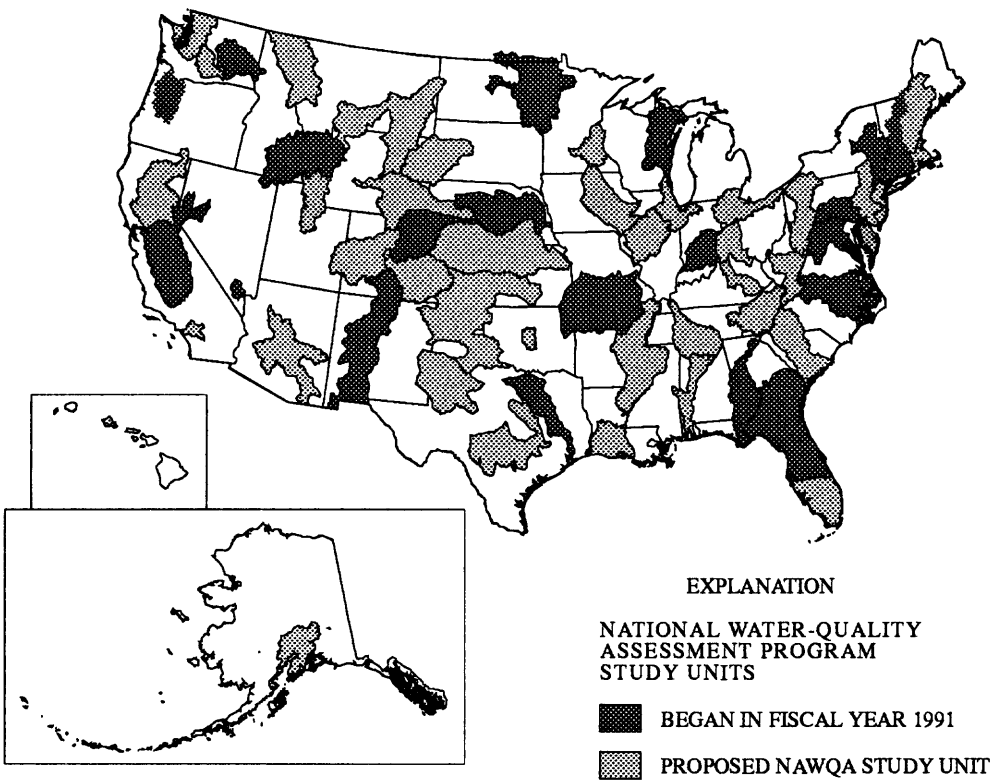
GUIDELINES FOR STUDIES OF CONTAMINANTS IN BIOLOGICAL TISSUES FOR THE NATIONAL WATER-QUALITY ASSESSMENT PROGRAM

By J. Kent Crawford and Samuel N. Luoma



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CONVERSION FACTORS
AND
ABBREVIATED WATER-QUALITY UNITS

Multiply	By	To obtain
centimeter (cm)	0.3937	inch
millimeter (mm)	0.03937	inch
gram (g)	0.03527	ounce, avoirdupois
kilometer (km)	0.6214	mile

Temperature is given in degrees Celcius (°C) which can be converted to degrees Farenheit (°F) by use of the following equation:

$$°F = 1.8\ (°C) + 32$$

Abbreviated water-quality units used in report:

micrograms per kilogram (µg/kg)
micrograms per gram (µg/g)

GUIDELINES FOR STUDIES OF CONTAMINANTS IN BIOLOGICAL TISSUES FOR THE NATIONAL WATER-QUALITY ASSESSMENT PROGRAM

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ABSTRACT

This report explains the concepts and field methods to be used by the U.S. Geological Survey's National Water-Quality Assessment (NAWQA) Program for evaluating contaminants in tissues of biological organisms. Laboratory methods for analysis of these contaminants will be detailed in a future report. Part 1 explains the rationale for analyzing contaminants in tissues and gives an overview of the approach. Part 2 describes the tissue-contaminant strategies of other agencies and compares them to the strategy used in NAWQA. Part 3 details the approach for the use of tissue analysis as an aid to interpreting quality of water in NAWQA study units. Concentrations of contaminants in tissues will complement measures of water and sediment chemistry, and ecological surveys in NAWQA, providing multiple lines of evidence for water-quality assessments. Individual sections in Part 3 provide detailed discussions of target contaminants, target taxa, and field procedures. Suggestions for interpretation of data are presented to facilitate consistency among NAWQA study units.

INTRODUCTION

Beginning in 1986, Congress annually appropriated funds for the U.S. Geological Survey (USGS) to test and refine concepts for a National Water-Quality Assessment (NAWQA) Program. The goals for a full-scale program are to:

(1) Provide a nationally consistent description of current water-quality conditions for a large part of the Nation's water resources;

(2) Define long-term trends (or lack of trends) in water quality; and

(3) Identify, describe, and explain, as possible, the major factors that affect observed water-quality conditions and trends.

This information, which will be obtained on a continuing basis, would be made available to water managers, policy makers, and the public to provide an improved scientific basis for evaluating the effectiveness of water-quality management programs and for predicting the likely effects of contemplated changes in land- and water-management practices. Concepts for a full-scale NAWQA Program are described by Hirsch and others (1988).

At present (1992), a pilot phase of the program is complete in seven project areas (study units) throughout the country that represent diverse hydrologic environments and water-quality conditions. Four pilot projects focused primarily on surface water, and three projects focused primarily on ground water. The surface-water pilot project areas were the Yakima River basin in Washington, the lower Kansas River basin in Kansas and Nebraska, the Kentucky River basin in Kentucky, and the Upper Illinois River basin in Illinois, Indiana, and Wisconsin. Now that the pilot phase is completed, surface-water and ground-water components will be integrated in each study unit for implementation of a full-scale NAWQA.

Biological measurements will be used in the surface-water component of NAWQA to assist in (1) determining the occurrence and distribution of waters contaminated by fecal material; (2) determining the occurrence of potentially toxic substances, including trace elements and organic compounds, through the use of tissue analyses; (3) assessing the relations between the physical and the chemical characteristics of streams and the functional or structural aspects of the biological community through ecological surveys; and (4) defining and quantifying biological processes that affect the physical and chemical aspects of water quality. Specific approaches to address these objectives have been developed and tested in the pilot program.

Purpose and Scope

This document describes the rationale, objectives, approach, and procedures to be used in the NAWQA Program for determining the occurrence, distribution, and trends in concentrations of trace elements and synthetic organic compounds in tissues (termed here tissue-contaminant studies). It is recognized that at least some of these approaches will evolve as additional experience is gained and as measurement and analysis techniques advance.

Acknowledgments

The authors gratefully acknowledge the assistance and contributions of many within and outside the USGS who contributed in various ways to this document. Outside the USGS, Donald W. Steffeck, Christopher J. Schmitt, and William G. Brumbaugh of the U.S. Fish and Wildlife Service (FWS), Gunnar G. Lauenstein, and Andrew Robertson of the National Oceanic and Atmospheric Administration (NOAA), Stephen Kroner of the U.S. Environmental Protection Agency (USEPA), and Barbara J. Koster, Rolf Zeisler, and Stephen A. Wise of the National Institute of Standards and Technology (NIST) offered advice and suggestions.

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Part 1: TISSUE ANALYSIS IN THE NATIONAL WATER-QUALITY ASSESSMENT PROGRAM

Rationale for Using Biological Tissues in Water-Quality Assessments

Determination of contaminant concentrations in biological tissues is widely used as a method to monitor and assess contaminant distributions and bioavailability in space and time (Phillips, 1980; Farrington and others, 1983; Bryan and others, 1985; Campbell and others, 1988; Schmitt and others, 1990). Phillips (1980) has identified three benefits of employing tissue analysis. Firstly, concentrations of contaminants may be greater in tissues than in water because of bioconcentration, bioaccumulation, or biomagnification. Therefore, tissue analysis increases the probability of detecting trace amounts of some contaminants in the environment. Secondly, measurements of contaminant concentrations in organisms provide a time-averaged assessment of the contaminant in question. Thirdly, concentrations of contaminants in tissues provide direct measurement of bioavailability of those toxicants that accumulate in biological tissues. Direct determination of bioavailability is especially important because the effects of contaminants on biota are not necessarily a simple function of their total concentrations in water and sediments (Sunda and Guillard, 1976; Phillips, 1980; Campbell and others, 1988; Luoma, 1989). A fourth benefit, not mentioned by Phillips, is that, where they are used together, tissue, water, and sediment analysis provide complementary lines of evidence in understanding the complexities of contaminant fate, distribution, and effects.

Several difficulties are inherent in any study of contaminants in biological organisms. However, successful tissue analysis programs have been conducted, most frequently in marine and brackish water environments, and conceptual methodologies for such programs are well established (Phillips, 1980; Farrington and others, 1983; Bryan and others, 1985; Campbell and others, 1988). These works have shown that, with rare exceptions, contaminant concentrations are comparable only within the same species at the same lifestage, reproductive condition, size, weight, and sex. Appropriate sample size (number of individuals), sample mass, and animal size and weight also must be considered. For most surveillance studies, relatively sedentary organisms whose exposure can be linked to a particular site are preferable to those that move or migrate. All these principles learned from marine and brackish-water environments appear to apply to freshwater environments, but they have not been thoroughly studied for freshwater species other than fish.

Establishing a national tissue analysis program that includes a variety of freshwater rivers and streams presents special challenges. Individual components of freshwater communities, such as fish, have limitations for many assessment applications when used alone (Weiner and Giesy, 1979). The diversity of the biological communities of freshwater streams and rivers also presents a challenge. No single taxon may occur everywhere, and the taxa of a locality can vary seasonally. Taxa can be very different in small, lower order streams than in large, higher order streams. Within a single river basin, distributions of taxa may be complex and taxa may vary in size.

In addition, the number of potential chemical contaminants is large and analytical costs are high. The contaminants of interest may vary from place to place. Decisions are required concerning which contaminants to target for analysis and whether to test for contamination in whole organisms or part of organisms.

Although a study of contaminants in biota presents special study design challenges, it is a logical component of an integrated assessment of existing water-quality conditions. As a data base develops describing the range of contaminant concentrations in specific taxa, comparisons of contamination among study units will be enhanced. Through careful sampling design, studies of contaminants in biological organisms will be employed to help explain what contaminants occur in each study unit, and how the important contaminants are distributed. These studies include an explicitly designed trend analysis component. Tissue analysis also can help explain how land use or other environmental factors influence contaminant bioaccumulation in selected biota. Finally, studies of tissue contaminants in NAWQA will advance the scientific understanding of water quality by helping development of this useful technique in fresh waters and by increasing understanding of how contaminants affect ecosystems in the nation's surface waters.

Overview of the National Water-Quality Assessment Program Tissue Analysis Approach

The specific objectives for the use of tissue analysis in the NAWQA Program include:

(1) *Contaminant occurrence*. Determine which potentially toxic trace elements and synthetic organic compounds occur in NAWQA study units.

(2) *Long-term trends*. Define long-term trends in the concentrations of selected contaminants in biological tissues at key locations within NAWQA study units and in a manner that will facilitate a national understanding of trends.

(3) *Spatial distribution of contaminants*. Assist in defining the spatial distribution of trace elements and synthetic organic compounds within study units and across broad geographic scales among the national set of study basins.

(4) *Develop biological data base*. Systematically develop a national, internally comparable data base on contaminant concentrations in a suite of common freshwater organisms.

(5) *Compare land use inputs*. Compare contaminant concentrations in biota from waters draining areas having different land uses or in gradients away from specific inputs.

(6) *Baseline concentrations*. Systematically develop an understanding of concentrations of contaminants in biota collected from waters of pristine or near-pristine quality in study units and regions of different geology.

(7) *Fate and bioavailability*. Increase knowledge of the fate, bioaccumulation, and biological availability of selected contaminants across study units with different geologic, hydrologic and human influences, in order to aid assessment of the potential for adverse biological effects.

(8) *Potential for human health effects*. Contribute information useful in assessing the levels of contamination in edible portions of selected game fish species.

(9) *Develop bioassessment procedures*. The experience gained in the use of tissue analyses in NAWQA will aid in the general development of freshwater bioassessment procedures.

Each of the above objectives has explicit requirements for choosing sampling stations, choosing what kinds of taxa to collect, and choosing which chemicals to analyze. The design required to meet each objective will yield specific predefined products. The objectives also are prioritized as listed, with the proviso that individual study units may alter this prioritization to meet local needs.

The overall NAWQA plan calls for a 9-year cycle of study in each study unit. Years 1 and 2 are reserved for staffing, planning, and gathering historical and background information. Years 3, 4, and 5 are for intensive data collection. Years 6-9 are for report writing and low-level data collection. Study units may differ in the number of objectives they achieve (or pursue) depending upon local issues and available expertise. However, all study units will undertake the first three objectives of the tissue bioassessment. Thus, every study unit will first do an exhaustive chemical analysis on samples from selected stations to assess what chemical contaminants are important in a study unit. Then, every study unit will establish and maintain sampling at a suite of stations for long-term trend analysis. Finally, in every study unit a spatial description of contaminant concentrations within main stem rivers and at the mouths of major tributaries will be conducted. If all nine objectives are not approached in the first cycle of intensive data collection, they will be accomplished in later cycles as understanding of the basin progresses.

An important product of tissue analysis in NAWQA is data that are comparable on local, regional, and national scales. Resident taxa will be collected for the NAWQA tissue bioassessment. In order to assure that analyses of taxa are comparable over the broadest areas possible, a NAtional Target Taxa (NATT) list will define the taxa that local teams should target in most collection efforts. The choice of NATT to be analyzed from each site will depend upon occurrence and abundance of resident taxa, whether the sample is to be analyzed for organics or trace elements, the objective being satisfied, the ability to obtain adequate mass for analysis, and whether samples of comparable taxa are being collected elsewhere within the basin (or in the

nation). The local study team will make the specific choices, within the national guidelines contained in this document. Although the details of the NATT list may change as experience increases, it will provide a national tissue contaminant data base for selected species widespread in freshwater environments.

The chemicals considered in the tissue contaminant studies are those for which analytical methodology exists, chemicals that are not rapidly metabolized to undetectable metabolites, and chemicals with high bioconcentration factors. NAWQA will use a two stage procedure to select chemicals that satisfy the above criteria. A comprehensive list of organic contaminants and trace elements will be targeted for analysis during the initial sampling effort associated with objective 1 (occurrence). Chemicals not detected during this reconnaissance, not found in water and sediment analyses, and not expected in the basin will not be analyzed during later sampling efforts. The goal of this procedure is to first ensure that all chemicals are considered in all study units, then to reduce costs of the more detailed study efforts eliminating analyses for chemicals whose concentrations are below detection limits. Examples of suites of chemicals that might be eliminated when approaching higher level objectives in the tissue analysis might include polynuclear aromatic hydrocarbons in some basins, dioxin-like chemicals in other basins, or the mercury-arsenic-selenium suite of trace elements that require special analytical procedures.

Table 1 summarizes the tissue bioassessment program planned for each NAWQA study unit.

Table 1.--Summary of tissue bioassessment sampling, by objective, planned for each study unit of the National Water-Quality Assessment Program

Objective	Number of sites	Taxa	Year of sampling	Contaminants analyzed
1. Contaminant occurrence	15 - 20	NATT [1], one taxon per station	3	Organochlorines, PAH's, PCB's trace elements
2. Long-term trend analysis	4 - 8	NATT, one taxon per study unit	3 - 9 1, 2	Local targets, National synthesis
3. Spatial distribution of contaminants	20 - 40	NATT, two taxa at 50 percent of stations	4, 5	Local targets
4. Biological data base	All	NATT	3, 4, 5	Local targets
5. Compare land use inputs	Variable	Comparable (NATT or local)	5	Local targets
6. Baseline concentrations	4	NATT	3, 4, 5	Organochlorines, PAH's, PCB's, trace elements, and local targets
7. Fate and bioavailability	Variable	NATT or food web	4, 5	Local targeted
8. Contamination of fish flesh	3 - 4	Game fish	5	Human health threats
9. Bioassessment procedures	All	All	3 - 9	All

[1] NATT - National Target Taxa.

Part 2: TISSUE-ANALYSIS ACTIVITIES OF OTHER AGENCIES

Biomonitoring has become an integral part of water-quality assessment programs in the United States. Since the implementation of the Federal Water Pollution Control Act of 1972 and the Toxic Substances Control Act (1976), large quantities of ambient water-quality data have been collected. Several Federal and many State agencies analyze tissues for concentrations of trace elements and synthetic organic compounds as part of their biomonitoring programs to assess the water quality and general health of aquatic ecosystems. The Federal agencies include the FWS, USEPA, NOAA, and the Tennessee Valley Authority (TVA). Tissue analysis programs of the FWS, USEPA, and NOAA are national in scope and thus, have requirements similar to the tissue analysis component of NAWQA. The experiences of these programs contributed to the development of the tissue analysis component of NAWQA. All are examined and compared below and in table 2.

The U.S. Fish and Wildlife Service's National Contaminant Biomonitoring Program

Since 1967, the FWS has maintained a biomonitoring network now known as the National Contaminant Biomonitoring Program (NCBP). The two major objectives of the NCBP are to assess differences in contaminant levels in fish and wildlife (birds) among geographic regions and to determine the changes occurring over time (Jacknow and others, 1986). Although the Program includes analyses of both fish and birds, only the fish component will be addressed here.

Originally referred to as the National Pesticide Monitoring Program, the early network consisted of 50 sampling stations located in the Great Lakes and in major rivers throughout the United States. Samples of three species of fish were collected twice annually, spring and autumn. In 1969, the frequency of sampling was reduced to once per year (autumn) and in 1970 the number of stations was increased to 100. In 1976, FWS added 17 new stations to the network and continued to collect samples annually. In 1984, sampling was reduced to even-numbered years.

In 1984, the program included 112 stations located on the Nation's major rivers and in the Great Lakes (fig. 1) (Schmitt and others, 1990). Most of these stations are near the downstream terminus of rivers and many of them are co-located with stations that are part of the National Stream Quality Accounting Network (NASQAN)(a fixed-station, fixed-interval, water-quality monitoring network of the USGS, established in 1973).

At each sampling site, the goal of FWS is to collect duplicate, composite samples of a representative bottom feeding fish and one composite sample of a predator fish species. These whole-fish samples are analyzed at the FWS National Fisheries Contaminant Research Center in Columbia, Mo. Originally, only organochlorine pesticides were analyzed. Now, the list of analytes is expanded to include a group of predefined organochlorine chemicals (both pesticides and industrial-related compounds) and seven inorganic trace elements (arsenic, cadmium, copper, lead, mercury, selenium, and zinc).

This is the only program of national scope that has monitored contamination in freshwater animals for any substantial length of time. Aside from presenting data in tabular form, the program interprets means, maxima, minima, and incidences of occurrence for selected contaminants. It has determined useful geographic rankings and temporal differences employing careful statistical analysis, and its products are published in the scientific literature. This program has set a high analytical standard and its methodological studies have helped advance the science of biomonitoring with fish. It provides a base of data that others can employ in comparative analyses, especially for a few of the more abundant fish species.

The NCBP has some limitations, mostly associated with the choice of geographic scales and the choice of only fish to study contamination in large river environments (Schmitt and others, 1983). The most important limitation is that the dispersal of stations limits differentiation of sources and understanding of the geographic scale of contamination. The NCBP monitors on a regional scale contamination that may commonly occur on sub-regional scales. Another important problem is that physical data, water chemistry

and sediment chemistry are not considered in NCBP publications. This limits explanatory capabilities. The NCBP data have geographic and temporal discontinuities because of the difficulty of collecting the same species of fish at every station and every time (Schmitt and others, 1983), but this problem is difficult to avoid in freshwater environments. Interpretations of space and time trends from NCBP have been limited to a large subset of the original stations. Finally, sensitivity of interpretations is limited for trace elements because whole fish are employed for analysis.

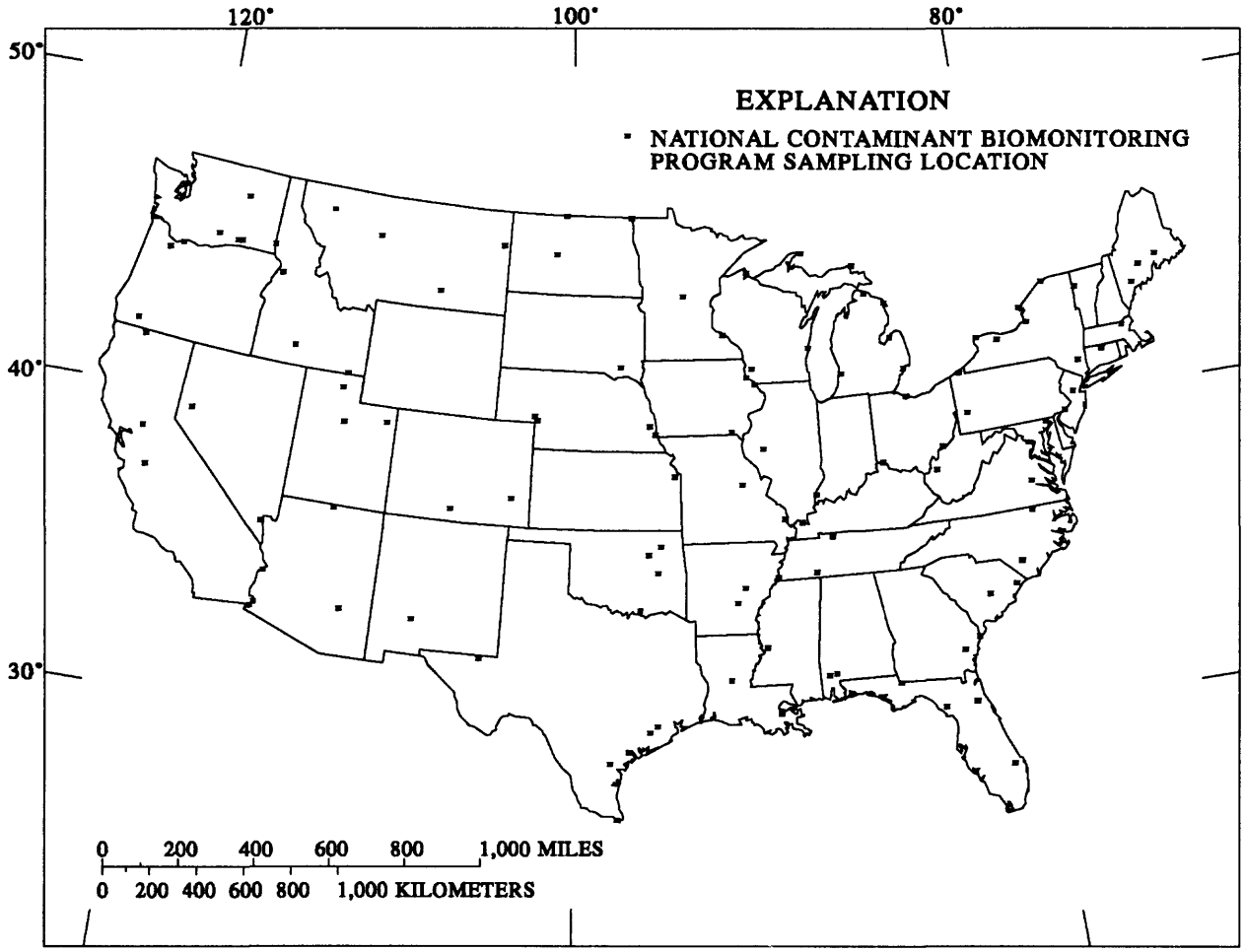

Figure 1.–Location of sampling sites for the National Contaminant Biomonitoring Program.

