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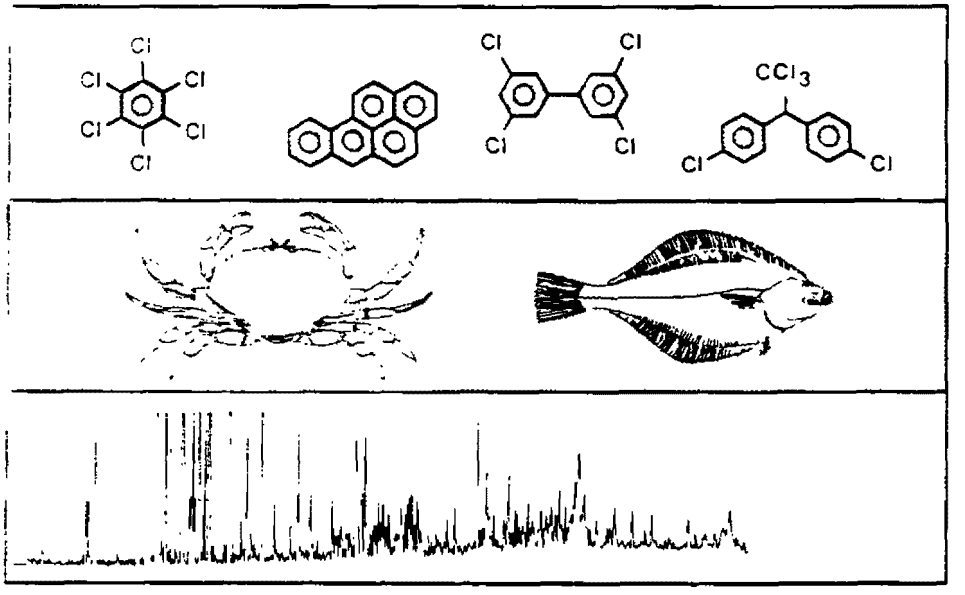
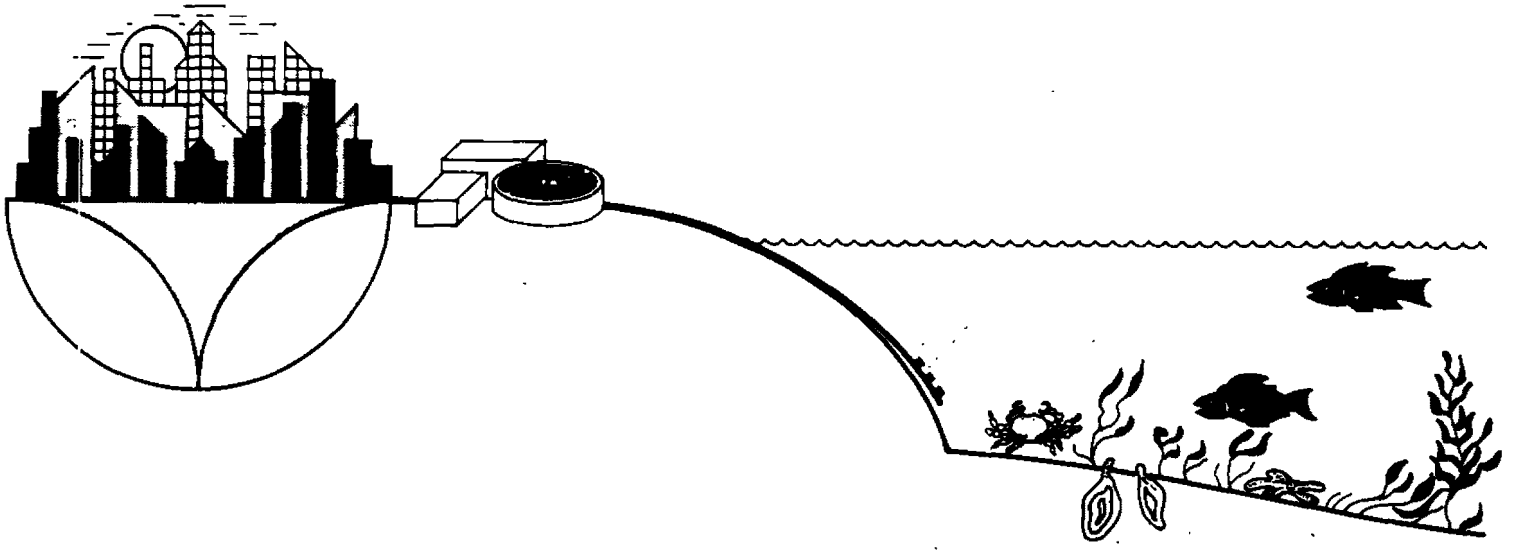
EPA

BIOACCUMULATION MONITORING GUIDANCE:

3. RECOMMENDED ANALYTICAL DETECTION LIMITS





BIOACCUMULATION MONITORING GUIDANCE:

RECOMMENDED ANALYTICAL DETECTION LIMITS

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PREFACE

This report is one element of the Bioaccumulation Monitoring Guidance Series. The purpose of this series is to provide guidance for monitoring of priority pollutant residues in tissues of resident marine organisms. These guidance documents were prepared for the 301(h) sewage discharge permit program under the U.S. EPA Office of Marine and Estuarine Protection, Marine Operations Division. Two kinds of monitoring guidance are provided in this series: recommendations for sampling and analysis designs, and aids for interpretation of monitoring data.

