQL[m]	MDL[n]	LOD[o]	LOQ[p]	MDL[q]	LOD[r]	Detection Limits[s]
Organochlorine Pesticides (continued)											
Heptachlor epoxide	10 ppb	N/I	N/I	N/R	2.5 ppb		<1-5 ppb	2-15 ppb	N/I	N/I	
Hexachlorobenzene	70 ppb	0.1-2 ppb	4 ppb	N/R	2.5 ppb		<1-5 ppb	2-15 ppb	N/I	N/R	
Lindane	80 ppb	0.1-2 ppb	4 ppb	N/R	2.5 ppb		<1-5 ppb	2-15 ppb	N/I	2 ppb	
Mirex	2,000 ppb	N/I	N/I	N/R	2.5 ppb		<1-5 ppb	2-15 ppb	N/I	N/I	
Toxaphene	100 ppb	3-15 ppb	60 ppb	N/I	N/I		60 ppb	153 ppb	N/I	100 ppb	
Organophoshate Pesticides						N/I					1-15 ppb
Chlorpyrifos	30,000 ppb	N/I	N/I	N/R	2.5 ppb		N/I	N/I	N/I	10 ppb	
Diazinon	900 ppb	N/I	N/I	N/I	N/I		N/I	N/I	N/I	50 ppb	
Disulfoton	500 ppb	N/I	N/I	N/I	N/I		N/I	N/I	N/I	N/I	
Ethion	5,000 ppb	N/I	N/I	N/I	N/I		N/I	N/I	N/I	20 ppb	
Terbufos	10,000 ppb	N/I	N/I	N/I	N/I		N/I	N/I	N/I	N/I	
Chlorophenoxy Herbicides											
Oxyfluorfen	800 ppb	N/I	N/I	N/I	N/I	N/I	N/I	N/I	N/I	N/I	N/I
PAHs[x]	10 ppb	20-100 ppb[y]	330 ppb[y]	N/I	N/I	N/I	N/I	N/I	N/I	N/R[z]	10 ppb
PCBs (total Aroclors)	10 ppb	(1-5 ppb)[aa]	(20 ppb)[aa]	N/R	(1.25-6.25 ppb)[bb]				50 ppb		
Aroclor 1016											20 ppb
Aroclor 1221											20 ppb
Aroclor 1232											20 ppb
Aroclor 1242		N/I	N/I	N/I	N/I		N/R	N/R	N/I	N/I	20 ppb
Aroclor 1248		N/I	N/I	N/I	N/I		62 ppb	167 ppb	N/I	50 ppb	20 ppb
Aroclor 1254		N/I	N/I	N/I	N/I		41 ppb	111 ppb	50 ppb	50 ppb	20 ppb
Aroclor 1260		N/I	N/I	N/I	N/I		61 ppb	155 ppb	50 ppb	50 ppb	20 ppb

See notes and references at end of table.

(continued)

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including those in Figure M-1, require uncorrelated data. Gilbert (1987) discusses several methods for performing the required analyses in these cases.

Temporal trends in contaminant concentrations may be detected by regression analyses, whereby the hypothesis is tested that concentrations are not changing in a predictable fashion (usually linear) over time. If the hypothesis is rejected, a trend may be inferred. States interested in performing regression analyses should consult statistics textbooks such as Gilbert (1987) or Snedecor and Cochran (1980).

M.3 REFERENCES

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Snedecor, G.W., and W.G. Cochran. 1980. *Statistical Methods.* 7th edition. Iowa State University Press, Ames, Iowa.

tion from any single station would not truly represent the potential contaminant exposure to fish consumers in the waterbody of concern.

M.2 TEMPORAL COMPARISON OF STATIONS

Both screening and intensive studies are often repeated over time to ensure that public health is adequately protected. By examining monitoring data from several time periods from a single site, it may be possible to detect trends in contaminant concentrations in fish tissues. Trend analysis data should never be used to conduct risk assessments. Procedures for conducting risk assessments are adequately covered elsewhere in this document (see Section 6.1.2.7). Trend analysis may, however, be useful for monitoring the effects of various environmental changes or policies on the contaminant concentrations in the target species. For example, a State may have issued a fish advisory for a contaminant for which the source is known or suspected. Source control for this contaminant is the obvious solution to the environmental problem. An evaluation of the effectiveness of the source control may be made easier by trend analysis. The State would still need to perform statistical calculations comparing data from each sampling site to the SV, but trend analysis could yield valuable information about the success of remediation efforts even if the fish advisory remained in place because of SV exceedances.

Trend analysis can be performed using the statistical framework outlined in Figure M-1, but complexities in pollution data collected over time may make this approach unsuitable in some instances. The types of complexities for which other statistical approaches might be warranted can be divided into four groups: (1) changes in sampling and/or analysis procedures, (2) seasonality, and (3) correlated data (Gilbert, 1987). Each of these subjects is discussed briefly here.

Changes in the designation of an analytical laboratory to perform analyses or changes in sampling and/or analytical procedures are not uncommon in long-term monitoring programs. These changes may result in shifts in the mean or variance of the measured values, which could be incorrectly attributed to natural or manmade changes in the processes generating the pollution (Gilbert, 1987). Ideally, when changes occur in the methods used by the monitoring program, comparative studies should be performed to estimate the magnitude of these changes.

Seasonality may introduce variability that masks any underlying long-term trend. Statistically, this problem can be alleviated by removing the cycle before applying tests or by using tests unaffected by cycles (Gilbert, 1987). Such tests will not be discussed here. States interested in performing temporal analyses with data for which a seasonal effect is hypothesized should consult the nonparametric test developed by Sen (1968) or the seasonal Kendall test (Hirsch et al., 1982).

Measurements of contaminant concentrations taken over relatively short periods of time are likely to be positively correlated. Most statistical tests, however,

A general statistical flowchart for comparing contaminant concentration data from several stations to each other is presented in Figure M-1. The cadmium data in Table M-1 may be additionally analyzed using the tests in Figure M-1. All of the statistical tests in Figure M-1 can be performed using commercial statistical software packages. By performing a spatial analysis of the data, the details of the risk assessment might be further refined. For example, one component of a fish advisory is often the establishment of risk-based consumption limits (see Volume II of this series). In order to calculate these limits, an estimate of the contaminant concentration in the target species must be available. In the example shown in Table M-1, there are three estimates of cadmium concentration. A spatial analysis of these data can help to identify which of the concentrations (if any) to use in establishing risk-based consumption limits.

The initial steps in the flowchart on Figure M-1 are to determine whether parametric or nonparametric statistical tests should be used. The first step is to test whether each of the three groups of data are from populations that are normally distributed. Three tests that may be used for this purpose are the Kolmogorov-Smirnov test for normality (Massey, 1951), Shapiro and Wilk's W test (Shapiro et al., 1968; Royston, 1982), and Lilliefors' test (Lilliefors, 1967). The results for the W test on each of the three groups of data indicate that each group was sampled from populations that are normally distributed (Table M-1). The next step is to test for homogeneity of variances between the three groups. Three tests that may be used for this purpose are Levene's test (Milliken and Johnson, 1984), the Hartley F-max test (Sokal and Rohlf, 1981), and the Cochran C test (Winer, 1962). The result of Levene's test indicates that the variances of the three groups of data are not significantly different from each other (Table M-1). These test results mean that parametric statistics (the left side of Figure M-1) are appropriate for this dataset.

An appropriate parametric test to perform to determine whether the three mean cadmium concentrations are significantly different from each other is a 1-way ANOVA. The result of this test indicates that the three means are significantly different (Table M-1). What this result does not show, however, is whether each mean concentration is significantly different from both of the other mean concentrations. For this answer, multiple comparison tests can be used to perform all possible pairwise comparisons between each mean.

Three tests that can be used to perform a multiple comparison are the Newman-Keul test (Sokal and Rohlf, 1981), Duncan's Multiple Range test (Hays, 1988; Milliken and Johnson, 1984), and the Tukey Honest Significant Difference test (Hays, 1988; Milliken and Johnson, 1984). Three pairwise comparisons are possible between three means (1 vs. 2, 1 vs. 3, and 2 vs. 3). The results of Duncan's Multiple Range test indicate that the mean concentration at station 1 (21.5 ppm) is significantly lower than the mean concentrations at both station 2 (29.4 ppm) and station 3 (31.3 ppm), which in turn are not significantly different from each other. Therefore, to be most conservative (i.e., protective), the State could use the mean of the 16 replicate samples from stations 2 and 3 to calculate risk-based consumption limits. In this example, use of the concentra-

each location and the statistical comparisons between the three groups are presented in Table M-1.

The mean cadmium concentration at each of three locations was more than twice the SV of 10 ppm (Table M-1). The most important statistical test, as indicated in Section 6.1.2.7, is a comparison of the mean target analyte concentration for each location with the appropriate SV for that target analyte using a *t*-test. These tests must be performed before any analysis of spatial trends is performed. The results of the *t*-tests indicate that each of the three mean tissue concentrations is significantly greater than the SV (Table M-1). By itself, these results indicate that a risk assessment is warranted.

Table M-1. Hypothetical Cadmium Concentrations (ppm) in Target Species A at Three River Locations

Replicate samples	Station 1	Station 2	Station 3
1	20	28	33
2	18	27	30
3	25	34	30
4	22	28	28
5	21	30	20
6	22	29	39
7	23	30	31
8	21	29	30
Mean	21.5	29.4	31.3
Standard deviation	2.07	2.13	3.45
p-Value for *t*-test with SV	<0.001	<0.001	<0.001
p-Value for W test	0.97	0.83	0.78
p-Value for Levene's test		0.52	
p-Value for ANOVA		<0.0001	
p-Value for Duncan's-1 vs. 2		<0.0001	
p-Value for Duncan's-1 vs. 3		>0.0001	
p-Value for Duncan's-2 vs. 3		0.17	

difference in mean concentrations between two group means can be further investigated using a multiple comparison test (Figure M-1). These tests indicate which specific means are significantly different from each other, rather than just indicating that one or more means are different, as the ANOVA does.

If the underlying assumptions for parametric testing are not met, nonparametric tests of significance can be employed. Nonparametric tests of significant differences in central tendencies are often performed on transformed data, that is, the ranks. Multiple comparison tests comparable to those used for parametric data sets are not available for nonparametric data sets. For data sets including three or more groups, a series of two-sample tests can be performed that can yield similar information to that derived from multiple comparison tests.

Because the concentrations of contaminants, particularly nonpolar organics, are often correlated with the percentage of lipid in a tissue sample (see Section 8.1.2), 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.

Intensive studies may include the collection of fish contaminant data from several locations within a region of interest or for multiple time periods (e.g., seasons or years) from a single location, or a combination of both. Data from intensive studies such as these may be used to perform spatial (i.e., between stations) or temporal (i.e., over time) analyses. It should be noted that these types of analyses, if performed, are performed in addition to the statistical comparisons of mean target analyte concentrations with SVs described in Section 6.1.2.7. It is only the latter type of comparison that should be used to make decisions regarding the necessity of performing risk assessments and the issuance of fish consumption advisories. Spatial and temporal comparisons of contaminant data, however, may yield important information about the variability of target analyte concentrations in specific populations of a particular target species.

M.1 SPATIAL COMPARISON OF STATIONS

Intensive studies also may involve the collection of contaminant data from multiple stations within a waterbody of interest. The stations could be located in different lakes within a single drainage basin, upstream and downstream of a point source of concern along a single river, or randomly located within a single waterbody if an estimate of random spatial variability is desired. The use of an example will serve to illustrate how a spatial analysis of contaminant data might be performed. In this example, a State has determined from a screening study on a river that cadmium is present in a target species at 20 ppm, which is two times the SV of 10 ppm (see Table 5-2). An intensive survey was undertaken in which eight samples were collected from three locations on the river of potential concern and analyzed for cadmium. The results of the analyses for