Table 2.--Overview of the tissue analysis component of the National Water-Quality Assessment Program compared to other tissue analysis programs of national scope in the United States

[SAS, Statistical Analysis System; STORET, Storage and Retrieval System; USEPA, U.S. Environmental Protection Agency; NIST, National Institute of Standards and Technology; NFCRC, National Fisheries Contaminant Research Center; x, included in the program; -, not included]

Element	Program					
		National Status and Trends Program [1]				
	National Water-Quality Assessment Program [2]	Benthic Surveillance Program	Mussel Watch	National Study of Chemical Residues in Fish [3]	Environmental Monitoring and Assessment Program, Surface Waters Component [4]	National Contaminant Biomonitoring Program [5]
Objectives						
Establish data base	x	x	x	-	-	-
Create specimen bank	-	x	x	-	-	x
Develop new techniques	-	x	x	-	-	-
Estimate environmental quality	-	-	x	-	x	-
Determine prevalence of contaminants	x	-	-	x	-	x
Identify chemicals of concern	-	-	-	x	-	-
Describe spatial variability	x	x	x	-	x	x
Identify regional variability	x	-	-	-	x	x
Identify areas needing more study	-	x	x	x	-	-
Evaluate contamination in vicinity of sources	-	-	-	-	x	-
Establish baseline concentrations	x	-	-	-	x	-
Detect temporal change	x	x	x	-	x	x
Determine contaminant fate	x	-	-	-	-	-
Determine contaminant bioavailability	x	x	-	-	-	-
Evaluate human health effects	-	-	-	x	x	-
Evaluate contaminants in game fish flesh	x	-	-	x	x	-
Resources targeted	Freshwater streams	Coastal and estuarine	Coastal and estuarine	Freshwater streams and lakes	Freshwater streams and lakes	Large rivers and Great Lakes
Geographic coverage	Nationwide	Nationwide	Nationwide	Nationwide	Nationwide	Nationwide
Number of sampling stations	~600	~50	~150	400	Subset of 6,400	112
Sampling frequency	Annual[6]	Annual	Annual	One-time only	Annual [7]	Bi-annual
Duration of program	-	1984 - present	1986 - present	1988	-	1967 - present

Table 2.--Overview of the tissue analysis component of the National Water-Quality Assessment Program compared to other tissue analysis programs of national scope in the United States--Continued

[SAS, Statistical Analysis System; STORET, Storage and Retrieval System; USEPA, U.S. Environmental Protection Agency; NIST, National Institute of Standards and Technology; NFCRC, National Fisheries Contaminant Research Center; x, included in the program; -, not included]

Element	Program					
		National Status and Trends Program [1]				
	National Water-Quality Assessment Program [2]	Benthic Surveillance Program	Mussel Watch	National Study of Chemical Residues in Fish [3]	Environmental Monitoring and Assessment Program, Surface Waters Component [4]	National Contaminant Biomonitoring Program [5]
Target organisms						
Bivalve mollusks	x	-	x	-	-	-
Bottom-feeding fish	x	x	-	x	x	x [8]
Sport/commercial/game fish	x	-	-	x	x	x [6]
Crayfish	-	-	-	-	-	-
Insects	x	-	-	-	-	-
Aquatic plants	x	-	-	-	-	-
Organs tested	Whole organism, fish livers, fish flesh	Fish livers, plus bile	Whole mollusk	Whole fish, fish fillets	Whole fish	Whole fish
Target variables						
Chlorinated organic compounds						
Organochlorine pesticides	x	x	x	x	x	x
PCB's	x	x	x	x	x	x
Dioxins	x	-	-	x	-	-
Polynuclear aromatic compounds	x	x	x	-	-	-
Trace elements	x	x	x	-	x	x
Additional data collected						
Water chemistry	x	x	x	-	x	-
Sediment chemistry	x	x	x	-	x	-
Sediment grain size	x	x	x	-	x	-
Fecal sterol	-	x	x	-	-	-
Coprostanol	-	x	x	-	-	-
Clostridium	-	x	-	-	-	-
Fish disorders	x	x	-	-	-	x
Lipid content	x	x	-	x	x	x

Table 2.--Overview of the tissue analysis component of the National Water-Quality Assessment Program compared to other tissue analysis programs of national scope in the United States--Continued

[SAS, Statistical Analysis System; STORET, Storage and Retrieval System; USEPA, U.S. Environmental Protection Agency; NIST, National Institute of Standards and Technology; NFCRC, National Fisheries Contaminant Research Center; x, included in the program; -, not included]

Element	Program					
		National Status and Trends Program [1]				
	National Water-Quality Assessment Program [2]	Benthic Surveillance Program	Mussel Watch	National Study of Chemical Residues in Fish [3]	Environmental Monitoring and Assessment Program, Surface Waters Component [4]	National Contaminant Biomonitoring Program [5]
Additional data collected --Continued						
Histopathology	-	x	x	-	x	-
Age	x	x	-	-	x	x
Gonadal index	-	-	x	-	-	-
Data storage	In-house distributed information	In-house microcomputer	In-house microcomputer	SAS data set and STORET	Unknown	U. of Missouri mainframe as SAS data set and NFCRC
Sample archival	In-house	NIST	NIST	USEPA lab, Duluth	Unknown	NFCRC

[1] National Oceanic and Atmospheric Administration (See Robertson and O'Connor, 1988, for an overview of the program).

[2] U.S. Geological Survey.

[3] U.S. Environmental Protection Agency (See U.S. Environmental Protection Agency, 1986, for an overview of the program).

[4] U.S. Environmental Protection Agency (See Whittier and Paulsen, 1992, for an overview of the program).

[5] U.S. Fish and Wildlife Service (See Jacknow and others, 1986, for an overview of the program).

[6] NAWQA will sample annually during a 3-year intensive phase, then reduce sampling for a 6-year non-intensive phase.

[7] EMAP will sample one-quarter of the 6,400 sites annually, rotating sites on a 4-year schedule.

[8] The National Contaminant Biomonitoring Program also targets birds for analysis.

The National Oceanic and Atmospheric Administration's National Status and Trends Program

In 1984, NOAA began the National Status and Trends Program (NS&T) for Marine Environmental Quality. The objectives of this program are to define geographic distribution of contaminant concentrations in biological tissues and sediments in coastal settings. The scale is regional and the goals are to determine trends, and to document biological responses to contamination (National Oceanic and Atmospheric Administration, 1987b). The program establishes a national contaminant data base by use of state-of-the-art sampling, preservation, and analysis methodologies. These data will be used to estimate environmental quality and develop a statistical basis for detecting spatial and temporal changes. The program consists of two components: (1) the Benthic Surveillance Project, and (2) the Mussel Watch Project. In contrast to the NCBP and the National Study of Chemical Residues in Fish (NSCRF)(discussed in the next section), which include only freshwater sampling sites, the NS&T sampling sites are located in estuarine and near shore areas of the Atlantic, Pacific, and Gulf coasts.

The Benthic Surveillance Project began in 1984. Bottom-dwelling fish are collected annually at about 50 sites and the livers are excised and analyzed for 16 elements, 18 aromatic hydrocarbons, and 15 chlorinated pesticides (Shigenaka and Lauenstein, 1988). The livers are composited for analysis of organic compounds, but are analyzed individually for trace elements (National Oceanic and Atmospheric Administration, 1987a). Sediment chemistry data are collected at both Benthic Surveillance sites and Mussel Watch sites.

The Mussel Watch Project was established in 1985 as an outgrowth of two previous "Mussel Watch" studies. The first national Mussel Watch study was coordinated by the Gulf Breeze Laboratory of the Bureau of Commercial Fisheries and operated from 1965 until 1972. The second Mussel Watch study was coordinated by USEPA from 1976 to 1978. The present NS&T Mussel Watch Project involves the annual collection of native bivalve mollusks at about 150 sites (fig. 2) and analysis of composites of the soft tissues for the same chemicals as in the Benthic Surveillance Project. Each collection consists of six separate composite samples. Composites consist of 30 mussels or 20 oysters (National Oceanic and Atmospheric Administration, 1987a).

Sampling sites for the NS&T are selected "at points known or expected to be depositional environments for sediments and habitats for the target bottom-feeding fish or bivalve mollusks." Sites are deliberately located away from known point sources and authorized dump zones. They are selected to represent accumulations of contaminants from multiple sources. This strategy is purported to lead to "characterization of entire estuaries or coastal regions" (National Oceanic and Atmospheric Administration, 1987a).

The NS&T program has only a limited explanatory component, and, like the NCBP, interpretations are limited by the dispersal of stations on a regional scale. The NS&T program should be successful in providing interpretable data on broad geographic trends in contaminants that bioaccumulate. However, sub-regional characterizations, delineation of specific sources of contamination, and understanding of transport or deposition are examples of problems that have proven difficult to understand from these data. Because estuaries and coastal zones commonly are characterized by multiple sub-regional scale contamination problems (Luoma and Phillips, 1988), these are important limitations. Important approaches developed in the older studies were designed into NS&T (sample size is large; animal size considered; sessile species employed as well as fish) and thus, the data base should be reliable and internally comparable. The older data base was employed in rankings to identify distributions and severity of contamination on regional scales in the coastal United States and provided a basis for setting priorities for management action. The broad set of national data also was useful to more spatially intensive state programs employing similar methodology (Hayes and others, 1986). Data from the older program were published in peer-reviewed scientific literature and provided a global standard.

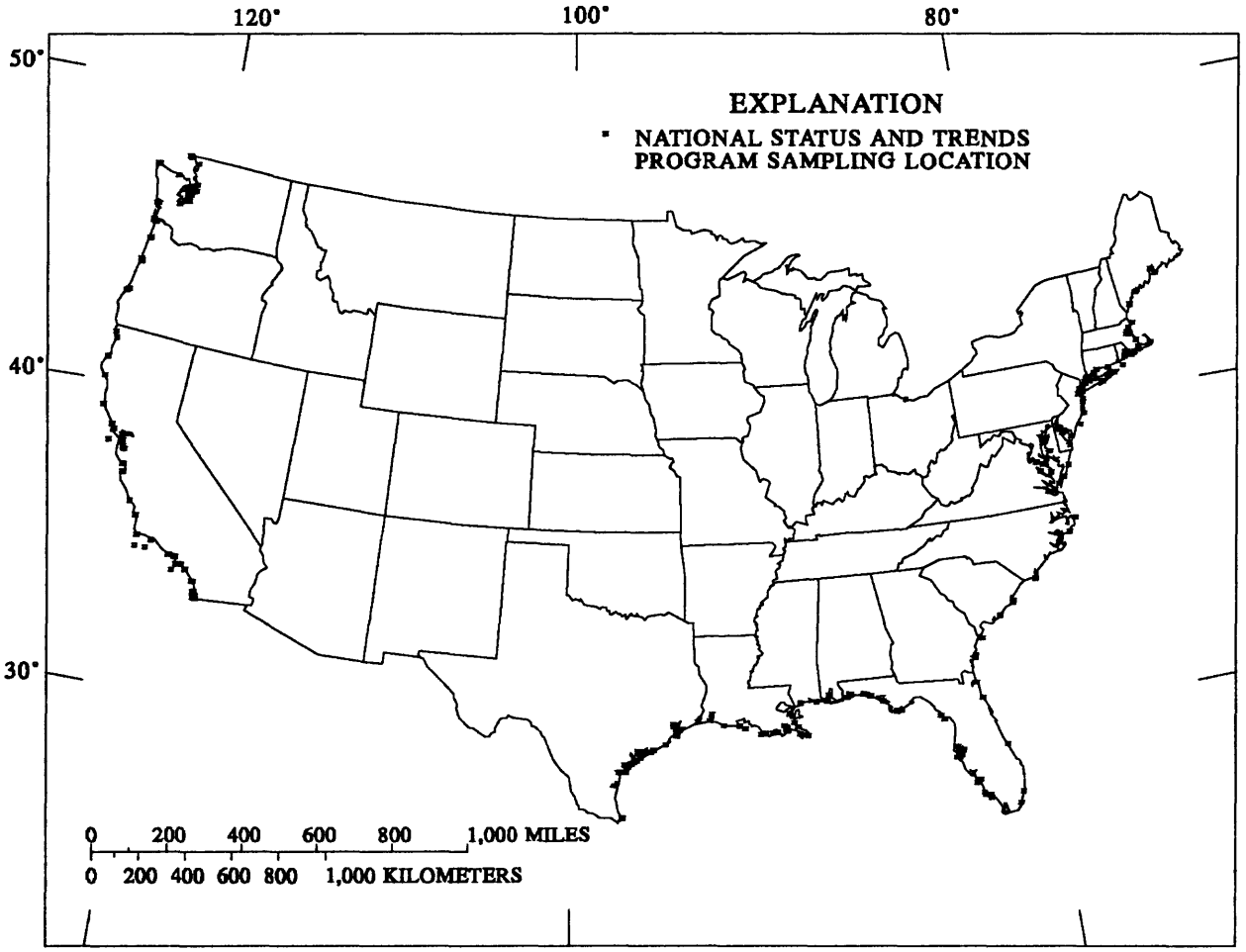

Figure 2.–Location of sampling sites for the National Status and Trends Program.

The U.S. Environmental Protection Agency's National Study of Chemical Residues in Fish

The NSCRF is a follow-up to the National Dioxin Study that was conducted in 1984 by the USEPA to examine the extent of contamination of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in soil, water, sediment, and fish tissue. The NSCRF was conducted because of concern that other pollutants with similar properties as dioxin might bioaccumulate in the aquatic food chain (U.S. Environmental Protection Agency, 1986). The major objectives of the NSCRF were to determine the prevalence and concentrations of selected contaminants in fish from undisturbed areas and from areas expected to have elevated concentrations of contaminants, and to correlate these concentrations with sources of these contaminants. A secondary objective was to identify areas that could have potential human health risks as a result of contaminated fish.

Approximately 400 sites across the Nation have been sampled in the NSCRF (fig. 3). The sites included 50 NASQAN and Hydrologic Benchmark Network stations operated by the USGS, potential problem sites near point and nonpoint sources, and other background sites.

Two samples were collected at each site, one a composite of whole bottom-feeding fish and the second a composite of sport/commercial fish fillets. Analysis was for a set of 60 organic chemicals selected by their bioaccumulation potential and threat to human health. The list included 15 chlorinated dioxins and furans, total PCB's and 10 PCB congeners, several chlorinated organic pesticides, and mercury.

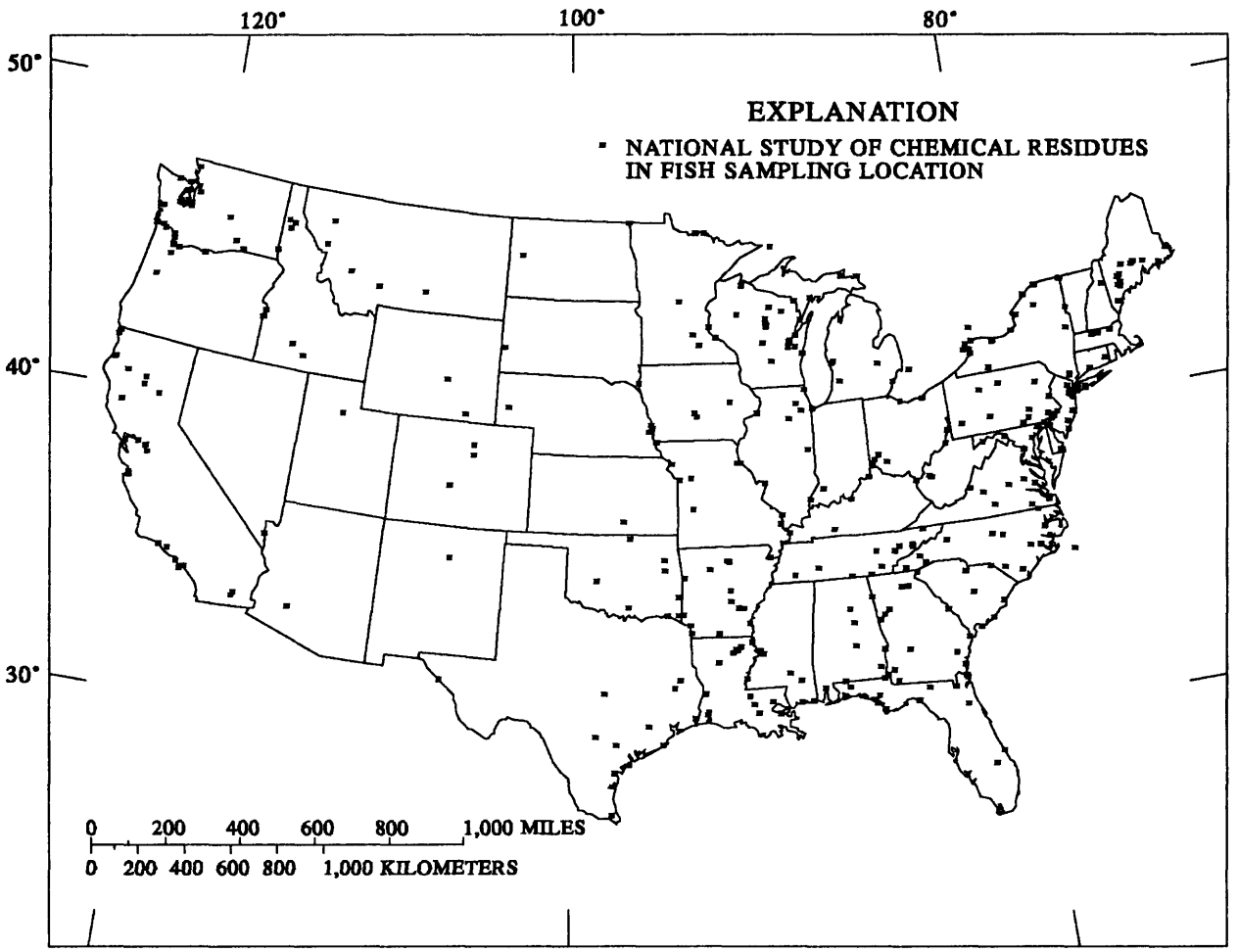

Figure 3.–Location of sampling sites for the National Study of Chemical Residues in Fish.

The final report for this project was published late in 1992 (U.S. Environmental Protection Agency, 1992). Upon evaluation of human health effects, chemicals warranting closer scrutiny will be identified and follow-up studies conducted (U.S. Environmental Protection Agency, 1986).

In contrast to the NCBP and the NS&T, the NSCRF is a one-time screening program oriented toward identifying concentrations of selected contaminants in fish tissue, their potential sources, and evaluating potential human health impacts. The NSCRF has a limited explanatory component (source correlation). While not part of the original design of the study, information will be evaluated for developing a data base for individual fish species.

The U.S. Environmental Protection Agency's Environmental Monitoring and Assessment Program

In 1988, the Science Advisory Board of the USEPA recommended that a program be developed and implemented "... to monitor ecological status and trends, and to develop innovative methods for anticipating emerging environmental problems ..."(U.S. Environmental Protection Agency, 1991). The Environmental Monitoring and Assessment Program (EMAP) is, in part, a response to that recommendation. EMAP will consist of an integrated monitoring network covering seven ecological resource groups: agroecosystems, arid lands, forests, Great Lakes, near coastal systems, inland surface waters, and wetlands. The inland surface waters component of EMAP most closely corresponds to the coverage of NAWQA; therefore, only the inland surface waters component of EMAP (EMAP-SW) will be reviewed here.

Objectives for EMAP-SW are to:

(1) estimate the current extent of inland surface waters;

(2) estimate the status and trends of indicators of the conditions of surface waters;

(3) monitor indicators of pollutant exposure and habitat condition and seek to identify causes of adverse effects; and

(4) report the findings.

To achieve these objectives, EMAP-SW will evaluate a number of indicators of ecosystem health (Whittier and Paulsen, 1992). One indicator to be examined in EMAP-SW is fish tissue contaminants.

Target analytes in fish tissues for EMAP-SW include several chlorinated insecticides, 17 PCB congeners and 12 trace elements. Analyses will be done on whole fish. Target fish are top predators, with largemouth bass being the first priority. Bottom-feeding fish are a secondary target group. Sixty-four hundred sampling sites for EMAP-SW will be randomly selected from a 40 km^2 grid covering the entire conterminous United States. One quarter of these will be sampled per year, giving a 4-year resampling cycle. Of the 6,400 sampling sites, only those exhibiting impaired ecological conditions will be selected for evaluations of fish tissue contaminants (U.S. Environmental Protection Agency, 1991).

EMAP-SW currently is in its infancy. Pilot studies are scheduled for 1992-93. Details of the program have not all been formulated and those that have been proposed will likely evolve as results from the pilot studies become available. However, the conceptual framework for the EMAP-SW is in place and, in many respects, is similar to the plans for NAWQA.

Regional and State Programs

In 1986, the Tennessee Valley Authority (TVA) began the Valley-Wide Fish Tissue Study to screen for potentially toxic levels of priority pollutant metals, pesticides, and PCB's in fish fillets (Dycus, 1989). The program coordinates tissue monitoring activities of the TVA and State agencies. State agencies collect fish from about half of the 83 sampling sites located throughout the Tennessee River Valley and parts of the Cumberland River Valley; the TVA collects samples from the others. The TVA does the laboratory analyses on composites of the collected fish fillets and places the data in USEPA's computerized data Storage and Retrieval (STORET) system. Frequency of sampling for each station is based on the type of station and on the results of analyses. Tributaries to reservoirs are sampled annually. Reservoir sites where the concentrations of contaminants in tissues are judged to be low are sampled every 3 years. Reservoir sites where the concentrations of contaminants in tissues are high undergo more intensive study. Channel catfish is the target species for the reservoir sampling. Game fish, catfish, and rough fish are all sampled in the inflows to the reservoirs. The TVA has conducted tissue analyses on selected reservoirs since the early 1970's, but this is the first attempt to establish a valley-wide, long-term monitoring program.

Other state and regional entities also maintain environmental monitoring programs that generally are consistent with Federal programs. California Department of Fish and Game, Illinois Environmental Protection Agency, the Ohio River Valley Water Sanitation Commission, and studies conducted through the Great Lakes Water Quality Agreement are examples. USEPA's Guidance for State Water Monitoring and Wasteload Allocation Program (U.S. Environmental Protection Agency, 1985) encouraged all states to scan fish and other tissue to detect local human health or environmental damages. Many states have established programs to study levels of specific pesticides and other contaminants in fish and other tissues, as well as in sediments and water. The state and regional programs tend to sample with more spatial intensity than Federal programs, and the data from some (Hayes and others, 1986) have been successful in identifying sources (some previously unsuspected) and geographic trends in contamination. However, individual programs tend to follow individual designs. Lack of continuity limits interpretations in many data sets, and comparability among programs is often difficult. Furthermore, results are not broadly disseminated, nor interpreted within a national context. In many cases, no serious attempts at interpretation have been made.