Some basic assumptions were made in developing the guidance presented in these documents: 1) each bioaccumulation monitoring program will be designed to meet the requirements of the 301(h) regulations, 2) tissue samples will be collected from appropriate locations near the sewage discharge and from an unpolluted reference site, 3) the initial chemicals of concern are the U.S. EPA priority pollutants and 301(h) pesticides, and 4) the monitoring data should be suitable for a meaningful evaluation of the potential hazards to living marine resources as well as human health. It should be recognized that the design of a monitoring program reflects the site-specific characteristics of the pollutant discharge and the receiving environment. Thus, site-specific considerations may lead to a modification of the generic recommendations herein. Finally, although these guidance documents were prepared specifically for monitoring of sewage discharges under the 301(h) program, their potential use extends to assessment and monitoring of bioaccumulation resulting from other kinds of pollutant discharges into marine and estuarine environments.

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ACKNOWLEDGMENTS

This document has been reviewed by the 301(h) Task Force of the Environmental Protection Agency, which includes representatives from the Water Management Divisions of U.S. EPA Regions I, II, III, IV, IX, and X; the Office of Research and Development - Environmental Research Laboratory - Narragansett (located in Narragansett, RI and Newport, OR), and the Marine Operations Division in the Office of Marine and Estuarine Protection, Office of Water.

This technical guidance document was produced for the U.S. Environmental Protection Agency under the 301(h) post-decision technical support contract No. 68-01-6938, Allison J. Duryee, Project Officer. This report was prepared by Tetra Tech, Inc., under the direction of Dr. Thomas C. Ginn. The primary authors were Ms. Ann C. Bailey, Mr. Robert C. Barrick, and Mr. Harry R. Beller. Ms. Marcy B. Brooks-McAuliffe performed technical editing and supervised report production.

RECOMMENDED ANALYTICAL DETECTION LIMITS

The accumulation of toxic substances in marine organisms that may lead to adverse biological effects or affect commercial or recreational fisheries is one of the major concerns in the 301(h) program related to evaluating the effects of sewage discharges into marine and estuarine waters. Evaluation of differences between body burdens in organisms from relatively uncontaminated reference areas and those from contaminated estuarine and marine environments potentially impacted by the discharge is an important part of bioaccumulation studies. Such comparisons will generally require data that are reliable at low part per billion concentrations. Therefore, low but practically attainable detection limits are a minimum requirement to ensure the usefulness of bioaccumulation monitoring data. This report reviews the factors that influence target pollutant detection limits and recommends minimum detection limits for bioaccumulation studies. Although this report is not designed to address specific analytical protocols, it serves as a companion document to the recommended analytical protocols in the Bioaccumulation Monitoring Guidance series.

Achieving low detection limits for all priority pollutants during bioaccumulation studies is difficult because a wide variety of techniques is required to achieve optimal detection of these numerous and chemically diverse compounds. The limited amount of tissue available for most samples and the need to detect and identify nanogram or picogram quantities of pollutants necessitates the use of sensitive instrumentation and complex analytical procedures.

Environmental analytical chemists have not universally agreed upon a convention for determining and reporting the lower detection limits of analytical procedures. Furthermore, the basis for detection limits reported in the literature is rarely given. Values reported as lower detection limits are commonly based on instrumental sensitivity, levels of blank contamination, and/or matrix interferences and have various levels of

statistical significance. The American Chemical Society's Committee on Environmental Improvement (CEI) defined the following types of detection limits in an effort to standardize the reporting procedures of environmental laboratories (Keith et al. 1983):

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

- Limit of Detection (LOD) -- the lowest concentration level that can be determined to be statistically different from the blank. The recommended value for LOD is 3σ, where σ is the standard deviation of the blank in replicate analyses.

- Limit of Quantitation (LOQ) -- the level above which quantitative results may be obtained with a specified degree of confidence. The recommended value for LOQ is 10σ, where σ is the standard deviation of blanks in replicate analyses.

- Method Detection Limit (MDL) -- the minimum concentration of a substance that can be identified, measured, and reported with 99 percent confidence that the analyte concentration is greater than zero. The MDL is determined from seven replicate analyses of a sample of a given matrix containing the analyte (Glaser et al. 1981).

