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Environmental Protection
Agency

Office of
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Washington DC 20460

Office of Research
and Development

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March 1991

Water

EPA Guidance on

Assessment and Control of Bioconcentratable Contaminants in Surface Waters

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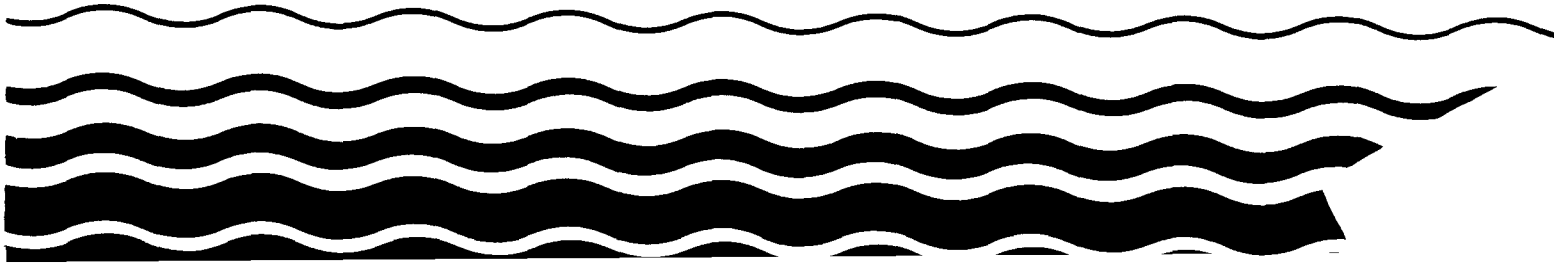

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

DEC 15 1991

OFFICE OF
WATER

Dear Colleague,

Enclosed for your information and review is a copy of EPA's draft guidance entitled "Assessment and Control of Bioconcentratable Contaminants in Surface Waters." Also enclosed is the Federal Register Notice that made this document available for public review and comment. A summary of the draft guidance document is provided in the Federal Register Notice.

The overall purpose of this draft guidance document is to provide guidance for identifying, and where necessary, controlling bioconcentratable organic compounds that may be present in effluents, nonpoint source runoff, receiving waters, bedded sediments, dredged material, and the tissues of aquatic organisms. Such compounds can cause adverse human health and aquatic life impacts. Identification of bioconcentratable and bioaccumulative compounds in water, tissue and sediment can serve as the basis for regulatory decisions concerning all sources of these pollutants.

EPA solicits comment on each chapter in this document relating to the overall applicability of the procedures described and the practical utility of the approach for addressing concerns about bioconcentratable pollutants in the Nation's waterways. In particular, EPA asks for public comment on two areas that are known to be of considerable interest to the public and where additional comment would be particularly helpful. First, comments on the selection of dischargers for assessment would be useful in determining the number and types of facilities which may be required to conduct this analysis. Second, comments are solicited on the capability and capacity of laboratories, both current and potential, which may be involved in applying the analytical methods described in this draft guidance.

The development of this document has been a cooperative effort of the Environmental Research Laboratory in Duluth, Office of Health and Environmental Assessment in Cincinnati, Office of Water Regulations and Standards, and the Permits Division of the Office of Water Enforcement and Permits. Should you have any questions regarding the document please contact me at 202/475-9545, or have your staff contact William Morrow at 202/475-9531.

Sincerely,

Cynthia C. Dougherty

Cynthia C. Dougherty, Director
Permits Division (EN-336)

Enclosures

GUIDANCE ON

ASSESSMENT AND CONTROL OF BIOCONCENTRATABLE CONTAMINANTS IN SURFACE WATERS

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DRAFT

Note:

This draft document contains procedures which are currently advisory and subject to validation. These include: 1) Appendix B, "Laboratory Procedures for Determining Bioconcentratable Chemicals in Aqueous Samples" and 2) the inclusion of bioaccumulation factors (BAFs) in the Chapter 3 formulas for calculating reference ambient concentrations (RACs).

United States Environmental Protection Agency

National Effluent Toxicity Assessment Center,
Environmental Research Laboratory - Duluth
Office of Water Enforcement and Permits
Office of Water Regulations and Standards
Office of Health and Environmental Assessment - Cincinnati

Foreword

Human consumption of fish and shellfish contaminated by exposure to industrial and municipal discharges is a potential toxic chemical exposure route of serious concern. Protection of human health is part of the full complement of water quality-based controls for toxic pollutants. This guidance document, "Assessment and Control of Bioconcentratable Contaminants in Surface Waters" (EPA 600/x-xx-xxx), provides guidance to State and Regional regulatory agencies in developing reference ambient criteria and determining necessary controls to protect against human health impacts due to consumption of contaminated fish and shellfish. It also contains the specific analytical procedures for use in determining the presence, identity and concentrations of bioconcentratable and bioaccumulative compounds in aqueous, fish tissue and sediment samples. **This document is agency guidance only. It does not establish or affect legal rights or obligations. It does not establish a binding norm and is not finally determinative of the issues addressed. Agency decisions in any particular case will be made applying the law and regulations on the basis of specific facts when permits are issued or regulations promulgated.**

This guidance will be revised periodically to reflect advances in the area of bioconcentratable pollutant control. Comments from users will be welcomed. They should be sent to the Office of Water Enforcement and Permits (EN-338), U.S. Environmental Protection Agency, 401 M. St. S.W., Washington, D.C. 20460.

Acknowledgements

This document results from the cooperative efforts of individuals at the Environmental Research Laboratory, Duluth (ERL-D); the Office of Water Enforcement and Permits (OWEP); the Office of Water Regulations and Standards (OWRS); and the Office of Health and Environmental Assessment (OHEA). The principle authors were Lawrence P. Burkhard (ERL-D), John R. Cannell (OWEP), Katharine Wilson Dowell (OWEP), William J. Morrow (OWEP), Barbara Riedel Sheedy (AScI Corporation, ERL-D), and Rick Brandes (OWEP).

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Donald I. Mount (AScI Corporation, ERL-D)
Hiranmay Biswas and Charles Delos (OWRS)
Linda R. Papa and Randall Bruins (OHEA)
Gilman D. Veith (ERL-D), Technical Advisor
Nelson A. Thomas (ERL-D), Project Leader
Robert Wood (OWEP), Validation Study Work Assignment Manager
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Glossary

aggregate risk	A methodology that estimates the total number of both cancer and non-cancer cases developed by populations exposed to pollutants from fish or shellfish.
analyte	Chemical of interest for a specific analytical procedure.
bioaccumulation	Uptake and retention of substances by an organism from its surrounding medium and from food.
bioaccumulation factor (BAF)	Measure of a chemical's tendency to bioaccumulate.
bioconcentration	Uptake of substances by an organism from the surrounding medium through gill membranes or other external body surfaces.
bioconcentration factor (BCF)	The measure of a chemical's tendency to bioconcentrate. The BCF is calculated by dividing the concentration of the chemical in the exposed organism's tissues by the concentration of the chemical in the exposure medium.
biomagnification	the process by which the concentration of a compound increases in different organisms, occupying successive trophic levels.
blank correction	Subtraction of the signal, output, or response observed for the method blank from that observed for the sample. A method blank is created by performing an analysis by using all the glassware, reagents, etc., that would be used with a sample.
carcinogenic potency factor (q1*)	A factor indicative of a chemical's human cancer-causing potential (upper 95% confidence limit of the slope of a linear dose-response curve). q1*'s (pronounced "que-one-star") are based on extrapolation of high dose levels to low dose levels and a lifetime exposure period. The q1* is expressed in units of reciprocal mg/kg/day

	and is also called the cancer potency slope factor and the oral slope factor.
chromatography	A method of separating and analyzing mixtures of chemical substances by preferential adsorption of chemical components in ascending molecular sequence onto a solid absorbance material.
corrected retention time	The corrected retention time for an analyte eluting from a chromatographic column is the retention time of that analyte minus the retention time of an unretained analyte. The corrected retention time is expressed in units of minutes.
depuration	The elimination of a chemical from an organism via a complex interaction of exchange processes across gills and excretion via kidneys or bile.
eluate	The chromatographic fluid (normally, an organic solvent) after passing through the chromatography column.
elution	The process of removing an absorbed chemical by means of a solvent in a chromatography column.
food chain	The scheme of feeding relationships by trophic levels which unites the member species of a biological community.
harmonic mean flow	A long term mean flow value calculated by retrieving several years of daily flow records, taking the reciprocal of each value, calculating the average, and taking the reciprocal of the average.
lowest observed effect concentration	The lowest dose that results in a level statistically significant effect in the test population.
no observed effect concentration	The highest dose tested at which no level effects are observed.
no observed adverse effect level	The highest dose tested at which there level is no statistically or biologically significant increase in adverse effects.