Relation of Other Programs to Tissue Analysis in the National Water-Quality Assessment Program

In total, currently existing and previous tissue monitoring programs have provided a bank of data, parts of which will be comparable to NAWQA data. While none of these programs used methodology exactly like any of the other programs, some of the data from the individual programs will be useful for preliminary identification of some geographical and temporal variations. Furthermore, the existing programs have been useful in refining the development of a tissue analysis programs such as that proposed for NAWQA.

NAWQA will differ from other national scope programs for tissue analysis in several ways. Firstly, some successful programs (for example, the NS&T Program) monitored exclusively marine and brackish water environments. NAWQA will employ tissue analyses in freshwater environments. Although it must target a larger set of species than NS&T Program, systematic development of a national data base will provide necessary comparability. The complexity of freshwater environments makes the design of a coherent program challenging, but the proposed plan is designed to develop information previously unavailable in a systematic fashion.

Secondly, existing national freshwater studies have been successful in broad regional monitoring, but cannot resolve contamination distributions on spatial scales smaller than thousands of kilometers. Existing freshwater programs do not include a component that seeks to explain the effects of specific processes on water quality; most are limited to one type of organism (NCBP and NSCRF employ only fish; Hayes and others (1986) employed one species of clam); and none have overtly set out to systematically develop a comparable national data base for individual species. By including a broader suite of organisms, and by including analyses of water and sediments, NAWQA's results will be applicable to a broad array of freshwater environments. The spatially intensive design, careful designation of objectives, and coordination with physical, chemical and ecological assessments will aid spatial, cause/effect and process interpretations.

Thirdly, among programs of national scope, only the NCBP is designed to monitor trends at the same freshwater stations over long periods of time. However, those stations are at the downstream end of rivers and are widely separated from one another. The dispersed nature of these sites adds greatly to difficulties in interpretation. NAWQA will assess trends at several stations located throughout 60 study units. This greater replication will allow a more sensitive, scientific determination of whether trends are single station anomalies compared to basinwide, regional, or national phenomena.

Fourthly, NAWQA should complement the NCBP. NAWQA will assess species other than fish in many basins, and its assessments will add information from a number of localities at a more detailed spatial scale in the study unit to complement the widely distributed spatial network of NCBP. Interpretations of data from each program will be improved by the existence of the other. Furthermore, the two together will provide a solid base of data and methodology from which local, state, or regional studies of specific problems (or assessment programs) can be developed.

Fifthly, none of the other national programs combine explanatory goals within individual basins with broader national goals for data interpretation. Furthermore, interpretation of tissue analyses within the context of other physical, chemical, and biological data gathered by the program is a fundamental goal of NAWQA. These unique aspects make tissue analyses in NAWQA part of an overall water-quality assessment rather than an isolated monitoring program.

One program, the surface waters component of EMAP, has many features similar to NAWQA. The major distinction between the two is the intensive river basin sampling approach proposed for NAWQA compared to the statistically-based sampling scheme for EMAP-SW.

Part 3: APPROACH OF TISSUE-ANALYSIS SURVEYS

General Approach

A systematic tissue analysis program involves the following steps. The objectives stated in this document must be clearly defined for the specific study unit. Those objectives will then be prioritized for the study unit and the timeline established for accomplishing specific objectives. A sampling strategy will be developed that will allow a rigorous consideration of each objective. Stations will be selected following the criteria established in this document with consideration given to the stratification scheme for the study unit and to national synthesis criteria. Goals will be established for the number of replicate samples necessary to implement the strategy and to achieve the stated objectives. Specific site selection for all tissue analyses will be made by the project chief of each study unit in consultation with study unit staff, regional staff and representatives of other agencies. The project chief will attempt to eliminate overlaps in sampling effort so that multiple objectives can be accomplished with optimal efficiency. Accomplishing each objective also will necessitate specific decisions about what chemicals to determine and what taxa to collect. Again, guidelines are established in the following discussion. Coordination among study units will be provided by regional and national coordinators. They will help adjust study designs to satisfy objectives and will help interpret data at scales larger than the individual study unit.

Objectives, Priorities, and Timeline

The general objectives of the tissue bioassessment in NAWQA are described below in order of priority. Each study unit will, as a minimum, accomplish the first three objectives, which are: (1) defining which contaminants are present in biological tissues (occurrence) in each study unit; (2) establishing a data set from which long-term temporal trends in contaminant concentrations can be determined locally and nationally; and (3) defining how contamination is distributed (spatial distribution) through individual NAWQA study units. As these immediate objectives are satisfied in individual basins, data will be aggregated to achieve objective 4: development of a comparative, national data base of contaminant concentrations in the tissues of a priority list of taxa (termed the NAtional Target Taxa list or NATT). This data base will allow determination of contaminant distribution among the nationwide collection of study units.

As study units proceed beyond the first set of objectives, additional goals will include (5) a comparison of contaminants in biota among waters draining areas having different land uses, or along gradients away from sources of contamination; (6) development of statistically rigorous data on background concentrations of contaminants in "unaffected" reference areas; (7) a comparison of partitioning of contaminants in water, sediment, and biota to give a measure of comparative bioavailability for those contaminants that bioaccumulate. Where appropriate, the program will (8) determine levels of contaminants in edible parts of selected game fish species. Knowledge gained from tissue analyses in NAWQA also is expected to (9) contribute to the refinement of existing and the development of new bioassessment procedures. The priority for these objectives may be altered to meet local study unit needs. Efficiencies will be sought in satisfying the nine objectives. For example, data to satisfy objectives 4, 6, and 7 will accumulate as the other higher priority objectives are satisfied. Aspects of objective 5 can be accomplished by embedding designs within aspects of the first three objectives, or by supplementing the design for the first three objectives. However, the design for every objective will be explicitly described in the workplan of every study unit.

The overall NAWQA plan calls for a 9-year cycle of study in each study unit. Years 1 and 2 are reserved for staffing, planning, and gathering historical and background information. Years 3, 4, and 5 are for intensive data collection. Years 6-9 are for report writing and low-level data collection. The tissue sampling effort will start in the third year with the reconnaissance for contaminant occurrence at selected stations. After that, fewer chemicals will be analyzed at more stations during the rest of the 3-year intensive data collection effort. In the second year of intensive study (year 4 of the NAWQA cycle) assessment of the spatial distribution of contaminants will begin in most study units. Every study unit will sample several stations on main stem rivers. Samples from major tributaries near their mouths also will be collected. Every study unit will develop a data base that can be used for long-term trend detection. This effort will begin in the third year of the NAWQA cycle at a subset of the sites used for the spatial distribution assessment. Where

feasible, the initial spatial characterization will be followed in year 5 of the NAWQA cycle by a more detailed spatial assessment, which will be tied in with the testing of explanatory hypotheses, such as the effects of land use or specified sources of contaminants. In many, but not all study units, the detailed spatial assessment and testing explanatory hypotheses will begin in the first cycle of intensive data collection. This is especially likely where pre-NAWQA data are available to help satisfy objectives 1-3.

Within any 1 year, all tissue sampling will be conducted during low flow, in the late summer or early fall. Because concentrations of contaminants in tissues may change seasonally (Phillips, 1980), it is important that sampling from year to year in a basin be conducted as near as possible to the time when samples were collected in previous years. Most objectives will require collection of comparable taxa among stations. Some later objectives will require collection of replicate composite samples of NATT taxa. The cost trade-offs between number of stations, level of sampling effort per station, and the number of analyses will be considered in determining the objectives each study unit will pursue. Ultimately, however, understanding of tissue contamination will develop in every study unit as objectives are progressively satisfied.

Table 3 shows examples of timelines for tissue analysis for a typical study unit. Some differences in pace of accomplishment are expected, depending upon availability of previous data, water-quality problems of interest in the study units, and the capabilities of the study teams. Thus, a high and a low level of accomplishment are shown. Tissue sampling will be coordinated with ecological survey sampling and will be concurrent with synoptic surveys for organic compounds and trace elements in bed material. Table 3 also shows an example of links with other components of NAWQA that must be considered when designing the tissue bioassessment. The table indicates that the most desirable sequence of events in most study units would have tissue sampling following a field reconnaissance. Experience gained in the field reconnaissance will aid station selection for the contaminant-occurrence, spatial distribution, and trends objectives of the tissue analysis. Table 3 implicitly assumes that sampling for the same objective can be done in more than 1 year. Some year-to-year variability is expected in chronically contaminated rivers and streams, but experience to date suggests that overall distributions and trends are consistent from year-to-year (Schmitt and others, 1990; Moore and others, 1991).

Table 3.--Suggested sequence for accomplishments of the tissue analysis component of the National Water-Quality Assessment Program

Year	Sequence	Accomplishment
1		Staffing, workplan
2		Retrospective literature review.
High Level of Accomplishment		
3	A	Field reconnaissance [1]
	B	Select and sample contaminant occurrence and trend stations using experience gained in reconnaissance.
4	A	Trend samples from four to eight stations.
	B	Complete spatial distribution survey (add necessary stations to initial "Occurrence" stations).
	C	Spatial characterization stations include stations to be employed in testing explanatory hypotheses.
5	A	Trend samples from four to eight stations.
	B	Add replicate, repeat, multispecies samples as necessary to complete statistically valid hypothesis testing.
6 - 9	A	Data analysis and report preparation.
	B	Trend samples from four to eight stations.
Minimum Level of Accomplishment		
3	A	Field reconnaissance.
	B	Collect samples for occurrence objective from approximately 10 stations.
	C	Select trend analysis stations.
4	A	Trend samples from four stations.
	B	Add samples to determine occurrence.
	C	Add stations for beginning of spatial characterization
5	A	Trend samples from four stations.
	B	Complete spatial coverage.
6	A	Data analysis and report preparation.
	B	Trend samples from four stations.

[1] See Cuffney, T.F., Gurtz, M.E., and Meador, M.R., written commun., 1991.

Study Strategies

Study strategies for each component of the tissue analysis will involve selection of station locations, selection of chemical analyses, selection of taxa to target, and determination of the product each objective will produce. Trade-offs are inevitable among the number of stations sampled, the intensity of field activity at each station, the number of chemical analyses and their cost, and the resources expended upon data storage and analysis, quality assurance and quality control, and sample archival. These trade-offs will place a ceiling on the number of sites sampled, the number of chemicals analyzed at each site, the number of species collected from each site and the number of replicate samples of each species. These trade-offs must be considered as the workplan for each study unit is developed. The present document provides guidance as to how the study teams might balance the trade-offs; but it is recognized that the balance may differ among study units with differences in resources, capabilities, and emphasis upon ecological problems.

Retrospective Analysis

The gathering of background information in the first 2 years is termed the retrospective analysis. The retrospective analysis should accomplish several goals specific to the tissue bioassessment. It should seek existing ecological data that will aid preliminary determinations of species to target for collection during the tissue bioassessment and it should assess what contaminants might be expected in the basin. The retrospective also should involve a thorough search for and interpretation of previous studies of contaminants in water, sediment, and tissues conducted in the study unit. In addition to a compilation of available data, the interpretation should include:

(1) A list of the contaminants that have been analyzed in tissues and those observed in enriched concentrations in the study unit.

(2) Maps showing where tissue analyses have been conducted.

(3) Maps of available information on spatial distributions of contaminants in water, sediment, and tissues. Spatial distributions should note localities where contamination was, and was not observed. All tissue data employed in interpretive products must be comparable (same species, similar size or age) and normalized for lipid content, if appropriate.

(4) Summaries of specific studies that assess influences of land use or point source inputs on bioavailable contaminant concentrations. Instances where studies indicate contaminants might influence ecological processes should also be noted, as should available data on partitioning of contaminants or contamination of fish flesh that exceeds existing standards.

Intensive Sampling Period (Years 3-5)

The intensive sampling period will focus on field sampling and laboratory analyses needed for addressing the objectives for the use of tissue analysis as part of NAWQA. Special strategies will be needed for each objective. Guidance for each objective follows.

Objective 1: What chemicals are present?

The primary objective of contaminant occurrence survey is to define what contaminants occur in the tissues of biota in each study unit. Results from the occurrence sampling will aid in selection of contaminants to be targeted in later samplings. The data collected in achieving this objective will contribute to the overall bank of data available for meeting other objectives. This initial sampling experience also will aid in selection of species to target for other objectives.

Samples will be collected from a limited number of sites (15-20) to satisfy this objective. Each sample will be analyzed for a broad suite of trace elements and organic compounds. Site-selection criteria are as follows. All sites should be contaminant occurrence sites for sediment analyses or fixed stations where trace elements and synthetic organic compounds have been analyzed at least in bottom sediments. Sites also should correspond to sites used for ecological survey sampling in NAWQA. Because the objective of contaminant occurrence tissue analysis sampling is to define which contaminants occur in biota in the study unit, most sampling sites (approximately 12-15) will be located where contamination is likely. The sites should be dispersed so that streams draining each major land use in the basin are sampled, in order to

obtain the widest view of the array of contaminants present. Enough stations should be sampled so the study unit team feels comfortable in eliminating contaminants from further consideration. Specific sites may be immediately downstream from large point sources, an urban area, an agricultural area, or wherever contaminants are suspected of being added to the stream. In addition, three to five reference sites should be selected where little or no contamination is expected. At least one reference site should be located in each ecological region, as defined by Hughes and Larsen (1988), in the study unit. The reference sites will allow detection of contaminants widely dispersed or naturally present in the study unit. Reference data also will aid interpretation of whether contaminants are enriched at other sites. Site-selection considerations include (a) knowledge of contaminant inputs; (b) maps of land use and basin management plans; (c) existing data on water flow, river morphology, and regional ecological characteristics; (d) prior studies of water quality and contaminant concentrations in biota, sediments, and water; and (e) aerial reconnaissance and on-site field reconnaissance if necessary to aid species selection.

The 15 to 20 locations selected in each study unit for the contaminant occurrence survey will be strong candidates for sites to be used to satisfy other objectives and will be chosen accordingly in the initial workplan. All collections need not necessarily be of comparable taxa to satisfy the occurrence objective. However, if the choice of species emphasizes NATT and comparability among stations is considered, the data are more likely to be useful for more than one objective. Therefore, sampling should emphasize collection of an abundant species from the NATT list. Replication of composite samples at each site is not necessarily important to satisfying this objective.

The products of this objective include a list of locally important contaminants from which analytical choices can be made for other objectives. Assessments of the presence or absence of contaminants in biological tissues on a national or regional scale also will be an important product.

Objective 2: Long-term-trend analysis

In every study unit, sampling for trend analysis will be initiated at four to eight stations in the third year of the first NAWQA cycle. The goal is to begin the data base necessary for long-term-trend detection. Long-term trends are defined on decadal time scales (that is, differences between NAWQA intensive study phases). Preliminary selection of trend stations will occur at the same time as stations are selected for objective 1. Thus, most trend sites will be sampled in all 3 years of the intensive data-collection phase. Samples from at least four stations also will be collected once per year through the nonintensive sampling phase to provide a continuous time series of bioavailable tissue concentrations. Such a time series at a few stations is essential for verifying and interpreting trends in contamination. That time series will be one product of this objective. If resources are available, the study units will also establish discrete 3-year data sets at additional stations (three to four). These data will be collected so that they can be statistically compared to similar data collected in later NAWQA cycles. The design for both aspects of the trend study must include enough replication so that trends on a 10- to 15-year time scale can be statistically verified. A statistically valid design must include adequate replication of composite samples at each station.

The taxa chosen for trend studies must be from the NATT list, but collection can be limited to one taxon per station. It is important that comparable taxa be collected every time a trend analysis station is re-sampled (either the same species or species with empirically established similar bioaccumulation capabilities). The individuals collected also should be comparable in size, age, and stage of reproductive cycle to previous samples from a station. Thus, any chosen taxon must be abundant and a permanent resident (as best as can be determined) of the trend analysis station. The station also should have a stable habitat, so as to support abundant populations of the target species through the years.

It is imperative that sites to be employed for analysis of trends in tissues correspond to sites employed to study trends in other aspects of water quality and ecological conditions. Thus, sediment sampling stations, fixed stations, or stations where other chemical analyses are being conducted will receive high priority. Some stations within the catchment-based trend analysis in the ecological survey component of NAWQA should also be given high priority. At least one uncontaminated or reference station from each study unit and the downstream-most fixed station (where water and sediment also are being continuously

monitored) will be included in the data collection effort for trends. If all of the above criteria can be met with a subset of sites selected to meet other objectives, the trend studies will be most efficient.

A broad suite of analyses will be conducted on samples from trend stations in the third year of every NAWQA cycle. In other years, analyses will be of chemicals initially chosen to be of interest in local or national synthesis efforts. Discretion will be employed in adding or subtracting chemicals from the initial list.

The products of this objective will be a perennial time series of contaminant concentrations in tissues at several stations in every study unit, and regional and national assessments of trends in contaminants of common biota.

Objective 3: Spatial characterization

Sampling to determine the spatial distribution of contamination in each study unit will begin during the field season following the contaminant occurrence survey. In most study units this effort will occur during years 4 and 5 of the NAWQA cycle (years 2 and 3 of the intensive sampling effort). Most study units will split the spatial description between the 2 years of study. The first year of such a staged effort might involve partitioning more effort toward areal coverage of subbasins and less effort toward collecting from main stem sites. The second year of sampling then could focus on main stem sampling locations. Replicate sampling should be adequate to statistically differentiate critical reaches in the study unit after this stage of study. Spatial sampling will allow every study unit to develop a minimal understanding of the spatial distribution of contamination in tissues during the first round of intensive data collection. In most study units this will be accomplished by sampling 20 to 40 stations. Distribution of contaminants among different species (for example a fish and an invertebrate or invertebrates from different trophic levels) is built into the spatial characterization at half the stations, where possible.

Two important guidelines define the minimum level of understanding that will be achieved in every study unit:

(1) Important tributaries to larger, higher-order streams will be sampled at least near their confluence. These samples will integrate the effects of activities within the basins of those tributaries and the effects of those activities on the larger stream.

(2) "Indicator" streams that drain small basins of known or homogeneous land use areas will be sampled near the downstream boundary of the basin.

The goal of the spatial study is an objective characterization of contaminant distributions in the study unit. Thus identifying areas with little contamination is as important as identifying contaminated localities. Site selection should not be dominated just by expectations of contamination. Ecological survey stations, fixed stations, and sediment synoptic contaminant occurrence sites will be given priority in site selection. Specific efforts should be made to identify perhaps five to eight areas where influence from human activities is minimal. These references are necessary to determine baseline levels of contaminants in each taxon and thus to provide a framework for interpreting observed differences among sites and changes over time, especially with respect to natural biological variability. In order to maximize efficiency, the stations selected for the spatial descriptions should complement those sampled during the contaminant occurrence survey. Of course, some resampling of occurrence stations will be necessary if taxa collected in the first effort are not comparable to those being employed in the spatial description. Similarly, stations, taxa and chemicals selected to test specific hypotheses will be included in the spatial description.

Taxa for the spatial study will be selected to maximize comparability within the study unit and among study units. Thus a strong preference will be given to organisms from the NATT list. More than one species (preferably both a fish species and an invertebrate species) will be collected at least at 50 percent of the stations. In many study units, no single taxon will occur in all streams and rivers. Spatial distributions in those instances will be derived from comparisons of streams and river segments with similar or overlapping taxa. Water and sediment data also can be considered in such comparisons.

Chemicals selected for the spatial study will be those defined as locally important by the retrospective literature survey, the tissue contaminant occurrence survey, and the sediment contaminant occurrence surveys. Chemicals targeted nationally for study will be included.

One product of the first stage of the spatial sampling will be an overview of the distribution of locally important contaminants in the study unit with a nationally consistent, minimum spatial scale of resolution. As the spatial study becomes more detailed, a conceptual model of contaminant distributions should progressively develop. Within and among basins, such models will take the form of reach comparisons, maps, and quantitative or qualitative descriptions. The products of the spatial study will set the stage for more intensive studies that explain contaminant problems and contaminant effects within the study unit.

Objective 4: National distribution of bioavailable contaminants

As data on contaminant tissue concentrations progressively accumulate in each study unit, satisfaction of regional and national objectives should begin to unfold with only minimal adjustment of study design. For example, as objectives 1-3 are satisfied, a data base will develop describing contaminant concentrations in a select group of species (the NATT list), each under a variety of conditions. From these data, comparisons will be possible of contamination in the different streams where each NATT species resides. An analysis of a NATT from any study unit can then be compared to analysis of the same taxon from other study units, providing a national perspective (or ranking) for the former. Ultimately national or regional maps should be possible showing the relative contamination of streams and rivers with similar fauna.

Objective 5: Explanatory studies: Effects of land use, point-source inputs, and hydrologic features

Some of the most interesting tissue analysis studies will be designed to evaluate and explain bioavailable contaminant distributions and effects. These studies will build on the base of understanding developed in the first four objectives. Local issues and regional or national comparisons (for example, the effects of specific land uses) will determine the choice of questions, stations, and chemicals to be studied. The first critical step in these studies is designation of a well-defined hypothesis. The statistical design for these comparative studies will be carefully considered before the study begins. Designation of a reference environment will be an important part of many comparisons. Most cases will require collecting replicate samples of comparable taxa from replicated, paired stations (indicator sites) or along a gradient. Comparability among sites is the most important criteria for the choice of species. Intensive sample collection and relatively large numbers of analyses will be necessary to answer most questions. Because the questions will be specific, most studies will involve analysis of only selected chemicals. Similar ecological region, stream size, drainage area, riparian vegetation, habitat, and substrate are preferable for comparisons between stations. However, the hydrologic similarity is less important in such comparisons than collection of comparable taxa (in terms of species, size, age, and life stage). Data from analyses of water and sediment will add to our understanding in these studies. Only a few hypotheses will be tested in each NAWQA basin during each cycle. However, through time, understanding developed from these studies will progressively increase and broad interpretations (within and among study units) will progressively develop. The products of this objective will then include reports describing local, regional and national influences of land use, effects of point sources, and effects of hydrologic processes or features on the distribution and effects of bioavailable contaminants.

Objective 6: Baseline concentrations

Determination of background, baseline, or reference concentrations of chemicals in tissues will be an important part of interpreting the extent and significance of tissue contamination. Reference stations will be explicitly selected for sampling in every NAWQA study unit as part of the contaminant occurrence survey, the spatial characterization, and the studies testing hypotheses. A regional and national goal is to develop a data base of reference concentrations for each contaminant and for each NATT as these reference stations are sampled. Understanding variability in baseline concentrations will also be essential for many statistical comparisons. Thus, it will be important in the early stages of NAWQA to identify the variance associated with reference concentrations for widely dispersed contaminants or those contaminants that occur naturally. Systematic study of sample variance within reference stations, within reference streams, and among reference streams for NATT species will be carried out in at least some study units in the first cycle of NAWQA.