The CEI recommended that results below 3σ should be reported as "not detected" (ND) and that the detection limit (or LOD) be given in parentheses. In addition, if the results are near the detection limit (3 to 10σ, which is the "region of less-certain quantitation"), the results should be reported as detections with the limit of detection given in parentheses.

The CEI definitions are useful for establishing a conceptual framework for detection limits, but are somewhat limited in a practical sense. The IDL does not address possible blank contaminants or matrix interferences and is not a good standard for complex environmental matrices, such as tissues. The LOD and LOQ account for blank contamination, but not for

matrix complexity and interferences. The high 10σ level specified for LOQ helps to preclude false positive findings, but may also necessitate the rejection of valid data. The MDL is the only operationally defined detection limit and provides a high statistical confidence level but, like the LOQ, may be too stringent and necessitate the rejection of valid data.

The detection limits recommended in this report are not strictly based on the CEI definitions. Instead, they are considered to be typically attainable values based on the best professional judgment and experience of analytical chemists who considered the instrumental sensitivity of affordable equipment, common problems with blank contamination and matrix interferences, and reasonable levels of laboratory analytical effort. The recommended values are not absolute, as analytical procedures and laboratory precision can affect attainable detection levels. The detection limits recommended herein fall between the IDL and MDL as defined by the CEI.

Several factors determine achievable detection limits for a specific priority pollutant, regardless of analytical procedure. The most important factors include

- Physical sample size available - In most cases, the more tissue available for analysis, the better the detection levels that can be achieved. Thus, for a given method, larger samples available for analysis will have lower detection limits than smaller samples.

- Presence of interfering substances - For example, because liver contains more salts than muscle, liver digestates may require matrix matching for trace metal analyses, while muscle digestates may not. Matrix matching may increase the detection limit.

- Range of pollutants to be analyzed - For example, if only one compound is of interest, a method can be optimized for that parameter without regard to potential effects on other parameters.

- Level of confirmation of results - For example, gas chromatography (GC) with electron capture detection (GC/ECD) is more sensitive than GC with mass spectrometry (GC/MS) for pesticide analysis. However, a single GC/ECD analysis does not provide positive identification of a compound, whereas GC/MS provides more information for molecular confirmation.

- Level of pollutant found in the field and in analytical blanks - For example, due to bottle preparation procedures, analytical blanks are often contaminated with varying concentrations of methylene chloride. This variation in contaminant level often precludes sensitive detection levels in tissue.

This review summarizes the detection levels generally achieved using methods commonly employed for tissue analysis in environmental laboratories. Because many of these levels are dependent on state-of-the-art technology, the detection levels can be expected to decrease as methods and instruments improve and become more commonly available.

For analytical purposes, the priority pollutant list of 126 chemicals can be divided into five categories: trace metals (13 parameters); volatile organic compounds (28 parameters); acid-extractable organic compounds (11 parameters); basic- and neutral-extractable organic compounds (47 parameters); and organochlorine pesticides (25 parameters). The organic pollutants included in each category are listed in Table 1. The remaining two priority pollutants, asbestos and cyanide, will not be discussed because significant bioaccumulation of these substances is not expected. Six additional pesticides are required for the 301(h) program (Table 1).

Procedures for chemical analysis of each analytical group consist of four sequential steps:

- Collection of organisms and preservation of tissue

- Physical preparation of tissue for analysis

TABLE 1. ORGANIC PRIORITY POLLUTANTS AND 301(h) PESTICIDES

Acid Compounds	Base/Neutral Compounds
2,4,6-trichlorophenol	acenaphthene
p-chloro-m-cresol	benzidine
2-chlorophenol	1,2,4-trichlorobenzene
2,4-dichlorophenol	hexachlorobenzene
2,4-dimethylphenol	hexachloroethane
2-nitrophenol	bis(2-chloroethyl)ether
4-nitrophenol	2-chloronaphthalene
2,4-dinitrophenol	1,2-dichlorobenzene
4,6-dinitro-2-methylphenol	1,3-dichlorobenzene
pentachlorophenol	1,4-dichlorobenzene
phenol	3,3'-dichlorobenzidine
	2,4-dinitrotoluene
Volatiles	2,6-dinitrotoluene
	1,2-diphenylhydrazine
acrolein	fluoranthene
acrylonitrile	4-chlorophenyl phenyl ether
benzene	4-bromophenyl phenyl ether
carbon tetrachloride	bis(2-chloroisopropyl)ether
chlorobenzene	bis(2-chloroethoxy)methane
1,2-dichloroethane	hexachlorobutadiene
1,1,1-trichloroethane	hexachlorocyclopentadiene
1,1-dichloroethane	isophorone
1,1,2-trichloroethane	naphthalene
1,1,2,2-tetrachloroethane	nitrobenzene
chloroethane	N-nitrosodiphenylamine
2-chlorethylvinyl ether	N-nitrosodimethylamine
chloroform	N-nitrosodi-n-propylamine
1,1'-dichloroethene	bis(2-ethylhexyl)phthalate
trans-1,2-dichloroethene	benzyl butyl phthalate
1,2-dichloropropane	di-n-butyl phthalate
cis- and trans-1,3-dichloropropene	di-n-octyl phthalate
ethylbenzene	diethyl phthalate
methylene chloride	dimethyl phthalate
chloromethane	benzo(a)anthracene
bromomethane	benzo(a)pyrene
bromoform	benzo(b)fluoranthene
bromodichloromethane	benzo(k)fluoranthene
chlorodibromomethane	chrysene
tetrachloroethene	acenaphthylene
toluene	anthracene
trichloroethene	benzo(ghi)perylene
vinyl chloride	fluorene
	phenanthrene
	dibenzo(a,h)anthracene
	indeno(1,2,3-cd)pyrene