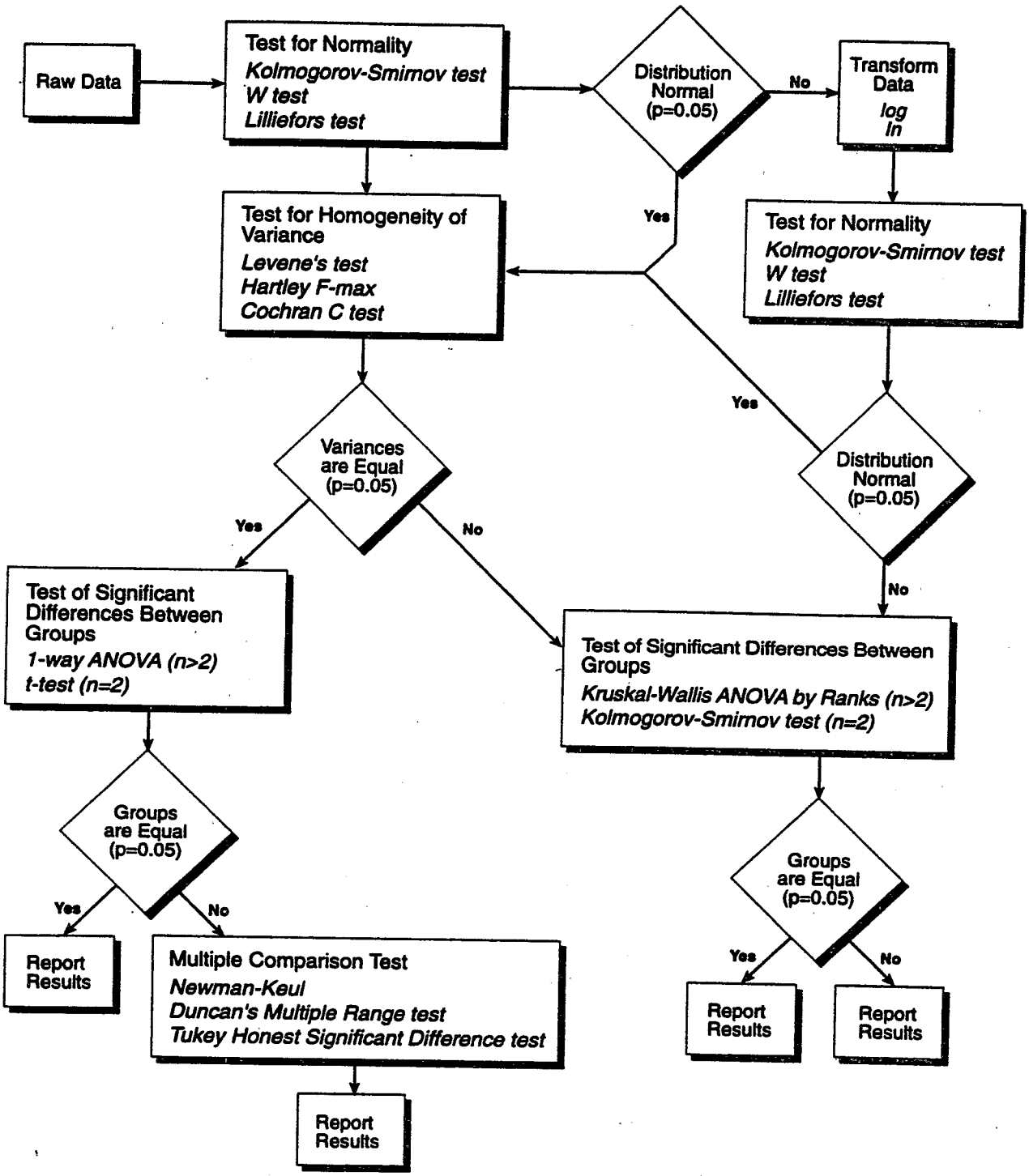

Figure M-1. Statistical approach to testing for significant differences between different groups of contaminant monitoring data.

APPENDIX M

STATISTICAL METHODS FOR COMPARING SAMPLES: SPATIAL AND TEMPORAL CONSIDERATIONS

The primary objective of Tier 2 intensive studies is to assess the magnitude and geographic extent of contamination in selected target species by determining whether the mean contaminant concentration exceeds the screening value (SV) for any target analyte. Secondary objectives of intensive studies may include defining the geographical region where fish contaminant concentrations exceed screening values (SVs), identifying geographic distribution of contaminant concentrations, and, in conjunction with historical or future data collection, assessing changes in fish contaminant concentrations over time. This appendix discusses some of the statistical methods that may be used to compare fish contaminant levels measured at different locations or over time.

The recommended statistical approach for comparing replicated contaminant measurements between two or more groups is outlined below and in Figure M-1. For each type of test, several options are provided, each of which may be appropriate in specific cases. State staff should consult a statistician as to the specific statistical tests to use for a particular data set.

Statistical tests of significant differences between means (or other measures of central tendency) can be divided into parametric and nonparametric types. Parametric tests assume that the contaminant concentrations in the population being sampled are normally distributed and that the population variances in the groups being tested are not significantly different from each other (Gilbert, 1987). If either of these assumptions is violated, a nonparametric test may be more appropriate. However, nonparametric tests should be used only when necessary because the power of parametric tests generally is greater than the power of nonparametric tests when the assumptions of the parametric test have been met (Sokal and Rohlf, 1981).

Because the populations of many environmental measurements are not normally distributed, logarithmic transformation is often performed on the sampled data (Gilbert, 1987). However, transformation may not be appropriate in all cases. If the data are sampled from a population that is normally distributed, then there is no need for transformation (Figure M-1).

If the assumptions of normality and equality of variance are met, parametric tests of significant differences between means, such as the one-way Analysis of Variance (ANOVA) and the *t*-test, should be performed. If three or more groups are compared using the ANOVA that results in a significant difference, the

APPENDIX M

STATISTICAL METHODS FOR COMPARING SAMPLES: SPATIAL AND TEMPORAL CONSIDERATIONS

RECOMMENDED PUBLICATIONS ON CERTIFIED STANDARDS AND REFERENCE MATERIALS

- **Standard and Reference Materials for Marine Science (NOAA, 1992). Available from**

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

  This catalog lists approximately 2,000 reference materials from 16 producers and includes information on their use, sources, matrix type, analyte concentrations, proper use, availability, and costs. Reference materials are categorized as follows: ashes, gases, instrumental performance, oils, physical properties, rocks, sediments, sludges, tissues, and waters. This catalog has been published independently by both NOAA and IOC/UNEP and is available in electronic form from the Office of Ocean Resources, Conservation, and Assessment, NOAA/NOS.

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

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

  This report contains approximately 2,700 analyte values for 117 analytes in 116 biological and 77 nonbiological environmental reference materials from more than 20 sources. Additional information on cost, sample size available, and minimum amount of material recommended for analysis is also provided.

REFERENCES

NOAA (National Oceanic and Atmospheric Administration). 1992. Standard and Reference Materials for Marine Science. Third Edition. U.S. Department of Commerce, Rockville, Maryland.

Toro, E. Cortes, R. M. Parr, and S. A. Clements. 1990. Biological and Environmental Reference Materials for Trace Elements, Nuclides and Organic Microcontaminants: A Survey. IAEA/RL/128(Rev. 1). International Atomic Energy Agency, Vienna.

RETAILERS OF EPA-CERTIFIED NEAT ORGANIC STANDARDS (Including the Former EPA Pesticide Repository Standards)

Absolute Standards
498 Russel Street
New Haven, CT 06513
Tel: 800-368-1131
FAX: 203-468-7407
Contact: Jack Ciscio

Accustandard
25 Science Park Road
New Haven, CT 06511
Tel: 203-786-5290
FAX: 203-786-5287
Contact: Mike Bolgar

Alltech Associates
2051 Waukegan Road
Deerfield, IL 60015
Tel: 708-948-8600
FAX: 708-948-1078
Contact: Tom Rendl

Ultra Scientific
250 Smith Street
North Kingston, RI 02852
Tel: 401-294-9400
FAX: 401-295-2330
Contact: Dr. Bill Russo

RETAILERS OF EPA-CERTIFIED INORGANIC QUALITY CONTROL SAMPLES

SPEX Industries, Inc.
3880 Park Avenue
Edison, NJ 08820
Tel: 1-201-549-7144 or 1-800-GET-SPEX
FAX: 1-201-549-5125

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Fisher Scientific
711 Forbes Avenue
Pittsburgh, PA 15219

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FAX: 203-468-7407
Contact: Jack Ciscio

Accustandard
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FAX: 708-948-1078
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Alameda Chemical and Scientific
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Phoenix, AZ 85034
Tel: 602-256-7044
FAX: 602-256-6566

Bodman Chemicals
P.O. Box 2221
Aston, PA 19014
Tel: 215-459-5600
FAX: 215-459-8036
Contact: Kirk Lind

Cambridge Isotope Laboratories
20 Commerce Way
Woburn, MA 01801-9894
Tel: 800-322-1174 or 617-938-0067
FAX: 617-932-9721

NSI Environmental Solutions, Inc.
P.O. Box 12313
2 Triangle Drive
Research Triangle Park, NC 27709
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FAX: 215-459-8036
Contact: Kirk Lind

Chemical Research Supply
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Addison, IL 60101
Tel: 708-543-0290
FAX: 708-543-0294
Contact: Nelson Armstrong

Crescent Chemical Corporation
1324 Motor Parkway
Hauppauge, NY 11788
Tel: 516-348-0333
FAX: 516-348-0913
Contact: Eric Rudnick

Curtis Matheson Scientific
P. O. Box 1546
9999 Veterans Memorial Drive
Houston, TX 77251-1546
Tel: 713-820-9898
FAX: 713-878-2221
Contact: Mitchel Martin

Environmental Research Associates
5540 Marshall Street
Arvada, CO 80002
Tel: 303-431-8454
FAX: 303-421-0159
Contact: Mark Carter

Restek Corporation
110 Benner Circle
Bellefonte, PA 16823
Tel: 814-353-1300
FAX: 814-353-1309
Contact: Eric Steindle

Supelco
Supelco Park
Bellefonte, PA 16823-0048
Tel: 800-247-6628 or 814-359-3441
FAX: 814-359-3044
Contact: Linda Alexander

Ultra Scientific
250 Smith Street
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FAX: 1-401-295-2330
Contact: Dr. Bill Russo

- EPA-certified inorganic quality control samples, including trace metals, minerals, and nutrients, are produced by:

  SPEX Industries, Inc.
  3880 Park Avenue
  Edison, NJ 08820
  Tel: 1-201-549-7144 or 1-800-GET-SPEX
  FAX: 1-201-549-5125

- EPA-certified solid matrix quality control samples, including standards for pesticides in fish tissue, are produced by:

  Fisher Scientific
  711 Forbes Avenue
  Pittsburgh, PA 15219

The most recent information on EPA-certified materials is available on the EPA Electronic Bulletin Board (Modum No. 513-569-7610). Names and addresses of retailers of EPA-certified CRADA QA/QC samples or standards as of February 20, 1991, are given below. When ordering these materials, specify "EPA Certified Materials."

APPENDIX L

SOURCES OF RECOMMENDED REFERENCE MATERIALS AND STANDARDS

SOURCES OF EPA-CERTIFIED REFERENCE MATERIALS

EPA-certified analytical reference materials for priority pollutants and related compounds are currently produced under five Cooperative Research and Development Agreements (CRADAs) for: organic quality control samples; organic solution standards; organic neat standards; inorganic quality control standards; and solid matrix quality control standards. The CRADA cooperators are listed below.

- EPA-certified organic quality control samples, including standards for pesticides in fish tissue, are produced by:

  Supelco, Inc.
  Supelco Park
  Bellefonte, PA 16823-0048
  Tel: 1-800-247-6628 or 1-814-359-3441
  FAX: 1-814-359-3044
  Contact: Linda Alexander

- EPA-certified organic solution standards for toxic and hazardous materials (formerly the EPA Toxic and Hazardous Materials Repository) are produced by:

  NSI Environmental Solutions, Inc.
  P. O. Box 12313
  2 Triangle Drive
  Research Triangle Park, NC 27709
  Tel: 1-800-234-7837 or 1-919-549-8980
  FAX: 1-919-544-0334

- EPA-certified neat organic standards, including neat pesticide standards (formerly the EPA Pesticide Repository), are produced by:

APPENDIX L

SOURCES OF RECOMMENDED REFERENCE MATERIALS AND STANDARDS

REFERENCES

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Table 2-11

PRECISION OF ARSENIC SPECIATION HYCO RESERVOIR
(February 1984)

	Sediment As, Sta. 5 $\mu g\ g^{-1}$ dry wt			Interstitial As, Sta. 5 $\mu g\ L^{-1}$			Water column, Sta. 4 $\mu g\ L^{-1}$		
Replicate	Total	As(V)	As(III)	Total	As(V)	As(III)	Total	As(V)	As(III)
1	38.33	25.15	13.18	75.8	41.1	34.7	1.222	1.128	0.094
2	36.61	21.74	14.87	67.1	29.9	37.2	1.082	0.983	0.099
3	25.27	15.24	10.03	77.2	32.0	45.2	1.186	1.079	0.107
4	21.28	12.75	8.53	--	--	--	--	--	--
5	29.49	17.26	12.23	--	--	--	--	--	--
6	28.71	16.97	11.74	--	--	--	--	--	--
N	6	6	6	3	3	3	3	3	3
$\bar{X}$	29.95	18.19	11.76	73.4	34.4	39.0	1.163	1.063	0.100
S	6.53	4.51	2.26	5.5	6.0	5.5	0.073	0.074	0.007
RSD	21.8%	24.8%	19.2%	7.5%	17.4%	14.1%	6.3%	6.9%	6.6%

Precision for Sediments and Water

The precision or reproducibility for replicate analyses of arsenic species in field samples is shown in Table 2-11. Collection of these field samples is described in Section 3 of this report. The sediment was analyzed for leachable As (III) and As (V). Interstitial water and water from Hyco Reservoir were also analyzed for As (III) and (V). The results indicate that the relative standard deviations (RSD) for arsenic (III) and (V) in sediment are approximately 20% while the RSD for these species in interstitial water and in the water column are approximately 15% and 7%.

CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER WORK

Arsenic speciation of a variety of materials in the limnological environment is simply and reproducibly achieved using selective hydride generation/low-temperature trapping techniques in conjunction with atomic absorption detection. The most difficult problem is the unambiguous determination of total arsenic in solids by this technique. Other related techniques which might be investigated include dry ashing, lithium metaborate fusion, and graphite furnace atomic absorption. An alternate method is to analyze select samples by X-ray fluorescence spectrometry.