n-octanol/water partition coefficient	The ratio at equilibrium, in a two-phase system of a chemical in the n-octanol phase to that in the water phase.
reference ambient concentration (RAC)	The concentration of a bioconcentratable chemical in water which will not cause adverse impacts to human health. The RAC is expressed in units of mg/L.
reference tissue concentration (RTC)	The concentration of a chemical in edible fish or shellfish tissue which will not cause adverse impacts to human health when ingested. The RTC is expressed in units of mg/kg.
reference dose (RfD)	An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure during a lifetime to the human population (including members of sensitive subgroups) that is likely to be without appreciable risk of deleterious effect. The RfD is expressed in units of mg/kg/day.
reverse-search	A library searching technique for identifying components detected during a GC/MS analysis. Identification is performed by comparing a mass spectrum of the unknown component to mass spectra stored in reference libraries on the GC/MS data system. With reverse searching, similarity between the unknown and reference spectra is measured by how well the unknown is included in the reference mass spectrum.
signal-to-noise ratio	The ratio between the amplitude of the analyte to the amplitude of the background noise signal at the same point.
trophic level	One of the successive levels of nourishment in a pyramid of numbers, food web, or food chain; plant producers constitute the first (lowest) trophic level, and dominant carnivores constitute the last (highest) trophic level.
wasteload allocation	The portion of a receiving water's total maximum daily pollutant load that is allocated to one of its existing or future point sources of pollution.

xenobiotic	Synthetic organic and organometallic chemicals foreign to living organisms.

Abbreviations

BAF	bioaccumulation factor
BCF	bioconcentration factor
CAS #	Chemical Abstract Services Registry Number
CHC	Chemicals of Highest Concern
FM	food chain multiplier
GC/MS	gas chromatography/mass spectrometry
HEAST	Health Effects Assessment Summary Tables
HPLC	high pressure liquid chromatography
IRIS	Integrated Risk Information System
LOAEL	lowest observed adverse effect level (human/animal toxicology)
log 10	logarithm base 10
log P	log of octanol/water partition coefficient (also known as log K_{ow})
LOEC	lowest observed effect concentration
MCL	maximum containment level
NOEC	no observed effect concentration
PHN	public health network
NOAEL	no observed adverse effect level (human/animal toxicology)
NPDES	National Pollutant Discharge Elimination System
QSAR	Quantitative Structure Activity Relationship
q1*	carcinogenic potency factor
RAC	reference ambient concentration
RTC	reference tissue concentration
RfD	reference dose
RT	retention time
RL	risk level
RWC	receiving water concentration
WLA	wasteload allocation
7Q10	seven day average low flow with a ten year return period
30Q5	thirty day average low flow with a five year return period

INTRODUCTION

The EPA surface water toxics control program is designed to provide the necessary regulatory controls to assure the protection of aquatic life, wildlife, and human health. To implement this program, EPA has developed whole effluent toxicity, chemical specific criteria, and bioassessment procedures to protect aquatic life. EPA has also initiated and continues to develop procedures to protect wildlife.

EPA has also developed criteria and control procedures to protect human health from adverse exposure to contaminants in drinking water and consumed fish or shellfish. It is this latter exposure from consumption of contaminated fish and shellfish which is the focus of this document.

During the past three decades, problems with chemical residues in fish, shellfish, and wildlife have been prominent throughout the country. Chemicals such as DDT, PCB's, mercury, tributyltin and 2,3,7,8-dibenzo-p-dioxin have received substantial attention and have resulted in fish consumption advisories and/or banning of commercial fish and shellfish harvesting.

Many studies have indicated that fish and shellfish consumption may be a major human exposure route to some bioconcentratable chemicals [1]. Ingestion of contaminated fish and shellfish poses serious health risks to the general public and this route of exposure currently has little if any specific controls.

Historically, water quality-based toxics control has relied on chemical-specific effluent limitations to protect human health. However, only those chemical-specific limits based upon human health water quality criteria for fish consumption can be usually preventive of chemical residue formation in fish or shellfish tissue. Procedures to amend EPA's 1980 human health water quality criteria for fish consumption have been developed and the criteria amended. The number of water quality criteria is small relative to the large number of toxic pollutants being discharged to surface waters.

Approach Description

This document provides guidance for the control of bioconcentratable pollutants in effluents including those not presently controlled by water quality criteria. This objective is accomplished by presenting analytical procedures to identify and quantify bioconcentratable pollutants in environmental samples, by presenting procedures for deriving criteria for aquatic organisms and criteria for receiving waters, and by

presenting permitting guidance for the control of these pollutants from point sources. Two options are described. For one, the effluent is screened for the presence of chemicals that bioconcentrate. If present, a determination is made as to whether the concentrations are sufficiently high to cause residue problems. Alternatively, residues in aquatic organisms can be measured and if they are above the permissible concentrations, the sources are traced using target analysis and then limited as for the above approach. If no residues of concern are found, no further action is needed except for periodic checks for emerging problems. By integrating these separate aspects, an unified approach has been developed for assessment and control of bioconcentratable chemicals in surface waters.

Detailed analytical procedures are provided for three environmental matrices, water, sediment, and tissue. Therefore environmental media such as effluents, biological tissues, sediments, dredge materials, stormwater, leachates, groundwater, and non-point runoff samples can be analyzed for the presence of bioconcentratable chemicals. These procedures are presented in a form suitable for dispersement to analytical laboratories for performing the analysis.

Approach Objectives

Application of this approach provides information on the presence of bioconcentratable chemicals which are present at concentrations which may cause problems from the consumption of fish by humans. Specifically, the results of this approach include the identity and concentrations of bioconcentratable chemicals in a sample for which human health criteria or risk information is available enabling the implementation of controls. Other bioconcentratable chemicals, for which human health risk information is not readily available, will also be identified. This information can be used to focus the development of human health risk information on chemicals shown to be present in the environment at potentially hazardous levels. The approach will also identify any chemicals present in a sample at very high concentrations, which represent a potential for bioconcentration, as well as any other chemicals which are present but which do not represent a significant potential for bioconcentration. This information will be useful for water quality regulatory authorities for better understanding the environmental risks from bioconcentratable contaminants and for determining the need for regulatory controls for these chemicals.

Approach Merits

The approach in this document complements the existing chemical-specific criteria approach and is recommended for the following reasons:

- A broad screen approach is achieved in a cost effective manner that does not require a prior knowledge of the presence of specific chemicals. It thus avoids over reliance on chemical by chemical screening for targeted pollutants which cannot provide as complete a characterization of bioconcentratable contaminants in an effluent.

- The approach focuses regulatory attention on those chemicals which bioconcentrate in the environment. In using this approach, patterns and trends may be discerned and new or previously unknown problem toxicants can be identified and controlled.

- The approach provides a standardized assessment methodology with broad applicability which offers the same sample handling, detection levels, and consistency of results for different applications (effluents, fish tissues, and sediments).

- The approach can be used to assess bioconcentration hazards for wildlife and for the protection of wildlife for which criteria are available.

- The approach provides a means to better prioritize permitting resources. If contaminants are not found at levels of concern, then that particular source can be considered a lower priority for establishing controls.

- The approach provides procedures for assessing and controlling complex mixtures. The identification of those chemicals present in a complex mixture such as an effluent, which pose a threat to human health, enables the additive risk from those chemicals to be addressed in the development of reference ambient concentrations.

Depending on the application, the analytical procedures which are presented may be conducted by either regulatory authorities or by the regulated community. The results will be useful to regulatory authorities in making decisions on the selection of control methods for both the remediation and the prevention of environmental impacts from bioconcentratable pollutants. For example, a dredged materials sample might be analyzed to determine if bioconcentratable contaminants are present which would pose a problem for a particular disposal

practice. This information would be useful in deciding on an appropriate regulatory action for those dredged materials.

Limitations of Approach

Although these analytical methodologies can be used for assessing a wide range of samples from different media and different sources, it was developed primarily for the assessment and control of bioconcentratable pollutants discharged in effluents of NPDES permittees. Guidance is provided for NPDES permitting authorities on selecting dischargers which may be required to conduct this analysis, for requiring and interpreting the results of the effluent and tissue residue procedures and for implementing permit requirements for the control of bioconcentratable contaminants. The document provides permittees and laboratories required to conduct these evaluations with the analytical procedures to identify, quantitate and confirm individual bioconcentratable pollutants. These procedures are appropriate for use in freshwater, estuarine and marine applications.