Table 1. (Continued)

Base/Neutral Compounds (Continued)	301(h) Pesticides
pyrene	Malathion
2,3,7,8-tetrachlorodibenzo-p-dioxin	Parathion
	Guthion
Pesticides	Demeton
	Mirex
aldrin	Methoxychlor
dieldrin	
α- + γ-chlordane	
4,4'-DDT	
4,4'-DDE	
4,4'-DDD	
α-endosulfan	
β-endosulfan	
endosulfan sulfate	
endrin	
endrin aldehyde	
heptachlor	
heptachlor epoxide	
α-HCH (hexachlorocyclohexane)	
β-HCH	
δ-HCH	
γ-HCH (lindane)	
PCB-1242 (mixture)	
PCB-1254 (mixture)	
PCB-1221 (mixture)	
PCB-1232 (mixture)	
PCB-1248 (mixture)	
PCB-1260 (mixture)	
PCB-1016 (mixture)	
toxaphene (mixture)	

- Chemical preparation of tissue for analysis

- Measurement of pollutant concentrations in the prepared samples.

Detailed recommendations for the above procedures are beyond the scope of this report and will be available in other reports of the Bioaccumulation Monitoring Guidance series. In general, it is noteworthy that collection of representative organisms is especially critical and that the samples must be protected against contamination and degradation. Sample volume and storage procedures are best determined after assessing specific compounds to be measured and detection levels to be obtained, as described in the monitoring guidance documents.

TRACE METALS

The detection of trace metals can be performed with several types of instrumentation (e.g., neutron activation analysis, x-ray emission spectrometry, and fluorescence spectrophotometry). However, the most widely used types of instrumentation are

- Atomic absorption spectrophotometry (AAS)
  - flame
  - graphite furnace
  - cold vapor
  - gaseous hydride
- Inductively coupled plasma emission spectrometry (ICP).

A combination of these instrumental techniques is typically used, since no single technique is best for all elements.

Approximate detection limits attainable with a sample size of 5 g (wet weight) diluted to 50 mL are presented in Table 2. Sample size can

TABLE 2. RECOMMENDED TRACE METAL DETECTION LIMITS FOR TISSUE SAMPLES[a]

Element	Detection Limit[b] (ug/g wet weight)			
	Atomic Absorption			
	Graphite Furnace	Flame	Gaseous Hydride	ICP
Antimony (Sb)	**0.02**	---	0.002	10
Arsenic (As)	**0.02**	---	0.01	3
Beryllium (Be)	**0.003**	0.1	----	0.03
Cadmium (Cd)	**0.01**	0.1	----	0.4
Chromium (Cr)	**0.02**	0.2	----	0.7
Copper (Cu)	**0.01**	0.1	----	0.6
Lead (Pb)	**0.03**	1.0	----	4
Mercury (Hg)	-------**0.01** (cold vapor)--------			---
Nickel (Ni)	**0.02**	0.5	----	1
Selenium (Se)	**0.02**	---	0.01	---
Silver (Ag)	**0.01**	0.1	----	0.7
Thallium (Tl)	**0.02**	1.0	----	4
Zinc (Zn)	**0.2**[c]	0.1	----	0.2

[a] Values in boldface type are detection limits recommended for metals in tissue samples. The most sensitive analyses for antimony, arsenic, and selenium are attained by gaseous hydride, but this instrumentation is not as widely available as graphite furance. When available, the use of gaseous hydride for these elements is recommended.

[b] Detection limits are based on 5 g (wet weight) of muscle tissue, digested, and diluted to 50 mL for the analysis of all elements.

[c] A lower detection limit of 0.02 ug/g for zinc is possible by graphite furance, but is not required because zinc is always detected at higher concentrations in tissues.

be varied, but a minimum of 25 mL of digestate is needed for multi-element flame AAS analysis. Sufficient dilution volumes are necessary not only to ensure complete dissolution of the tissue but also to ensure that "dissolved salts" have been diluted to a maximum of 2 percent of the digestate (wt/vol) (U.S. Food and Drug Administration 1979). Thus, a maximum of 10 g of tissue (containing 10 percent ash) could be dissolved and diluted to 50 mL for analysis. To avoid possible matrix interferences, half of the maximum weight (i.e., 5 g) is recommended for dissolution.