Interlaboratory Comparison

An interlaboratory comparison exercise was conducted between Battelle-Northwest (BNW) and Dr. M. O. Andreae of Florida State University (FSU) to demonstrate the effectiveness of the sample storage and shipping procedure and varify the accuracy of the anlaytical technique for determination of arsenic species in fresh water. Three samples were prepared as follows: (1) Dungeness River water (DRW) was filtered, (2) filtered DRW was spiked with nominally 0.45 μg L^{-1} of As (V) and 2 μg L^{-1} each of DMA and MMA, and (3) coal fly ash, standard reference material NBS-1633, was leached with DRW then filtered. All solutions were frozen immediately after preparation in liquid nitrogen then transferred and stored at -80°C. Samples were shipped on dry ice. Samples were analyzed at BNW and FSU the same week approximately two months after preparation. The results in Table 2-10 show good agreement between these two laboratories even for concentrations below 0.1 μg L^{-1}. We believe this interlaboratory exercise has demonstrated that these storage and shipping procedures are appropriate for freshwater samples and the analytical method used for arsenic speciation is sensitive and accurate for concentrations of inorganic arsenic greater than approximately 0.05 and for organic arsenic concentrations greater than 0.2 μg L^{-1}.

Table 2-10

ARSENIC SPECIATION INTERCOMPARISON EXERCISE

	μg ℓ^{-1}							
	As (III)		As (V)		MMA		DMW	
Sample	BNW	Andreae	BNW	Andreae	BNW	Andreae	BNW	Andreae
DRW	0.061 ±0.004	0.067	0.042 ±0.008	0.023	<0.01	0.002	<0.01	0.067
SDRW	0.061 ±0.005	0.066	0.468 ±0.028	0.421	1.96 ±0.11	1.67	1.92 ±0.13	1.82
FA	0.052 ±0.006	0.031	12.9 ±0.2	12.0	<0.01	ND	<0.01	ND

Intercomparison exercise results with Meinrat O. Andreae for arsenic speciation in limnological samples. DRW is filtered Dungeness River water; SDRW is Dungeness River water spiked with nominally 0.45 μg·ℓ^{-1} As (V), and 2 μg·ℓ^{-1} each DMA and MMA. FA is the filtrate of 1000 mg·ℓ^{-1} NBS coal fly ash leached with DRW. BNW results are the mean of (3) determinations. ND means not detected. ± = one standard deviation.

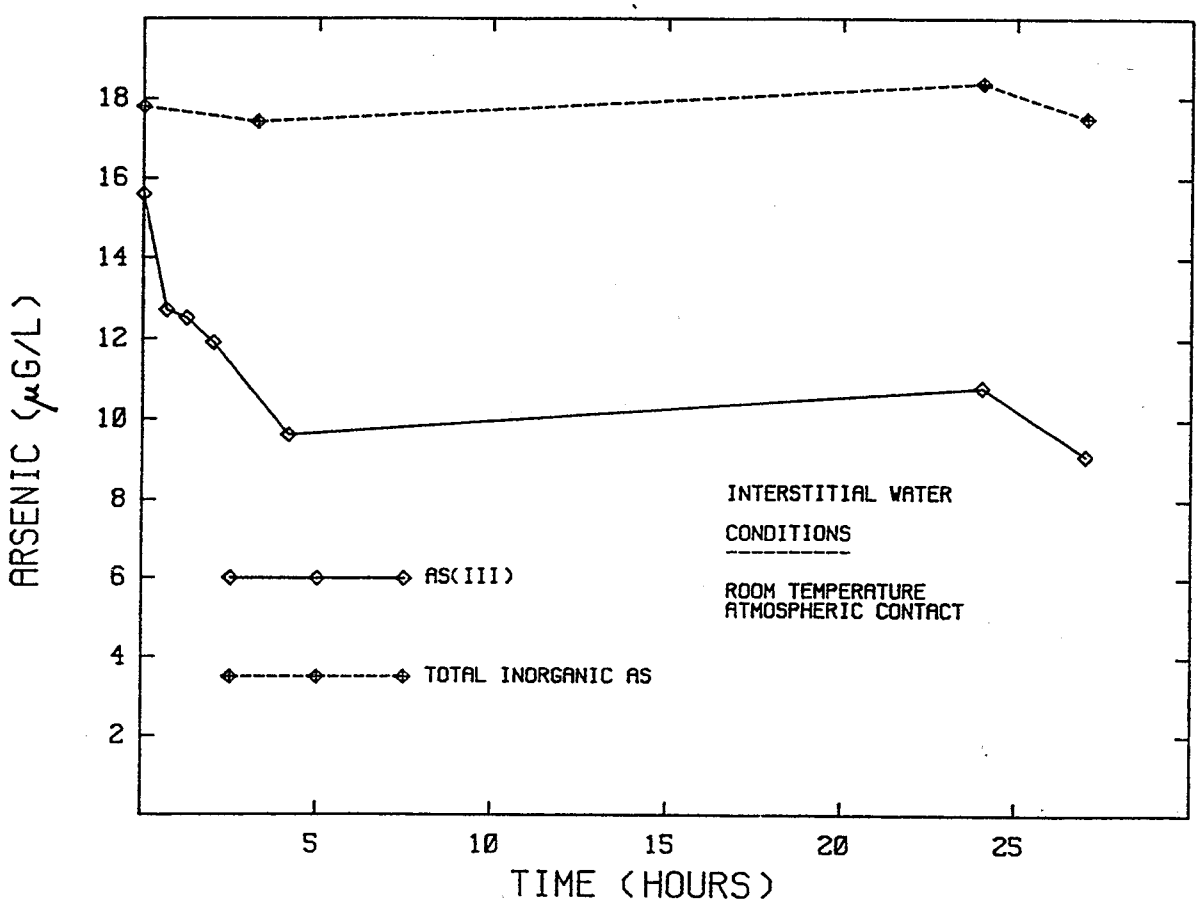


Figure 2-7. Plot of the concentration of AsIII and total inorganic arsenic versus storage time in interstitial water.

Interstitial Water. Interstitial water is collected from mud by pressure filtration under nitrogen. An aliquot (~100 g) of mud is placed into a plastic pressure filtration vessel with 1.0 μ acid-cleaned filter, and tapped down to remove air bubbles. The system is pressurized to 75 psi, and after discarding the first 1 to 2 ml of filtrate, the interstitial water is collected into a 30-ml polyethylene bottle under nitrogen. The As(III) stability curve in Figure 2-7 was generated on a sample in contact with air. Within 5 minutes, the sample had changed from colorless to brown, indicating that Fe(II) had oxidized to Fe(III), and precipitated as colloidal $Fe(OH)_3$. If an aliquot of sediment is filtered under nitrogen and then frozen at -196°C, as for water samples, within 5 to 10 minutes, minimal changes in the As(III)/As(V) ratio should have taken place.

Using the above technique, a sample of spiked, Lake Washington sediment was analyzed for interstitial water arsenic speciation 30 days after spiking with arsenic. This data is presented in Table 2-9 and shows that the distribution coefficients (K_d) of the various species between the solid and aqueous phases increase in the following order: DMA<<MMA<As(III)<<As(V). In fact, a sizable fraction (4.3%) of the DMA is in the interstitial water in a given sample, a fact which is important considering the intimate interaction of the interstitial water and living creatures.

Table 2-9

ARSENIC SPECIATION OF SPIKED LAKE WASHINGTON MUD INTERSTITIAL WATER. K_d VALUES REPRESENT [As (DRY WEIGHT SEDIMENT]/[As (INSTERSTITIAL WATER)]

	Arsenic concentration $\mu g \cdot g^{-1}$		
Species	Dry sediment	Interstitial water	K_d
AsV	20	<0.002	>10,000
AsIII	5.2	0.014	371
MMA	40	0.11	364
DMA	38	1.72	23

mud (LWM) and spiked LWM were placed into polyethylene bottles and frozen at -18°C, while three aliquots were kept refrigerated at 0 to 4°C. After 30 days these samples were analyzed for arsenic species, the results of which are shown in Table 2-8. These data indicate that small changes in the concentrations of the various species may be occurring, with significant decreases (20-30%) in the organic species being seen. These changes are small enough, however, that if the samples were analyzed as soon as possible after collection, they should not be of great importance.

Table 2-8

THIRTY-DAY STORAGE RESULTS FOR ARSENIC SPECIATION IN SEDIMENTS

Lake Washington mud

Arsenic species	$\mu g \cdot g^{-1}$ Arsenic, dry weight basis		
	Initial concentration	Concentrations after 30-day aging	
		Refrigerated, 0-4°C	Frozen, -18°C
As(III)	2.2 ± 0.3	2.2 ± 0.4	2.3 ± 0.3
As(V)	4.4 ± 0.3	5.2 ± 0.4	5.4 ± 0.4
MMA	<0.8	<0.8	<0.8
DMA	<0.8	<0.8	<0.8

Spiked Lake Washington mud

Arsenic species	$\mu g \cdot g^{-1}$ Arsenic, dry weight basis		
	Initial concentration	Concentrations after 30-day aging	
		Refrigerated, 0-4°C	Frozen, -18°C
As(III)	8.2 ± 1.4	7.1 ± 2.7	9.9 ± 1.3
As(V)	13.5 ± 1.7	13.8 ± 1.0	16.0 ± 0.5
MMA	51.3 ± 6.0	39.9 ± 1.6	46.2 ± 3.5
DMA	47.0 ± 4.2	46.5 ± 3.2	40.0 ± 2.4

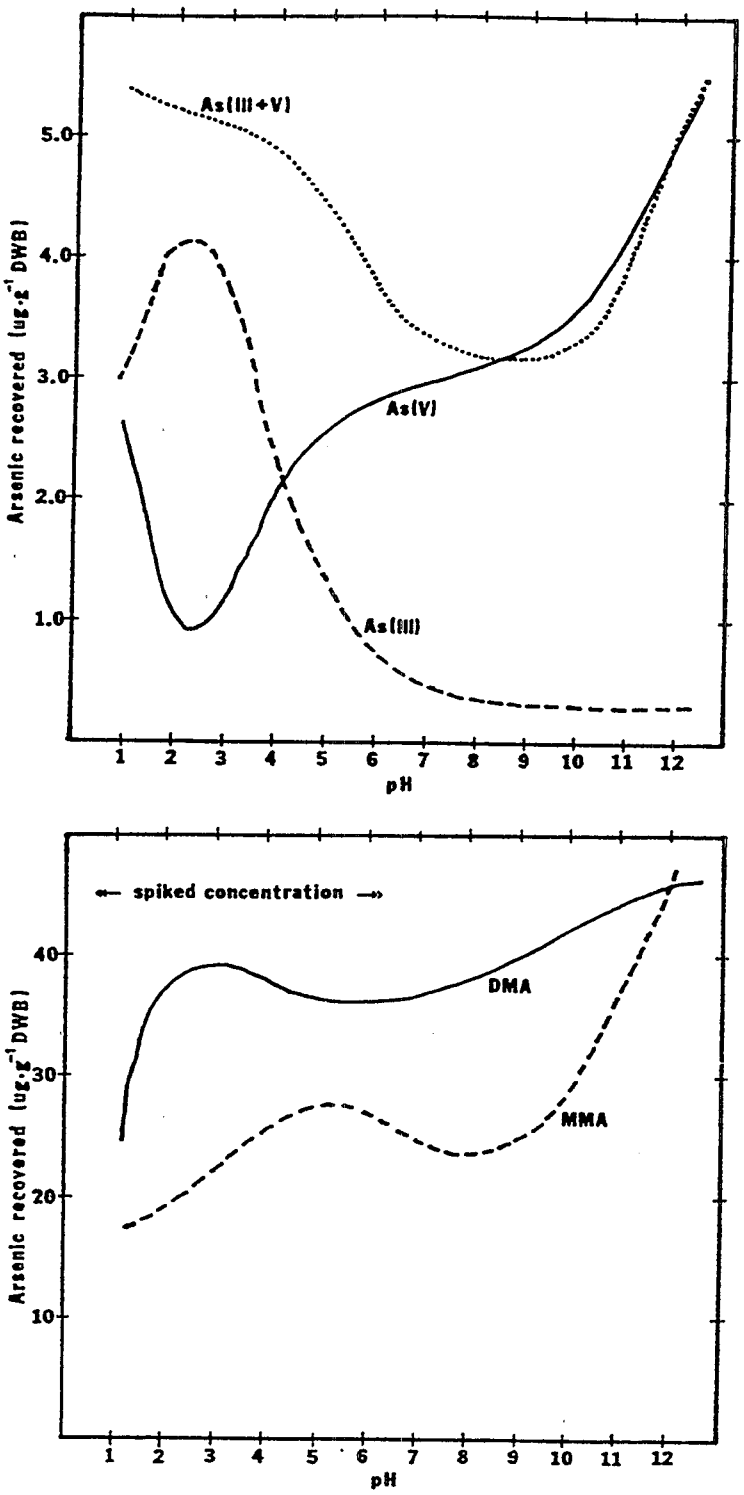


Figure 2-6. Arsenic species released from sediments as a function of solution pH. Plot of arsenic in sediment leached, μg g^{-1} dry weight basis (DWB), versus pH of leachate.