The analytical procedures contained in this document will not detect all bioconcentratable chemicals which form residues in aquatic organisms. The analytical procedures will only detect nonpolar organic chemicals which can be successfully analyzed using GC/MS. Some of the general limitations of the approach are the sensitivity of the analytical method selected and the ability to identify unknown GC/MS components. Analytical limitations unique to each option; tissue, effluent, and sediment; are described in each of the corresponding sections of Chapter 3 (Sections 3.1.14, 3.2.16, and 3.4.10).

This document does not provide for an entire human health risk assessment for bioconcentratable pollutants, rather it focuses only on the fish consumption exposure route. The procedures to address human health effects of contaminated fish consumption that are described within this document are consistent with existing EPA risk assessment methodology.

This guidance document is intended to supplement EPA's water quality based toxics control program. As such, the guidance and recommendations found in this document are not mandatory.

Contents of Document

Chapter 1, Approach to Assessment and Control of Bioconcentratable Contaminants, defines the scope of this guidance document and discusses the assessment options.

Chapter 2, Principles of Bioconcentration Control, provides background information and introduces bioconcentration, terminology, processes, and methods.

Chapter 3, Assessment Options for Bioconcentratable Contaminants, provides a detailed description of tissue and effluent options and their analytical procedures. Chapter 3 also provides discussions on generating chemical analytical data, quality assurance, and sediment analysis.

Chapter 4, Reference Concentration Derivation, provides procedures for deriving RTCs and RACs for bioconcentratable pollutants identified by the analytical procedures used for the assessment. RTCs and RACs are used to determine the need for controls and for the derivation of effluent limitations.

Chapter 5, Exposure Assessment and Wasteload Allocations, discusses approaches to wasteload allocation development under a variety of discharge situations. Discussions focus on receiving water characteristics such as available dilution and mixing, type of pollutant(s) being discharged, data availability, and data generation.

Chapter 6, Permit Limits for Control of Bioconcentratable Chemicals, provides guidance on determining reasonable potential for exceedence of RACs, and the development of NPDES permit limits specific to the control of bioconcentratable chemicals and the protection of human health.

The appendices contain the technical specifications for the laboratory procedures along with other supporting information.

Chapter 1

Approach to Assessment and Control of Bioconcentratable Contaminants

A generalized flowchart for this approach to the assessment and the control of bioconcentratable contaminants in surface waters is presented in Figure 1.1. This flowchart presents a conceptual overview of the major steps and decision points contained in the approach described in this document. Each of the components of this overall process are described in detail in the corresponding sections of the document.

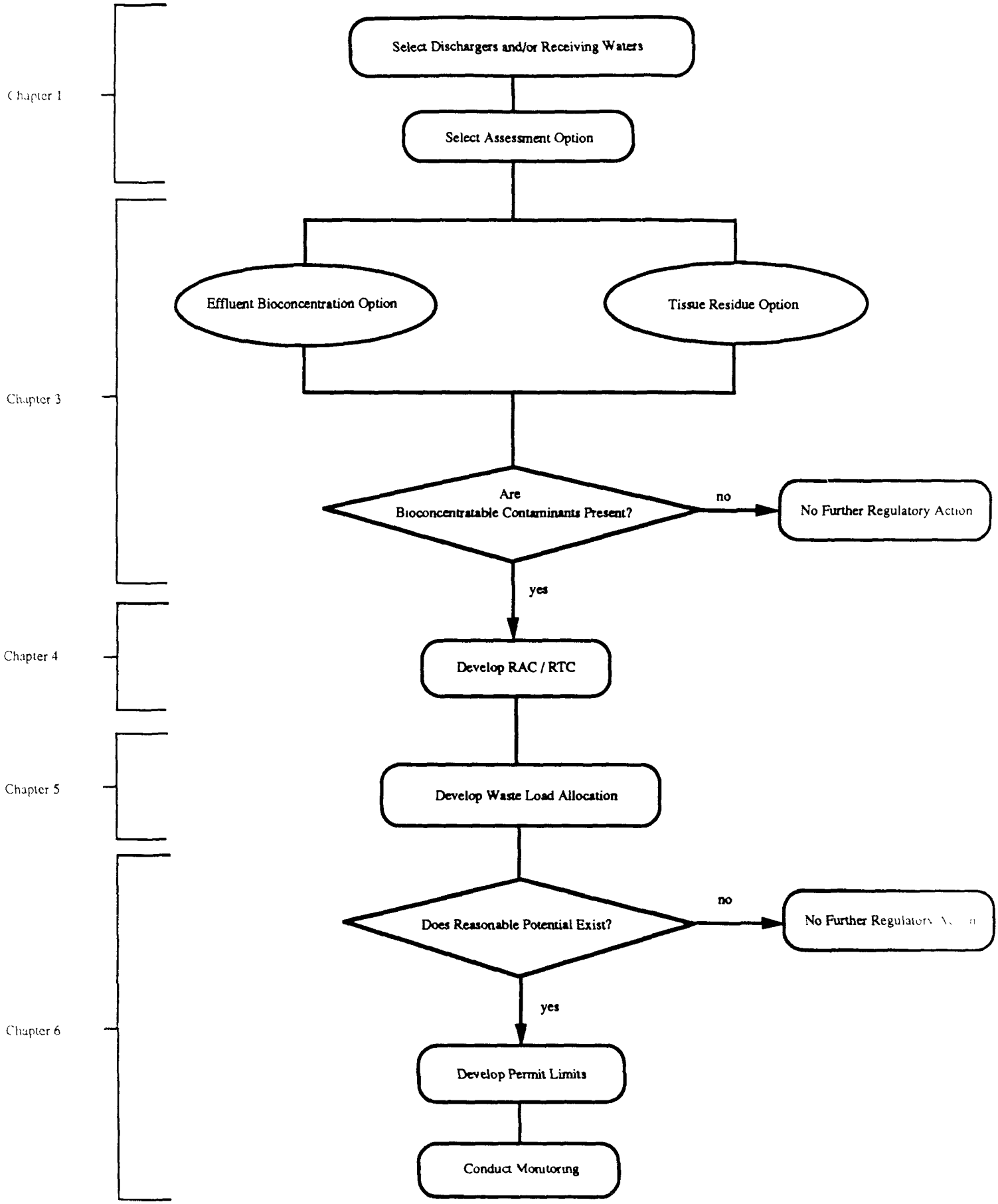
The approach illustrated in Figure 1.1 is a seven step procedure. These steps are: 1) selection of dischargers or receiving waters for assessment, 2) selection of the appropriate assessment option, effluent bioconcentration or tissue residue option, 3) analysis of tissue or effluent samples for bioconcentratable chemicals, 4) calculation of reference tissue concentrations (RTCs) and/or reference ambient concentrations (RACs) for the identified bioconcentratable contaminants, 5) development of wasteload allocations, 6) determination if concentrations are present which have the reasonable potential to pose health risks for human consumers of fish and shellfish, and if so, 7) permit limit development.

Depending on the application of this approach, the regulatory authority may require a discharger to conduct step 3, the effluent or tissue residue assessment options, or these assessment options may be utilized by the regulatory authority. An analytical chemistry laboratory with residue chemistry and GC/MS capability will be needed to conduct the analytical methods for effluent and tissue bioconcentratable chemical identification and the confirmation of the identified chemicals. The specific step-by-step laboratory method instructions are contained in the appendices to this document.

The recommended data interpretation procedures to be followed by the regulatory authority in reviewing the reported chemical analytical results are contained in the discussion of the assessment options in Chapter 3. In requiring a discharger to conduct these assessments the regulatory authority should specify what information and results the discharger needs to generate and report. This should include information on sampling and sample handling as well as the other QA/QC information that is specified in the methods appendices.

Figure 1.1

Procedure for Assessment and Control of Bioconcentratable Contaminants in Surface Waters



Once compounds that bioconcentrate are identified, several pieces of information may need to be determined if water quality standards for those compounds are not in place. For the tissue residue option the Reference Tissue Concentration (RTC) for each contaminant must be developed to determine if unacceptable concentrations are present. For the effluent option, a Reference Ambient Concentration (RAC) is developed. The RAC is similar to a water quality criterion for human health. The RTCs and RACs are based on each specific bioconcentratable chemical's toxicity and risk to humans. Chapter 4 provides a discussion of the information needed, and the procedures for development of RTCs and RACs.

If the tissue residue option rather than the effluent screening option has been used, the RTC is first used to screen for the presence of potentially hazardous concentrations of the bioconcentratable chemicals in fish tissue. (For the sake of simplicity, the remainder of this document the term fish is generally used to mean both fish and shellfish). If this proves to be the case, then target chemical analysis for those chemicals must be done on effluent samples and the RAC calculated and utilized as described above.