For analysis by AAS or ICP methods, tissue samples must be in solution. A wide range of wet- (acid digestion) or dry- (ashing) oxidation methods (U.S. EPA 1977) is available to decompose and solubilize tissue samples (Plumb 1984). Nitric acid in combination with perchloric acid is the most effective wet-oxidation mixture for tissue dissolution. However, hydrogen peroxide is often used instead of perchloric acid, due to the extraordinary care required to avoid explosions when working with perchloric acid. Although wet-oxidation methods are less prone to loss of analytes by volatilization, they also use more reagents and are thus more likely to result in sample contamination than dry-ashing methods. Low-temperature or programmed-temperature ashing furnaces have been used to minimize loss of analytes during dry-ashing. Because dry-ashing is not appropriate for all elements, elemental recovery after dry-ashing should be monitored.

The specific analytical technique to use on digested tissue samples depends upon the required level of sensitivity. Flame AAS is generally the least sensitive method, but it may be adequate to analyze certain elements (e.g., zinc) at ambient levels found in tissue samples. Graphite furnace AAS is more sensitive than flame AAS, but is subject to more matrix and spectral interferences. Because of its high sensitivity, graphite furnace AAS requires particular caution with regard to laboratory contamination. For some trace elements (e.g., cadmium, lead, silver), graphite furnace AAS is the best analytical method because other procedures are not sensitive enough to detect the typically low ambient tissue concentrations. In both AAS methods, the concentration of each element is determined by a separate analysis, making the analysis of the entire scan of priority pollutant metals labor-intensive and relatively expensive compared to ICP. By using

ICP for trace element analyses, several elements can be measured simultaneously. However, detection limits achieved with ICP are higher than those achieved with graphite furnace AAS. Thus, ICP detection is not recommended for any of the trace metals with the possible exception of zinc.

Recommended detection limits for trace metals are listed in Table 2. These detection limits are based on 5 g (wet weight) of fish tissue, digestion with minimal elemental loss and contamination, and analysis with minimal interference. The detection limit that may be attained for a sample depends on the type of tissue, the digestion technique, and the choice of instrumentation.

In most cases, the lowest detection limit listed in Table 2 for each element is recommended. The most sensitive instrumental techniques listed for beryllium, cadmium, chromium, copper, lead, nickel, silver, and thallium is graphite furnace AAS. Graphite furnace detection of antimony is appropriate and recommended if gaseous hydride instrumentation is unavailable. Arsenic and selenium can be analyzed with roughly equivalent sensitivity by graphite furnace AAS or gaseous hydride AAS. Because graphite furnace is a widely available technique, it is recommended for analysis of arsenic and selenium. Environmental concentrations of zinc are typically high enough for detection by either graphite furnace AAS, flame AAS, or ICP. For mercury, cold vapor AAS analysis is the only recommended technique.

For mercury analyses, sample dissolution with sulfuric acid and potassium permanganate is often performed on a separate sample aliquot (Plumb 1984). However, a separate dissolution for mercury is not necessary if precautions are taken to prevent analyte volatilization. For the remaining elements, wet-acid digestion using nitric acid in combination with either perchloric acid or hydrogen peroxide is recommended. Dry-ashing is not recommended because analytes of concern may be lost by volatilization.

For purposes of comparison with recommended detection limits (Table 2), minimum and maximum detection limits reported in past studies of trace metals concentrations in tissues of marine organisms are listed in Table 3.

TABLE 3. MINIMUM AND MAXIMUM TRACE METAL DETECTION LIMITS REPORTED FOR TISSUE SAMPLES

Element	Detection Limit (ug/g wet weight) Minimum	Maximum
Antimony	0.01[a]	1.0[a]
Arsenic	Always detected[b] (minimum = 0.72)	
Beryllium	0.003[a]	0.25[a]
Cadmium	0.001[b]	0.75[b]
Chromium	0.005[b]	1.29[b]
Copper	Always detected[b] (minimum = 0.052)	
Lead	0.030[b]	1.6[b]
Mercury	0.0004[b]	0.09[b]
Nickel	0.019[b]	1.0[b]
Selenium	Always detected[b] (minimum = 0.29)	
Silver	0.001[b]	0.27[b]
Thallium	0.01[a]	0.5[a]
Zinc	Always detected[b] (minimum = 1.42)	

[a] Detection limits are based on a summary of Gahler et al. (1982), Martin et al. (1984), and Tetra Tech (1985b).

[b] Detection limit ranges are summarized from Tetra Tech (1985a, Appendix D).

The detection limits in Table 3 were compiled from data in another report of the Bioaccumulation Monitoring Guidance series (Tetra Tech 1985a, Appendix D). The recommended detection limits tend toward the lower range of reported detection limits.