Arsenic Speciation of Sediments. Maher (4) has shown that various arsenic species that may be removed from solids at different pH values. This approach was tested on a sample of spiked Lake Washington mud, over a wide range of pH using phosphate buffers. The results of these experiments, shown as arsenic recovered versus pH for all four species, are illustrated in Figure 2-6. Notice that the maximum recovery of As(III) occurs at about pH = 2.8 and that the maximum for As(V), MMA and DMA occur at pH >12. From these data, the two convenient buffers of 0.1 M H_3PO_4 (pH = 1.5) and Na_3PO_4 (pH = 12) were chosen to selectively extract the arsenic species from sediments. Samples extracted with H_3PO_4 (final pH = 2.3) are analyzed only for As(III) whereas those extracted with Na_3PO_4 (final pH = 11.9) are analyzed only for total As, which gives As(V), MMA and DMA, as As(III) is not extracted at this pH. On untested sediment types it would be wise to test this relationship to be sure it holds true before instituting an analytical regime.

Recovery of arsenic species from spiked Lake Washington mud is illustrated in Table 2-7. The calculated spike was added to the mud, which was then aged 14 days at 4°C before analysis. All analysis were carried out in quintuplicate. The yields are good and within the day-to-day variability for the respective species.

Table 2-7

RECOVERY OF ARSENIC SPECIES FROM SPIKED LAKE WASHINGTON MUD BY SELECTIVE LEACHING

	$\mu g \cdot g^{-1}$ Arsenic, dry weight basis			
Arsenic species	Lake Washington mud	Spike added	Total recovered	Percent recovery
As(III)	2.2 ± 0.3	5.8	8.2 ± 14	103%
As(V)	4.4 ± 0.3	9.5	13.5 ± 17	96%
MMA	<0.8	58.0	51.3 ± 6.0	88%
DMA	<0.8	54.0	47.0 ± 4.2	87%

The values of the above analysis were then taken as the time zero values, and the mud divided and stored in one of two ways. Three aliquots each of Lake Washington

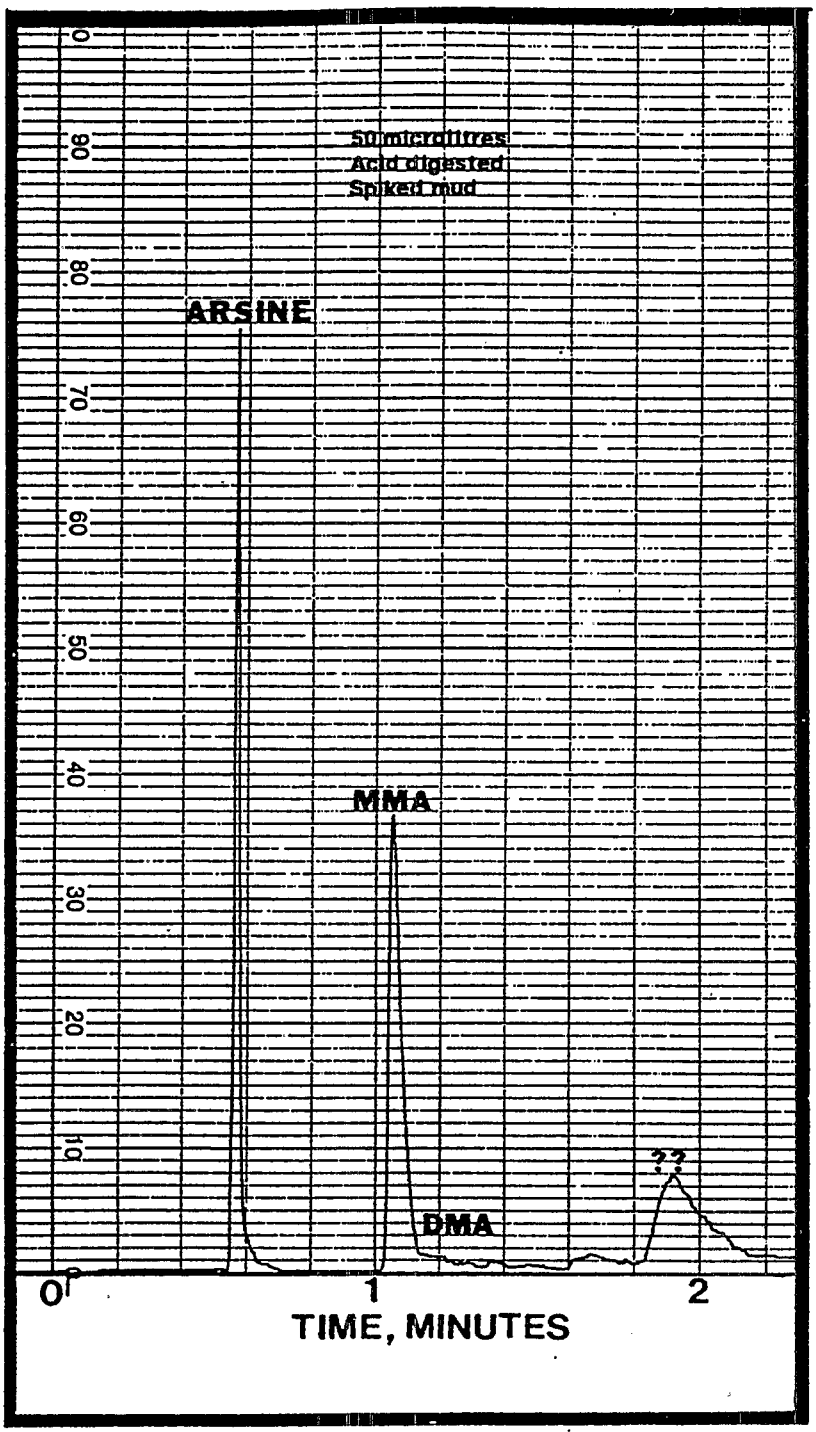


Figure 2-5. Chromatogram of digested (HNO_3/H_2SO_4) spiked Lake Washington mud. Vertical axis absorbance, horizontal axis time. Note absence of DMA peak and presence of unidentified higher boiling compound.

Table 2-6

COMPARISON OF X-RAY FLUORESCENCE SPECTROSCOPY AND HYDRIDE GENERATION AA IN THE DETERMINATION OF TOTAL ARSENIC ENVIRONMENTAL SEDIMENTS. ALL REPRESENT TOTAL INORGANIC ARSENIC BY HOT ACID DIGESTION EXCEPT (*) SLWM, WHICH IS THE SUM OF SPECIES BY LEACHING

Type of Sediment	Total Arsenic, $\mu g \cdot g^{-1}$ dry weight basis			
	XRF		Hydride AA	
Lake Washington (silt)	14.6 ± 0.1	n=3	14.5 ± 1.1	n=6
Spiked Lake Washington (silt)	124.1 ± 3.4	n=3	120.0 ± 7.5	n=5*
BCSS-1, clean estuarine (mud)	11.7 ± 0.7	n=3	9.9 ± 1.0	n=5
Contaminated Puget Sound (sandy)	108.0 ± 24.0	n=3	93.0 ± 21.0	n=3
Duwamish River (sand)	8.0	n=1	2.6	n=1

However, when Lake Washington sediment spiked with inorganic as well as organic forms was analyzed by this method, the following was observed:

1. All of the MMA was recovered as MMA.
2. All of the inorganic arsenic was recovered as inorganic arsenic.
3. None of the DMA was recovered, but an unidentified higher boiling peak was generated.

This peak is clearly illustrated in Figure 2-5. Even after the above samples were re-digested to near-dryness (white fumes) in HNO_3 plus $HClO_4$, the same results were obtained. Therefore, at this point we recommend no hydride generation method to determine total arsenic in sediments, though this may be achieved using either neutron activation analysis or X-ray fluorescence spectroscopy. On the other hand, since no organic forms have been detected in any natural sediment and since both MMA and DMA give observable peaks if they are present, it is safe to assume as a general guideline that if only an inorganic arsenic peak is generated by a given sample, then it probably represents close to the total arsenic content of the sample.

Determination of Arsenic Species in Sediments

Two procedures were investigated in the determination of arsenic in sediments. One, a wet-acid digestion was used to determine total arsenic. The second was a mild, pH-selective leach to remove various arsenic species intact.

Total Arsenic. In applying the hot HNO_3/H_2SO_4 digestion to standard sediments and air particulate matter, good agreement was attained between the established values and the measured values (Table 2-5). Also, in the case of estuarine and riverine sediments collected in the Puget Sound area, there was good agreement between X-ray fluorescence spectroscopy and this method (Table 2-6). In either case, all observed arsenic was in the inorganic form.

Table 2-5

TOTAL INORGANIC ARSENIC IN STANDARD SEDIMENTS BY HNO_3/H_2SO_4

	Total (inorganic) arsenic $\mu g \cdot g^{-1}$ dry weight basis			
Replicate	MESS-1 Estuarine sediment	BCSS-1 Estuarine sediment	NBS-1646 Estuarine sediment	NBS-1648 Air particulate matter
1	8.9	10.9	9.8	123.0
2	8.8	8.5	10.0	136.0
3	8.8	9.4	9.8	115.0
4	9.6	9.8	8.5	-
5	10.1	10.7	11.0	-
N	5	5	5	3
$\bar{X}$	9.2	9.9	9.8	125.0
S	0.6	1.0	0.9	11.0
RSD	6.5%	10.1%	9.2%	8.8%
Certified	10.6	11.1	11.6	115.0
±	1.2	1.4	1.3	10.0