Following the development of RTCs and/or RACs, the approach described in this document proceeds through the wasteload allocation and, if needed, permit limit development. These more traditional pollutant control procedures follow the guidance provided in the Technical Support Document and are discussed in the context of this approach to the control of bioconcentratable contaminants in Chapters 5 and 6.

1.1 Scope of Approach to Bioconcentration Assessment and Control

The approach in this document identifies and controls contaminants in effluents, and contaminants in other aqueous samples, capable of forming fish tissue residues based upon the tendency of the compound to bioconcentrate. Chemicals that bioconcentrate include organic compounds, and a small number of metals and organometals. With the tissue residue option, the approach described in this document is limited to nonpolar organic chemicals which produce measurable chemical residues in aquatic organisms. With the effluent option, the approach is limited to nonpolar organic chemicals with characteristics which cause these compounds to bioconcentrate, i.e. log P values greater that 3.5. This threshold value of log $P > 3.5$ is discussed in Section 3.2.4. This approach <u>does not</u> address other types of chemicals known to bioconcentrate, such as metals (e.g. mercury, selenium) and organometals (e.g. tributlytin). Also,

this approach may not detect the presence of some compounds, such as dioxin, which can form unacceptable residues at very low exposure concentrations (i.e. below the method detection level, see discussion in Section 2.7).

1.2 Selection of Dischargers for Assessment

Guidelines are necessary to help NPDES permitting authorities prioritize dischargers for assessment. At this time, the EPA is soliciting comments on the selection of point source dischargers for assessment. The final document will provide recommendations for the selection process.

1.3 Tissue Residue Option

The tissue residue option measures the concentrations of organic bioconcentratable chemicals in tissue samples of indigenous organisms from the receiving water. This analysis involves the collection of fish or shellfish samples, the extraction of the organic chemicals from the tissue and the analysis of these extracts with GC/MS to identify and quantify the bioconcentratable contaminants. The procedure provides recommendations to sort the results of this screening analysis in order to determine which of the contaminants pose a hazard and require regulatory action. The approach recommends that the identity of those contaminants then be confirmed prior to taking subsequent action.

In order for a tissue residue analysis to accurately assess the effects of a given discharge of bioconcentratable contaminants in an effluent it is essential for the tissue sample analyzed to be representative of a long term exposure to the effluent. For this reason the ambient sampling for this option must be carefully designed and the tissue residue option also recommends target chemical analyses of the associated effluents for the specific residue chemicals identified in the tissue samples from the receiving water. The tissue residue option may be applied to measure residues in organisms which arise from other sources of the chemical to the receiving water. These sources may include nonpoint sources, sediments, and any other upstream point source dischargers.

1.4 Effluent Option

The effluent option measures the concentrations of organic bioconcentratable chemicals in effluent samples from point source dischargers. This analysis involves the collection of effluent

samples, the extraction of the organic chemicals from the effluent sample, and the separation of the chemicals which have characteristics known to result in bioconcentration from the other chemical components of the effluent sample. This separation is achieved by way of an analytical chemistry methodology called high pressure liquid chromatography (HPLC). The use of HPLC also enables the fractionation of the effluent sample into three sub-samples or "fractions". These three fractions would contain chemicals with increasing potential to bioconcentrate with the third fraction containing those chemicals with the highest bioconcentration rates. Following HPLC fractionation, each fraction is then analyzed with GC/MS to identify and quantify the bioconcentratable contaminants. The effluent procedure also provides recommendations to sort the results of the initial screening analysis in order to determine which of the contaminants pose a hazard and require subsequent regulatory action. The approach then recommends that the identity of those contaminants then be confirmed prior to taking further regulatory action.

It is important to recognize that these effluent bioconcentration analysis procedures are subject to a number of basic principles and assumptions. These principles and assumptions, described in Chapter 2, provide a number of constraints on the application of the analytical procedure and should be recognized and understood in order to appropriately conduct and interpret the results of the procedure. These underlying principles also hold for the application of this approach to other sources (i.e. dredged materials) from which aqueous samples can be extracted. It is also important to note that the collection of effluent samples is subject to the effects of effluent variability. In order to accurately assess an effluent with high variability, it may be necessary to collect and perform this analysis on a greater number of samples.

1.5 Selection of Assessment Option

While either of the assessment options described above may be utilized for a given discharger, generally one of these options will be preferred by the regulatory authority for an initial assessment. The regulatory authority should select the assessment approach based on the available site and facility specific information and the objectives of each application.

In general, EPA recommends that a discharger be required to conduct the effluent option if existing fish tissue and/or facility information suggests the potential presence of bioconcentratable contaminants. Examples of this are waters under a fishing ban due to bioconcentratable pollutants, or an organic chemical facilities known to manufacture

bioconcentratable chemicals. In these cases, there exists a strong possibility for the bioconcentration of pollutants in fish tissues to unsafe levels and the effluent option might be used to determine if a point source discharger is in fact a contributing source of these types of pollutants.

EPA recommends that the tissue residue option be required if the objective of the regulatory authority is to assess existing ambient bioconcentration or bioaccumulation problems in the absence of existing water body or facility information on the presence of these contaminants. In these cases, an overall assessment of ambient exposure is needed. The tissue residue option allows for a direct assessment of the ambient conditions which may include the effects from multiple sources. For example, for certain waterbodies one species of fish may be of predominant concern (e.g. salmon) and this option might be selected to determine the identities of any bioconcentratable contaminants which may be present. It may also be used for trend analysis in determining the effectiveness of any previous controls.

The selection by the regulatory authority of an assessment option for a given discharger will, to a large extent, be determined by the site specific circumstances of each application and the specific objectives or questions which the assessment is being required to address. The selection of the appropriate option will greatly increase the utility of the analytical data generated. The trade offs inherent in the options must be understood in order to make this selection. The following discussion compares these options and is intended to assist in this selection.

The tissue residue option tends to assess problems due to bioconcentration on a receiving water basis and the effluent option on a discharge by discharge basis. The tissue residue option measures existing residues in indigenous organisms, while the effluent option examines effluents for chemicals with the known potential to bioconcentrate. Both approaches will provide information on the presence and identity of bioconcentratable chemicals and may be used to base controls on these contaminants.

The tissue residue option measures existing chemical residues in indigenous organisms sampled from the receiving water for an effluent discharge. The residues measured in these organisms may arise as a result of some or all of the sources of a particular chemical to the receiving water. This could include loadings from multiple point source discharges, any nonpoint sources of the chemical and sediments. Consequently, an existing residue found in the tissue of the indigenous organism might have no relationship to a given discharger or this discharger may be

only partially responsible for the presence of the contaminant in the tissue sampled. In order to tie a specific discharger to those chemical residues found, the tissue residue option includes the recommendation to conduct follow up target analyses of effluent samples for those specific chemicals.

The effluent option begins with a selected discharger and directly determines the presence and concentrations of bioconcentratable chemicals in the effluent. This assessment option does not integrate multiple point sources discharges, nor does it incorporate nonpoint sources and sediments. If the regulatory authority's primary objective is to assess the cumulative effects of these sources then the tissue residue option is the more appropriate initial approach. In this way the total amount of the contaminants from these sources which result in tissue residues can be determined and the total loading can be controlled by allocation among these multiple sources.

The effluent option may also be used to assess multiple point source discharges by requiring each discharger to conduct the analyses. The results of these assessments could then be used in setting controls, either through the traditional single source wasteload allocation process (which may not adequately account for the multiple source loadings) or by developing a multiple source wasteload allocation for those selected dischargers. This approach would not directly incorporate loadings from nonpoint sources or sediments (unless these assessments are performed separately) and therefore in some cases, may not result in controls which are stringent enough to totally prevent the formation of tissue residues. However, this is not to say that this approach would not be effective in developing controls for the selected discharges, only that the level of control which is set may not factor in the other sources mentioned.

Another distinction between the two assessment options concerns whether the objective is primarily to determine if there are existing problems in a waterbody or if a specific discharger is causing, or may in the future cause such a problem. The tissue residue option is limited to those contaminants already existing in indigenous organisms which are sampled and which can be identified in the target chemical effluent analyses. The tissue residue option cannot prevent residue problems due to new chemicals, either new to the receiving water or new to the organism sampled, because the option can only detect chemicals which have had time to form a residue. For most chemicals, a continuous laboratory exposure of 28 days is used to determine measured bioconcentration factors. The effluent option may identify these compounds as well as any additional chemicals in the effluent with the potential to bioconcentrate. Because of this, the effluent option may prevent tissue contamination from

occurring as well as assessing existing problems. Whichever option is selected, setting controls on point source discharges will require the calculation of an RAC based on the chemical's BCF and a food chain multiplier which are described in Chapters 2 and 4.