ORGANIC COMPOUNDS

Although nationally standardized analytical protocols have been established for organic priority pollutants in water and wastewater, no such standardized protocols have yet been developed for tissues. Therefore, various laboratories use different analytical procedures, which can vary significantly in their sensitivity and minimum attainable detection limits.

Analysis of volatile organic pollutants in water is usually performed by a vapor-stripping technique, commonly referred to as the purge and trap technique (U.S. EPA Method 624), with subsequent GC/MS detection and quantification (U.S. EPA 1979). However, variations of this technique used for tissue samples often produce low spike recoveries and high detection limits. A more successful adaptation of U.S. EPA Method 624 involves a device that vaporizes volatile organic compounds from the tissue sample under vacuum and then condenses the volatiles in a super-cooled trap (Hiatt 1981). The trap is then transferred to a purge and trap device, where the concentrate is diluted to 5 mL and treated as a water sample. Using this technique, the average recovery of volatile compounds from tissue samples spiked with 25 ng/g was found to be 74 percent (Hiatt 1981).

Analysis of semi-volatile organic compounds involves a solvent extraction of the sample, cleanup of the characteristically complex extract, and GC analysis and quantification. Extraction for acidic, basic, and neutral organic pollutants in tissue often involves an initial extraction with methylene chloride and/or methanol (Plumb 1984; Boehm 1984; MacLeod et al. 1984). This results in an extract containing a wide range of chemicals, including many substances that are not of concern (e.g., fats and glycerides). For the most sensitive analysis, extracts must be cleaned up by removing the interfering compounds. Ideally, chemically distinct fractions (i.e., acids, bases, and neutrals) should be separated before detection and quantifi-

cation, although this is often prohibitively expensive. Efficient extract cleanup and careful handling to minimize contamination throughout the procedure result in optimum detection limits. For a given kind of tissue and sample size, variation in cleanup and extraction procedures, which differ widely among laboratories, produces a broad range of detection levels. For example, tissue extractions can be performed either by grinding the sample with the solvent, refluxing the solvent through the tissue, or digesting the tissue in a basic solution prior to solvent extraction. A comparative study of the relative efficiency of these extraction techniques was not reported in the literature reviewed for this report. Cleanup of the extract can be achieved by liquid-liquid partitioning, gel permeation chromatography, and/or normal phase liquid chromatography. The chosen methods must be easily reproduced and must allow for a high recovery for compounds of interest.

The minimum and maximum organic compound detection limits reported in past studies of organic compound concentrations in tissues of marine organisms are listed in Table 4. This information was summarized from data in another report of the Bioaccumulation Monitoring Guidance series (Tetra Tech 1985a, Appendix D). For some chemical groups with limited historical data for target species, detection limit ranges were determined from a review of selected references (i.e., Gahler et al. 1982; Martin et al. 1984; Tetra Tech 1985b). The chemical groups in Table 4 are arranged such that compounds with similar chemistry and similar detection limits are grouped together. The range of detection limits within each group in Table 4 is large, indicating a wide variability among laboratories and techniques.

The selection of organic compound minimum analytical detection limits for 301(h) bioaccumulation monitoring should be guided by tissue contaminant levels in reference areas. This guideline will not be practical for very clean reference areas that have undetectable contamination in the low part per billion range. From data on the median concentrations of compounds reported in the reference areas (Tetra Tech 1985a, Tables 3-22 and Appendix D), concentrations for most compounds are in the low part per billion range. Thus, optimal detection limits should be near the low end of the range of detection limits summarized in Table 4. Another factor to consider

TABLE 4. MINIMUM AND MAXIMUM TRACE ORGANIC COMPOUND DETECTION LIMITS REPORTED FOR TISSUE SAMPLES

Priority Pollutant Group	Detection Limit (ug/kg wet weight)	
	Minimum	Maximum
Phenols	0.69[a]	4,100[a]
Organonitrogen compounds	1.72[a]	500[a]
Aromatic hydrocarbons (low and high molecular weight)	0.08[b]	1,320[b]
Chlorinated hydrocarbons	0.015[b]	41[b]
Halogenated ethers	0.86[a]	200[a]
Phthalates	3[a]	50[a]
PCBs	0.40[b]	253[b]
Pesticides	0.015[b]	95[b]
Volatile compounds (halogenated alkanes and alkenes; aromatics, carbonyl compounds; ethers)	0.69[a]	200[a]

[a] Detection limits are based on a summary of Gahler et al. (1982), Martin et al. (1984), and Tetra Tech (1985b). Detection limits summarized for Martin et al. (1984) were recommended by the authors, and are not necessarily attainable by available methods.