Stations selected as references for tissue analysis should coincide with ecological survey reference stations wherever possible. One product of this objective then will be chemical verification of the reference status of the ecological reference sites. Conversely, for stations having concentrations of contaminants exceeding reference concentrations, influences of contaminants on communities of fish and invertebrates must be considered. The reference concentrations will be critical for local, regional, and national interpretation of the severity of contamination. A data base for such comparisons will be an important product of this objective. The influence of land use changes (for example, logging) or hydrologic differences on tissue concentrations of contaminants will also be assessed as reference data accumulates, providing another product of this objective.

Objective 7: Contaminant bioavailability

Contaminant concentrations in tissues will aid interpretations concerning the fate and bioavailability of many contaminants. One way of assessing processes that determine contaminant bioavailability is to determine partitioning between sediments, water, and tissues in different hydrologic regimes. Comparisons of contaminant partitioning between different species within a food web also might enhance such understanding. Objective 7 is an interpretive goal, in which such partitioning will be calculated where the appropriate data are available at a station or series of stations. Some specific sampling to accomplish this goal will occur, guided by explicit national synthesis questions. However, most data will come from efforts directed toward other objectives. Fixed-station and sediment contaminant occurrence sites will be good choices for tissue analysis sampling stations that will meet this objective. Use of taxa from the NATT list will be essential for comparisons over broad geographic scales. At some stations more extensive sampling of food webs may be undertaken. Some replication of composite samples will be necessary to assure the statistical validity of the results.

Local, regional, or national comparisons of partitioning data can all contribute to the study of processes controlling contaminant bioavailability. Thus the products of this objective will be local explanations of contaminant partitioning and advances in general process understanding that will benefit regional and national interpretations of water quality.

Objective 8: Contaminants in edible fish flesh

Samples of fish collected for analysis of organic compounds in NAWQA typically will consist of composites of eight whole fish. Therefore, any sampling of game fish for objectives 1, 2, and 3 will not be applicable for an assessment of contaminants in fish flesh. Samples from a limited number of stations (three to four) will be needed to meet this objective. These stations will be sampled in the final year of intensive data collection (the fifth year of the NAWQA cycle). Samples will be collected from stations where fish are harvested in sport or commercial fishing and where prior study showed contamination is present or likely. Contamination will be interpreted relative to state, national, and international criteria for toxicants in edible tissues. Analyses will be restricted to those contaminants that concentrate in fish muscle and that have potential effects on human health. Examples include the chlorinated pesticides or mercury. Samples for this objective will not be collected in study units where local entities adequately assess human health threats in fish flesh.

Nonintensive Sampling Period (Years 6-9)

During the nonintensive phase of a NAWQA cycle (years 6-9), study-unit activities will focus on data management and analysis and report preparation. However, limited field sampling will continue, one time per year and only at those four to eight stations selected for trend analysis. These data will be used to bolster the existing trend data collected during the intensive sampling phase and to provide a means for detecting step trends.

Factors Affecting Selection of Target Chemicals

NAWQA will use a two-stage procedure to select chemicals. Several bioaccumulative contaminants including synthetic organic compounds and trace elements will be targeted for analysis during contaminant occurrence sampling. The contaminants selected for this broad analysis will be determined by

the availability of analytical methods, contaminant toxicity, and bioaccumulative potential; and the capacity of target taxa to metabolize them. Chemicals not detected during the occurrence survey, not found in water and sediment analyses, and not expected in the basin will not be analyzed during sampling for the later objectives. The goal of this procedure is to first insure that all chemicals are considered in all study units, then to reduce costs of the more detailed study efforts by eliminating analyses for contaminants occurring at concentrations less than the limits of detection. Examples of suites of chemicals that might be eliminated at some cost savings when approaching other objectives include polynuclear aromatic hydrocarbons, dioxin-like chemicals, or the mercury-arsenic-selenium suite of trace elements that require separate samples and analytical procedures different from other trace elements. Chemicals included in national synthesis studies will be determined in all samples whether or not they are found during the reconnaissance. The following discussion explains in detail how contaminants are selected for inclusion in the tissue analysis studies.

Analysis of tissues in the NAWQA Program will include potentially toxic trace elements and synthetic organic compounds. For purposes of this document, the term synthetic organic compounds includes a variety of pesticides, polynuclear aromatic hydrocarbons, chlorinated dioxins and furans, and methyl mercury, even though some of these also may be considered naturally occurring.

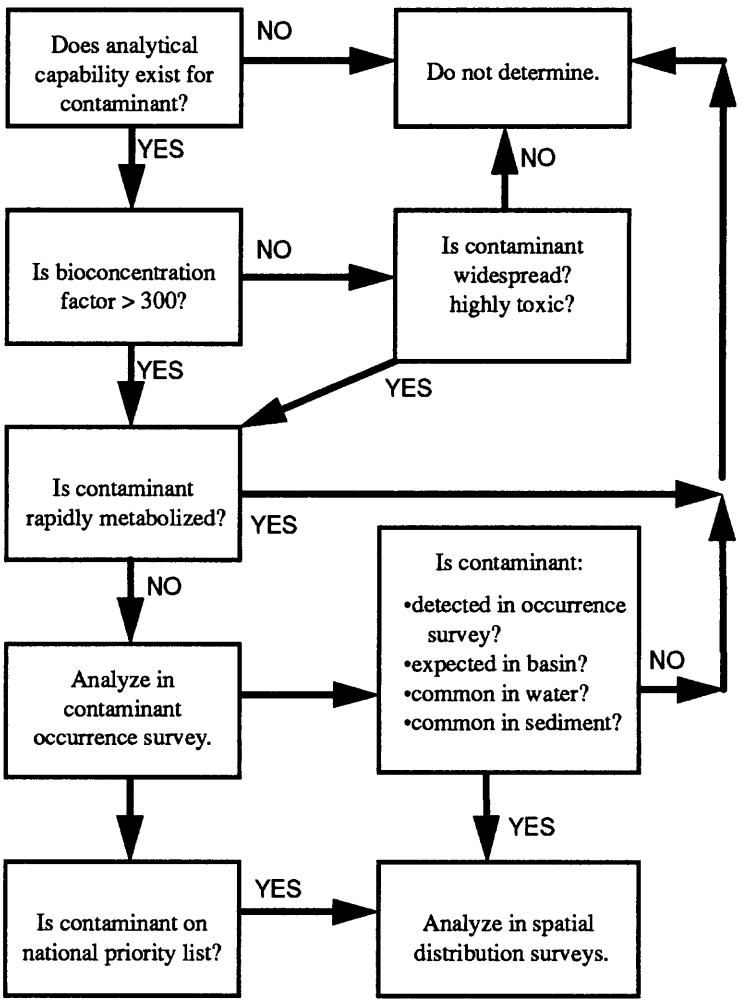

Figure 4.--Decision tree for selecting target synthetic organic compounds for analysis in tissues collected in the National Water-Quality Assessment Program.

Analysis of contaminants can be a major expense in a water-quality assessment program, especially organic contaminants. Six principles have been defined to guide a cost-effective choice of contaminant analyses in tissues for NAWQA. The process that guides that choice is outlined in figure 4 and the principles are listed below.

(1) Analytical methodology must be available to detect the contaminant at biologically meaningful concentrations.

(2) The compounds with highest priority for analysis are those with the greatest potential for bioconcentration (that is, those with the highest hydrophobicity or octanol:water partition coefficient).

(3) Targeted compounds will be toxic to biota or to humans.

(4) Biological factors will be considered in the choice of contaminants for analysis and the methods of analysis. Compounds will not be determined in taxa that readily metabolize them although the metabolic products are candidates for analysis. All tissue analyses of organic contaminants will be accompanied by lipid analyses.

(5) Samples collected from the first year contaminant occurrence sampling will be analyzed for a wide suite of compounds including all those on the national target list. Analyses in the following years of spatial distribution, trends, and low-intensity sampling will be limited to compounds shown important in either the contaminant occurrence tissue sampling, the sediment/water sampling, or prior studies in the basin.

(6) Organic compounds chosen for analysis in tissue samples in the spatial distribution and trends sampling should be a subset of those chosen for study in sediments within each basin, so the data are complementary. Compounds that are important in the programs of other agencies also will be given strong consideration.

Analytical Capabilities

Assurance of adequate methods and careful attention to the precision and accuracy of those methods are essential. Interlaboratory comparisons in other programs have demonstrated the reliability of available methods for analysis of many organochlorine pesticides, polychlorinated biphenyls, and some polynuclear aromatic hydrocarbons in tissues (Farrington and others, 1988). Useful determinations of other halogenated hydrocarbons also have been conducted in tissues (Pereira and others, 1988). The availability of reliable, sensitive methods may be the ultimate limiting step for study of many new organic compounds in tissues. If such methods are not available, a compound will not be determined in NAWQA. However, on-going development of such analytical methods will part of research conducted complementary to the NAWQA program.

Another analytical consideration is that limits of detection must be low enough to find biologically meaningful concentrations in tissues. Limits of analytical detection are a function of the tissue mass included in a sample, the ultimate volume into which that mass is dissolved, the extraction efficiency of the solvent, and the detection capabilities of the instrumentation. Therefore, field collections will have as much influence on whether a contaminant is detectable as laboratory processing. An important aspect of interpreting occurrence data is determining whether sample mass has been adequate to achieve desired detection limits. As general guidance, meaningful limits of detection should be 5 µg/kg for polynuclear aromatic hydrocarbons, 5 µg/kg for chlorinated pesticides, 1.0 µg/kg for polychlorinated biphenyl congeners, and 1.0 µg/kg for chlorinated dioxins and furans. Methods used for analysis of mercury should be capable of detecting the compound at 0.1 µg/g. Another trace element that is often difficult to detect is cadmium. In general, if sample mass for inductively coupled plasma (ICP) analysis is adequate to detect cadmium at 0.1 µg/g, other trace elements also will be detectable. A sample mass of 5 g wet weight is adequate if all trace elements are to be analyzed. One-half this mass can be employed if only ICP or only arsenic, selenium, and mercury are to be analyzed (for example, in spatial distribution study).

Bioconcentration

The concentration of an organic compound that may accumulate in an organism is determined by the physical and chemical properties of the compound in question, its concentration in the environment, and metabolism, excretion, and other physiological rates in the organism. An important characteristic is the compound's hydrophobicity--how strongly it is repelled by water. This characteristic typically is measured by the *n*-octanol:water partition coefficient (K_{ow}) which measures the extent a compound dissolves in the solvent *n*-octanol, compared to the extent the compound dissolves in water (Chiou and others, 1977; Moriarty, 1983). In general, the higher the n-octanol:water partition coefficient, the more hydrophobic the compound, and the more likely it is to partition into aquatic organisms. (The octanol:water partition coefficient is a good predictor of accumulation potential up to a certain point; chemicals with high coefficients ($\log K_{ow} > 6$) tend not to accumulate because their large molecular size limits absorption.) Thus, the hydrophobic compounds with bioconcentration factors greater than 300 will be the focus of organic analyses for tissue studies in NAWQA.

Other Biological Factors

Biota have varying capabilities for metabolizing contaminants; therefore, consideration of these capabilities is a prerequisite for selecting target organic compounds for analysis. Some organic compounds are not bioaccumulated because they are rapidly metabolized. Chemicals will not be targeted if rapid (within a month's time) metabolism occurs in a species, or if analytical methodology is not available for determination of metabolites. For example, fish have readily inducible mixed function oxidase systems that oxidize polynuclear aromatic hydrocarbons (PAH's). Because it is well established that PAH's do not bioaccumulate in fish, analyses for these compounds will not be conducted in fish samples. On the other hand, invertebrates also possess such enzymes but are generally less efficient in oxidizing PAH's. Mollusks have been successfully employed in studies of such compounds elsewhere (Pearson and others, 1980; Hale, 1988; Pereira and others, 1988). Therefore, PAH's will be analyzed in mollusks in NAWQA. Fish tissues and invertebrate tissues both can be valuable in studies of refractory organics such as organochlorine pesticides, polychlorinated biphenyls and other halogenated hydrocarbons. Because of the relation between lipid content and concentrations of organic contaminants in tissues (Chiou, 1985), lipid analysis is essential on all samples. Similarly, animal size and age are important in interpreting data for some types of refractory organics and trace elements and these data will be collected for all fish in NAWQA tissue samples.

Target Compounds for Contaminant Occurrence

Organic Compounds

The NAWQA list of organic analytes will be similar to the analytes targeted by other programs. In the NS&T, analyses are conducted for polynuclear aromatic hydrocarbons (PAH's), chlorinated pesticides, and polychlorinated biphenyls (PCB's). In their NCBP, the FWS analyzes for chlorinated pesticides and industrial chemicals. The NSCRF list includes dioxins and furans because of their extreme toxicity. From these, a pattern emerges of measuring PAH's, PCB's, DDT and its derivatives, dioxins and furans, and an array of chlorinated hydrocarbon pesticides.

Methods used by the USEPA to select the list of target chemicals for the NSCRF are a starting point for selecting target organic compounds for tissue analysis in NAWQA. The approach was to assemble a list of candidate compounds on the basis of their bioaccumulation potential and their effects on human health. This resulted in a list of 403 compounds (U.S. Environmental Protection Agency, 1986). The list was narrowed by dropping all compounds having bioconcentration factors (BCF's) less than 300 because the projected human exposure from fish consumption for these compounds would be less than the projected exposure from drinking water. Of the remaining compounds, some were eliminated from consideration because they are quickly transformed in the environment. Others were removed because of potential analytical problems or because they were judged to pose no threat to humans.

The final NAWQA occurrence list (table 4) includes several chlorinated pesticides, total PCB's and several PCB congeners, 15 chlorinated dioxins and furans, and PAH's (invertebrates only). Desirable PCB congeners to be analyzed will include a combination of the most toxic ones and the ones most commonly found in environmental samples, based primarily on the works of McFarland and Clarke (1989), Duinker and others (1988), Jones (1988), Jones and others (1989), and Smith and others (1990). However, congener analysis will be determined by available methodology. A few chemicals (for example, heptachlor epoxide, hexachlorocyclohexanes (BHC), and oxychlordane) also are included on the NAWQA reconnaissance list because of their toxicity, history of widespread usage, or because they have been frequently analyzed in tissues collected in other programs, even though some have bioconcentration factors of less than 300. Some dioxins and furans are included because of their extreme toxicity. However, because of the high cost of their analysis, analyses for these compounds should be limited to selected stations in study units where USEPA or other dioxin studies either were not conducted or showed dioxin to be present.

Table 4.--Synthetic organic compounds targeted for analysis in tissues collected in the National Water-Quality Assessment Program and currently analyzed in existing tissue analysis programs of national scope

[USGS, U.S. Geological Survey; NOAA, National Oceanic and Atmospheric Administration; USEPA, U.S. Environmental Protection Agency; FWS, U.S. Fish and Wildlife Service; +, included in the program]

Chemical name	Program				
	National Water-Quality Assessment Program (USGS)	National Status and Trends Program (NOAA)	National Study of Chemical Residues in Fish (USEPA)	Environmental Monitoring and Assessment Program, Surface Waters Component (USEPA)	National Contaminant Biomonitoring Program (FWS)
Polynuclear aromatic hydrocarbons[1]					
Acenaphthene	+	+			
Acenaphthylene	+				
Anthracene	+	+			
Benz(a)anthracene	+	+			
Benzo(b)fluoranthene	+				
Benzo(k)fluoranthene	+				
Benzo(g,h,i)perylene	+				
Benzo(a)pyrene	+	+			
Benzo(e)pyrene		+			
Biphenyl		+	+		
Chrysene	+	+			
Dibenz(a,h)anthracene	+	+			
2, 6-Dimethylnaphthalene	+	+			
Fluoranthene	+	+			
Fluorene	+	+			
1-Methylnaphthalene		+			
2-Methylnaphthalene		+			
1-Methylphenanthrene		+			
Naphthalene	+	+			
Perylene		+			
Phenanthrene	+	+			
Pyrene	+	+			
Organochlorine insecticides					
Aldrin		+		+	+
Chlorbenzilate			+		
Chlordane, cis	+	+	+	+	+
Chlordane, trans	+		+		+
Chlorpyrifos			+		+
o, p'-DDD	+	+		+	+
p, p'-DDD	+	+		+	+
o, p'-DDE	+	+		+	+
p, p'-DDE	+	+	+	+	+
o, p'-DDT	+	+		+	
p, p'-DDT	+	+		+	
Dicofol (Kelthane)	+		+		+
Dieldrin	+	+	+	+	+
Diethylhexylphthalate (DEHP)					+

Table 4.--Synthetic organic compounds targeted for analysis in tissues collected in the National Water-Quality Assessment Program and currently analyzed in existing tissue analysis programs of national scope--Continued

[USGS, U.S. Geological Survey; NOAA, National Oceanic and Atmospheric Administration; USEPA, U.S. Environmental Protection Agency; FWS, U.S. Fish and Wildlife Service; +, included in the program]

Chemical name	Program				
	National Water-Quality Assessment Program (USGS)	National Status and Trends Program (NOAA)	National Study of Chemical Residues in Fish (USEPA)	Environmental Monitoring and Assessment Program, Surface Waters Component (USEPA)	National Contaminant Biomonitoring Program (FWS)
Organochlorine insecticides--Continued					
Diphenyldisulfide					+
Endrin	+		+	+	+
Heptachlor	+	+	+	+	+
Heptachlor epoxide	+	+	+	+	+
Hexachlorocyclohexane(HCH), alpha	+	+	+		+
Hexachlorocyclohexane(HCH), beta	+	+	+		+
Hexachlorocyclohexane(HCH), delta	+	+	+		+
Hexachlorocyclohexane(HCH), gamma (Lindane)	+	+	+	+	+
Hexachlorocyclopentadine	+				
Kepone (Chlordecone)	+		+		+
o, p'-Methoxychlor	+		+		+
p, p'-Methoxychlor	+		+		+
Mirex	+	+	+	+	+
n-alkanes					+
Nitrofen			+		+
Nonachlor, cis	+		+		+
Nonachlor, trans	+	+	+	+	+
Oxychlordane	+		+		+
Pentachloroanisole	+		+		+
Perthane	+		+		+
Toxaphene					+
Trichloronate	+				+
Herbicides					
Butachlor	+				
Isopropalin	+		+		+
Trifluralin	+		+		+
Fungicides					
Hexachlorobenzene (HCB)	+	+	+	+	+
Pentachloronitrobenzene (PCNB)	+		+		+
Miscellaneous industrial compounds					
Octachlorostyrene	+		+		+
Pentachlorobenzene	+		+		+
Pentachlorophenol (PCP)	+		+		+
1,2,4,5-Tetrachlorobenzene	+		+		+
1,2,3,4-Tetrachlorobenzene	+		+		+
1,2,3,5-Tetrachlorobenzene	+		+		+

Table 4.--Synthetic organic compounds targeted for analysis in tissues collected in the National Water-Quality Assessment Program and currently analyzed in existing tissue analysis programs of national scope--Continued

[USGS, U.S. Geological Survey; NOAA, National Oceanic and Atmospheric Administration; USEPA, U.S. Environmental Protection Agency; FWS, U.S. Fish and Wildlife Service; +, included in the program]

Chemical name	Program				
	National Water-Quality Assessment Program (USGS)	National Status and Trends Program (NOAA)	National Study of Chemical Residues in Fish (USEPA)	Environmental Monitoring and Assessment Program, Surface Waters Component (USEPA)	National Contaminant Biomonitoring Program (FWS)
Miscellaneous industrial compounds--Continued					
1,2,3-Trichlorobenzene			+		+
1,2,4-Trichlorobenzene	+		+		+
1,3,5-Trichlorobenzene	+		+		+
Triphenyl phosphate	+				+
Polychlorinated biphenyls					
Monochlorobiphenyls			+		
Dichlorobiphenyls		+	+		
Trichlorobiphenyls		+	+		
Tetrachlorobiphenyls		+	+		
Pentachlorobiphenyls		+	+		
Hexachlorobiphenyls		+	+		
Heptachlorobiphenyls		+	+		
Octachlorobiphenyls		+	+		
Nonachlorobiphenyls		+	+		
Decachlorobiphenyls			+		
Total PCBs	+		+		
PCB congeners (selected)	+			+	
Aroclor 1242					+
Aroclor 1248					+
Aroclor 1254					+
Aroclor 1260					+
Chlorinated Dioxins and Furans					
2,3,7,8-TCDD	+		+		
1,2,3,7,8-PeDD	+		+		
1,2,3,6,7,8-HxDD	+		+		
1,2,3,7,8,9-HxDD	+		+		
1,2,3,7,8-HxDD	+		+		
1,2,3,4,6,7,8-HpDD	+		+		
2,3,7,8-TCDF	+		+		
1,2,3,7,8-PeDF	+		+		
2,3,4,7,8-PeDF	+		+		
1,2,3,6,7,8-HxDF	+		+		
1,2,3,7,8,9-HxDF	+		+		
1,2,3,4,7,8-HxDF	+		+		
2,3,4,6,7,8-HxDF	+		+		
1,2,3,4,6,7,8-HpDF	+		+		
1,2,3,4,7,8,9-HpDF	+		+		

[1] Not targeted for analysis in vertebrates.

Trace Elements

Eighteen trace elements will be analyzed in tissues collected in NAWQA study basins during the chemical reconnaissance (table 5). These constituents include 16 of the 20 major metals and trace elements selected as target variables for the pilot phase of NAWQA (Hirsch and others, 1988, p. 18). Boron, iron, and manganese do not normally pose toxic threats to aquatic animals or humans, and are therefore not candidates for tissue analysis. However, iron and manganese can be used as normalization factors to account for the amount of other elements that were found in the organism as a result of sediment in the gut. Therefore, they are included in the target list. Fluoride has a low bioaccumulative potential (Wright, 1977), eliminating it as a candidate for analysis in tissues.

Many inorganic elements are regulated by aquatic organisms. Several of these are required in biochemical reactions or are required in enzymes, vitamins, or hemoglobin-like molecules. However, when the ability of an organism to metabolize these elements is overwhelmed, the element can act as a toxicant. Some are poorly bioaccumulated. For example, many trace elements that behave as cations (with the exception of mercury) are not bioaccumulated in fish muscle. Thus, trace element analyses will be conducted on fish livers, but not on fish muscle.

Table 5.--Major metals and trace elements targeted for analysis in tissues collected in the National Water-Quality Assessment Program and currently analyzed in existing tissue analysis programs of national scope

[USGS, U.S. Geological Survey; NOAA, National Oceanic and Atmospheric Administration; USEPA, U.S. Environmental Protection Agency; FWS, U.S. Fish and Wildlife Service; +, included in the program]

Major metals and trace elements	Program				
	National Water-Quality Assessment Program (USGS)	National Status and Trends Program (NOAA)	National Study of Chemical Residues in Fish (USEPA)	Environmental Monitoring and Assessment Program, Surface Waters Component (USEPA)	National Contaminant Biomonitoring Program (FWS)
Aluminum	+	+		+	
Antimony	+	+			
Arsenic	+	+		+	+
Barium	+				
Beryllium	+				
Cadmium	+	+		+	+
Chromium	+	+		+	
Copper	+	+		+	+
Iron	+	+		+	
Lead	+	+		+	+
Manganese	+	+			
Mercury	+	+	+	+	+
Molybdenum	+				
Nickel	+	+		+	
Selenium	+	+			+
Silicon		+			
Silver	+	+		+	
Thallium		+			
Tin		+		+	
Vanadium	+				
Zinc	+	+		+	+

Only one tissue sample is needed to determine all the elements in table 5. The laboratory will split the sample for the different analytical procedures. One part will be used exclusively for analysis of mercury by cold vapor atomic absorption spectrophotometry with hydride analysis and one part for inductively coupled plasma mass spectrometry and inductively coupled plasma emission analysis. Inorganic constituents may be added to the target list to address specific problems in individual study units. Trace elements targeted for analysis in tissues also should be analyzed in water and bottom sediments.