The tissue residue option may provide greater sensitivity than the effluent option for those chemicals with large BCFs and which are present at very low concentrations in a given effluent. This enhanced sensitivity for the residue option exists due to the organism concentrating those chemicals over time from the receiving water. Of course this increased concentration will only occur in organisms which have been exposed to the chemicals from a discharge and requires the development of a sampling requirements with this point in mind. For example, for discharges confined to small streams and rivers, a time period of one to two months may be necessary for the residue concentration in the organism to reach equilibrium. This time period could be much greater for discharges of a chemical to larger bodies of water.

The tissue residue option may detect a wider range of residue forming chemicals than the effluent option. This is due to the analytical techniques required in the effluent option to simplify the sample and remove the non residue forming chemicals from the effluent extract. Unfortunately, these procedures may also cause some chemicals which do form residues in organisms to decompose. This clean up of the sample extract is not required for the tissue option since the organism itself, via the uptake, depuration and metabolic processes, will have eliminated the non-residue forming chemicals from the tissue prior to extraction. For this reason the effluent option may detect a narrower range of residue forming chemicals.

Another limitation of the effluent option also arises as a result of the analytical methods used. Hydrocarbons, such as those found in lubricants, oils and gasoline, are not removed by the aforementioned clean up step. These chemicals rarely form residues in aquatic organisms but do cause interferences in the analyses. Specifically, these types of compound prevent successful GC/MS analysis of the third fraction of the effluent extracts. For this reason, application of this option to discharges expected to contain very large numbers of hydrocarbons, such as refineries, is not recommended. However, since this type of chemical does not form residues, the tissue residue option is not subject to this analytical interference and may be applied.

A final consideration in the selection of the assessment option is the complexity for implementation of the two options. The analytical procedures used in the tissue residue option are

somewhat less extensive than those for the effluent option since the extraction method is simpler and the use of HPLC fractionation is not required. However, this is somewhat offset by the more elaborate field sampling design and implementation which may be required for the tissue residue option in comparison to the collection of effluent samples for the effluent option.

1.6 Timing and Mechanisms for Assessment

EPA recommends that for an initial assessment the effluent bioconcentration evaluation and/or fish tissue evaluation be conducted by the selected permittees from one to four times over a period of a year. If the effects of seasonality or effluent variability are of relatively low concern, then a sampling frequency of once per year would be appropriate. On the other hand, if seasonal or effluent variability are of concern, these assessments should be scheduled accordingly more frequently, four times per year, to address this variability. The sampling results should be recorded and used for the effluent characterization step of the permitting process (described in Chapter 6). Since average concentrations are of most concern, composite rather than grab samples should be used in the assessment.

In order for the regulatory authority to make a determination on the need to develop permit limits for bioconcentratable contaminants for a given facility at the time of permit reissuance, the permittee would need to be required to conduct these assessments one year in advance of permit reissuance. This would allow time for the required samples and analyses to be conducted and the results submitted to the regulatory authority prior to the time of permit reissuance.

Alternatively, the requirement to conduct these assessments may be placed in the permit at the time of reissuance and if limits are determined to be needed, then the permit may be reopened or the limits may be placed in the permit at the next reissuance. Effluent or fish tissue evaluations may also be required in permits annually if the regulatory authority has reason to believe a change in process or discharge may occur which would result in the appearance of new chemicals not found in the initial screening.

The regulatory authority should determine which of these time frames is most appropriate for a given facility based on the site specific information available for that discharge. For dischargers that are considered of high priority for this assessment, EPA recommends dischargers be required to begin to conduct these analyses in advance of permit reissuance and provide the results for review at the time of permit reissuance.

1.7 Field Validation of Bioconcentration Protocol

Because the regulatory application of this assessment procedure will direct regulatory decisions on the control of bioconcentratable pollutants, EPA has designed and implemented a series of field applications to establish the validity of this approach. The field validation study is designed to show that the bioconcentration procedures are correlated to the bioconcentratable contaminants identified in the effluent discharges, and with the approximate concentrations in organisms collected at the associated discharge sites. The validation studies will be carried out at a series of sites with both saltwater and freshwater receiving waters. A more detailed description of the study designs and of the results of these field validations is contained in Appendix I.

EPA initiated these studies to show that this methodology can predict, with reasonable accuracy, the concentrations of bioconcentratable pollutants in fish tissues when organisms are exposed to these pollutants in the environment. The reasonable demonstration of accurate predictions in several situations will be considered to establish the correlation between effluent release of bioconcentratable contaminants and tissue contamination.

1.8 Evaluation of Contaminants in Sediments

The assessment of sediment for bioaccumulative contaminants, described in Chapter 3, can determine the presence, identity, and concentrations of pollutants in sediment samples subjected to contamination from different sources. Since sediments can accumulate these types of pollutants over relatively long periods of time, the bioaccumulative chemicals may be present in greater concentrations in sediment than in a given effluent sample. In some cases, this may facilitate detection of contaminants which are present in an effluent or other sources at very low concentrations or are only released periodically. For point and non-point sources, the results of the sediment evaluation can help influence the investigation of potential problem areas. Data from sediment evaluations may also be used to determine the spatial extent of a remediation area, monitor the benefits derived from remediation activities, help pinpoint responsible parties, evaluate the impacts of depositing contaminated sediments in aquatic environments, and evaluate the success of remediation activities.

CHAPTER 2

Principles of Bioconcentration Control

2.1 Concept of Bioconcentration and Bioaccumulation

Fish, shellfish, and wildlife act, in a sense, like magnets for certain types of chemicals. Like the attraction of iron filings to a magnet, organisms, when exposed to certain types of chemicals, will collect and retain these chemicals in their bodies. The amount of chemical collected in an organism can become very high and on a concentration basis the tissues of an organism can achieve concentrations which are orders of magnitude larger than those for the chemical in the environment.

The accumulation process, i.e., the collection and retention of the chemical in the organism, occurs with all concentrations of the chemical in the environment. For aquatic organisms, this accumulation process is referred to as either bioconcentration and/or bioaccumulation. Chemicals which have the propensity to accumulate in aquatic organisms are, in general, called bioconcentratable.

In this document, the definitions relating to bioconcentratable chemicals, as proposed by Brungs and Mount [3] and summarized by Murty [4], are used. These definitions are:

> "Bioconcentration is the process by which a compound is absorbed from water through gills or epithelial tissues and is concentrated in the body; bioaccumulation is the process by which a compound is taken up by an aquatic organism, both from water and through food; and biomagnification denotes the process by which the concentration of a compound increases in different organisms, occupying successive trophic levels."

In this document, these terms will always be used according to this definition. In the literature, these terms are often used interchangeably and may cause some confusion.

In comparing the bioconcentration and bioaccumulation processes, concentrations of chemicals in aquatic organisms resulting from bioaccumulation will always be equal to or greater than the tissue concentrations caused by the bioconcentration process above. For some predatory fishes, the difference in tissue concentrations can approach two orders of magnitude. The structure of the food chain for the organism and n-octanol/water partition coefficient of the residue forming chemical significantly influence the level of bioaccumulation. Further information about the bioconcentration and bioaccumulation is available in the literature [4-9].

2.2 Concern for Bioconcentration and Bioaccumulation

Chemical residues caused by bioconcentration and bioaccumulation processes in fish and shellfish can cause serious health problems for their predators, i.e., humans and wildlife. These processes occur at exposure concentrations that are not by themselves toxic to the aquatic organisms. Thus, ingestion of contaminated fish by humans and wildlife can result in toxic doses of the residue forming chemicals even though perfectly healthy looking fish are consumed. This route of exposure is direct and cannot be controlled for wildlife after a chemical is released into the environment. For human consumers, this exposure can be limited by banning commercial fishing and issuing fish advisories. Currently, the issuance of such bans and advisories by States is increasing significantly.

2.3 Bioconcentration Factors

The potential for a chemical to bioconcentrate in aquatic organisms is quantitatively expressed using the bioconcentration factor (BCF). The BCF is defined as the ratio of the concentration of the chemical in the organism to the concentration in water surrounding the organism.

BCFs can be calculated from experimental measures by dividing the measured concentration of the chemical in the exposed tissue by the measured concentration of the chemical in the exposure water, after a steady-state condition is reached [10]. In equation form:

$$BCF = \frac{\text{Concentration in Tissue}}{\text{Concentration in Water}}$$

Bioconcentration factors can also be calculated by dividing the uptake rate, k_1, by the elimination rate, k_2 [11]. In equation form:

$$BCF = k_1/k_2$$

BCFs can also be estimated using structure-activity relationships based upon the relationship between the BCF and the n-octanol/water partition coefficient (log P) for organic chemicals [10,12-14].