[b] Detection limit ranges are summarized from (Tetra Tech 1985a, Appendix D).

when selecting optimal organic pollutant detection limits for 301(h) monitoring is that tissues need to be analyzed for many pollutants having different chemical characteristics. Dedicated analyses developed specifically for one group of compounds (e.g., aromatic hydrocarbons) would not be applicable to the analysis of all compounds of concern. Some of the minimum detection limits in Table 4 are from dedicated analyses for selected compound classes and may not be achieved by full-scan analysis. Selection of appropriate methods must therefore be based on a trade-off between full-scan analyses, which are economical and feasible for a large group of users but cannot provide optimal sensitivity for some compounds, and alternate methods that are more sensitive for specific compound groups but can result in higher analytical costs and large sample size requirements if multiple extractions are required. This trade-off has been considered in the review of available methods and associated detection limits for analyses of trace organic compounds in tissues.

Based on a review of current extraction and detection methods for a broad range of organic priority pollutants in tissues, detection limits listed in Table 5 are recommended for 301(h) bioaccumulation monitoring. Compounds that could have substantially different detection limits within a compound class, or are difficult to recover, are footnoted in the table. Except for volatile organic compound analyses, which are based on a separate sample of 5 g (wet weight), the limits in Table 5 are based on the extraction of 25 g (wet weight) of tissue. This quantity of tissue was chosen for the detection-limit recommendations, since 25 g of tissue can be obtained easily [reported initial wet-sample weights for tissue analyses ranged from 3 g (MacLeod 1984) to 100 g (Boehm 1984)] and extracted efficiently. In addition, a 25-g sample provides adequate tissue for appropriate detection levels.

As previously discussed, extraction procedures can vary, but must efficiently recover the broad range of compounds of interest (i.e., acids, bases, and neutrals). Compound recovery should be carefully evaluated for all proposed extraction procedures. A specific analytical procedure, including sample extraction and extract cleanup, is not recommended in this report but will be presented in another report of the Bioaccumulation

TABLE 5. RECOMMENDED ORGANIC PRIORITY POLLUTANT DETECTION LIMITS FOR TISSUE SAMPLES[a]

Priority Pollutant Group	Gas Chromatography Detection Limits[b] (ug/kg-wet weight)	
	Mass Spectrometry	Electron Capture Detection[c]
Phenols, substituted phenols	**20**[d]	e
Organonitrogen compounds	**20**[f]	0.1-1[g]
Aromatic hydrocarbons (low and high molecular weight)	**10**	e
Chlorinated hydrocarbons	**10-20**[h]	0.1-5[i]
Halogenated ethers	**10-20**	e
Phthalates	**10**	1-5[g]
PCBs	e	**20**
Pesticides	50	**0.1-5**[i]
Volatile compounds (halogenated alkanes and alkenes; aromatics, carbonyl compounds; ethers)	**5-10**[j]	e

[a] Values in boldface type are detection limits recommended for organic compounds in tissue samples.

[b] Except for the volatile compounds, detection limits are based on a 25-g (wet weight) tissue sample extracted, concentrated to 0.5 mL after gel permeation chromatography cleanup, and 1-uL injected. For volatile compounds a separate 5 g (wet weight) of tissue would be used for analysis. Bonded, fused silica capillary GC columns, which provide better resolution than packed columns, are assumed for analyses of semi-volatile compounds.

[c] Extract cleanup (e.g., removal of polar interferences by alumina column chromatography) is assumed.

[d] Substantially increased detection limits are observed for:

4-nitrophenol	100
2,4-nitrophenol	100
pentachlorophenol	80

TABLE 5. (Continued)

[e] No detection limits provided since methodology does not allow adequate recovery and/or detection.

[f] Benzidine and 3,3'-dichlorobenzidine may be unreported because of analytical recovery problems.

[g] Use of electron capture detection for these compounds would require dedicated analytical protocols.

[h] Substantially increased detection limits are observed for:

hexachloroethane	40
hexachlorobutadiene	40
hexachlorocyclopentadiene (typically not reported because of its lability in heated injection ports)	

[i] The higher range of detection limits are appropriate for pesticides such as mirex, methoxychlor, the DDTs, and endosulfans, and for chlorinated butadienes. Compounds such as lindane, aldrin, heptachlor, and hexachlorobenzene can be detected at the lower limit. Toxaphene (a mixture) may require a higher detection limit than the other organochlorine pesticides, 20 ppb.

The nonchlorinated, organophosphorous 301(h) pesticides (Malathion, Parathion, Guthion, and Demeton) should not be analyzed with the same procedures as the organochlorine pesticides. They require dedicated protocols (e.g., one- or two-step extract cleanup and GC/phosphorous specific flame photometric or alkali flame ionization detection) for appropriate detection limits of approximately 1-15 ppb.