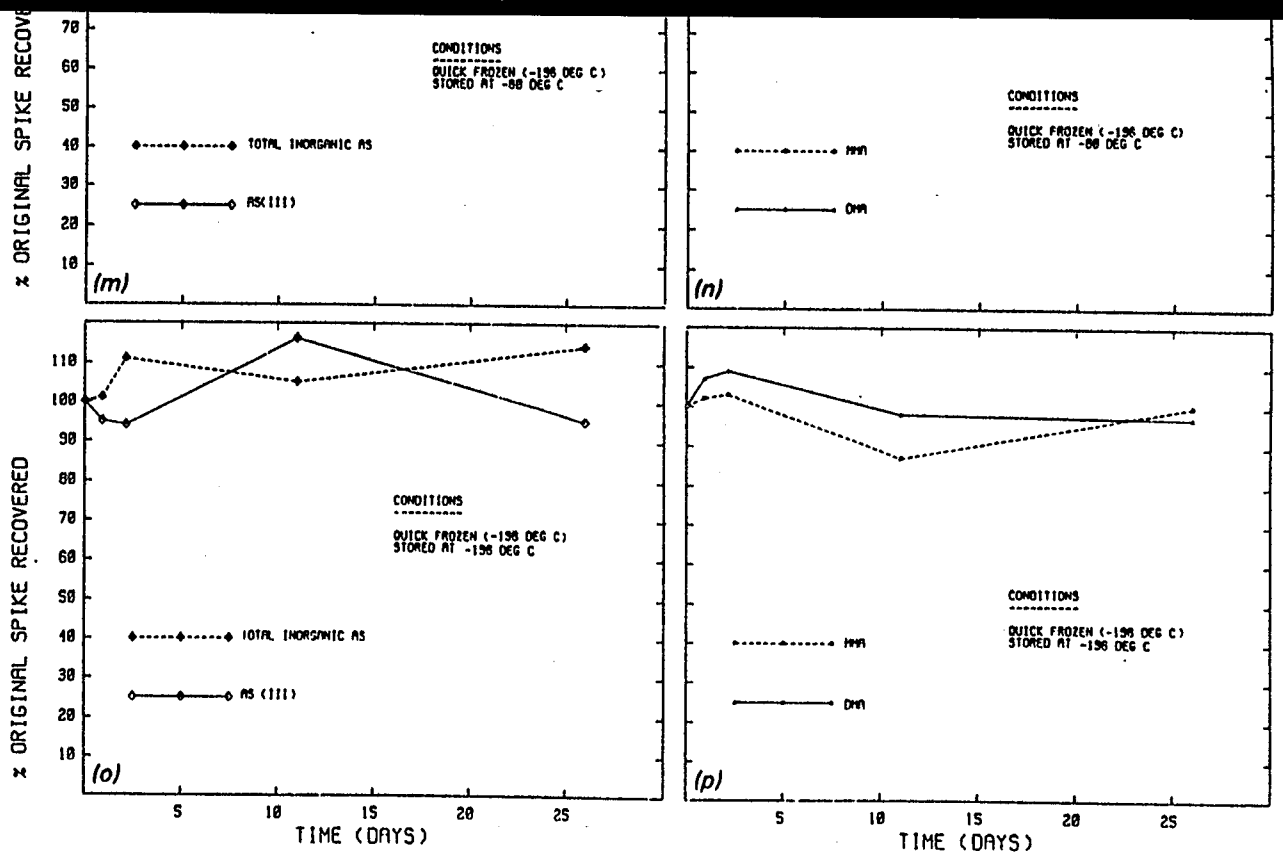
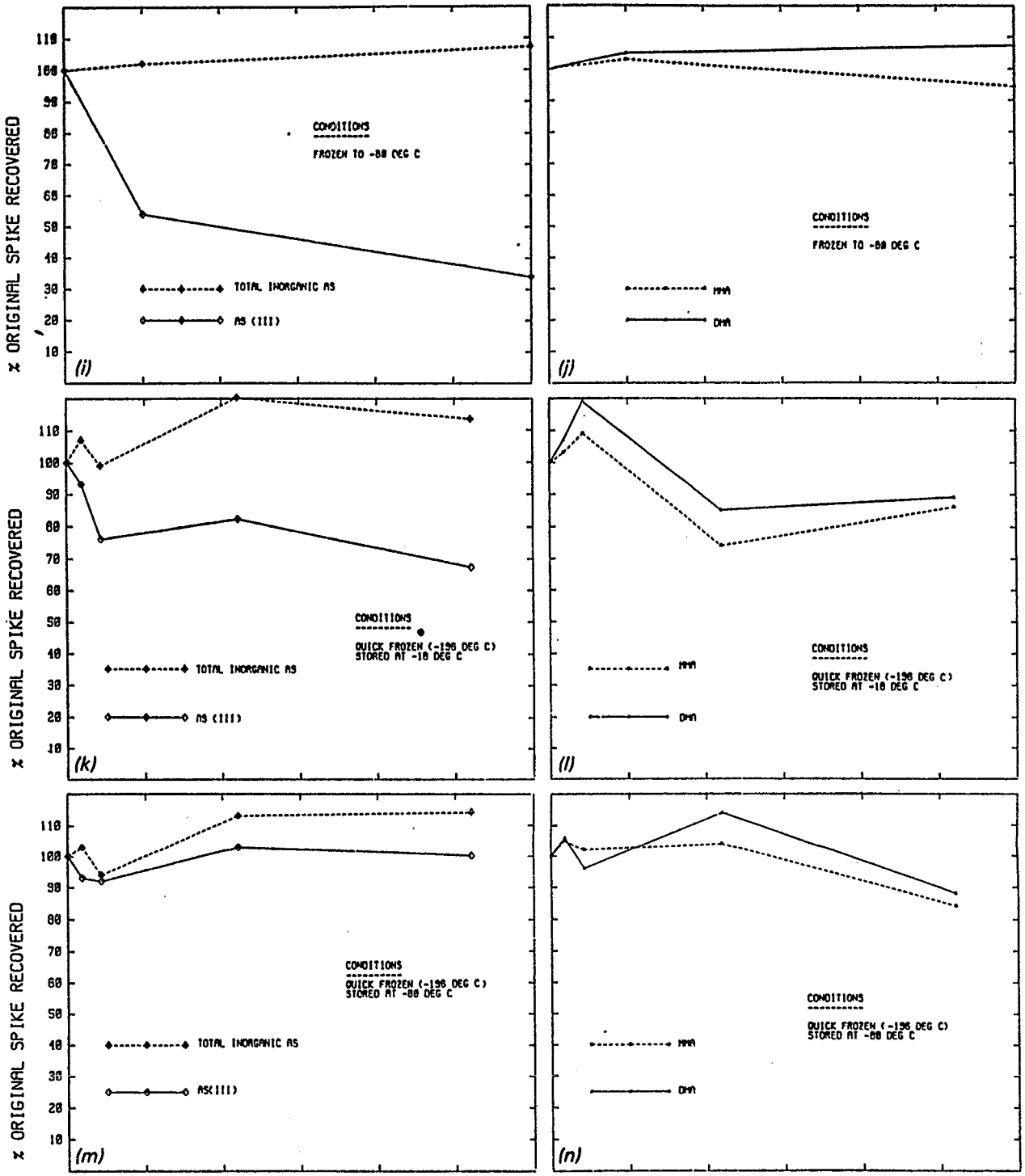


Figure 2-4a-p, continued.


% ORIGINAL SPIKE RECOVERED
CONDITIONS
FROZEN TO -80 DEG C
TOTAL INORGANIC AS
AS (III)
(i)
MMA
DMA
(j)
QUICK FROZEN (-196 DEG C)
STORED AT -10 DEG C
(k)
(l)
STORED AT -80 DEG C
(m)
(n)

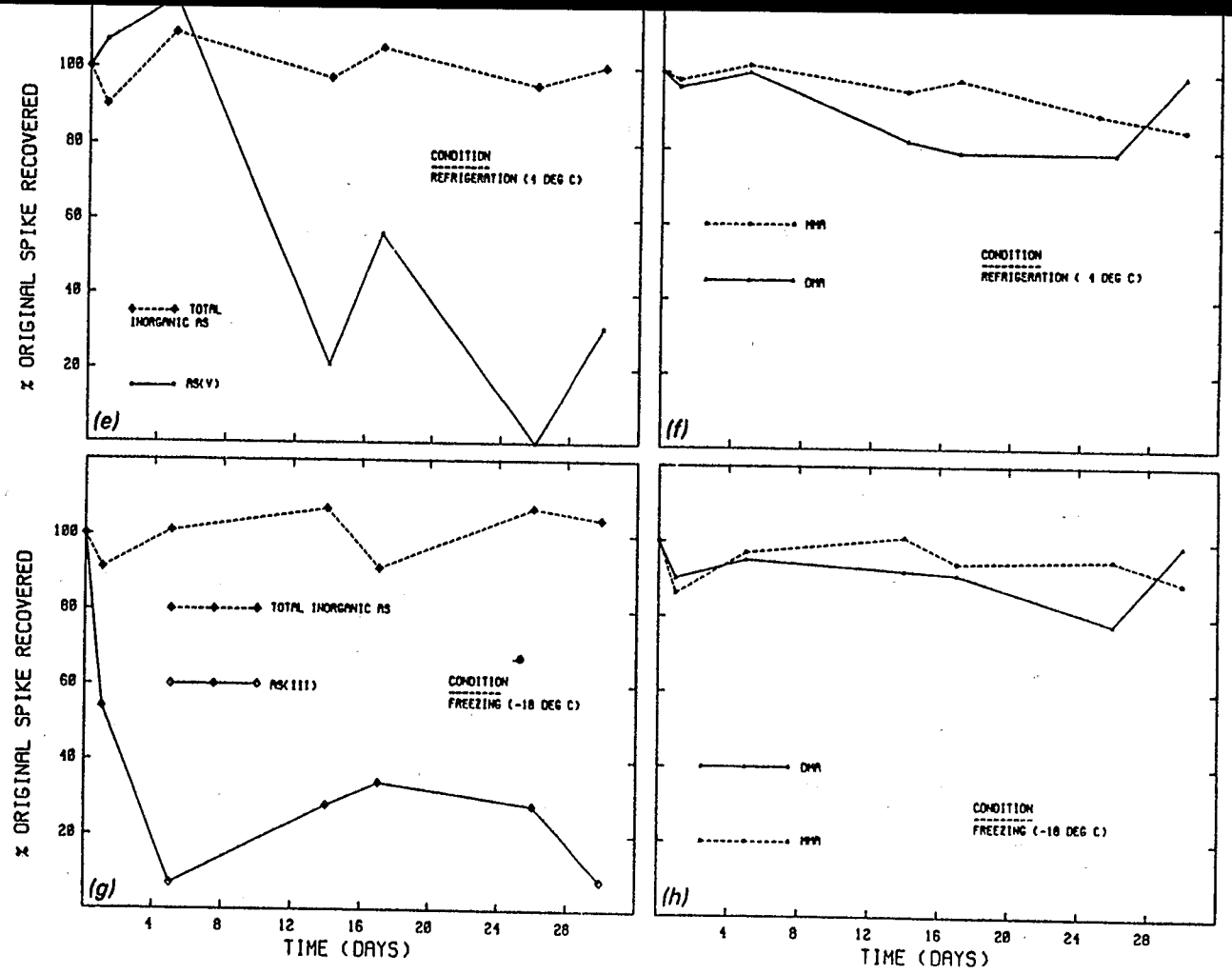
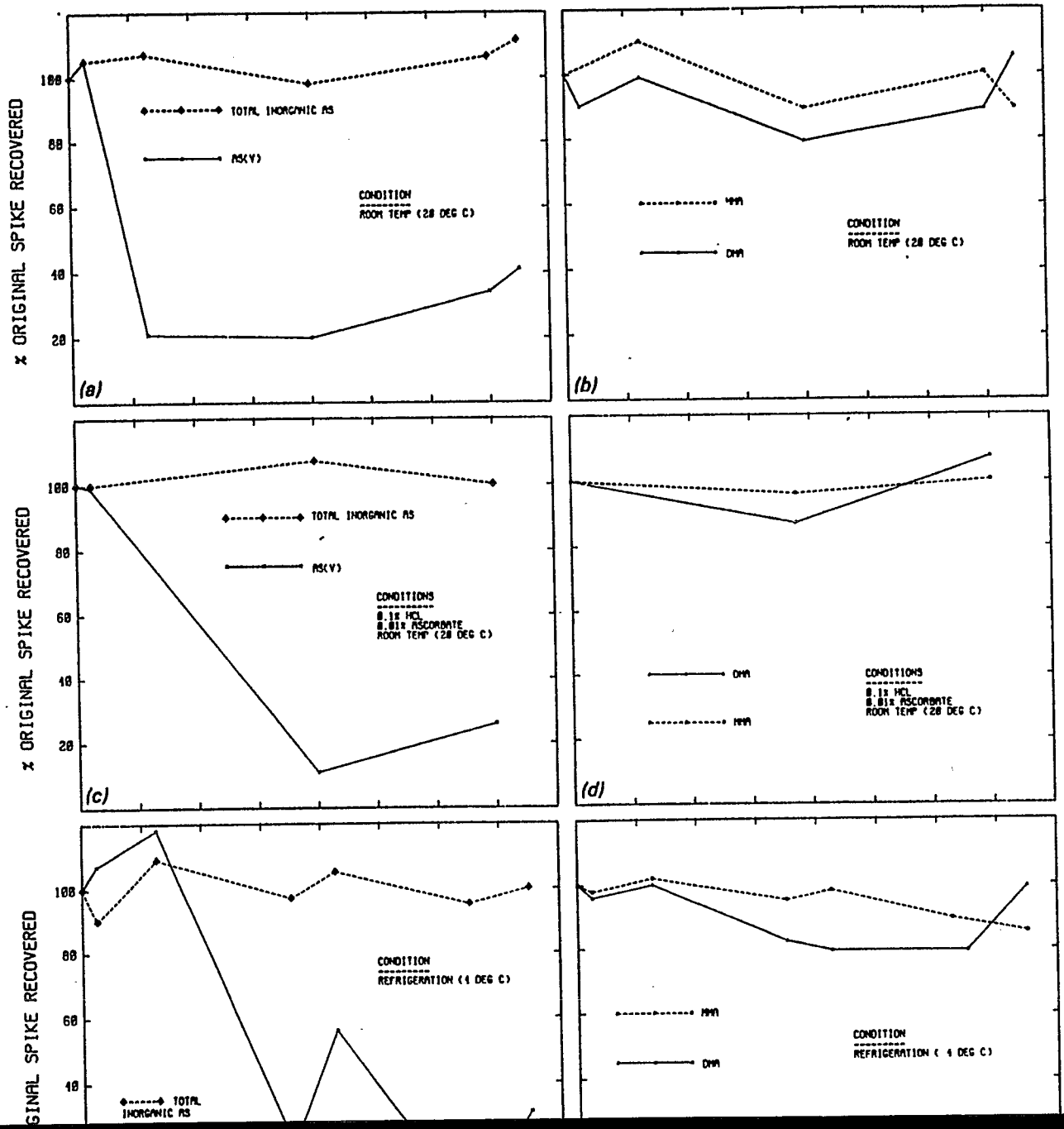


Figure 2-4a-p. Results of aqueous arsenic species storage tests. Plotted are the percentages of soluble arsenic species remaining versus storage time.


% ORIGINAL SPIKE RECOVERED
100
80
60
40
20
TOTAL INORGANIC AS
AS(V)
CONDITION
ROOM TEMP (20 DEG C)
(a)
MMA
DMA
CONDITION
ROOM TEMP (20 DEG C)
(b)
% ORIGINAL SPIKE RECOVERED
TOTAL INORGANIC AS
AS(V)
CONDITIONS
0.1% HCL
0.01% ASCORBATE
ROOM TEMP (20 DEG C)
(c)
DMA
MMA
CONDITIONS
0.1% HCL
0.01% ASCORBATE
ROOM TEMP (20 DEG C)
(d)
% ORIGINAL SPIKE RECOVERED
CONDITION
REFRIGERATION (4 DEG C)
TOTAL INORGANIC AS
MMA
DMA
CONDITION
REFRIGERATION (4 DEG C)

concentration of these parameters was within ±20% of the initial in all cases. The noise in the data is due mostly to the day-to-day analytical variability, which has been observed to be about twice that of same-day replicate analysis. On the other hand, these data also show that it is very difficult to preserve the original As(III)/As(V) ratio in samples, even for a short time. Two major observations are made: first, river water (Dungeness River water) tends to spontaneously reduce As(V) to As(III), even though the water has been filtered to 0.4 μ, thus removing most living creatures. This is also curious, as the natural equilibrium As(III)/As(V) ratio is about 0.2 in Dungeness River water. It is surmised that dissolved organic materials in the water are responsible for its reducing properties, a conclusion that is supported by work involving the reduction of Hg(II) to Hg(0) by humic acids (Bloom, unpublished work). The second observation is that the freezing of water inexplicably, but reproducibly causes the oxidation of As(III) to As(V) (Figure 2-4-g, i), except in the case of very rapid freezing by immersion in LN_2 (Figure 2-4-m, o).