BCFs for organic chemicals cover a wide range of values, depending upon the characteristics of the individual chemicals. Some chemicals have BCFs of one million or greater. BCFs for most compounds have been found to be constant over a wide range of exposure concentrations [15]. The BCFs of non-metabolized, highly persistent, lipophilic organic chemicals are well-correlated with their n-octanol/water partition coefficients [10, 12-14]. Compounds with low BCFs reach steady-state residue concentrations relatively quickly [16], whereas compounds with high BCFs may never reach steady-state. Compounds with low BCFs

are more water soluble and have shorter retention times on a reverse phase high performance liquid chromatography (HPLC) column than compounds with higher BCFs.

2.4 Bioaccumulation Factors

The potential for a chemical to bioaccumulate in aquatic organisms is quantitatively expressed using the bioaccumulation factor (BAF). The BAF can be calculated from experimental measures by dividing the total uptake rate from water and food, k_1, by the elimination rate of the chemical, k_2 [11]. In equation form:

$$BAF = k_1/k_2$$

The BAF is dependent upon the structure of the food chain for the organism of concern and the log P value of the chemical. For ecosystems with different food chains, the same organism may have substantially different BAFs due to differences in feeding habits of the organism, the feeding habits of their prey, the feeding habits of prey that their prey eats, etc. [17-19].

For chemicals with log P values below 5.0, BAFs and BCFs are equal regardless of the ecosystem structure. For these chemicals, the bioconcentration process is more important than the bioaccumulation process from food. For chemicals with log P values ranging from 5.0 to 7.0, bioaccumulation from food becomes more important with increasing log P value and complexity of the food chain [17,18]. For chemicals with log P values greater than about 7.0, there is some uncertainty regarding the degree of bioaccumulation, but generally, food chain structure appears to become less important due to slow uptake rates, low bioavailability, and "dilution" by growth for these types of chemicals.

In this document, rather than attempting to define BAFs, bioaccumulation is accounted for by "adjusting" the BCF using a food chain multiplier (FM) for the organism of concern. The bioaccumulation and bioconcentration factors for a chemical are related as follows [17,18]:

$$BAF = FM * BCF$$

By incorporating the FM and BCF terms into the equations for development of reference concentrations, bioaccumulation is included. FMs are provided in tabular form as a function of log P and food chain position (trophic level) of the organism.

2.5 Log P-Log BCF Relationship

For organic chemicals, bioconcentration is a partitioning process between the lipids of the organisms and the surrounding water. This mechanism, proposed by Hamelink et al. [20], has gained general acceptance because the BCF and the n-octanol/water

partition coefficient (P) are strongly correlated [10,12-14,21-23]. The general form of this correlation is:

Equation 1.1) $\log BCF = A \log P + B$

where, A and B are constants derived using measured experimental data.

However, for chemicals with log P values higher than approximately 6.0, the measured BCFs are often lower than those predicted. Gobas et al. [24] have attributed this over-estimation of the BCF to violations of the conditions required for a BCF determination. These violations are caused by slow uptake rate, low bioavailability, and "dilution" by growth for the chemical of interest.

Numerous log BCF-log P correlations have been developed and reported in the literature for small groups of chemicals for many species of aquatic organisms [25]. In this guidance, a correlation based on 122 BCF values for 13 species of freshwater and saltwater species is used [22]. Zaroogian et al. [26] have shown that the correlation is the same for both freshwater and saltwater species. This correlation predicts BCFs for tissues with 7.6% lipid content. The equation expressing the relationship is:

Equation 1.2) $\log BCF = 0.79 \log P - 0.40 \qquad (r^2 = 0.86)$

Since the BCF is in part dependent on the lipid content, a correction for lipid content is needed for different species or for different edible portions. Equation 1.3 incorporates this correction for organisms and tissues with a 3.0% lipid content:

Equation 1.3) $\log BCF = 0.79 \log P - 0.40 - \log (7.6/3.0)$

In this guidance document, BCF values will be presented and discussed on a 3.0% lipid content, typical of fillets, unless otherwise noted. Equation 1.3 can be used for prediction of BCF values for other lipid contents by replacing the 3.0% with the desired value lipid content (in percent).

The equation derived by Veith et al [22] has 95% confidence limits for the prediction of an individual BCF of approximately one order of magnitude and has 95% confidence limits for the predicted mean BCF value of approximately 5%. Thus, for a chemical with an estimated BCF of 100, the 95% confidence limits for this value would range from approximately 10 to 1000. For BCFs of extremely hydrophobic chemicals, i.e., chemicals with log Ps greater than 6.5, over estimation of the BCF value by log P regression equations will be greater as the log P increases above 6.5 [24].

2.6 Measured versus Calculated Bioconcentration Factors

EPA recommends that BCF values calculated from the log P - log BCF relationship be used in the calculation of the reference tissue and ambient concentrations. Use of calculated BCF values will be necessary in most cases because carefully measured values will not be available and the cost to measure these properly will be high. However, since the methods for calculating BCF values do not include metabolism (which will reduce the BCF), these values will be conservative and measured values may be necessary to get more precise values for chemicals that metabolize.

When measured BCF values are used, the utmost caution is necessary when selecting an appropriate BCF value. For most chemicals great variation in measured BCF values exists in the literature. This variability arises from inappropriate experimental conditions and/or poor analytical measurements. Questionable BCF values exist when either of these conditions exist during the BCF determination. Many of the literature BCF values will be inappropriate for use in the guidance procedures due to the above problems. Unfortunately, detection of incorrect BCF values is made difficult because experimental conditions are often incomplete. Methods used should follow ASTM's "Standard Practice for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Mollusks, 1022-84" [27]. Experimental measurements should include: control residues, measured exposure concentrations, analytical recoveries for both tissue and exposure water quantification methods, wet weight tissue concentrations, lipid content of the tissues, use of flow-through exposures, and demonstrated attainment of steady-state conditions. The ASTM method recommends that the exposure duration continue for 28 days or until apparent steady-state is reached. Because steady-state can depend on the species, life stage, physiological condition, test conditions, etc., it is difficult to set exposure time to a uniform length. The ASTM method also recommends that all organisms be of uniform size and age. Use of a juvenile or older life stage organisms is recommended.

2.7 Analytical Chemistry and Bioconcentration Control

The analytical methods provided in this document have a fundamental difference from other EPA methods. The methods described in this document look for a certain type of chemical in the sample and when a component with the proper characteristics is detected by the GC/MS, it is identified and quantified. In essence, these methods survey/screen/inspect the sample and provide a listing of the "bioconcentratable" chemicals in the sample. In contrast, other EPA methods are chemical specific and these methods are designed to quantify a specific predetermined chemical. Chemical specific or target chemical analyses will only provide information about the individual chemicals of interest.

This fundamental difference requires that the data generated by the assessment methods be viewed in a different light than the data generated by target chemical analysis. With target chemical analyses, the identity of the chemical is known and concentration of the chemical is measured accurately. With the assessment methods described herein, the reported identity and concentration of a chemical are less certain. This occurs because model compounds are used to quantify the identified chemical and because mass spectral algorithms for identifying unknown chemicals are, currently, imprecise.

The use of model compounds for quantifying the identified chemicals is required since we do not know a priori what chemicals are in the sample. Quantifications based upon the model compounds assume that analytical recoveries and mass spectral responses are the same for the model and identified chemicals. These assumptions can be expected to cause error in the quantification of no worse than one order of magnitude. The largest part of the overall error in quantification is caused by the wide differences in mass spectral responses among the individual compounds [28].

These uncertainties in quantification and identification of the GC/MS components are eliminated in later steps in the guidance approach. With the tissue and effluent assessment options, confirmation analyses are required **before** development of a RAC, wasteload allocation and when necessary, permit limits for a chemical. Confirmation analyses provide conclusive identification and substantially more accurate quantification for the GC/Ms component of interest. In addition, with the tissue option target chemical analyses on the effluent will be required for the chemical of interest prior to developing wasteload allocations and permit limits. In general, target chemical analysis techniques have much smaller quantification errors than the analytical procedures included in this guidance. For example, EPA method 1625 has initial method quantification accuracy requirements for bioconcentratable chemicals which are typically no worse than a factor of 2.

Mass spectral library searching algorithms are used to assign tentative identifications to components detected in the GC/MS analysis of the prepared sample extracts. Two libraries of mass spectral data are used in the assessment methods, the Chemicals of Highest Concern (CHC) and the EPA/NIH/NBS mass spectral libraries. These algorithms compare the mass spectra of the GC/MS component to those in the libraries and the ten best fitting/matching tentative identifications with fits/matches of 70% and greater are reported. These identifications are considered tentative because a mass spectra by itself is not enough information to conclusively identify a GC/MS component/peak. Multiple tentative identifications are provided for each component because the correct identification is often not the best matching tentative identification. This imprecision in the searching algorithms has important implications for evaluation of the reported data.