[j] Substantially increased detection limits are observed for:

acrolein	100
acrylonitrile	100
2-chloroethylvinyl ether	100
methylene chloride	100

Monitoring Guidance series. At a minimum, one- or two-step cleanup should be performed following extraction to obtain adequate compound resolution. The detection limits recommended in Table 5 are based on extract cleanup by gel permeation chromatography and by alumina column chromatography for ECD analyses (e.g., U.S. EPA 1984). After cleanup, the sample extract can be concentrated to volumes usually ranging from 0.1 to 3.0 mL. The recommended detection limits assume a final extract volume of 0.5 mL and a minimum instrument injection volume of 1 uL.

Recommended detection limits (Table 5) are listed for either mass spectrometry or electron capture detection. Because of the greater sensitivity of GC/ECD relative to GC/MS for chlorinated compounds, PCBs and chlorinated pesticides should be quantified with GC/ECD. However, analysis by GC/ECD does not provide positive compound identification. Problems with false readings due to interferences have been commonly reported. Thus, confirmation of PCBs and pesticides on an alternative GC column phase (on GC/ECD), or preferably by GC/MS if analyte concentrations are sufficiently high, is essential for reliable results. All other organic compound groups are recommended for analysis by GC/MS.

A review of observed concentrations of organic compounds in marine organisms from reference areas (Tetra Tech 1985a, Tables 3-22) indicates that the recommended detection limits for organic compounds (Table 5) may result in a number of "undetected" values. These levels are nonetheless useful for purposes of comparison. By removing interferences with a one- or two-step cleanup and using mass spectrometry confirmation (as recommended in this report), the recommended detection limits will reliably detect substantial elevations in organic pollutants in the vicinity of a wastewater discharge.

As a specific monitoring program progresses, certain compounds or compound groups may be consistently undetected near wastewater discharge sites even with low detection limits. Such findings may justify the discontinued analysis of these compounds on a site-specific basis. Focusing on selected compound groups enables analytical methods for critical compound groups to be optimized, and typically results in improved detection limits.

Furthermore, if non-target organic pollutants are found to occur frequently and at significant concentrations in tissue samples such that they are major peaks in GC/MS reconstructed ion chromatograms, and if these compounds can be reliably identified by comparison of their mass spectra to those of the U.S. EPA/NIH computerized library, they should be added to the list of 301(h) target compounds on a site-specific basis.

SUMMARY OF RECOMMENDATIONS FOR DETECTION LIMITS

Detection limits for each sample analyzed are required to be reported with all data sets. In general, the detection limits recommended in this report (Tables 2 and 5) are the most sensitive that may be feasibly attained under the requirements for full scan analyses of U.S. EPA priority pollutant metals and organic compounds.

Detection limits for trace metals in tissue are based on a minimum sample size of 5 g (wet weight) (Table 2). An additional 1 g (wet weight) of tissue may be used for a separate analysis of mercury. A detection limit of 0.003 ug/g (wet weight) is recommended for beryllium. Detection limits of 0.01 are recommended for cadmium, copper, mercury, and silver. Detection limits of 0.02 ug/g (wet weight) are recommended for antimony, arsenic, chromium, nickel, selenium, and thallium. A detection limit of 0.03 ug/g (wet weight) is recommended for lead. A less sensitive detection limit of 0.1 ug/g (wet weight) is recommended for zinc.

Detection limits for organic pollutants in tissue are based on a minimum sample size of 25 g (wet weight), with an additional 5 g (wet weight) of tissue recommended for a separate analysis of volatile organic compounds (Table 5). For the majority of the volatile organic compounds, detection limits between 5 and 10 ug/kg (wet weight) are recommended. Detection limits of 10 ug/kg (wet weight) are recommended for aromatic hydrocarbons and phthalates. Detection limits ranging from 10 to 20 ug/kg (wet weight) are recommended for chlorinated hydrocarbons and halogenated ethers. Detection limits for the chlorinated pesticides range from 0.1 to 5 ug/kg (wet weight) with GC/ECD. In areas where high concentrations occur, mass spectrometric detection (with a detection limit of 50 ug/kg) will provide compound confirmation. If GC/MS confirmation is not possible, GC/ECD analysis with an alternative GC column should be performed. PCBs should be analyzed by GC/ECD with a detection limit of 20 ug/kg (wet weight). PCB confirmation

on an alternative GC column, or by GC/MS if concentrations permit, is strongly recommended.

To attain the recommended detection limits, a total sample size of 35 g is recommended for a complete analysis of priority pollutant trace metals, semivolatile, and volatile organic compounds (i.e., 5 g for trace metals, 25 g for semivolatile organic compounds, and 5 g for volatile organic compounds). If individual organisms selected will not provide roughly 35 g of tissue, the Region may need to evaluate modification of the monitoring program to either reduce the scope of the analyses (e.g., eliminate volatile organic compound analysis), raise the recommended detection limits, or composite tissue from several organisms. To satisfy requirements for quality assurance of the data, an additional 35 g tissue is recommended for each replicate set of analyses conducted. Typically, replicate analyses (including matrix spike analyses) are conducted on 5 to 10 percent of the total number of samples.

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