In light of these observations, the following storage regimes are recommended for arsenic in aqueous solution:

1. If only total inorganic arsenic plus MMA and DMA are to be determined, the sample should be stored at 0 to 4°C in polyethylene bottles until analysis. No chemical preservative is needed or desired and the analysis should be carried out as soon as possible.

2. If the As(III)/As(V) ratio is to be maintained, the sample must be quick-frozen to -196°C in liquid nitrogen, and then stored at at least -80°C until analysis. Note that Figure 4-k shows that even in the case of rapid freezing to -196°C, followed by storage at -18°C, a definite oxidation of As(III) to As(V) was observed.

A convenient and safe way to quick-freeze samples is to place 55 ml of sample into a 60-ml narrow-mouth polyethylene bottle, screw on the cap (which has a 2 mm diameter hole) tightly, and drop into a Dewar flask full of liquid nitrogen. These bottles have been shown not to crack if less than 58 ml of water is placed in them, and not to float in the LN_2 if more than 50 ml is placed in them. After returning to the laboratory, the bottles may be placed into a low temperature freezer until analysis. Note of caution, if a small hole is not placed in the lid of the bottles, which are frozen in liquid nitrogen, the bottles may explode when removed from the liquid nitrogen.

Table 2-4

PRECISION DATA FOR THREE ARSENIC SPECIES, ILLUSTRATING THE DECREASE IN PRECISION WITH INCREASING BOILING POINT OF SPECIES. THESE SAMPLES WERE SPIKED RIVER WATER USED IN WATER STORAGE TESTS

	Arsenic concentrations, $ng \cdot l^{-1}$		
Replicate	Inorganic arsenic	MMA	DMA
N (8-24-83)	3	3	3
X	937	2483	2173
S	44	79	181
RSD	4.7%	3.2%	8.3%
N (9-11-83)	3	4	4
X	800	2342	2393
S	24	165	260
RSD	3.0%	7.0%	10.9%

The detection limit of this technique has not been explored to the extreme as the usual environmental sample benefits from less, not more sensitivity. For a chart recorder expansion of 600 mau full scale, and the parameters given in the text, and for a 30-ml sample aliquot, the following approximate detection limits are found: As(V), 0.006 $\mu g \cdot l^{-1}$ (twice the standard deviation of the blank); As(III) 0.003 $\mu g \cdot l^{-1}$ (0.5 chart units); MMA, 0.010 $\mu g \cdot -^{1}$ as As (0.5 chart units); DMA, 0.012 $\mu g \cdot l^{-1}$ as As (0.5 chart units). For As(III), MMA and DMA, no contribution to the blank has been found due to reagents, except for the As(III) present in the river water used as a dilutant. As for As(V) a small contribution is found, mostly from the $NaBH_4$, and to a smaller extent from H_3PO_4. These may be minimized by selecting reagent lots of reagents found to be low in arsenic.

Water Storage Experiments

From the many experiments undertaken to determine a storage regime for arsenic species, the following general conclusion can be made: Almost any storage scheme will preserve the total arsenic, MMA, and DMA concentrations of river water in the $\mu g \cdot l^{-1}$ range. This is illustrated in the Figures 2-4a-p, where the final

As arsenic response is quite sensitive to the H_2/O_2 ratio in the flame, it is necessary to restandardize the instrument whenever it is set up. Usually, however, the response is quite constant and stable over the entire day.

Precision, Accuracy and Detection Limits

Precision and accuracy are the greatest and the detection limits the lowest for inorganic arsenic. The precision and accuracy of the inorganic arsenic determination is illustrated at two concentrations in Table 2-3. The standard seawater, NASS-1 (National Research Council of Canada) was run in 5.0-ml aliquots and the "standard river water" (National Bureau of Standards) was run in 100-µl aliquots. In either case, both the precision (RSD) and accuracy were about 5%. Precision begins to decrease, as the boiling point of the compound increases, as is illustrated in Table 2-4, for spiked river water. No standard reference material has been found for the organic species.

Table 2-3

REPLICATE DETERMINATIONS OF TOTAL INORGANIC ARSENIC IN SOME STANDARD WATERS

	Total (inorganic) arsenic, $\mu g \cdot l^{-1}$	
Replicate	NASS-1 Seawater	NBS River water
1	1.579	81.5
2	1.556	74.5
3	1.591	71.8
4	1.493	79.0
5	1.529	79.3
$\underline{N}$	5	5
$\bar{X}$	1.550	77.2
S	0.040	4.0
RSD	2.6%	5.2%
Certified	1.65	76.0
±	0.19	7.0

$\underline{M}$ - number of replicates.
$\bar{X}$ - mean
S - ± one standard deviation
RSD - relative standard deviation

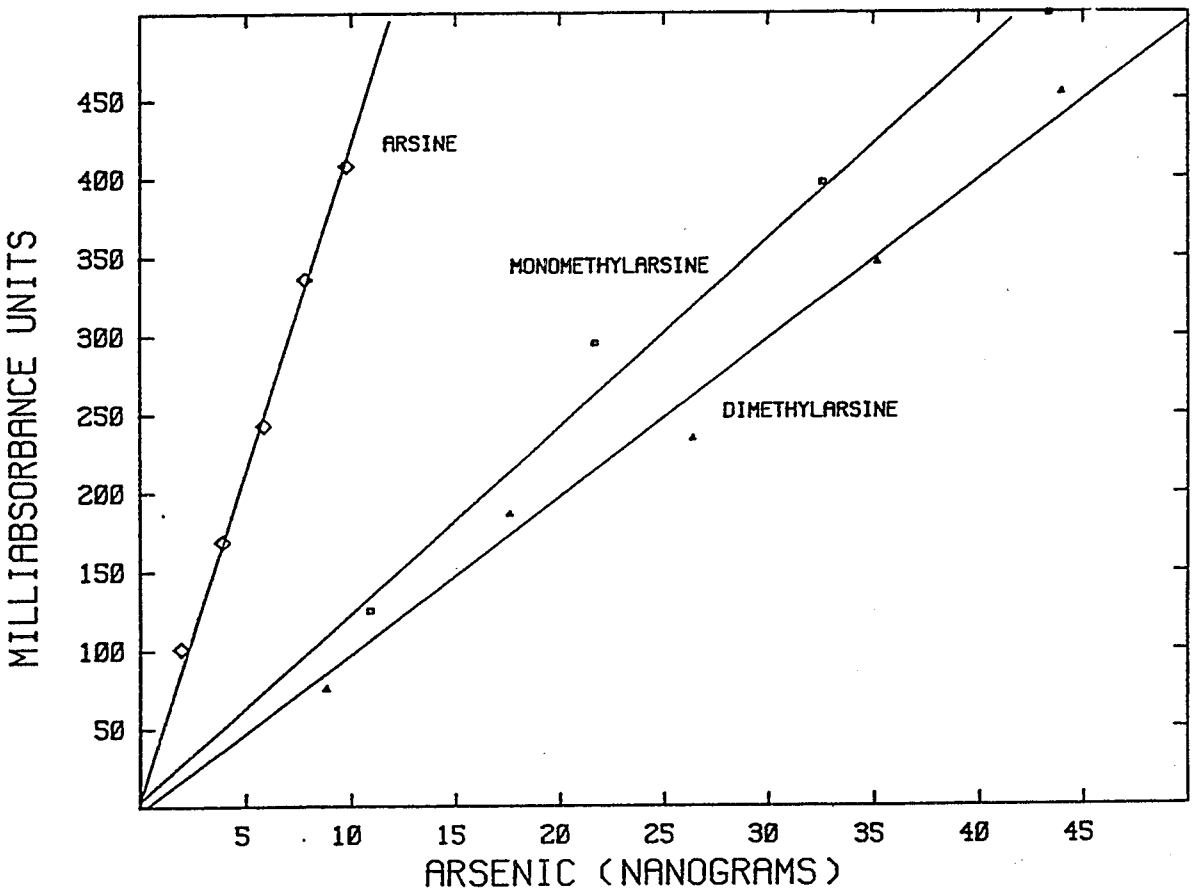


Figure 2-3. Standard curves, absorbance versus concentration for arsenic hydride species, atomic absorption detector.

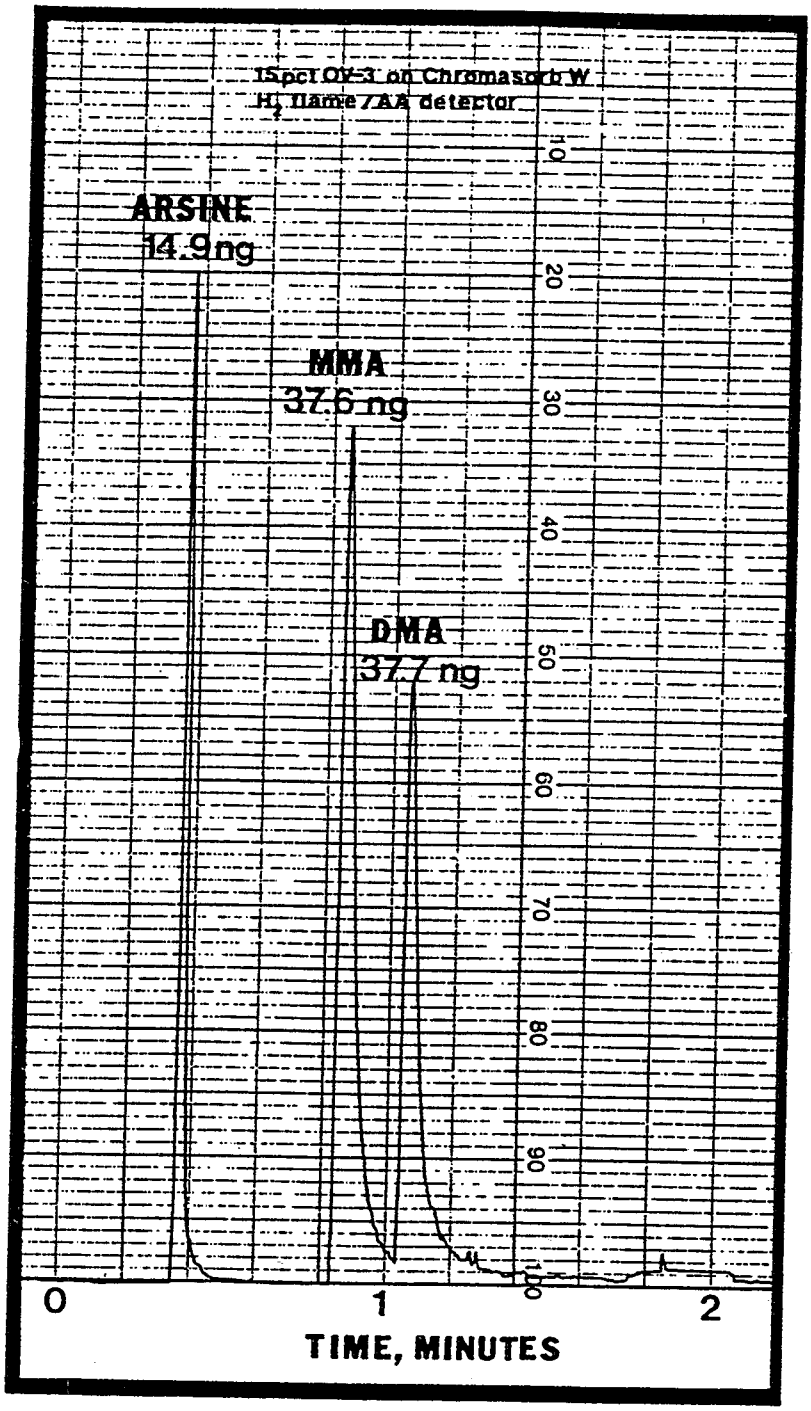


Figure 2-2. Typical chromatogram of arsenic hydride species. Vertical axis absorbance, horizontal axis time.

Conditions of temperature ranging from 20°C to -196°C were assessed, as well as preservation with HCl and ascorbic acid. Storage tests were carried out over a period of one month for water samples.

The stability of the As(III)/As(V) ratio in interstitial water at room temperature, in the presence of air was carried out over a 24-hour period to determine the feasibility of the field collection of interstitial water.

Because of the time-consuming nature of sediment analysis, a two-point storage test was carried out with triplicate samples analyzed for two sediments at two temperatures (0°C and -18°C). Mud samples were stored in polyethylene vials and analyzed at time zero and one month.