Computer algorithms for identifying unknown mass spectra via library searching are often categorized as either forward or reverse searching. In general, reverse searching algorithms have demonstrated advantages for identifying unknown mass spectra when the unknown is not chemically pure [38]. With GC/MS analyses, mass spectral data can never assumed to be pure and thus, the use of reverse searching algorithms is recommended when available. Unfortunately, some GC/MS systems do not have reverse searching algorithms. In these cases, library searching should be performed using the default algorithm provided by the manufacturer of the GC/MS system.

To evaluate the data generated by the assessment methods, **all tentative identifications must be evaluated for each component.** This requirement is **absolutely necessary** since the best matching (fitting) tentative identification is often not the correct identification for the component. Analyses to confirm the true identity of the chemical are performed **after evaluation** of the analytical data. A chemical would be considered confirmed when the retention time on the GC/MS column and mass spectra of the component are identical between the sample and a standard that is made from the pure chemical.

The analytical methods provided in this document have been designed to achieve low levels of detection. Minimum levels of detection are assured in these methods by the use of surrogate compounds. These chemicals are placed into the sample at low concentrations at the start of the analysis, 100 ng/l and 5 ng/g for the effluent and tissue procedures, and detection of these chemicals in the GC/MS analysis of the prepared extracts ensured that these levels of detection are achieved. Detection limits for the methods are estimated to be approximately 10 ng/l and 1 ng/g, respectively. These levels of detection will require substantially better analytical technique than currently used by many contract laboratories which perform standard EPA methods. These methods can be performed successfully, on a routine basis, with the use of good low level residue techniques.

2.8 Chemicals of Highest Concern

The analytical methods for the residue and effluent options determine the presence of bioconcentratable chemicals in tissues and effluents. To identify compounds, GC/MS analyses are performed on sample extracts and all peaks/components in the data are compared to two libraries of mass spectral data. These libraries are the Chemicals of Highest Concern (CHC) and EPA/NIH/NBS mass spectral libraries.

The CHC library consists of approximately 30 chemicals which pose serious risks to human health due to high toxicities and high potential to bioconcentrate. These characteristics cause residues in fish and shellfish which are of concern even when these chemicals are present at very low concentrations in the receiving water. With either assessment option, detection of

these chemicals will be difficult. To increase the chance of detecting these chemicals, all components in the GC/MS data are compared to the CHC library to determine if any of these chemicals are present. If any of the chemicals in the CHC library are found, efforts to evaluate and control these chemicals should be of the highest priority. The CHC library (Table 2.1) was compiled by selecting chemicals which produce residues of concern at very low ambient concentrations.

TABLE 2-1

CHEMICALS OF HIGHEST CONCERN LIST

CAS number	chemical name
50-29-3	p,p'-dichlorodiphenyltrichloroethane (DDT)
57-74-9	chlordane
58-89-9	hexachlorocyclohexane (lindane)
60-57-1	dieldrin
70-30-4	hexachlorophene
72-54-8	p,p'-dichlorodiphenyldichloroethane (DDD)
72-55-9	p,p'-dichlorodiphenyldichloroethylene (DDE)
76-44-8	heptachlor
91-94-1	3,3'-dichlorobenzidine
95-94-3	1,2,4,5-tetrachlorobenzene
101-61-1	4,4'-methylene bis(N,N'-dimethyl) aniline
115-32-2	dicofol
117-81-7	bis(2-ethylhexyl)phthalate (BEHP)
118-74-1	hexachlorobenzene
309-00-2	aldrin
319-84-6	alpha-hexachlorocyclohexane (alpha-HCH)
319-85-7	beta-hexachlorocyclohexane (beta-HCH)
608-73-1	technical-hexachlorocyclohexane (t-HCH)
608-93-5	pentachlorobenzene
924-16-3	N-nitroso-di-n-butylamine
1024-57-3	heptachlor epoxide
1746-01-6	dioxin (2,3,7,8-TCDD)
2104-64-5	ethylp-nitrophenylphenylphosphorothioate(EPN)
2385-85-5	mirex
8001-35-2	toxaphene
39515-41-8	danitol
11096-82-5	polychlorinated biphenyl 1260
11097-69-1	polychlorinated biphenyl 1254
11104-28-2	polychlorinated biphenyl 1221
11141-16-5	polychlorinated biphenyl 1232
12672-29-6	polychlorinated biphenyl 1248
12674-11-2	polychlorinated biphenyl 1016
53469-21-9	polychlorinated biphenyl 1242

CHAPTER 3

Assessment Options for Bioconcentratable Contaminants

In Figures 3.1 and 3.2, detailed flowcharts for the assessment process are presented for the tissue and effluent options. These flowcharts diagram the data generation, decision points, and evaluation logic for each option. In this chapter, the discussion presented will start with sample collection and proceed as if one was performing the assessment process. The outputs from this process for either option are lists of identified bioconcentratable chemicals which require reference concentration development (Chapter 4), waste load allocation (Chapter 5), and when necessary, permit limits (Chapter 6).

The discussion presented in this chapter will provide detailed information on the decision points, analytical procedures, and underlying rational used by the assessment options. Some of the section titles in this chapter are the same as the blocks in the flowcharts. This one to one correspondence is intentional and is intended to aid readers of this document to quickly find discussion relevant to the flowcharts. Also included in this chapter is an abbreviated discussion on the analytical procedure for assessing the presence of bioconcentratable chemicals in sediments. The detailed analytical procedures for the tissue, water, and sediment matrices are provided in Appendices A, B, and C.

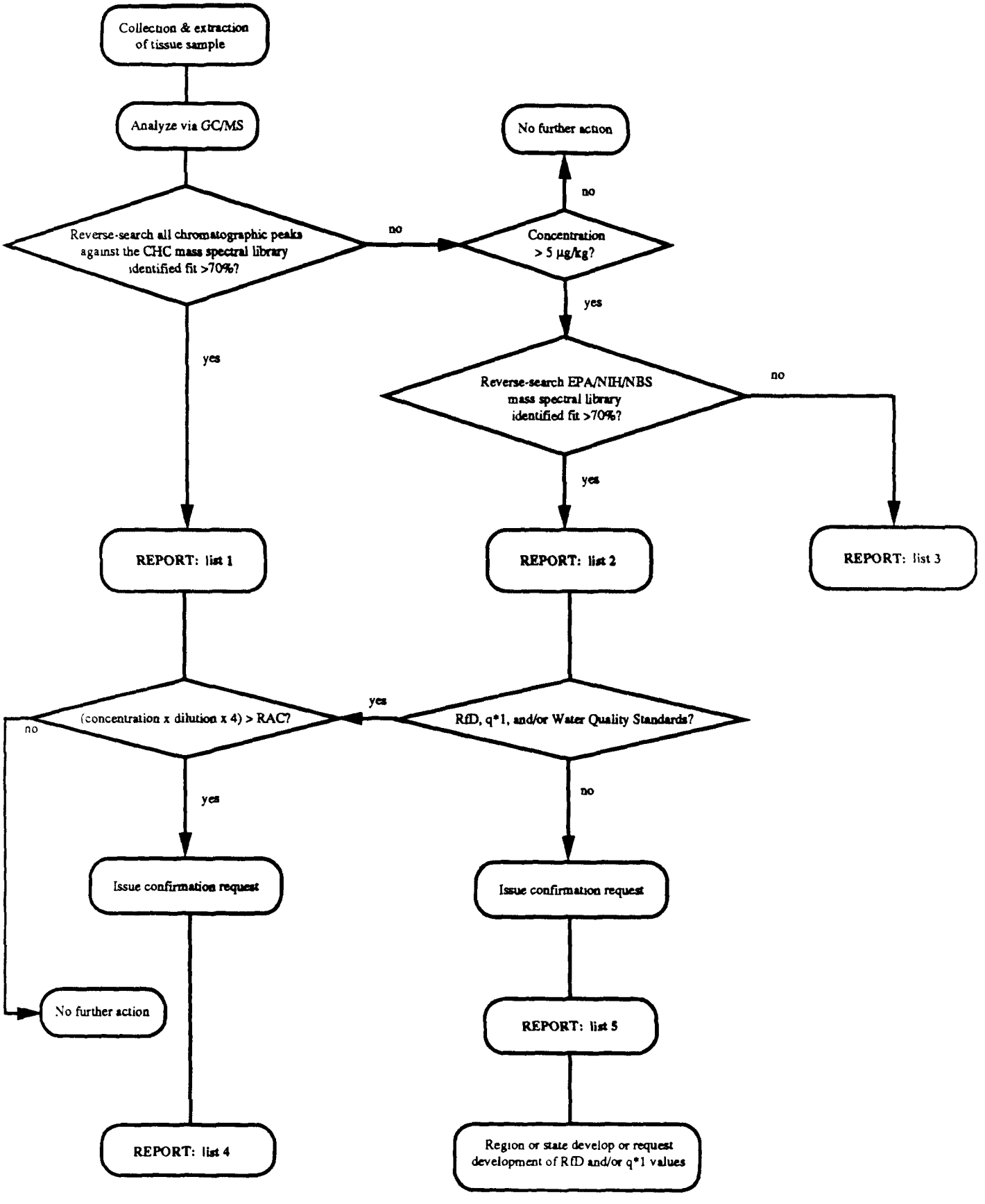
3.1 Tissue Residue Option

3.1.1 Sampling Considerations

For successful implementation of the tissue option, samples which are representative of the organisms in the receiving water are necessary. Analyses performed on representative samples allows the determination of the mean concentrations for residue forming chemicals in the receiving water population. Due to natural variation, haphazard sample collection can result in effluent permitting situations which are not protective for bioconcentratable chemicals because the assessment was performed on unrepresentative tissue samples.