Targeted Chemical Analyses Following the Contaminant-Occurrence Survey

Chemicals may be eliminated from spatial distribution samples and trends samples if not found in the contaminant occurrence phase samples. Little cost savings will be realized unless an entire suite of elements (for example, those analyzed by ICP or those analyzed by hydride analysis, dioxins, or PCB's) is eliminated, however. Reducing the number of contaminants studied during the later phases of study raises a risk that an important contaminant will be missed in the basin. This risk is more than offset by the possibility of eliminating expensive "below detection" results that would take resources away from other aspects of NAWQA. If a cost savings can be realized and there is no reason to suspect that a compound is of interest in a particular study unit, then there will be no analysis for the compound in question during the more detailed studies. Of course, compounds should not be eliminated from consideration if no cost savings is realized. The results of the contaminant occurrence survey, results from water and sediment chemistry studies in the first year of the NAWQA cycle, and prior studies in the study unit will be employed to define the compounds selected for the more detailed sampling. However, all the compounds identified as important by other components of NAWQA are not necessarily appropriate for tissue analysis. For example, water samples will be analyzed for the more polar, hydrophilic compounds including the chlorophenoxy acids and triazine herbicides, organophosphorus, and carbamate insecticides, and in some cases, methylene-chloride-extractable and purgeable compounds. Analyses of most of these compounds in tissues is not necessary because many are readily metabolized or poorly bioconcentrated. Thus, each compound should be given careful scrutiny by the study unit team when targeting chemicals for each objective. While there needs to be consistency in the list of target compounds from year to year, the list should also be flexible. For example, a compound of concern should be added to the target list if its initial use in the basin occurs following establishment of the list. The best opportunity for modifying the target contaminant list will come in each NAWQA cycle when the results of the chemical reconnaissance are available.

Selection of Taxa for Analysis

Resident taxa will be collected for the NAWQA tissue bioassessment. The choice of taxa to be analyzed from each site will depend upon occurrence and abundance of resident taxa, whether the sample is to be analyzed for organics or trace elements, the objective being satisfied, and whether samples of comparable taxa are being collected elsewhere within the basin (or in the nation). The local study team will make the specific choices, within nationally consistent guidelines. The NATT list defines the taxa that local teams should target in most collection efforts. A decision tree establishes priorities within the list. Although the details of the NATT list may change as experience increases, it will provide a national tissue contaminant data base for a limited number of species widespread in freshwater environments. Focusing on species on the NATT list will help maximize the number of sites and basins across the nation that can be compared.

Bioassessments of contaminants in tissues can employ transplanted individuals of one species (Hayes and others, 1986), or targeted taxa resident at each sampling site (National Oceanic and Atmospheric Administration, 1989). The use of transplanted individuals involves moving individuals of the selected species from a common site to each site of interest, and holding those individuals over a pre-determined period of exposure. The most important advantage of this strategy is that all data are (in theory) directly comparable, because the same species is used everywhere. The strategy also has inherent disadvantages: deployment in a foreign environment may affect behavior of the organism and contaminant bio-accumulation; results are dependent upon short-term bioaccumulation kinetics rather than an integrated lifetime of exposure; the transplanted individuals may never reach tissue concentrations of contaminants similar to those found in local resident populations (Cain and Luoma, 1985), and transplantation studies

involve a risk that unwanted nonnative taxa will become established and be harmful to the resident taxa. The disadvantages from the use of transplanted taxa to simulate contaminant bioaccumulation are especially significant in freshwater environments because of the diversity of the environments that must be studied. Successful transplantation of a single species over the range of habitats included in NAWQA is highly improbable.

Given the limitations of transplanting organisms and because NAWQA is an assessment of actual conditions in the nation's freshwaters, resident taxa better fulfill the program's objectives than do deployed species. Only resident taxa will be employed in tissue biomonitoring in NAWQA.

The use of resident taxa in the study of contaminant bioaccumulation also has disadvantages. The same taxa will not be present in all environments because of the diversity of freshwater systems included in NAWQA. The availability of taxa for analysis may be additionally constrained in the most contaminated environments because of toxicity. Nevertheless, those taxa collected will represent the integrated lifetime exposure that is actually occurring in nature. Special design considerations will be included to aid comparability among environments. The simultaneous consideration of contaminants in water, sediment, and biota, and the option of conducting local intensive studies, should aid interpretations where biota are totally absent because of extreme contamination. Additional special studies may be warranted when all biota are absent from a site. Even when biota are present, but no target taxa are available, study-unit staff may choose to omit biological sampling for contaminants in tissues (see section on "Target Organisms for NAWQA Tissue Analysis," page 42).

Criteria for Choice of Taxa

Several authors (Butler and others, 1971; Haug and others, 1974; Phillips, 1976a, 1976b, 1980; Farrington and others, 1983; Bryan and others, 1985; Campbell and others, 1988) have outlined important characteristics for biological indicators that are employed in tissue analysis studies. A summary of these characteristics, with some modifications appropriate for NAWQA, is shown below.

(1) Concentrations of chemicals in the test organism should be responsive to environmental exposures.

(2) Uptake of contaminants by the test organism should be rapid relative to release or metabolism of the contaminants.

(3) Contaminants should be concentrated in the organism above the ambient concentrations in water.

(4) The organism should not be so highly sensitive as to be killed by low levels of the contaminant to be measured.

(5) Concentrations in the test organism should possess low variability within sites.

(6) The organism should be sufficiently sedentary to reflect contaminant concentrations in the study area.

(7) The organism should be abundant and widespread in the study area (and in the region for a national study such as NAWQA).

(8) The organism should be of reasonable size, to provide sufficient tissue for analysis.

(9) The organism should be sufficiently long-lived to integrate environmental exposures of at least several months.

(10) The organism should be easy to sample and hardy enough to survive in the laboratory, allowing excretion of material in the digestive tract before analysis (if desired) and permitting laboratory studies of pollutant uptake.

(11) Data employed in comparisons of tissue contaminant concentrations must be from the same species and the effects of size or age on the data must be recognized. Comparisons among species and size/age classes can be conducted if comparability is empirically demonstrated in the scientific literature.

These criteria were used as guidelines in evaluating groups of organisms as targets for NAWQA tissue analysis sampling. Fish, mollusks, insects, crayfish, and plants were evaluated to determine their suitability for use as target taxa in NAWQA. Because different organisms respond differently to trace elements and synthetic organic compounds, the discussion will specifically differentiate between the two classes of chemicals.

Selection of target taxa also needs to be reconciled with the different objectives of the tissue bioassessment. Whichever taxa are chosen for an analysis in a basin, that choice must be defensible on the basis of satisfying one or more of these objectives.

NAtional Target Taxa List

No single species can satisfy all the above objectives, given the diversity of freshwater environments among the NAWQA study units. Thus, a suite of species will be employed. A NATT list has been developed in order to achieve comparability among environments. Targeting taxa for collection will help focus the efforts of study teams in the field. It also will reduce analytical expenses by limiting the number of analyses conducted on organisms for which few comparable data are available. Through time, and from existing literature, a data base for each national target taxon should develop showing the range of contaminant concentrations characteristic of each taxon in a variety of contaminated and uncontaminated settings. As the data base grows, the severity of contamination in any single analysis of a NATT can then be evaluated relative to other observations for that taxon. Relative bioavailable contamination can then be assessed in each NAWQA basin (or at each sampling site) compared to other basins (or other sampling sites) with a similar NATT.

Taxa from the NATT list must be given first priority when study teams select which species will be collected at a NAWQA study site. Collection of national target taxa is especially critical to satisfy the goals of spatial distribution, across basin comparisons, characterizing reference sites, and long-term trend determinations within a study unit. If no NATT is sufficiently abundant in the basin, then other appropriate species can be employed to meet more local objectives (for example, comparisons of land use), but the loss of information of national utility must be recognized. The following discussion will suggest taxa for the NATT list, but the list is expected to grow or be modified as experience with NAWQA grows.

Mollusks

Mollusk tissues are widely used in water-quality assessments, especially in marine environments, estuaries and lakes (Phillips, 1980; Farrington and others, 1983; Bryan and others, 1985). Of the mollusks, the bivalves (Bivalvia) and snails (Gastropoda) have several characteristics that make them well suited for use in NAWQA. They have limited mobility and, once located, are easily captured. Many are large, providing adequate mass for analysis.

Snails are common in freshwater, but they have disadvantages that limit their use as NATT. Although a few authors have studied contaminant responses of freshwater snails; (Everard and Denny, 1984; Nebeker and others, 1986), no systematic study of their use in tissue bioassessment programs is available. This may be because their populations commonly are scattered and a high diversity of species appears to occur (the comparability of bioaccumulation among these species is unknown). Many species of freshwater snails also are small and cleanly extracting them from their shells for tissue analysis may be problematic in a routine assessment program. Until further experience is gained, snails will be low priority as a choice for collection in a study unit and for the NATT list.

Bivalve mollusks have been used extensively in tissue analysis studies for both trace elements and trace organic compounds in freshwaters (Bedford and others, 1968; Smith and Green, 1975; Rodgers and others, 1979; Adams and others, 1981; Graney and others, 1983). Because it is unlikely that adequate numbers of larger bivalves can be found in headwater streams and mid-order streams (Pennak, 1989), they will be most useful for tissue analysis in larger streams and rivers. Not all bivalves are suitable for inclusion in the NATT list. For example, fingernail clams (Sphaeriidae) are too small for efficient removal of soft tissues and analysis. Many species of freshwater mussels are apparently undergoing a decline in North America, with several species now on endangered or threatened species lists. This decline may point to the sensitivity of

mussels to contaminants, which could limit their availability in streams having poor water quality. Mussels of the family Unionidae (especially the genus *Anodonta*) have been employed in studies of contaminant bioaccumulation (Hemelraad and others, 1986; Riccardi and Ravera, 1989) and may be candidates for collection. However, because of the possibility of collecting species which are endangered or threatened, they are not considered good candidates for the NATT list. If unionids are targeted, the status of resident populations (that is, is the population depauperate or the species endangered?) should be considered by the study teams before mussels are collected. Species having viable populations but limited distributions within a basin should not be collected because (1) there would be few other samples of this species available for making comparisons essential to satisfying the objectives of NAWQA, and (2) they may represent relict populations. Also, because of the high chance of disturbing depauperate populations of bivalves, it is mandatory that the FWS be notified prior to sampling for bivalves. This notification should allow at least 90 days for the FWS to respond to the collection request.

A bivalve taxon that is thriving in North American waters and increasing its range is the introduced Asiatic clam *Corbicula fluminea*. Except in headwater streams, this species is very widespread and is as close as any North American species to being ubiquitous (McMahon, 1983). Turgeon and others (1988) recognize only one species in the genus, *C. fluminea* (Müller). Pennak (1989) equates this species with *C. manilensis* and *C. laena*, but also recognizes only one species. Previous studies describe methodologies for employing *Corbicula* in tissue bioassessments and interpreting the data collected (Graney and others, 1983; Foe and Knight, 1987; Johns and others, 1988; Luoma and others, 1990; Leland and Scudder, 1990). The wide distribution of *Corbicula*, and its prior use as an indicator, coupled with the inherent advantages of all mollusks, makes *Corbicula* a first priority candidate for collection and for the NATT list. *Corbicula* is a good choice for both trace element and synthetic organic compound analysis. Other bivalves have characteristics making them good choices for tissue bioaccumulation studies, but the danger of disturbing threatened or endangered species means they will not be targeted in NAWQA.

Fish

Fish are the most widely used organisms in tissue analysis programs, having been integral parts of the NS&T Program, the NCBP, the NSCRF, TVA's Valley-Wide Fish Tissue Study, and several state monitoring programs. The appeal for the use of fish in biomonitoring lies in their economic importance, their large size, and their position at the top of the trophic scheme of aquatic systems. They also are the primary source of food for important terrestrial animals such as fish-eating birds, and they provide a direct pathway for human exposure to contaminants.

Although fish are understandably popular monitoring organisms, they have certain drawbacks that must be recognized. Firstly, some species of fish are especially sensitive to contaminants, and thus may be absent in the streams where tissue bioassessments are most important (Nehring, 1976; Spehar and others, 1980). Rainbow trout (*Oncorhyncus mykiss*) is an example of an otherwise widely distributed species that is more sensitive to metals than other trout species and is commonly absent in metal-contaminated waters. Such species would be poor choices as a tissue biomonitor in a study unit.

Secondly, mobility of fish is a concern, although long-distance movement is not characteristic of all species. Anadromous and catadromous species would be unsuitable for collection in NAWQA because their tissue concentrations may not reflect conditions at the sampling site. Fish such as walleye (*Stizostedion vitreum vitreum*) travel up to tens of miles in the Mississippi River (Holzer and Von Ruden, [1984?]) and channel catfish (*Ictalurus punctatus*) may travel more than 100 miles in the Mississippi River (Hubley, 1963). Some suckers also may migrate large distances. Other species in these studies traveled smaller distances. Walleye migration occurred during the spawning season (early Spring). The migrations of other species also may be related to the spawning cycle or changes in stage of the life cycle. If fish tissues are used in water-quality assessments, sampling during the migratory cycle of the species (for example, the spawning season) should be avoided. Study teams also must recognize that many species will integrate contamination over large areas, and interpret their results from such fish accordingly. It is essential that the study team biologist be cognizant of the life history characteristics of the fish targeted for collection in a specific basin. Selection of species and interpretation of data must be guided by that knowledge.

Thirdly, concentrations of contaminants vary among different organs of fish. Concentration in the gills can be highly variable (Hughes and Flos, 1978; Saltes and Bailey, 1984). Polar substances (cadmium, copper, lead, zinc) concentrate strongly in the liver and kidney (Weatherly and others, 1980; Wachs, 1985) but are regulated to very low concentrations in the muscle (Bollingberg and Johansen, 1979; Wiener and Giesy, 1979). Less polar compounds, for example methyl mercury and some organics, are lipid soluble and are concentrated more readily in muscle tissue (Wachs, 1985) or other fatty tissues where lipids are present, including the gonads and the liver. Phillips (1980) noted that concentrations of organic compounds vary among organs and even within organs in association with variable lipid concentrations. Normalization of concentrations of low polarity compounds to lipid content is sometimes effective in aiding interpretations of differences between tissues, individual organisms, and species (Clark and others, 1988) but is not the only biological factor that affects bioaccumulation (Schmitt and others, 1990).

The mass of a whole fish is overwhelmingly dominated by muscle. Thus, whole fish will be insensitive monitors of polar trace elements, which concentrate in various organs, especially the liver. Given the above, NAWQA will analyze fish livers for trace elements. For assessing organic compounds in fish, various agencies use whole fish, fish fillets, or sections from fish fillets. The use of whole fish is a logical choice for those studies such as the NCBP, where the protection of birds eating these fish is one of the objectives. The use of fish fillets or edible parts of fish is logical where human health is an objective. The use of standardized sections from fish fillets also yields information valuable for assessing the human health question, and it avoids the uncertainties of defining exactly what constitutes an edible portion. That is, does an edible portion mean a fillet with skin on or skin off; belly flap included or not included; lateral line fat included or not included; epipleural ribs extracted or remaining with the filet? NAWQA will use composites of whole fish for evaluating organic contaminants in fish. This decision reflects the need for information relevant to the NAWQA objectives of contaminant occurrence and spatial distribution of contaminants, while minimizing the potential for contamination of the sample. This means two separate composite samples will be required to conduct all the analyses needed. One composite of whole fish will be employed for GC-analyzed trace organics and dioxin-furan analysis. A second composite of fish livers will be required for trace element analysis. A separate sample of fish fillets from game fish will be analyzed for the specific objective of assessing the potential for contamination to humans (table 1).

Fourthly, concentrations of contaminants vary with size and age of fish (Schmitt and others, 1983). Larger and older fish have higher levels of some contaminants in contaminated environments, where net bioaccumulation may occur over a lifetime. Therefore, in the NAWQA tissue analysis studies, composite samples of fish should be composed of similar sized individuals and length and weight for each fish in the composite noted on a field data sheet. This will allow future sampling at the same site to target similar sized organisms. If more than one composite is collected, each might represent a different size group so a range of sizes can be compared. Scale samples or spines also will be collected to provide age information to aid in data interpretation. In interpreting data, composites having similar sized individuals will provide the best comparisons. Collecting a size range at selected sites may aid comparison of locations where different sized individuals dominate.

Fifthly, many organisms exhibit seasonal changes in the concentrations of organic compounds in their tissues in response to seasonal physiological changes (for example, changes in lipid content). Seasonal physiological changes in fish occur related to the sexual cycle. The lipid content of fish increases prior to spawning as lipid-containing gametes multiply and grow. Increases in lipids are usually accompanied by a corresponding increase in concentrations of hydrophobic organic compounds. After spawning, lipid content, and hence concentrations of trace organic compounds, may sharply decrease. This factor also must be considered in interpretations. Tissue sampling in NAWQA will be limited to late summer and early fall, at low flow, to minimize seasonality in the data. As noted earlier, lipid analyses must be run on all samples analyzed for organics.

Finally, the difficulty of obtaining an adequate number of fish of the same species (or demonstrably comparable taxa) from a number of sites should not be underestimated. Optimally, eight individual fish (five individuals is a bare minimum) of comparable size must be analyzed for each suite of contaminants from each site. At some sites, finding eight fish (or even five fish) of the same species and of similar size will

be difficult. Study teams should be prepared in advance for this challenge. While collecting, it is advantageous to remember that samples of more species can be collected than analyzed. If uncertainty exists about which of two species to collect at a site, both might be collected and frozen. Then the final decision about which to analyze can be made after all stations have been sampled.

Selection of fish species for the NATT list must be done with recognition of the diversity of fish species that occur in North American streams. Hocutt and Wiley (1986) provide an overview of fish distribution and abundance across the continent. Also, collections for NCBP samples give an indication of the availability of different species in larger rivers (Schmitt and others, 1981; May and McKinney, 1981; Schmitt and others, 1985; Schmitt and others, 1990). The fish that have been collected with the highest frequency in this study were carp, white sucker, largemouth bass, channel catfish, largescale sucker, and yellow perch. Thus, if the NATT list for NAWQA begins with these six fish species, successful sampling could be expected at the vast majority of larger river stations. No comparable data set is available from smaller rivers, but limited experience suggests adding brown trout, brook trout and longnose sucker to this list could include many smaller streams. Fish diversity and abundance decreases with decreasing stream order (Karr and others, 1986), adding to the challenge of collecting comparable taxa in these small-stream environments.

The fish taxa suggested for the NATT list include predators and several bottom feeding species. Carp (*Cyprinus carpio*), white sucker (*Catostomus commersoni*) and channel catfish (*Ictalurus punctatus*) are widespread bottom-feeders and commonly are employed in contaminant surveys and studies (for example, McFarlane and Franzin, 1980; Schmitt and others, 1983; Munkittrick and Dixon, 1988; Rada and others, 1986). All predators on the list also have been included in prior contaminant studies (Abernathy and Cumbie, 1977; Wiener and Giesy, 1979; Gillespie and Baumann, 1986; Bendell-Young and others, 1986; Johnson, 1987; Hamilton and others, 1987; are examples). Largemouth bass (*Micropterus salmoides*) and bluegill (*Lepomis macrochirus*), are widely sought-after sport fish whose range extends through much of the continental United States. Trout are the most commonly sought-after game fish in coldwaters, and they monopolize the list of species studied from coldwater environments. Rainbow trout (*Oncorhyncus mykiss*) are widely studied, but because of their sensitivity to contaminants, may not be a good choice for the NATT list. Brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) are widespread even in smaller streams and are relatively tolerant to contaminants. Brook trout move only short distances during the spawning season, but brown trout may migrate large distances. Both brook trout and brown trout are fall spawners and should be collected before spawning migrations begin, if they are chosen.

The priority for collecting NATT fish in a NAWQA basin is determined by the expected national abundance and the extent of prior use of the taxa in contaminant studies. First priority is given to five bottom-dwelling species that are spread through a variety of environments: carp, white sucker, longnose sucker, largescale sucker, and channel catfish (table 6). If adequate numbers of the bottom dwellers cannot be collected, then second priority is given to predators: largemouth bass, bluegill, brook trout and brown trout. Third priority is given to a species that is widespread, but not as desirable as a target taxon in contaminant studies: rainbow trout.

Modification of the NATT list for fish species will occur with time. Most objectives of tissue analysis in NAWQA could be met by efforts focused on one or more of the nine species discussed above (table 6). Confining efforts to these taxa will reduce analysis expense and collection effort that would have little national or regional comparability.

The objective of identifying contaminants in game fish flesh will be met by targeting game fish for sampling. Game fish on the NATT list should be top priority for this objective, although locally important game fish are also acceptable.

Aquatic insects

Aquatic insect larvae appear to have several advantages for use in tissue analysis, especially in smaller cobble bottom streams where suitable mollusks might be rare. Insect larvae are relatively easy to collect, they can be abundant, most move only short distances, and some live long enough and are large enough to be suitable for a tissue analysis program. Prior studies show that they accumulate metals in proportion to

Table 6.–NAtional Target Taxa list for tissue bioassessment in the National Water-Quality Assessment Program

Group	Taxon
Mollusks	
	Corbicula fluminea (Asiatic clam)
Insect larvae	
	Trichoptera (Caddisflies)
	Hydropsyche sp.
	Brachycentrus sp.
	Limnephilus sp.
	Chironomidae (Midges)
	Chironomus sp.
	Plecoptera (stoneflies)
	Perlidae
	Perlodidae
	Pteronarcyidae
Fish	
	Carp (*Cyprinus carpio*)
	White sucker (*Catostomus commersoni*)
	Longnose sucker (*Catostomus catostomus*)
	Largescale sucker (*Catostomus macrocheilus*)
	Channel catfish (*Ictalurus punctatus*)
	Largemouth bass (*Micropterus salmoides*)
	Bluegill (*Lepomis macrochirus*)
	Brook trout (*Salvelinus fontinalis*)
	Brown trout (*Salmo trutta*)
Vascular plants	
	Pondweed (*Potamogeton* sp.)
	Hydrilla (*Hydrilla verticillata*)
	Waterweed (*Elodea* sp.)

concentrations existing in the environment (Nehring, 1976; Spehar and others, 1978; Shuman and others, 1977; Nehring and others, 1979; Colborn, 1982; Luoma and others, 1989). Few studies of trace organic contamination in aquatic insects have been found, and their small size will preclude most species from use in routine trace organic assessments (it is not practical to obtain enough mass for analysis). Therefore, aquatic insects will not be used for evaluations of organic contaminants in NAWQA.