RESULTS AND DISCUSSION

Data Output

Using the procedures outlined above, and a mixed standard containing As(V), MMA, and DMA, standard curves were prepared for each of the arsines generated. A typical chromatogram from this procedure is illustrated in Figure 2.2. Under the conditions described in this paper, the elution times for the various arsines are as follows: AsH_3, 24 ± 2 s; CH_3AsH_2, 53 ± 2 s and $(CH_3)_2AsH$, 66 ± 2 s. Notice that the peaks are broadened and that the sensitivity decreases as the boiling point of the compound increases. The small amount of signal after the DMA peak is probably a higher boiling impurity in the DMA, or some DMA that is lagging in the system during elution. We had previously noted much larger, multiple peaks in this region when water was allowed to condense between the trap and the detector. Such peaks were effectively eliminated and the DMA peak sharpened with the addition of the heating coil between the trap and the detector.

The typical standard curves in Figure 2.3 are prepared from the mean of two determinations at each concentration. Arsenic peak-height response appears to be linear to at least 600 mau (milliabsorbance units), which is the full scale setting used on our chart recorder. Andreae (3) shows that arsenic response is extremely non-linear above this for the peak height mode, and recommends the use of peak area integration to increase the linear range. We have chosen to simply use a small enough sample aliquot to remain within 600 mau.

settle overnight. An appropriate-sized aliquot of the supernatant liquid (25-100 µl) is added to 20 ml of deionized water and run as for total arsenic.

Leachable Arsenite

An aliquot (~1-2 g) of fresh or freshly thawed wet homogeneous sediment is weighed to the nearest 10 mg directly into a 40-ml acid-cleaned Oak Ridge type centrifuge tube. To this is added 25 ml of 0.10 M H_3PO_4 solution and the tubes are agitated with the lids on. Periodic agitation is maintained for 18 to 24 hours, at which time the tubes are centrifuged for 30 minutes at 2500 RPM. Twenty milliliter aliquots of the supernatant liquid are removed by pipetting into cleaned polyethylene vials and saved in the refrigerator until analysis. Analysis should be accomplished within the next couple days.

For analysis, an appropriate-sized aliquot (10-100 µl) is added to 20 ml of well-characterized filtered river water (or other nonoxidizing/nonreducing water). Enough 1.0 M NaOH solution is added to approximately neturalize the H_3PO_4 (1/3 the volume of the sample aliquot), and then 1.0 ml of Tris buffer is added. The sample is then analyzed as for As(III).

Leachable Arsenate, MMA and DMA

An aliquot (~1-2 g) of wet sediment is weighed into a centrifuge tube, as above. To this are added 25 ml of 0.1 M Na_3PO_4 solution, and the tubes agitated periodically for 18 to 24 hours. After centrifugation the supernatant liquid (dark brown due to released humic materials) is analyzed as for total arsenic using an appropriate-sized aliquot in 20 ml of deionized water. The total inorganic arsenic in this case should be only As(V), as As(III) is observed to not be released at this pH. No pre-neutralization of the sample is necessary as the HCl added is well in excess of the sample alkalinity.

Interstitial Water Analysis

Interstitial water samples may be treated just as ordinary water, except that as they are quite high in arsenic, usually an aliquot of 100 to 1000 µl diluted in deionized water or river water is appropriate in most cases.

Storage Experiments

Storage experiments designed to preserve the original arsenic speciation of samples were carried out for a wide variety of conditions. For water samples, 30-ml and 60-ml polyethylene bottles precleaned in 1 M HCl were used.

Table 2-2

REDUCTION PRODUCTS AND THEIR BOILING POINTS OF VARIOUS AQUEOUS ARSENIC SPECIES

Aqueous form	Reduction product	B.P., °C
As(III), arsenous acid, $HAsO_2$	AsH_3	-55
As(V), arsenic acid, H_3AsO_4	AsH_3	-55
MMA, $CH_3AsO(OH)_2$	CH_3AsH_2	2
DMA, $(CH_3)_2AsO(OH)$	$(CH_3)_2AsH$	35.6

Arsenic (III) Determination

The same procedure as above is used to determine arsenite, except that the initial pH is buffered at about 5 to 7 rather than <1, so as to isolate the arsenous acid by its pKa (1). This is accomplished by the addition of 1.0 ml of Tris buffer to a 5- to 30-ml aliquot of unacidified sample. (If the sample is acidic or basic, it must be neutralized first, or the buffer will be exhausted.) For the As(III) procedure, 1.0 ml of $NaBH_4$ is added in a single short (~10 seconds) injection, as the rapid evolution of H_2 does not occur at this pH.

Small, irreproducible quantities of organic arsines may be released at this pH and should be ignored. The separation of arsenite, however, is quite reproducible and essentially 100% complete. As(V) is calculated by subtracting the As(III) determined in this step from the total inorganic arsenic determined on an aliquot of the same sample previously.

SEDIMENTS

Total Inorganic Arsenic

A 1.00-g aliquot of freeze-dried and homogenized sediment is placed into a 100-ml snap-cap volumetric flask. Five milliliters of deionized water is added to form a slurry and then 7 ml of the acid digestion mixture is added. After 5 minutes, the caps are replaced and the flasks heated at 80 to 90°C for 2 hours. Upon cooling the samples are diluted to the mark with deionized water, shaken, and allowed to

Tris Buffer. 394 g of Tris·HCl (tris (hydroxymethyl) aminomethane hydrochloride) and 2.5 g of reagent grade NaOH are dissolved in deionized water to make 1.0 liter. This solution is 2.5 M in tris and 2.475 M in HCl, giving a pH of about 6.2 when diluted 50-fold with deionized water.

Sodium Borohydride Solution. Four grams of >98% $NaBH_4$ (previously analyzed and found to be low in arsenic) are dissolved in 100 ml of 0.02 M NaOH solution. This solution is stable 8-10 hours when kept covered at room temperature. It is prepared daily.

Phosphoric Acid Leaching Solution. To prepare 1.0 liter of 0.10 M phosphoric acid solution, 6.8 ml of reagent grade 85% H_3PO_4 are dissolved in deionized water.

Trisodium Phosphate Leaching Solution. To prepare 1.0 liter of 0.10 M trisodium phosphate solution, 6.8 ml of 85% H_3PO_4 and 12 g of reagent grade NaOH are dissolved in deionized water.

Acid Digestion Mixture. With constant stirring, 200 ml of concentrated reagent grade H_2SO_4 are slowly added to 800 ml concentrated HNO_3.

METHODS

Total Arsenic Determination

An aqueous sample (5-30 ml) is placed into the reaction vessel and 1.0 ml of 6M HCl is added. The 4-way valve is put in place and turned to begin purging the vessel. The G.C. trap is lowered into a Dewar flask containing liquid nitrogen (LN_2) and the flask topped off with LN_2 to a constant level. A 2.0-ml aliquot of $NaBH_4$ solution is then introduced through the silicone rubber septum with a disposable 3-ml hypodermic syringe and the timer turned on. The $NaBH_4$ is slowly added over a period of about 1 minute, being careful that the H_2 liberated by the reduction of water does not overpressurize the system or foam the contents out of the reaction vessel.

After purging the vessel for 8 minutes, the stopcock is turned to pass helium directly to the G.C. trap. In rapid order, the LN_2 flask is removed, the trap heating coil is turned on, and the chart recorder is turned on. The arsines are eluted in the order: AsH_3, CH_3AsH_2, $(CH_3)_2AsH$ according to their increasing boiling points given in Table 2.2 (1).

Detector. Any atomic absorption unit may serve as a detector, once a bracket has been built to hold the quartz cuvette burner in the wave path. This work has been done using a Perkin-Elmer Model 5000® spectrophotometer with electrodeless discharge arsenic lamp. An analytical wavelength of 197.3 nm and slit width of 0.7 nm (low) are used throughout. This wavelength has been shown to have a longer linear range, though about half the sensitivity of the 193.7 nm line (2). Background correction is not used as it increases the system noise and has never been found necessary on the types of sample discussed in this paper.

Standards and Reagents

Arsenite (As(III)) Standards. A 1000 $mg \cdot l^{-1}$ stock solution is made up by the dissolution of 1.73 grams of reagent grade $NaAsO_2$ in 1.0-liter deionized water containing 0.1% ascorbic acid. This solution is kept refrigerated in an amber bottle. A 1.0 $mg \cdot l^{-1}$ working stock solution is made by dilution with 0.1% ascorbic acid solution and stored as above. Under these conditions this solution has been found stable for at least one year.

Further dilutions of As(III) for analysis, or of samples to be analyzed for As(III), are made in filtered Dungeness River water. It has been observed both here and elsewhere (Andreae 1983) that deionized water can have an oxidizing potential that causes a diminished As(III) response at low levels (1 $\mu g \cdot l^{-1}$ and less). Dilute As(III) standards are prepared daily.

Arsenate (As(V)) Standards. To prepare a 1000 $mg \cdot l^{-1}$ stock solution, 4.16 g of reagent grade $Na_2HASO_4 \cdot 7H_2O$ are dissolved in 1.0 liter of deionized water. Working standards are prepared by serial dilution with deionized water and prepared monthly.

Monomethylarsonate (MMA) Standards. To prepare a stock solution of 1000 $mg \cdot l^{-1}$, 3.90 g of $CH_3AsO(ONa)_2 \cdot 6H_2O$ is dissolved in 1.0 liter of deionized water. Working standards are prepared by serial dilution with deionized water. Dilute standards are prepared weekly.

Dimethylarsinate (DMA) Standards. To prepare a stock solution of 1000$mg \cdot l^{-1}$, 2.86 g of reagent grade $(CH_3)_2AsO_2Na \cdot 3H_2O$ (cacodylic acid, sodium salt) is dissolved in 1.0 liter deionized water. Dilute standards are handled as for MMA.

6M Hydrochloric Acid. Equal volumes of reagent grade concentrated HCl and deionized water are combined to give a solution approximately 6M in HCl.

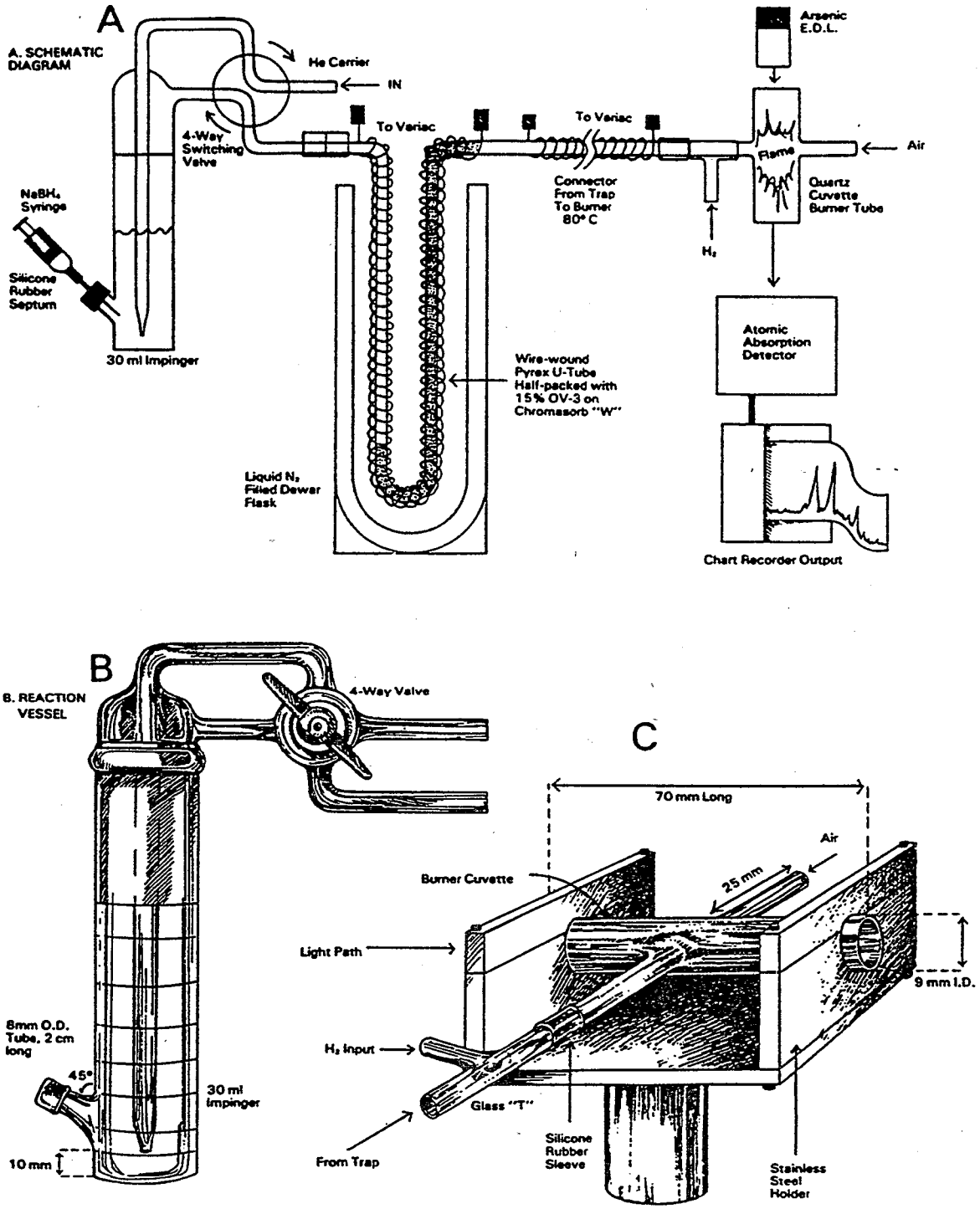


Figure 2-1. Arsenic Speciation Apparatus: (a) Schematic Diagram, (b) Reaction Vessel, (c) Quartz Cuvette Burner Tube.