Feeding habits and animal size influence metal concentrations in aquatic insects (Smock, 1983a; Smock, 1983b). Smock (1983a) found that larger aquatic insects had lower tissue concentrations of cobalt, chromium, iron, antimony, and scandium than smaller insects and attributed these differences to differences in surface area and surface adsorption. Cain and others (1989) found lower concentrations of arsenic in larger animals than in smaller animals in a range of species when the metal was precipitating from solution with iron oxides. However, in less geochemically active areas, organism size had less influence on metal concentrations in stoneflies and caddisflies (Cain and others, 1992). Mean animal weight in composites should be recorded for all insect samples and considered in interpretations.

Elwood and others (1976) determined that gut contents have a significant effect on the analysis of metals in small insects. A depuration period may be necessary to allow the undigested material to pass through the gut prior to analysis, although it can be argued that predators eat the gut content of these organisms as well as the body tissue. A short depuration period will be included as part of the NAWQA sample processing procedure.

Small sizes, short life cycles, taxonomic diversity, and sensitivity to trace contaminants limit the taxa of insect larvae that are suitable for the NAWQA tissue bioassessment program. For example, midges (Diptera, Chironomidae) and mayflies (Ephemeroptera) are some of the most abundant taxa in small streams. However, the number of species of both can be large, species distributions are complex, and life cycles may be completed within a few months. Furthermore, many free-living mayflies are highly sensitive to trace metals. Thus, most taxa from these two groups will be unsuitable choices for the NAWQA tissue bioassessment (although their presence or absence will be useful data for the NAWQA ecological surveys). However, a few taxa have been studied that appear ideal for the NAWQA NATT list. As experience grows, other suitable taxa may be found. Those that appear useful at present, include the following:

(1) Net-spinning and free-living caddisflies (Trichoptera) from the family Hydropsychidae and case-bearing caddisflies of the family Brachycentridae appear to be widespread in distribution (Bradt and Williams, 1990) and abundant in many cobble-bottom streams. Some species in these families live for a year or more in larval form and are present throughout the year. Separation of these taxa to genus seems adequate for most sampling (Cain and others, 1989); the genera *Hydropsyche* and *Brachycentrus* demonstrate sensitive responses to changes in bioavailable metal concentrations (Moore and others, 1991; Cain and others, 1992). These species also are relatively tolerant of metal contamination. Because of their widespread occurrence, tolerance to moderate contamination, relatively large size, and ability to produce dense populations, the *Hydropsyche* are amenable to easy sampling and are the primary target insects for NAWQA tissue studies. The closely related genera *Arctopsyche* and *Cheumatopsyche* have characteristics similar to *Hydropsyche* and are substitutes for *Hydropsyche* when the latter cannot be found (D.J. Cain, U.S. Geological Survey, oral commun., 1990).

(2) The large encased caddisfly *Limnephilus* also appears to be tolerant of contaminants and widespread in cobble and some soft-bottom environments (Cain and others, 1989; Moore and others, 1991).

(3) Individuals of the chironomid genus *Chironomus* are large, multivoltine, and common in soft-bottom environments (see metal studies of Krantzberg and Stokes, 1989; Krantzberg, 1989). This genus may prove to be a useful target for tissue sampling in some NAWQA study units. Some are found in streams enriched with sewage or other organic material and may be the only organisms available from these stressed environments.

(4) Large stoneflies of the families Perlidae (mostly predators), Perlodidae (predators), and Pteronarcyidae (mostly detritus feeders or shredders) may live 2 to 3 years in nymph form. A few taxa from each of these families appear to be common over a range of cobble-bottom streams. Several species also may be relatively tolerant of metal contamination (Luoma and others, 1989; Cain and others, 1992). Taxa within these families must be analyzed separately, at least to the genus level (Cain and others, 1992). The stoneflies should be considered of lower priority than the caddisflies because their distribution may be more patchy, they appear to be less tolerant of contamination, and they may regulate some metals (Luoma and others, 1989a).

Moore and others (1991) targeted six species from the above group for collection in the Blackfoot River. They composited individuals from each targeted taxon present at each site, and used replicate composites, replicate stations and repeated sampling from year-to-year to estimate variances for individual species. No one species was present at all stations sampled, but a consistent picture of contamination could be drawn when overall trends in the five species were compared. Where aquatic insects are employed, a multi-species strategy of this nature may be necessary to define spatial distributions or achieve within-basin comparisons. This approach is more complex than if a single cosmopolitan species were available; however, it has an added benefit of contributing information about food web interactions of contaminants.

Crayfish

Crayfish (decapod crustaceans) have some advantages for use in tissue bioassessment programs. They are large enough to provide adequate mass for analysis, numerous enough in many environments to provide adequate numbers for replication, and widespread enough to offer the opportunity for comparisons between environments. The group is taxonomically rather small, with only 6 genera and about 350 species known in the United States (Pennak, 1989). Crayfish also are economically important in some

environments and they are sufficiently abundant to be collected relatively easily by use of kicknets or kicknets in conjunction with electroshocking gear. In other environments, obtaining an adequate sample may require traps or other methods more difficult to implement. They are mobile, but at least some stream-dwelling crayfish have a home range of less than 30 meters (Pennak, 1989), so the chemical composition of an individual is likely to be indicative of the water quality at the site where it was collected. However, crayfish are scavengers, and in some situations, their diet may consist of material that did not originate in the stream. Other disadvantages of employing crayfish are much the same as those with fish.

Metal bioaccumulation is well studied in crayfish and related decapods (Luoma, 1976; Gillespie and others, 1977; Anderson and Brower, 1978; Thorp and others, 1979; Knowlton and others, 1983; Bryan and others, 1986). These studies show that decapods may regulate tissue concentrations of zinc and perhaps copper, but bioaccumulate nonessential metals in proportion to exposure. Concentrations of metals vary among tissues, with the highest levels occurring in the green gland, hepatopancreas, the alimentary tract and the exoskeleton. Except for mercury, concentrations in muscle tissue are relatively insensitive to metal exposures (Stinson and Eaton, 1983). Dissection of hepatopancreas from crayfish would probably be the best strategy for their use for metal analyses (analogous to use of fish livers). However, obtaining enough dissection tissue mass for analytical work would be very time consuming and would require many organisms to be collected. Therefore, the use of crayfish for trace element analysis in NAWQA is not proposed.

Decapods have been successfully employed to study a variety of organic contaminants distributions in marine and estuarine environments (McLeese and Metcalfe, 1980; Pearson and others, 1980; Hale, 1988; Pereira and others, 1988). Some metabolism of compounds such as PAH's occurs, but metabolic rates are not as great as in fish. Extensive studies of trace organic bioaccumulation specifically in crayfish in fresh water environments have not been found, and crayfish are not proposed as candidates for the NAWQA NATT list for synthetic organic compounds.

Aquatic plants

Whitton (1984) listed several advantages of aquatic plants for use as monitoring organisms:

- They are easily transported from one site to another (for on-site studies at different sites),
- They have the ability to concentrate trace elements in their biomass at levels three to four orders of magnitude greater than levels in the ambient environment, thereby increasing the sensitivity over analysis of water,
- They have the ability to give a time-integrated picture of contaminant concentrations,
- They reflect the bioavailable fraction as opposed to analysis of water, and
- They are easy to store and preserve.

These positive characteristics of plants for tissue analysis studies must be weighed against significant negative aspects.

Monospecific samples of phytoplankton are not practical to obtain and planktonic algae are, by definition, not stationary in flowing waters. These types of plants are not satisfactory for use in the NAWQA tissue bioassessment.

Attached periphytic algae are ubiquitous and sedentary; however, the taxa comprising the periphyton are highly variable. Furthermore, sediments accumulate in association with periphyton, and cannot readily be separated. This problem appears to be insurmountable (Newman and others, 1983; Newman and others, 1985) and means that these plants are inappropriate for NAWQA tissue sampling.

Attached macroalgae also are stationary. However, many macroalgae are abundant only in streams having substantial enrichment and most are too limited in distribution to serve as good test organisms for the nationwide assessment envisioned for NAWQA. As with periphyton, sediments become associated with the fronds of filamentous algae that are virtually impossible to separate from the algae.

Vascular aquatic plants appear to have all the advantages for tissue bioassessment listed above. The rooted forms are stationary; some species are widespread; and some of the most common species have been studied in tissue biomonitoring of metal contamination (Hutchinson, 1975; Campbell and others, 1988). Vascular plants have the advantage of being perennial, but some may be available only during a relatively short growing period. For most, the growth period coincides with low flow, the time period when most tissue bioassessments will be conducted in NAWQA.

The suitability of vascular plant taxa for use in tissue bioassessments is, in part, determined by their growth form. Vascular plants can have emergent or submerged leaves, and can have roots or be free floating. Rooted plants obtain at least trace elements from both bottom sediments and water (Mayes and others, 1977; Cushing and Thomas, 1980; Hart and others, 1983). Free-floating plants presumably obtain their metals from water and suspended sediment. Translocation of contaminants from one part of the plant to another occurs to varying degrees in all vascular plants; thus, some emergent plants can be as indicative of water quality as submersed species (Heisey and Damman, 1982). However, some elements are very poorly translocated from roots to leaves, and concentrations of some elements are fairly well controlled in plant tissues.

If vascular plants are employed in the tissue analysis program, it is advisable to compare contaminant concentrations only in a particular structure. Roots and shoots concentrate many sediment-associated contaminants more strongly than leaves and stems (Heisey and Damman, 1982; Brix and Lyngby, 1983). However, concentrations in structures below the water-sediment interface may be biased by precipitated iron oxides, and attached fine sediment grains with their associated contaminants. Leaves and stems are especially important in the cycling of contaminants, acting as a source of those contaminants to food webs and to the system as a whole during periods of decomposition. Therefore, leaves and stems are more representative indicators of bioavailable contaminants. Epiphytes and microorganisms on the surfaces of leaves and roots can bioaccumulate contaminants and should be removed prior to analysis (Hart and others, 1983). Thus, rooted aquatic plants will be a target for analysis of trace elements, and for NAWQA collections, leaves and stems from the apical 5 cm of the plant will be used for analysis.

Two additional factors must be considered if leaves from vascular plants are to be employed in space/time comparisons. Contaminant concentrations may differ as much as two fold (Brix and Lyngby, 1983) with the age or degree of senescence of the leaf. By collecting only the apical 5 cm of the plant, it is anticipated that older, senescent plant material will be avoided. Nevertheless, age differences between samples may affect comparisons, and should be considered in interpretations. Also, some seasonal variability in concentration may be driven by seasonal differences in growth rates (Heisey and Damman, 1982). Again, this could affect comparisons if not considered. These factors can be controlled in comparisons within a basin, but may affect interpretive comparisons among study units or temporal trend analyses.

Vascular plants may have advantages in assessing tissue concentrations of trace organics. Although studies are limited, it appears that plants routinely bioconcentrate organic compounds (Lockhart and others, 1983; Boyle, 1984). However, the relatively low lipid content of aquatic plants means their ability to bioconcentrate organic contaminants may be limited. Chemical clean-up in preparation for analysis of organic contaminants and separation of natural organic material from vascular plants may be much simpler than with animals (W.E. Pereira, U.S. Geological Survey, oral commun., 1991). The usefulness of plants in assessments of trace organics is presently a focus of research adjunct to NAWQA and they are not recommended for study of trace organics at this time.

From this discussion, it follows that planktonic algae, periphyton, and attached macroalgae are not acceptable as target organisms for NAWQA tissue studies. Rooted aquatic plants have important advantages over nonvascular forms and should be considered for the NAWQA NATT list for trace element studies. Several widespread species of vascular aquatic plants have been successfully employed in surveillance of metal contamination in freshwaters and will be used in NAWQA. However, given the low lipid content of plants, they are not to be used for organics surveillance in NAWQA.

Taxa successfully employed in previous studies of trace elements include species of the genus *Potamogeton* (submerged pondweeds)(for example, Cushing and Thomas, 1980; Heisey and Damman, 1982; Campbell and others, 1988); the emergents *Pontederia cordata* (pickerelweed) (Heisy and Damman, 1982) and *Typha latifolia* (cattails) (Evans and Giesey, 1980); and the floating species *Lemna perpusilla* (Duckweed) (Guthrie and Cherry, 1979) and *Eichhornia crassipes* (water hyacinth) (Scudder and Leland, 1988). Other possible candidates include submersed taxa such as *Hydrilla* or *Elodea*. The possibility of uptake from the atmosphere reduces the suitability of emergent species. Floating species are mobile and therefore, are not suitable. Therefore, the highest priority vascular plants for NAWQA trace element analyses are the submersed genera listed above (*Potamogeton, Hydrilla,* and *Elodea*) (table 6).

Target organisms for NAWQA tissue analysis

The preceding discussion indicates that no single organism is a perfect choice for tissue analysis sampling. Bryan and others (1985) arrived at the same conclusion in their review of the use of biological indicators for assessments of heavy-metal contamination. They state: "Any reasonable monitoring programme should therefore include the analysis of several species ... to try and assess different forms of contamination and determine the possibility of food-chain biomagnification." The approach of the use of more than one species for analysis will be retained in NAWQA tissue analysis studies, specifically in the sampling to meet the spatial distribution of contaminants objective.

The selection of taxa for NAWQA sampling will involve local flexibility of choice within a structured, nationally consistent procedure. The national consistency will be provided by the NATT list of recommended taxa (table 5) and decision trees (figs. 5 and 6) that will guide selection from that list. Judgmental decisions by the study team, as advised by the regional biology team (see discussion of ecological surveys in NAWQA), will ultimately be necessary in determining which specific taxa to collect at each site. These decisions will be guided by consideration of the nationally consistent objectives, understanding of the advantages and disadvantages of the different taxa in tissue bioassessment, and assessment of the local availability of taxa. Where no species from the NATT list are available, study teams should carefully consider choices of taxa so that comparisons can be made that at least satisfy local objectives. They also should report taxa that might be candidates for the national list.

The decision trees provide a systematic approach to selecting taxa for collection from the NATT list. The purpose of the NATT list and the decision trees is to force local decisions toward a few taxa and thus provide a maximum number of comparable stations across the nation. Ultimately, taxa higher in the decision tree should be collected at more stations across the nation than taxa lower in the tree. Nonetheless, over time, some comparability will develop for those taxa lower on the NATT decision tree. The number of taxa collected at each station will be determined by the study team. In most study units, collection and especially, analytical costs, will limit the choice. In some cases interpretations may benefit from increasing the number of taxa analyzed. In other cases, analysis of replicate composites of the same taxa may best meet the statistical requirements of some objectives. These judgments will be made most effectively if the specific objective being satisfied is clearly defined by the study team before each station is sampled.

Different decision trees will be used for trace elements and synthetic organic compounds because organisms concentrate these constituents differently. For synthetic organic compounds (fig. 5), the extensive experience in employing mollusks in tissue bioassessments and the ability of mollusks to meet the criteria for good biological indicators make mollusks the first priority for collection. The widely abundant Asiatic clam, *C. fluminea,* will be the mollusk of highest priority. Other mollusks, while ideal for evaluating contaminants, are not targeted in NAWQA because of the need to protect threatened and endangered species. If *C. fluminea* are not present at a site, then fish will be the second priority for organisms to collect. If no target taxa from these two choices is available, then no sample for organics analysis should be collected. This last step in the decision tree, the decision to omit collection of a sample from a site where suitable target organisms are not present, should be made with consideration of local objectives. Where a nontarget taxon is available, it may be desirable to collect a sample of this taxon. Certainly, some data from a site, even from a nontarget taxon, can yield valuable information. However, the sample should be taken with an understanding that little or no comparative use on a national scale can be made of the results from

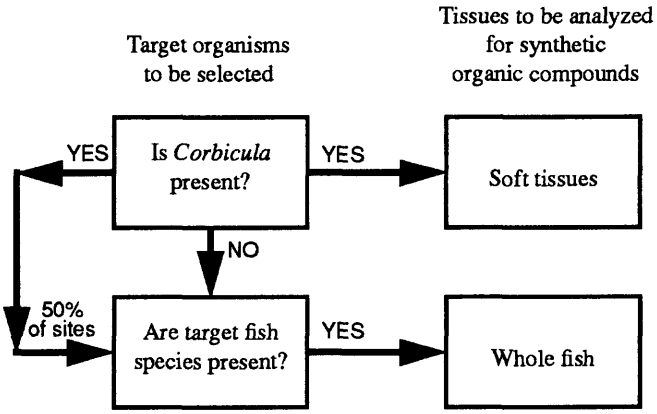

Figure 5.--Decision tree for selecting target organisms for analysis of synthetic organic compounds in tissues collected in the National Water-Quality Assessment Program.

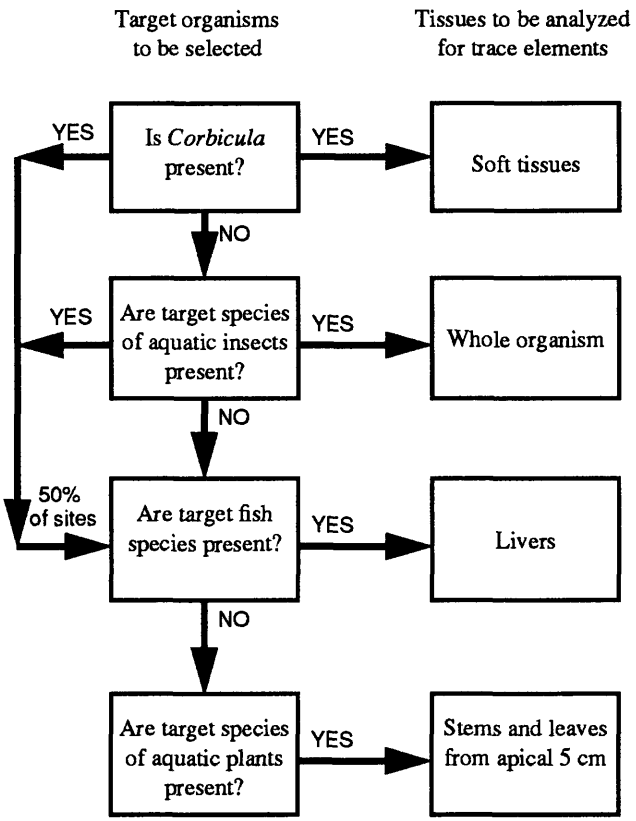

Figure 6.--Decision tree for selecting target organisms for analysis of trace elements in tissues collected in the National Water-Quality Assessment Program.

this sample. At approximately 50 percent of the sites, both a bivalve sample and a fish sample are to be collected during the spatial distribution survey. These sites should be key sites in the study unit, such as sites in a known contaminant gradient.

For trace elements (fig. 6), *Corbicula* is again the first priority target organism. *Corbicula* may not be abundant in cobble-bottom streams and in upstream reaches. Where a sufficient sample of *Corbicula* cannot be found, aquatic insects are the next choice for trace element analysis. The choice of taxa will be determined by considering the NATT list, by abundance at the specific site, and within comparable reaches of the watershed as a whole.

In environments where neither *Corbicula*, nor insect larvae are available in adequate numbers, trace element assessments in NAWQA will depend upon analysis of livers of fish from the NATT list. Every effort should be made to sample only one fish taxon in all samples collected throughout the study unit. Where one taxon is not distributed throughout the study unit, additional taxa should be sampled, with the goal of achieving study-unit-wide coverage with as few different taxa as possible. Interspecific comparisons of contaminants in fish are relatively undocumented in the scientific literature, so if more than one taxon is collected, collection of both taxa at stations where both are found is recommended.

Vascular plants will be selected for trace element analysis only if *Corbicula*, insects, and fish are not available, or in those local circumstances where knowledge of exposure of plants is of special interest.

Collection of both invertebrate and fish samples at 50 percent of stations within each study unit is suggested for the spatial distribution survey. This strategy will assure that fish data will be obtained from every study unit for comparisons with other programs and will help provide information of greatest interest to the general public.

The above taxa selection strategy will be used to guide the sampling in occurrence, trends, and spatial distribution of contaminants. The field reconnaissance and occurrence-sampling efforts will be critical times to assess distribution of taxonomic groups across the basin and develop the general choices for taxa selection to be employed during the following years of trends and spatial sampling. Prior to the contaminant-occurrence field sampling, an examination of historical data from the basin concerning the distribution of biota, and the field reconnaissance conducted in the first year of the ecological survey will provide valuable information on probable target species. As the trends and spatial sampling proceed, selection of taxa at each station should be consistent from year-to-year so trends can be assessed.

Optimal approaches for improving taxa selection, and providing protocols for interpretation efforts will continue to be studied in research adjunct to NAWQA as will effects on bioaccumulation of animal age, animal size, lipid content, taxonomic level, feeding type, gut content, life cycle stage, and river-specific geochemistry/hydrology.

Field Procedures for Collecting and Processing Tissue Samples

All USGS safety procedures are to be followed when collecting and processing samples for tissue analysis. In addition, safety should be a prime concern for any field activity. Collection of organisms will require appropriate collecting permits. The field crew chief will be responsible for obtaining the necessary permits from state agencies prior to sampling. Even though NAWQA will not target endangered species of bivalve mollusks, the requirement to notify the FWS prior to initiating sampling remains in effect because threatened or endangered mollusks occur in the targeted NAWQA watersheds.

Mollusks

Bivalve mollusks, including *Corbicula*, can be collected by any means practical, as long as the specimens are not injured or contaminated in the collection process. A clam rake, which resembles a garden rake with long tines and a basket attached, is frequently used and is effective in sandy bottomed locations. Another effective method in clear shallow water is to simply observe the animals in the water and pick them up by hand. Underwater viewing devices can be used to assist in spotting the organisms. A combination of raking to disturb the gravel or sand, then viewing can be effective. Divers, dredges, or grabs are sometimes needed

at deep water sampling sites, but the latter are inefficient because of their small sampling area. All collecting and processing will be accomplished while wearing disposable unpowdered vinyl, polyethylene, or latex gloves, in order to avoid possible contamination of the sample.

The ideal sampling scheme is to collect replicate composite samples from each station. Such an ideal situation would provide maximum confidence in the analytical results. However, the paucity of target organisms at some sites, the limited collection time available, and the high cost of analytical work will undoubtedly make that ideal unattainable for most study units. However, triplicate composite samples for the less expensive trace element analysis is a goal to shoot for. The tremendous expense of analyses for organic compounds dictates that one composite for each species targeted per sampling site is a realistic goal for organic samples.

Therefore, *Corbicula* samples from each station will ideally consist of 3 composites of 10 individuals each for trace element analyses and at least 1 composite of approximately 50 individuals for trace organics. Fifty *Corbicula* are needed for a sample for organic contaminants because typically, the soft tissues from one *Corbicula* weigh about a gram, and a minimum of 50 g wet weight of tissue is needed for analysis of organic compounds. One hundred grams is the optimal sample size. At least 5 g wet weight is needed for all the trace elements of interest to be analyzed with 10 g being optimal. Even if the appropriate mass of tissue is collected with only 2 or 3 individual organisms, 5 to 10 individuals are to be included in each sample to account for individual variability. All composited individuals should be of the same species, and each composite should be composed of similar sized individuals. The size of each individual in the composite must be noted on a field data sheet. Replicate samples should be collected from at least 10 percent of the sampling sites.