Atomizer. The eluted arsines are detected by flame atomic absorption, using a special atomizer designed by Andreae (2). This consists of a quartz cross tube as shown in Figure 2-1-c. Air is admitted into one of the 6-mm o.d. side tubes (optimal flows are given in Table 2-1), while a mixture of hydrogen and the carrier gas from the trap is admitted into the other. This configuration is superior to that in which the carrier gas is mixed with the air (Andreae, personal communication 1983) due to the reduction of flame noise and possible extinguishing of the flame by microexplosions when H_2 is generated in the reaction vessel. To light the flame, all of the gases are turned on, and a flame brought to the ends of the quartz cuvette. At this point a flame will be burning out of the ends of the tube. After allowing the quartz tube to heat up (~5 minutes) a flat metal spatula is put smoothly first over one end of the tube, and then the other. An invisible air/hydrogen flame should now be burning in the center of the cuvette. This may be checked by placing a mirror near the tube ends and checking for water condensation. Note that the flame must be burning only inside the cuvette for precise, noise-free operation of the detector.

Table 2-1

OPTIMAL FLOWS AND PRESSURES FOR GASES IN THE HYDRIDE GENERATION SYSTEM

Gas	Flow rate $ml \cdot min^{-1}$	Pressure $lb \cdot in^{-2}$
He	150	10
H_2	350	20
Air	180	20

Precision and sensitivity are affected by the gas flow rates and these must be individually optimized for each system, using the figures in Table 2-1 as an initial guide. We have observed that as the O_2/H_2 ratio goes up, the sensitivity increases and the precision decreases. As this system is inherently very sensitive, adjustments are made to maximize precision.

septum (Ace Glass #9096-32) to allow the air-free injection of sodium borohydride. The standard impinger assembly is replaced with a 4-way Teflon stopcock impinger (Laboratory Data control #700542) to allow rapid and convenient switching of the helium from the purge to the analysis mode of operation.

GC Trap. The low temperature GC trap is constructed from a 6 mm o.d. borosilicate glass U-tube about 30-cm long with a 2-cm radius of bend (or similar dimensions to fit into a tall widemouth Dewar flask. Before packing the trap, it is silanized to reduce the number of active adsorption sites on the glass. This is accomplished using a standard glass silanizing compound such as Sylon-Ct® (Supelco Inc.). The column is half-packed with 15% OV-3 on Chromasorb® WAW-DMCS (45-60 mesh). A finer mesh size should not be used, as the restriction of the gas flow is sufficient to overpressurize the system. After packing, the ends of the trap are plugged with silanized glass wool.

The entire trap assembly is then preconditioned as follows: The input side of the trap (non-packed side) is connected via silicone rubber tubing to helium at a flow rate of 40 $ml \cdot min^{-1}$ and the whole assembly is placed into an oven at 175°C for 2 hours. After this time, two 25-µl aliquots of GC column conditioner (Silyl-8®, Supelco Inc.) are injected by syringe through the silicone tubing into the glass tubing. The column is then left in the oven with helium flowing through it for 24 hours. This process, which further neutralizes active adsorption sites and purges the system of foreign volatiles, may be repeated whenever analate peaks are observed to show broadening.

Once the column is conditioned, it is evenly wrapped with about 1.8 m of nichrome wire (22 gauge) the ends of which are affixed to crimp on electrical contacts. The wire-wrapped column is then coated about 2-mm thick all over with silicone rubber caulking compound and allowed to dry overnight. The silicone rubber provides an insulating layer which enhances peak separation by providing a longer temperature ramp time.

The unpacked side of the column is connected via silicone rubber tubing to the output from the reaction vessel. The output side of the trap is connected by a nichrome-wire wrapped piece of 6-mm diameter borosilicate tubing to the input of the flame atomizer. It is very important that the system be heated everywhere (~80°C) from the trap to the atomizer to avoid the condensation of water. Such condensation can interfere with the determination of dimethylarsine. All glass-to-glass connections in the system are made with silicone rubber sleeves.

Section 2

DETERMINATION OF ARSENIC SPECIES IN LIMNOLOGICAL SAMPLES BY HYDRIDE GENERATION ATOMIC ABSORPTION SPECTROSCOPY

INTRODUCTION

This section describes the analytical methods used to determine the arsenic species in waters and sediments. Also, sample storage tests were conducted to select methods of storing and shipping environmental samples that would minimize changes in speciation. Based on results of previous studies we selected hydride generation coupled with atomic absorption spectroscopy as the method of quantification of arsenic. In this technique arsenate, arsenite, methylarsonic acid, and dimethylarsinic acid are volatilized from solution at a specific pH after reduction to the corresponding arsines with sodium borohydride (1). The volatilized arsines are then swept onto a liquid nitrogen cooled chromatographic trap, which upon warming, allows for a separation of species based on boiling points. The released arsines are swept by helium carrier gas into a quartz cuvette burner cell (2), where they are decomposed to atomic arsenic. Arsenic concentrations are determined by atomic absorption spectroscopy. Strictly speaking, this technique does not determine the species of inorganic arsenic but rather the valence states of arsenate (V) and arsenite (III). The actual species of inorganic arsenic are assumed to be those predicted by the geochemical equilibrium model described in Section 1 of this report.

EXPERIMENTAL SECTION

Apparatus

The apparatus needed for the volatilization, separation and quantitation of arsenic species is shown schematically in Figure 2-1-a. Briefly, it consists of a reaction vessel, in which arsenic compounds are reduced to volatile arsines, a liquid nitrogen cooled gas chromatographic trap, and a H_2 flame atomic absorption detector.

Reaction Vessel. The reaction vessel is made by grafting a side-arm inlet onto a 30-ml "Midget Impinger" (Ace Glass #7532-20), as illustrated in Figure 2-1-b. The 8-mm diameter side arm may then be sealed with a silicone rubber-stopper type

APPENDIX K

A RECOMMENDED METHOD FOR INORGANIC ARSENIC ANALYSIS

Extracted from:

Crecelius, E.A., N.S. Bloom, C.E. Cowan, and E.A. Jenne. 1986. *Speciation of Selenium and Arsenic in Natural Waters and Sediments.* Volume 2: Arsenic Speciation, Section 2, in EPRI report #EA-4641, Vol. 2, pp. 2–1 to 2–28.

APPENDIX K

A RECOMMENDED METHOD FOR INORGANIC ARSENIC ANALYSIS

Table J-1 (continued)

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Table J-1 (continued)

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(continued)

Table J-1 (continued)

[r] LOD = Limit of detection. The lowest concentration that is statistically different from a blank. Determined according to the IUPAC method in Long and Winefordner (1983).

[s] From U.S. EPA (1985). Based on detection levels normally achieved in methods commonly used for tissue analyses in environmental laboratories. These detection limits are generally between the instrument detection limit (IDL) and method detection limit (MDL) (see Section 8.3.3.3) and are based on the expertise and best professional judgment of experienced analysts. Detection limits for metals based on 5 g (wet weight) of muscle tissue digested and diluted to 50 mL. Detection limits for organics based on 25 g (wet weight) of muscle tissue extracted, concentrated to 0.5 mL after gel permeation chromatography cleanup, and 1 µL injected. Bonded, fused silica capillary GC columns, which provide better resolution than packed columns, are assumed for analysis of semivolatile compounds.

[t] Inorganic arsenic was not included in any of the major monitoring programs (see Appendix C). Detection limits for the analysis of inorganic arsenic by hydride generation atomic absorption (HAA) and high-performance liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-GC/MS) are on the order of 5 -50 ppb and 50-100 ppb, respectively (E. Crecelius, Battelle Pacific Northwest Laboratories, Marine Sciences Laboratory, Sequim, WA, personal communication, June 1995).

[u] LOD = Limit of detection. No procedure given for determining the LOD.

[v] GFAA method used is not specific for tributyltin. Mono-, di-, and tetrabutyltin as well as other alkyltins may be included in the analysis. Method detection limit for analysis by gas chromatography/flame photometric detection (GC/FPD), which is specific for tributyltin, is 2-5 ppb (E. Crecelius, Battelle Pacific Northwest Laboratories, Marine Sciences Laboratory, Sequim, WA, personal communication, June 1995).

[w] The higher detection limits are appropriate for pesticides such as mirex, the DDTs, and endosulfans. Compounds such as lindane and hexachlorobenzene can be detected at the lower limits. Toxaphene (a mixture) may require a higher detection limit than the other organochlorine pesticides.

[x] All tabulated detection limits for PAHs are based on low-resolution GC/MS. Detection limits of less than 1 ppb can be achieved using HRGC/HRMS.

[y] Values given are for semivolatile organics in general.

[z] Dry weight detection limits for 24 PAHs in fish tissue ranged from 0.1 to 0.2 ppb.

[aa] Aroclors not determined. Values given are for individual mono- through decachlorobiphenyls.

[bb] Aroclors not determined. PCBs reported by total congener at the following levels of chlorination (TQLS in parentheses): 1-3 (1.25 ppb); 4-6 (2.5 ppb); 7-8 (3.75 ppb); 9-10 (6.25 ppb).

[cc] Detection and quantitation limits obtained from a survey of 10 laboratories with expertise in dioxin/dibenzofuran analyses by HRGC/HRMS ranged from 0.04-10 ppt and 0.2-100 ppt, respectively.

References:

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(continued)

Table J-1 (continued)

[e] U.S. EPA (1991). Analysis of total arsenic, cadmium, and selenium by ICP. Analysis of mercury by CVAA. MDLs determined in fish tissue matrix.

[f] U.S. Fish and Wildlife Service National Contaminant Biomonitoring Program (Schmitt and Brumbaugh, 1990; Schmitt et al., 1990). Analysis of cadmium by GFAA. Analysis of mercury by CVAA. Analysis of total arsenic and selenium by hydride generation atomic absorption (HAA). Analysis of organochlorine pesticides and PCBs by GC/ECD.

[g] Pollock et al. (1991). Composited fish samples extracted and analyzed for organics by GC/ECD using FDA Method PAM 211.1 in the *Pesticide Analytical Manual*–Vol. I (U.S. FDA, 1978). This method has been validated in interlaboratory studies and is an official method of the Association of Official Analytical Chemists (AOAC) for DDT, chlordane, and PCBs in fish. Mercury was determined using the AOAC flameless atomic absorption method (CVAA) (Williams, 1984). Analysis of tributyltin by GFAA.

[h] California Department of Fish and Game. (1990). Metals methods based in part on EPA SW-846 methods (U.S. EPA, 1986b). Analysis of cadmium by flame AA and GFAA. Analysis of mercury by CVAA. Analysis of total arsenic and selenium by HAA. Analysis of tributyltin by GFAA. Organics methods based on FDA methods (U.S. FDA, 1975) and EPA 301(h) methods (U.S. EPA, 1986a). Analysis of organochlorine and organophosphate pesticides and PCBs by GC/ECD. Analysis of PAHs by gas chromatography/flame ionization detection (GC/FID).

[i] U.S. EPA (1985, 1986a, 1987b). Analysis of total arsenic by GFAA, ICP, or HAA. Analysis of cadmium by GFAA or ICP. Analysis of selenium by GFAA or HAA. Analysis of mercury by CVAA. Analysis of organochlorine pesticides and PCBs by GC/ECD. Analysis of organophosphate pesticides by GC/phosphorus specific flame photometric or alkali flame ionization detection. Analysis of PAHs by GC/MS. Extract cleanup (e.g., removal of polar interferences by alumina column chromatography) assumed.

[j] LOD = Limit of detection. Method detection limit as defined in 40 CFR 136 using a minimum of three replicates.

[k] PQL = Practical quantitation limit. Defined in the Puget Sound Estuary Program as the minimum concentration of an analyte required to be measured and allowed to be reported without qualification as an estimated quantity for samples without substantial interferences. 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):

$$\text{PQL } (\mu g/g;\text{ppm}) = \frac{C\,(\mu g/mL)\cdot V(mL)}{W(g)} .$$

[l] MLD = Minimum level of detection. Concentration predicted from ratio of baseline noise area to labeled internal standard plus three times the standard error of the estimate from the weighted initial calibration curve.

[m] TQL = Target quantitation limit. Specific detection limits were not determined for individual samples, so were operationally set at zero.

[n] MDL = Method detection limit. Minimum concentration of an analyte that can be identified, measured, and reported with 99 percent confidence that the analyte concentration is greater than zero. Determined according to the procedure in 40 CFR 136 using seven replicates.

[o] LOD (for metals) = $3\{S_b^2 - S_s^2\}$, where S_b^2 and S_s^2 are variances of concentrations measured for procedural blanks and a low-level sample, respectively. LOD (for pesticides) = Mean method blank plus three times the standard deviation. Determined according to Keith et al. (1983).

[p] LOQ = Limit of quantitation. Mean method blank plus 10 times the standard deviation. Determined according to Keith et al. (1983).

[q] MDL = Method detection limit. Determined according to procedure in 49 CFR 209, except for tributyltin, which was determined as 2 times the standard deviation of the sample blank.

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