Once collected, the individual *Corbicula* will be rinsed with ambient stream water to remove any attached algae or debris. Following the recommendation of Uthe and Chou (1988), the organisms will then be held in stream water from the collection site at approximately 10 °C for a 24-hour depuration period. If held in a cooler, do not let water from melted ice contaminate holding water. *Corbicula* to be used for trace element analyses should be kept in a holding vessel of precleaned glass, polypropylene, or polyethylene. Those to be used for analysis of synthetic organic compounds should be kept in a holding vessel of precleaned glass or stainless steel.

Following the depuration period, the *Corbicula* shell lengths (greatest anterior-posterior dimension) will be measured to the nearest millimeter and the entire organisms, including the shells, from all the organisms in a composite placed in precleaned, glass jars, vials, or new plastic bags, and labeled. The sample is then placed inside a labeled zipper-seal type plastic bag, frozen using dry ice, and shipped frozen (packed in dry ice) to the laboratory for further processing and analysis. Shells from three to five additional organisms are to be dried and saved for voucher collections.

Fish

As with the other target organisms, collection of fish for tissue analysis need not be quantitative so any convenient method may be used with the caution that the contaminant content of the fish not be altered. For example, electroshocking or seining would be acceptable, as would hoop nets or other traps. Devices such as gill nets that injure or kill the captured fish and may result in deterioration of the specimen prior to removing the fish from the nets, should be avoided. However, gill nets may be an acceptable alternative provided they are checked frequently.

Each sample will consist of a composite of whole fish (for organic compounds) or livers (for trace elements) from adult individuals of the same species, all approximately the same size. Composite samples from five or more fish are acceptable, although every reasonable effort should be made by the field crew to collect eight fish for each composite. If fewer than five fish are collected for a composite, then that sample should <u>not</u> be sent to the laboratory for analysis. The reason for this is that "the confidence in the estimate of the mean concentration of contaminant in tissue increases as the number of individual samples in the composite increases" (U.S. Environmental Protection Agency, 1989b, p. 45). This relation has been quantified by Tetra Tech (1986). Their analysis shows that for an environmental sample having moderate

variability, 6 to 10 individuals per composite sample are needed to detect a difference between treatments (that is, between sampling locations) equal to 100 percent of the overall mean for all treatments (locations) (fig. 7). This analysis assumes five composite samples are collected per treatment, a level of sampling intensity that will not be achieved in NAWQA. Thus, if fewer than five individuals are collected for composite sample, the confidence associated with that sample would be so low as to reduce its practical utility. Therefore, NAWQA will strive for eight individuals contributing to each composite sample. Replicate samples should be collected from at least 10 percent of the sampling sites. Two or three additional fish of each sampled species should be collected as voucher specimens. (See the Voucher Collections and sample Archival section of this report.).

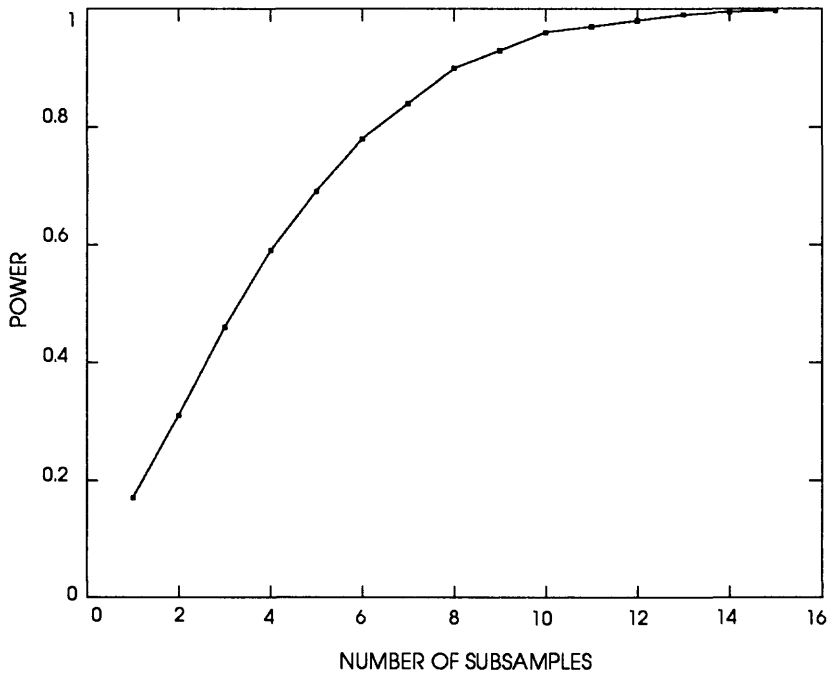

Figure 7.--Statistical power (probability of detecting a difference among means) compared to the number of subsamples in composite samples for samples containing moderate variability (Number of composites = 5; number of stations = 5; significance level = 0.05) (Modified from Tetra Tech, 1986).

Once collected, each individual fish should be sacrificed by a sharp blow to the base of the skull and rinsed in the field with native water to remove sediment, detritus, vegetation, or any foreign substance. After rinsing, each fish should be weighed to the nearest gram and measured for total length to the nearest millimeter and the data recorded on the field data sheet. Either a scale sample or a bony spine should be taken for age determination. For soft-rayed fishes, a scale sample of about 20 scales should be taken from the side of the fish, above the lateral line, and slightly anterior to the midpoint of the length of the fish. For spiny-rayed fishes, the scale sample should be taken from the area below the lateral line, near the tip of the oppressed pectoral fin. The scales should be placed in a scale envelope (a coin envelope) that is labeled with date and location of collection, the species, length, and weight of the fish, and the NAWQA sample identification number (explained later in this report). Some fish have no scales. Others, such as the suckers, have scales which are notoriously difficult to age. For these fish, a pectoral fin spine should be clipped from the fish and placed in the scale envelope. Age determinations are recommended, but are considered optional for organisms used for tissue analysis in NAWQA. Knowing the ages of organisms in a composite sample may help in interpreting contaminant concentrations.

The processing procedures differ depending on whether the sample will be used for trace elements or synthetic organic compounds. For trace elements (fig. 8), only the livers will be used in the analysis. While wearing vinyl gloves, first open the body cavity of each fish with a precleaned stainless steel scalpel and

blade or stainless steel scissors, with care taken to avoid touching the liver. Record the sex and maturity of each individual fish. By use of plastic or teflon-coated stainless steel forceps, expose the liver. Dissection of the liver is done by use of a second surgically cleaned stainless steel scalpel and stainless steel or plastic forceps that have been rinsed with high purity distilled water. Ceramic instruments may be substituted for stainless steel. It is important that a different set of instruments is used in dissecting the liver than the set used to open the body cavity. The exterior surface of the fish is assumed to be contaminated so instruments used to open the body cavity become contaminated in use. Caution is needed when excising the liver to avoid puncturing the gall bladder. The liver is then placed in the precleaned and tared glass sample container and covered with a teflon-lined cap while livers from the other specimens are dissected. Once all eight livers have been dissected, they are weighed and the weight is recorded. For the analysis of trace elements, at least 5 g of sample material is needed by the laboratory. Ten g is optimal. If eight livers in the composite do not provide the 5 g of mass needed, more livers will have to be added to the composite. The sample jar is labeled with the sample identification number, date, location, species of fish, and the analyses to be preformed, placed in a zipper-seal plastic bag which also is labeled, and frozen in the field with dry ice. The sample is shipped frozen to the laboratory.

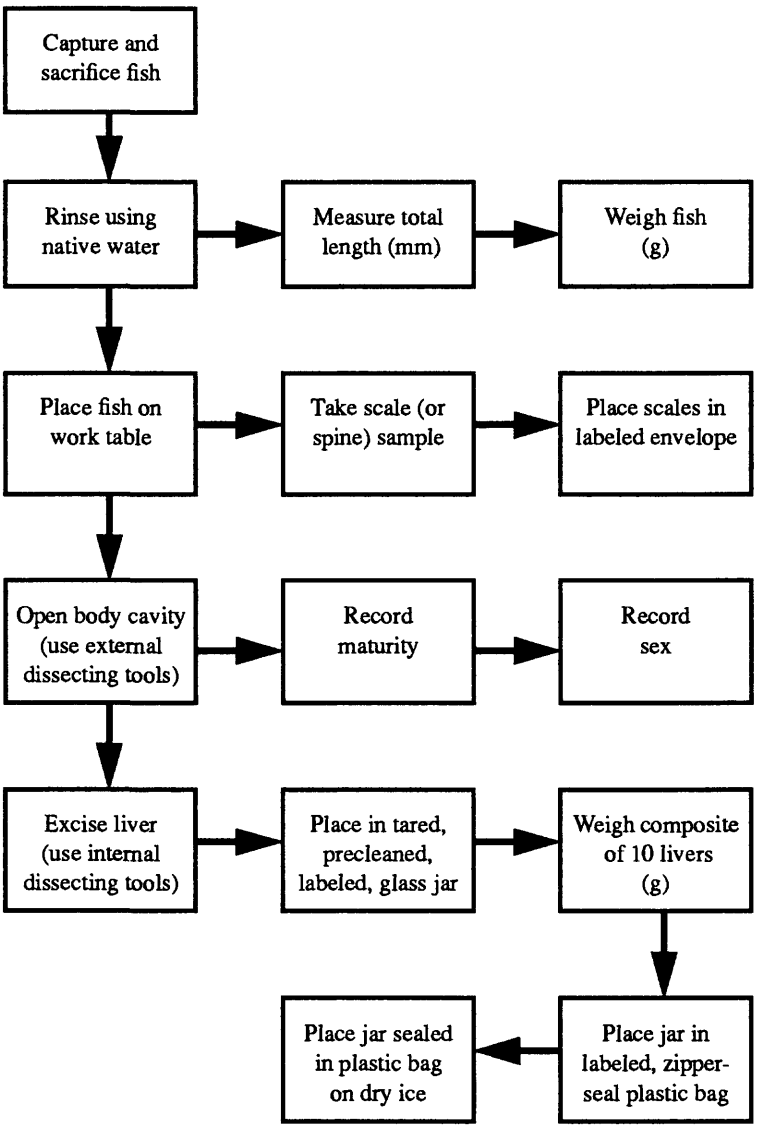

Figure 8.--Dissection procedure for excising liver from fish for trace-element analysis in the National Water-Quality Assessment Program.

For synthetic organic compounds, a composite of eight whole fish of one species is used as the sample. In the field, each fish is sacrificed, rinsed, weighed, and measured. A scale sample or spine sample is taken. The body cavity is opened to determine the sex of the fish. For large fish, each individual is wrapped in heavy-duty aluminum foil with the dull side in contact with the specimen. All foil-wrapped specimens are placed in a polyethylene bag along with an appropriate label for the sample. The bag also should be labeled so that there are eight individually-wrapped fish and one label inside the polyethylene bag and one label on the outside of the bag. Then the sample is placed in a second bag, to make sure individuals in the sample stay together. All eight wrapped and bagged specimens representing the composite sample from a site are field frozen on dry ice and held frozen at -20 °C or colder (U.S. Environmental Protection Agency, 1982) for shipment to the analytical laboratory. For small fish, all 10 fish can be wrapped in one aluminum foil packet, rather than having each specimen wrapped individually.

A composite sample of at least eight fish fillets is to be used for addressing the potential for human health effects. Fillets are to be collected from the left side of the fish. Schmitt and Finger (1987) have demonstrated that contamination of fish flesh samples is likely unless the most exacting clean dissection procedures are used, so extreme care is warranted.

Begin the dissection procedure (fig. 9) by sacrificing the fish. Weigh and measure each individual specimen, and take a scale sample. For fish having scales, the scales are removed and the fillet is submitted

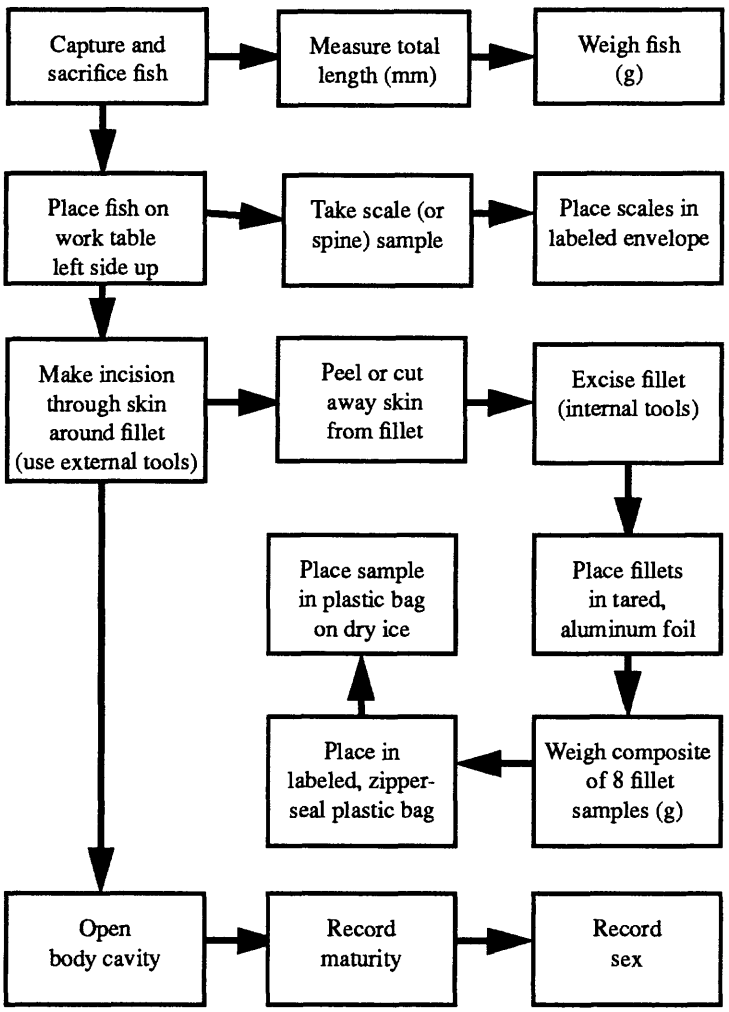

Figure 9.--Dissection procedure for excising fillets from game fish for synthetic organic compound analysis in the National Water-Quality Assessment Program.

for analysis with the skin on. For fish without scales (for example, catfish) the fillet is submitted with skin removed. In order to extract the fillet, first position the fish with its left side up on a precleaned dissecting board. Using precleaned stainless steel instruments, scale the fish. Alternatively, for scaleless fish, peel the skin back to expose the underlying muscle tissue. With a second set of precleaned stainless steel instruments, remove the fillet, including the belly flap, from the left side and wrap the fillet in aluminum foil. Repeat this process for all eight (or more) fish in the composite sample. If the specimens are small, or if a dioxin sample is required, an additional fillet may be collected by the same procedures on the right side of the fish. Again, the ideal sample mass needed for synthetic organic compound analyses is 100 g of tissue with 50 g being the minimum amount required. Label the aluminum foil-wrapped fillets and place them in a labeled, zipper-seal plastic bag. Again, double bagging is desirable. Freeze the sample with dry ice and hold at a temperature of -20 °C or colder (U.S. Environmental Protection Agency, 1982) for shipment to the laboratory for further processing and analysis.All equipment (weighing pans, measuring boards, buckets, and containers) that come in contact with the fish to be submitted for organics analyses should be made of stainless steel that has been cleaned with a laboratory detergent, followed by copious rinsing with tap water, then rinsed with methanol (distilled in glass (DIG) or pesticide grade (GR)), and allowed to air dry in a protected place. Field personnel are responsible for collecting hazardous-waste chemicals in approved containers and transporting these chemicals to the laboratory for proper disposal. Currently, no known source exists for supplying stainless steel measuring boards; therefore, plastic or wooden measuring boards, thoroughly cleaned with detergent, and distilled water, and rinsed with native water, will be substituted.

Aquatic Insects

Insects may be collected by any method practical so long as the organisms are captured alive and uninjured. For most conditions, a kick net will be the sampling device of choice. For rivers too deep to wade safely, a bottom sampler such as an Ekman dredge may be used. In some instances, divers may be needed for the collection of aquatic insects.

The insect sample from each station will consist of a composite of 20 or more individuals, all of one targeted taxon. Twenty or more individuals per composite should account for individual organism variability. However, because some insects are small, most composite samples will require many more individuals to provide the mass needed for analysis. At least 5 g wet weight of tissue is necessary if all trace elements are to be analyzed with 10 g wet weight considered optimal. This will require about 100-200 individuals for small organisms such as *Hydropsyche* or *Brachycentrus* caddisflies (removed from their case). Fewer individuals of the larger taxa will satisfy the mass requirement. Triplicate composite samples of each targeted taxon are ideal, where possible, and each composite should be composed of similar sized individuals. A few (6-10) additional individuals are to be collected and preserved for a voucher sample.

Once collected, insects should be removed from their cases (if any) and rinsed copiously with ambient stream water to remove attached particles. Then they will be held live in stream water from the site of collection in precleaned glass, polyethylene, or polypropylene containers in a laboratory setting (or in an ice chest in the field) at approximately 10 °C (± 2 °C) for a 4-6-hour purging period to allow ingested sediments to pass through the gut. Hare and others (1989) have shown that trace elements in the gut of mayfly nymphs can account for as much as 22 percent of the entire body burden of trace elements. Therefore, a period for purging the gut is warranted. Experience in the pilot phase of NAWQA has shown that holding the organisms in a labeled, plastic bag is an easy, efficient way to hold insects for this purging period. The animals are not to be fed during purging.

Following the purging period, the insects can be frozen on dry ice in the labelled, polyethylene, zipper-seal bags. Drain as much water as possible from the specimens before freezing. The sample vial or bag will then be placed in a second labeled zipper-seal plastic bag, and frozen with dry ice. Before shipping the samples for analysis, they must be cleaned of debris, taxonomically verified, and composited. This is done in the office laboratory after field collections are complete. Each sample should be thawed and the individuals quickly rinsed free of debris in uncontaminated, distilled water. Note the number of individuals in the composite, then weigh the composite sample and calculate the average weight per individual. Ship the refrozen sample in a clean bag or vial to the laboratory for analysis.

Aquatic Plants

Collection of rooted aquatic plants will be accomplished while wearing disposable vinyl gloves, by cutting or by handpicking the plant stems and leaves from the apical 5 cm of several individual plants. Stems and leaves should be washed thoroughly in ambient stream water, then soaked in filtered ambient stream water for 1 hour to minimize attached sediments, debris, algae, and organisms. The water should then be changed and the plant material soaked for 1 additional hour. The stems and leaves should then be frozen using dry ice in a pre-labeled, chemically cleaned glass jar or in a zipper seal bag and held in a frozen condition for shipment to the laboratory. A minimum of 5 g (wet weight) of plant material is needed for analysis of trace elements with 10 g being optimal. Replication is desirable where possible. Voucher specimens of submersed aquatic plants should be pressed in the field for later identification and cataloging.

Field Records

As each sample for tissue analysis is collected in the field, a unique identification number is assigned to the sample. The identification number will have a uniform format for all NAWQA tissue samples. It will be 16 characters long, composed of 6 fields (fig. 10). The first field will represent the study unit from which the sample is taken. It will be four characters long and will be alphanumeric. Codes for each study unit are listed in table 7. The second field will be a two-character numeric field representing the month in which the sample was collected. The third field is also a two-character numeric field. It represents the year of collection. The fourth field is a three-character alphanumeric field representing the sample type. The first character of this field is always a "T" representing a sample of tissues. The second character indicates the type of organism in the sample. This character will be a "C" if the sample consists of *C. fluminea*, an "F" if the sample consists of fish, an "I" if the sample consists of insects, or a "T" if the sample consists of plants. The third character will indicate the analysis type needed for the sample, "O" for organic compounds or "T" for trace elements. The fifth field is a four-digit numeric field consisting of a consecutive numbering of the tissue samples collected in the study unit. Finally, a one-character alphanumeric field indicates the container number for the sample. This is applicable where more than one container is needed to hold one entire sample. It will be used frequently in the ecological survey where more than one jar will typically be used for invertebrate samples. Tissue contaminant samples will normally be limited to only one container, so this character will be "A". For example, the nine thousand nine hundred and ninety-ninth sample from the Yakima River study unit which is composed of bridgelip suckers collected in November 1991 and is to be analyzed for organic compounds would be identified by the number "YAKI1191TFO9999A." Similar 16-digit codes will be used to identify invertebrate and algal samples collected for the ecological survey component of NAWQA.

A field data sheet (figs. 11-14) is required for each sample, containing a record of the sample identification number, location, date, time, type of organism collected, tissues collected, composite information, chemicals to be analyzed, and personnel in the field crew. For collections of fish, total length and standard length (in millimeters), weight (in grams), and sex of individual fish in composite samples also should be recorded on the field sheet along with any external anomalies. Anomalies are defined as the presence of externally visible skin or subcutaneous disorders or parasites (Ohio Environmental Protection Agency, 1987). The presence of external anomalies may indicate sublethal environmental stresses, behavioral stresses, or exposure to chemical contaminants. External anomalies include deformaties, eroded fins, lesions, tumors, diseases, and parasites. Each anomaly on each fish in the composite sample should be recorded on the field data sheet using the two-letter codes listed in table 8. The field data sheet for fish includes a column to record the age of individual fish. This is an optional measurement which may help in interpreting the contaminant data. Fish age cannot be determined in the field; but once it is determined, age should be added to the field data sheet so all pertinent ancillary data are available on this one data sheet. Length of bivalve mollusks shells (in millimeters) should be recorded on the field sheet for bivalve samples.

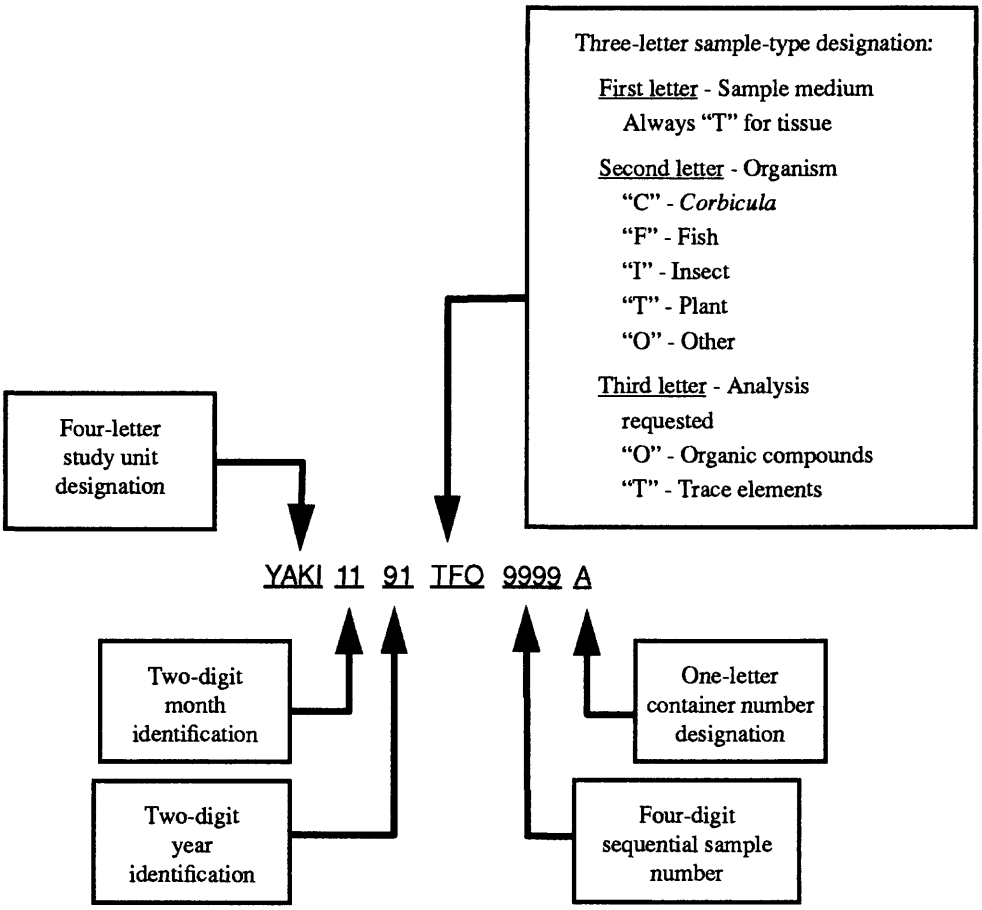

Figure 10.--Composition of unique identification numbers for tissue samples collected in the National Water-Quality Assessment Program.

Table 7.--Four-letter abbreviations for the National Water-Quality Assessment Program study units

ALBE	Albemarle-Pamlico Drainage	NHME	New Hampshire-Southern Maine Basins
ALGH	Allegheny and Monongahela Basins	NPLT	North Platte Basin
ACFB	Apalachicola-Chattahoochee-Flint River Basin	NROK	Northern Rockies Intermontane Basins
BALC	Balcones Fault Zone	NVBR	Nevada Basin and Range
CHEV	Chicot-Evangeline	OAHU	Oahu
CHEY	Cheyenne and Belle Fourche Basins	OZRK	Ozark Plateaus
CHPL	Central High Plains	POTO	Potomac Basin
CCPT	Central Columbia Plateau	PUGT	Puget Sound Drainages
CNBR	Central Nebraska Basins	REDN	Red River of the North
COKL	Central Oklahoma	RIOG	Rio Grande Valley
CONN	Connecticut Valley Drainage	SACR	Sacramento Basin
COOK	Cook Inlet Basin	SANA	Santa Ana Basin
DELR	Delaware Basin	SANJ	San Joaquin-Tulare
DLMV	Delmarva Peninsula	SANT	Santee Basin and Coastal Drainage
EIWA	Eastern Iowa Basins	SENE	Southeastern New England
GAFL	Georgia-Florida Coastal Plain	SHPL	Southern High Plains
GRSL	Great Salt Lake Basins	SOAZ	Southern Arizona
HDSN	Hudson Basin	SOFL	Southern Florida
KANA	Kanawha Basin	SPLT	South Platte Basin
KANS	Kansas River Basin	TRIN	Trinity River Basin
KNTY	Kentucky River Basin	UARK	Upper Arkansas River Basin
LERI	Lake Erie-Lake Saint Claire Drainage	UCOL	Upper Colorado Basin
LINJ	Long Island	UIRB	Upper Illinois River Basin
LIRB	Lower Illinois River Basin	USNK	Upper Snake River Basin
LSUS	Lower Susquehanna River Basin	UTEN	Upper Tennessee River Basin
LTEN	Lower Tennessee River Basin	WHIT	White River Basin
MIAM	Great and Little Miami River Basins	WILL	Willamette Basin
MISE	Mississippi Embayment	WMIC	Western Lake Michigan Drainage
MNSP	Minneapolis-St. Paul Basin	YAKI	Yakima River Basin
MOBL	Mobile River and Tributaries	YELL	Yellowstone Basin

Table 8.--Two-letter codes used to record external anomalies of fish

Code	Description
AA	No anomalies
DE	Deformities of the head, skeleton, fins, and other body parts
ER	Eroded fins
LE	Lesion, ulcers
TU	Tumors
AL	Anchor worms
BL	Black Spot
CL	Leeches
FU	Fungus
IC	Ich
NE	Blind - one or both eyes, includes missing and grown over eyes (does not include eyes missing due to popeye disease
PA	Other external parasites (not previously specified)
PE	Popeye disease

NATIONAL WATER-QUALITY ASSESSMENT (NAWQA) PROGRAM

FIELD DATA SHEET FOR CONTAMINANTS IN MOLLUSKS

16-digit NAWQA Sample ID Number: _____

Sample source:
(stream name and location) _____

USGS downstream-order station number: _____

Latitude: _____

Longitude: _____

NAWQA study unit name: _____

Date of collection: _____ (mo/day/yr)

Time of collection: _____ (24-hr clock)

Time gut-purging began: _____ (mo/day/yr) _____ (time)

Time gut-purging ended: _____ (mo/day/yr) _____ (time)

Data on individuals in composite sample: Species: _____

Organism number	Total length (in mm)	Organism number	Total length (in mm)	Organism number	Total length (in mm)	Organism number	Total length (in mm)	Organism number	Total length (in mm)
1		11		21		31		41	
2		12		22		32		42	
3		13		23		33		43	
4		14		24		34		44	
5		15		25		35		45	
6		16		26		36		46	
7		17		27		37		47	
8		18		28		38		48	
9		19		29		39		49	
10		20		30		40		50	

Composite weight
Average weight

Total wt. (g)
- Tare wt. (g)
Sample wt. (g)

Note: All individuals in composite must be same taxon.

Field crew: _____

Tissues collected (circle one):
- Soft tissues only
- Whole organism (lab dissection)
- Other _____

Analyses requested (circle one):
- Organic compounds (Lab code 2100)
- Trace elements (Lab code 2200)
- Other _____

Figure 11.--Field data sheet to be completed for each mollusk sample collected in the National Water-Quality Assessment Program.

NATIONAL WATER-QUALITY ASSESSMENT (NAWQA) PROGRAM

FIELD DATA SHEET FOR CONTAMINANTS IN INSECTS

16-digit NAWQA Sample ID Number: _____

Sample source: _____
(stream name _____
and location) _____

USGS downstream-order station number: _____

Latitude: _____

Longitude: _____

NAWQA study unit name: _____

Date of collection: _____ (mo/day/yr)

Time of collection: _____ (24-hr clock)

Time gut-purging began: _____ (mo/day/yr) _____ (time)

Time gut-purging ended: _____ (mo/day/yr) _____ (time)

Species collected: _____

Number of organisms in sample: _____

Wet weight of organisms in composite sample (g): _____

Average weight of organisms in composite sample (g): _____

Note: All individuals in composite must be same taxon.

Field crew: _____

Tissues collected (circle one):

Whole organism

Other _____

Analyses requested (circle):

Trace elements (Lab code 2200)

Other _____

Figure 12.--Field data sheet to be completed for each insect sample collected in the National Water-Quality Assessment Program.

NATIONAL WATER-QUALITY ASSESSMENT (NAWQA) PROGRAM

FIELD DATA SHEET FOR CONTAMINANTS IN FISH

16-digit NAWQA Sample ID Number: _____

Sample source:
(stream name and location) _____

USGS downstream-order station number: _____ Latitude: _____

Longitude: _____

NAWQA study unit name: _____

Date of collection: _____ (mo/day/yr) Time of collection: _____ (24-hr clock)

Data on individuals in composite sample: Species: _____

Organism number	Standard length (in mm)	Total length (in mm)	Weight (in g)	Gender (m or f)	External anomalies (use code)	Age (in yrs)	Comments
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
	Composite weight Average weight					Total wt. (g) - Tare wt. (g) Sample wt. (g)	

Note: All individuals in composite must be same taxon.

Field crew: _____

Tissues collected (circle one):
- Whole organism
- Liver
- Fillet
- Other _____

Analyses requested (circle one):
- Organic compounds (Lab code 2100)
- Trace elements (Lab code 2200)
- Other _____

Scale sample collected? yes ___ no ___ Spine sample collected? yes ___ no ___

Figure 13.--Field data sheet to be completed for each fish sample collected in the National Water-Quality Assessment Program.

NATIONAL WATER-QUALITY ASSESSMENT (NAWQA) PROGRAM

FIELD DATA SHEET FOR CONTAMINANTS IN AQUATIC PLANTS

16-digit NAWQA Sample ID Number: _____

Sample source: _____
(stream name and location) _____
_____

USGS downstream-order station number: _____

Latitude: _____

Longitude: _____

NAWQA study unit name: _____

Date of collection: _____ (mo/day/yr)

Time of collection: _____ (24-hr clock)

Species collected: _____

Wet weight of organisms in composite sample (g): _____

Note: All individuals in composite must be same taxon.

Field crew: _____
_____

Tissues collected (circle one):

Apical 5 cm

Other _____

Analyses requested (circle one):

Trace elements (Lab code 2200)

Other _____

Figure 14.--Field data sheet to be completed for each aquatic plant sample collected in the National Water-Quality Assessment Program.

Laboratory Procedures for Analyzing Tissue Samples

Tissue samples will be analyzed by the USGS's Central Analytical Laboratory in Lakewood, Co. The samples are to be held at a temperature of -20 °C or below, until ready for analysis. Analyses should be conducted within 6 months of the time a sample was collected.

Trace Elements

At the lab, samples (composites of soft tissues in the case of mollusks; composites of whole organisms in the case of insects; composites of livers in the case of fish; composites of leaves and stems in the case of aquatic plants) will be dried, digested, and analyzed. Percent moisture will be calculated for each sample. Three separate analysis techniques are expected for trace element analysis; inductively coupled plasma emission spectrophotometry, inductively coupled plasma mass spectrometry, and cold vapor atomic absorption (for total mercury). Pre-concentration may be needed to achieve analysis at the prescribed detection levels for some elements analyzed by the inductively coupled plasma techniques.

Synthetic Organic Compounds

At the lab, all individuals in a composite sample will be ground together to form one homogeneous mass, from which subsamples will be taken as needed for analysis of target compounds and lipids. Procedures will resemble those used by NOAA for the NS&T program (MacLeod and others, 1985) as adapted by the National Water Quality Laboratory of the USGS. The process will involve homogenization; extraction by use of methylene chloride in a soxhlet apparatus; clean-up by use of gel permeation chromatography; fractionation by use of alumina/silica gel; and finally, analysis. Analysis will be by gas chromatography with two dissimilar capillary columns coupled with an electron capture detector. Confirmation of target compounds will be performed by gas chromatography/mass spectrometry for about 20 percent of the samples.

Quality Assurance and Quality Control

Data quality is critical to the success of the tissue analysis part of NAWQA. However, analysis of chemicals in tissues, particularly organic compounds, poses special problems in that the analytical methodology is long and involved, the compounds of interest must be extracted from a matrix of naturally occurring organic compounds that are part of the biological monitoring organism itself, and the potential for contamination during the collection, shipment, sample preparation, and storage is high.

Quality assurance and quality control (QA/QC) procedures will be strictly followed at all stages of the data collection process: sample collection, field preparation, sample shipping, laboratory sample processing, chemical analysis, and data storage. Strict attention must be paid to the possibility of contamination during all processing steps. Sample collection and field processing and shipping will be governed by the protocol already detailed in this document. However, an important part of the quality control process will involve training field personnel. The USGS will offer special training courses specifically geared for those who will be conducting biological studies as part of the NAWQA program. Routine quality assurance measures in the lab will be defined by lab protocols and will include analysis of reagent blanks, reference samples from NIST or other reference sample providers, spiked samples, duplicate samples, and interlaboratory comparisons. Results from blanks, duplicates, and spikes will be placed in the National Water Information System (NWIS) along with results from the actual samples. This will allow those interpreting the data to make an informed review of the analyses provided. Sample archival, as outlined in the next section, will be part of the QA/QC process.

Voucher Collections and Sample Archival

Voucher or reference collections are needed for verifying the identifications of organisms used for NAWQA tissue studies. These collections will be permanently maintained for use as needed in future NAWQA studies. Separate collections will be needed for each group of organisms used for tissue analysis such as mollusks, fish, insects, and plants.

A voucher collection of several individuals is required for each species used in the tissue analysis study in each study unit at each collection time. Where possible, the voucher should consist of individuals of various sizes and maturity, both female and male. These voucher specimens will be processed, labeled, and preserved according to procedures currently being developed. It is anticipated these procedures will be similar to those outlined by Haedrich (1983) for fish, by Klemm and others (1990) for invertebrates, and by Porter (1967) or Robertson (1980) for plants.

Consideration of several options for housing and maintaining voucher collections for NAWQA is currently underway. It is anticipated that collections made in connection with the tissue analysis component of NAWQA will be stored at the U.S. Geological Survey's biological QA/QC unit in Arvada, Co., which is currently in the planning stage.

Sample archival, or specimen banking, is important for any long-term biological assessment program and archival will be an integral part of the tissue analysis component of NAWQA. Archiving biological material will allow future analyses--

- for chemicals not presently thought to be important,
- for new chemicals,
- using improved analytical techniques, and
- to compare analytical methodologies employed at different times (Moriarty, 1983).

Most sample archival is handled by the collecting agency. While this allows more direct supervision of quality-control measures and more flexibility in future analysis of archived samples, it also adds a burden to the collecting agency for long-term maintenance of the samples and requires additional personnel to administer the archival program. The USGS does not currently have an agency-wide archival program for biological samples; however, development of an archival program within the USGS's National Water Quality Laboratory is underway.

Data Management

Data generated by the tissue-analysis studies of NAWQA will be stored in a widely accessible relational data base maintained by the USGS as part of its NWIS. These data will be available for use in other aspects of NAWQA and by other agencies or nongovernmental researchers. There is currently no existing, easily accessible, and fully documented data storage facility comparable to the WATSTORE of the USGS or STORET of USEPA that has been designed for storage of biological data of the kind to be generated by the tissue analysis component of NAWQA. However, by about October 1993, NWIS will contain such a facility, and this is where the NAWQA tissue-contaminant data will be stored.

The existing tissue analysis monitoring programs of other agencies have their own individual data storage procedures. Although the data from these programs are available upon request, procedures for obtaining the data are not publicly documented. Data generated from the NS&T Program are stored and managed in a microcomputer environment with widely available commercial software. The system is efficient and can be instituted rather easily by other agencies, and, although the data are available upon request, there is no formal process for data transfer. No published documents describing the system currently exist.

For the NCBP, data are manually transferred from the analytical instruments to an in-house minicomputer at the National Fisheries Contaminant Research Center and are then stored permanently on the mainframe computer at the University of Missouri, Columbia. Data are provided to other users upon request in any medium and any format desired. Again, although the data are available on request for legitimate uses, there is no published and widely distributed user documentation that tells how to access the data. Several alternatives are presently being pursued to make these data more generally accessible.

Data for the NSCRF are stored in a modified subsystem of USEPA's STORET data storage system. These data also are available for use by anyone but there has been no publication detailing what parameter codes are used or how to access the data.

There are currently two national storage systems being developed specifically to handle biological data, one in the USGS and one in the USEPA. The USGS's system is expected to include provisions for handling tissue residue data. It will be an integral part of the NWIS and will have published documentation and wide accessibility. However, an in-place, functioning facility is not expected until late 1993.

USEPA's biological data handling system currently under development is known as BIOS. It is a component of STORET and will eventually consist of three parts; the Field Survey File, the Toxicity Testing File, and the Tissue Residue File. Presently, only the Field Survey File is operational. Development of the other two parts of BIOS is currently under contract. In addition to storage in NWIS, NAWQA tissue analysis data will be stored in the BIOS system, once the system is fully operational.

The primary repository for NAWQA tissue data will eventually be the USGS's biological data storage system within NWIS. The data will be available to anyone and the retrieval system will be fully documented. Until that system is operational, the data will be placed on the USGS's existing Distributed Information System. Anyone wishing access to the data should contact the Office of Water Quality, 12201 Sunrise Valley Drive, Reston, VA 22092. The data also will be placed in STORET on a temporary basis until BIOS is fully operational.

Data Interpretation

The study teams and regional ecology teams will be responsible for initial interpretation of data produced by NAWQA's tissue bioassessment program. Broader interpretations will be addressed by National Synthesis Teams within the USGS. The data interpretation process should systematically progress through the objectives, employing statistics, graphics, and tables to answer questions inherent in those objectives. The following specific goals should be the minimum interpretation obtained from each data set.

Contaminant Occurrence

Data from occurrence, trends, and spatial sampling can be employed in defining which contaminants occur in a study unit. However, immediate interpretation of the data collected during the occurrence sampling must be completed in time to select contaminants, biota, and long-term stations in the second field season. The following questions, at a minimum, should be asked first of the occurrence-sampling data alone and later of all data collected during the 3 years of sampling.

(1) What are characteristic reference values (mean and confidence interval or median and range for non-normally distributed data) for each contaminant for each taxon in each ecological region of the basin?

(2) Which contaminants exceed reference values at the stations sampled?

(3) With what frequency does enrichment (values in excess of reference) of each contaminant occur?

(4) Which contaminants were found to be enriched in the basin in prior studies of biota, sediment and water or in the NAWQA studies of sediment and water? How do these compare to occurrence in tissue bioassessment phase of NAWQA?

(5) What are the most important contaminants to target for investigation within the basin?

(6) What groups of species are most appropriate to target for collection in further studies within the basin?

Spatial Distribution of Contaminants

The goal here is to illustrate distributions of contaminants found in the basin. In the early stages of the program, analyses may be limited to graphical displays with comparative statements being made without benefit of uncertainty estimates. As more comparable data become available, statistical tests (t-tests and analyses of variance for parametric data, Wilcoxon (also known as the Mann-Whitney test) and Kruskal-Wallis tests for nonparametric data) can be applied to quantify the confidence attributable to perceived

differences between concentrations at different sampling sites. Presence or absence and quantitative values have both been successfully employed to illustrate distributions. All values must be judged relative to reference stations. Important questions include:

(1) What is the distribution of a contaminant in each taxon within the basin? When all taxa are considered are consistencies evident in distribution of the contaminant?

(2) How do distributions in tissues of biota compare to distributions in water and sediment observed in other phases of NAWQA?

(3) Can contaminant anomalies be explained by specific human activities, water-quality data, natural processes?

(4) What is the general description of contaminant distribution in the basin (regional, sub-regional, small patches)? What is the evidence for this conclusion? How does this characterization compare to other study units where this contaminant is present?

(5) Do general differences in concentrations of specific contaminants occur between ecoregions in the study unit? Between areas of broadly different land use? Between smaller streams and larger (integrator) systems?

Long-Term Trends

The goal of this sampling in each cycle of NAWQA is to establish a systematic data base from which statistically significant trends are determined. The continuous time series data will require replication of samples collected each year and collection of comparable taxa in each year. Schmitt and others (1981) employed analysis of variance to determine differences in concentrations among different locations and different times in NCBP assessments of temporal variability. This approach included a two-by-two factorial design, with location and time as main effects variables and tissue concentrations of contaminants as the dependent variable. Such a design is appropriate for NAWQA, assuming the data meet the assumptions of having a normal distribution, equality of variances, and independence. If these assumptions are not met, nonparametric statistical tests such as the Kruskal-Wallis test or computing analyses of variance on the ranks of the data will be employed to distinguish differences among stations and times (Helsel, 1987). In each cycle the following questions should be asked of the data:

(1) What is the between year variance? Is it significantly different than the variance within years?

(2) What are the characteristic values for contaminant concentrations in water and sediments at the long-term sites (for example, annual mean concentrations and confidence interval at fixed stations)?

(3) Are significant differences evident between NAWQA cycles? Are trends similar at all stations within the study unit and within the region? If not, why not?

Comparison of Effects of Land Use

The field experiments designed to test influences of land use will require replication in control and treatment. Then questions about the statistical significance of the effects of the land use can be approached. Significance can be judged relative to upstream site(s) or relative to established reference values. Comparisons envisioned will involve tests of differences between sample means such as t-tests or the nonparametric equivalent, the Wilcoxon test. Comparisons of the effects of similar land uses in other basin studies also should be made.

Assessment of Fate and Bioavailability of Contaminants

Widespread collection of data on contaminants in tissues offers opportunities to assess the response of several taxa to contaminants in sediments and water and assess differences in bioaccumulation within or among species across a range of environments. This can be done by regression techniques with concentrations or by studying bioconcentration factors (concentrations in tissues of a taxon relative to those in sediments or water). Bioconcentration factors may be indicative of bioavailability changes among

environments. The following questions are appropriate to meeting this goal:

(1) Do contaminant concentrations in sediments (or water) correlate significantly with concentrations in individual taxa? If not, does the range of available data affect the correlation? Do the correlations improve if concentrations in sediments are normalized to organic carbon content of the sediments?

(2) Do the above correlations differ among taxa or among contaminants? Explain.

(3) Do concentrations in taxa collected from the same site correlate significantly?

(4) Do enrichment factors differ for different contaminants?

(5) Do enrichment factors in the tissues of individual taxa differ among environments within the basin? Are there systematic differences with ecoregion, geology, source of contamination, land use, and so forth?

(6) How do enrichment factors in this NAWQA basin compare with those from other basins?

Development of a Biological Data Base

The NATT list provides an opportunity to compare the level of contamination in taxa from one basin to concentrations observed in the same taxa elsewhere (both contaminated and uncontaminated situations). The results of each analysis from a basin should, therefore, be added to the nationwide data base for that taxa. Then questions such as the following can be asked:

(1) How severe is contamination at each site relative to the national average (or median) for the taxa in question?

(2) How severe is contamination in the study unit as a whole? Do data from a variety of taxa support this conclusion?

(3) How do reference concentrations from this study unit compare to other study units? Is widespread contamination indicated?

Assessment of Contaminants in Game Fish

After 2 years of study, areas within a basin can be identified where greatest contamination of sport taxa is likely. Assessments will involve comparisons of contaminant concentrations in edible tissue of game fish with state, national, and international standards.

Assessment of the Effects of Contaminants on Biota

Assessment of contaminant distributions and bioavailability can help focus questions about the effects of contamination on the biological communities within a basin. That is, the results from the tissue analysis studies can assist in interpreting results from the ecological surveys conducted in NAWQA study units. Some examples of appropriate questions include:

(1) Were sites chosen as references for the ecological survey relatively free of contamination (were they true references, chemically)?

(2) Did the taxa within a study unit change coincident with high levels of contamination in sediment, water, or resident biota? If so, how?

(3) At which locations might effects of contamination be expected based upon observations of contamination in biota? What might be appropriate contaminant-related questions about biological effects within this study unit?

(4) Were contaminant concentrations found in tissues that indicate a potential for toxicological effects in organisms, on the basis of threshold concentrations derived from previous studies?

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