Sampling designs which result in representative tissue samples must consider: 1) when to sample (time of year and frequency), 2) how to sample (number of species, age, size, type of species, number of organisms per sample), and 3) where to sample (number of sampling stations and to consider exposure to

Figure 3.1
TISSUE ANALYSIS



sources and effects of dilutions and mixing zones). Complete descriptions of individual programs for tissue residue monitoring programs are available from many States and other groups [44].

When to Sample

Chemicals that form residues in aquatic organisms have, in general, a longer half-life in the organisms than in effluents and receiving waters. This difference occurs in part due to the relative time scales associated with bioconcentration and bioaccumulation processes, i.e., weeks to years, in comparison to hydrologic processes associated with effluents and receiving waters, i.e., hours to days. Consequently, residue measurements on indigenous organisms from a receiving water provides information on chemical exposure for a longer time frame, i.e., weeks to months, than does chemical analyses on effluent samples which are typically 24 hour composites.

Since the objective of this guidance is to protect humans from unacceptable residue consumption, a prime consideration for time of sampling is the period when most organisms are harvested for consumption. In the absence of other evidence, sampling should be done after periods of normal or sub-normal dilution of effluents and if possible, include at least <u>two</u> seasons of the year. Seasonal variation in residues concentrations may occur from changes in food type, feeding level, changes in metabolism, and reproductive stages for the species. There should be no need to sample organism residues more than monthly; one sample each quarter is probably more inclusive than four samples taken in consecutive months.

How to Sample

Two species of organisms which are consumed by humans should be collected from a receiving water and these species should have different feeding habits, occupy different habitat niches and represent different physiology. One species at least should be a predator since bioaccumulation via the food chain may be important for the receiving water. When possible, a vertebrate and invertebrate species is advised since major differences in metabolic activity exist between these groups [30].

In general, small but fully adult organisms are most likely to contain the highest residue for a given exposure (if a correction is made for lipid content) and thus, are recommended. It is not necessary to sample organisms actually consumed by humans; however, it is important to include information for species consumed by humans in the subsequent assessments of the residue data. In some situations, the problems of collecting or availability of organisms will dictate the species to be collected. When possible, 10 organisms should be collected per sample. By using 10 organisms per sample, a better estimate of the average residue concentrations in the receiving water organisms can be obtained than with samples based upon 1 or 2 organisms.

Where to Sample

The location of sampling is a site-specific decision and there are trade-offs to consider with regards to proximity to the sources and complexity of the receiving water. In general, one would expect the highest residues closest to the outfall. However, if bioaccumulation via the food chain is important, depositional zones downstream of discharge might produce higher residues in the indigenous organisms. Collection of organisms in the mixing zone is <u>not</u> recommended since these organisms might have unusually low residue concentrations. These organisms may avoid the mixing zone or reside in it for very short time periods. Additionally, if food chain accumulation is an important source for the residue forming chemical, sufficient time for bioaccumulation may not occur there. On the other hand, if locations too distant from the discharge are sampled, dilution, degradation, or sorption may cause residues to be lower.

The ideal sampling design would consist of sites where the organisms are confined by the geography of the receiving water, where the exposure of the organisms to the discharge is well established, and where the site is close to the discharge. Geographical features which could confine organisms include dams and waterfalls on streams and rivers and salinity gradients in marine estuaries. The sampling of sessile organisms can also be used to define exposure as well as limit mobility of organisms.

With well defined sampling sites, one well placed site is probably enough for a successful assessment with the tissue option. As the complexity of the receiving water increases, i.e., more dischargers and mixing in the receiving water becomes less defined, additional sites might be necessary. These additional sites should, possibly, be placed downstream of the first site. If additional sites are required, increased costs for performing this option will occur. A possible alternative to evaluating these additional sites at the same time would be to perform assessments on a rotating basis, i.e., each quarter a different site is assessed.

3.1.2 <u>Compositing of Organism Samples for Analytical Procedure</u>

Concentrations of the bioconcentratable chemicals in the receiving water organisms will not be exactly the same among individual organisms due to natural variation. To obtain the best estimate of the average residue concentration for an individual species, it is recommended that a tissue sample consist of 10 organisms and that equal portions of each organism be used in the tissue composite. If more than one species of organisms is collected for a sampling site, **each species should be composited separately.**

If fish fillets are to be composited, equal portions by mass from each fillet would be combined. For whole fish, two options are available. One of these approaches would consist of grinding each fish separately and then, subsampling equal portions by mass of the ground tissues to obtain the composite sample. The other approach would consist of selecting fish of equal mass and compositing them. For this approach, the coefficient of variation for the masses of the whole fish should not exceed 10%. With whole fish, removal of the guts and possibly the head before grinding and/or subsampling may be desirable since human consumers, in general, rarely ingest these parts of the organisms.

For composites from the fillets and whole fish, thorough grinding of the tissue to yield homogenous tissue samples is essential. In general, homogeneous grinding of tissue samples is obtained by passing the tissue composite through a grinder three times. Ground tissues are stored at -10°C in solvent rinsed glass jars sealed with aluminum foil or Teflon lined lids until analysis.

If less than 10 individuals per sample were collected, compositing would be performed as described except with fewer individuals per composite sample.

3.1.3 Extraction of Composite Tissue Samples

The tissue sample is extracted using standard residue chemistry techniques. This process consists of mixing 20 grams of the tissue after thawing with enough anhydrous sodium sulfate, 100 to 140 grams, to dry the sample. Half of this mixture is placed into a Soxhlet extraction thimble and three surrogate chemicals are spiked onto the tissue in an extraction thimble. The remaining sodium sulfate/tissue mixture is then placed into the extraction thimble and the extraction thimble is placed into a Soxhlet extractor body. The tissue is extracted for a minimum of 18 hours using a 50:50 mixture of methylene chloride and hexane. The extract is concentrated using a Kuderna-Danish concentrator and its volume is adjusted to 10.0 mls. A portion of the extract, i.e., 0.50 or 1.0 ml, is removed from the extract and is placed into a tared weighing pan. After evaporation of the solvent in the weighing pan, the pan is placed into a 105°C oven to dry the sample. The dried sample is reweighed and percent lipid content of the tissue is determined.

The surrogate chemicals, d_{10}-biphenyl, $^{13}C_6$-1,2,4,5-tetrachlorobenzene, and $^{13}C_6$-hexachlorobenzene, are added to the tissue at a 5.0 µg/kg concentration prior to extraction of the sample. These chemicals are referred to as surrogates because their behavior mimics that of bioconcentratable chemicals in the analytical procedure. The surrogate chemicals are used for quality control and quantification in this analytical procedure. This procedure is presented in detail for laboratory use in Appendix A.

3.1.4 Gel Permeation Chromatography/Silica Gel Cleanup of Tissue Extract

The remaining volume of the extract, i.e., 9.0 or 9.5 mls, is adjusted to a suitable volume for gel permeation chromatography (GPC), e.g. 5.0 ml. The extract is injected onto the GPC column and lipids are removed from the extract. The GPC process might have to be performed multiple times using aliquots of the extract because some GPC columns can handle limited amounts of lipid before coming overloaded. GPC is typically performed using automated analytical instrumentation. This instrumentation injects the extract onto the GPC column, performs the chromatography process, and collects the proper eluate from the GPC column which contains the chemicals of interest.

After the lipids are removed from the extract, the extract is concentrated to 0.5 ml. Silica gel chromatography is then performed on the extract and this process removes cholesterol like chemicals from the extract. Silica gel chromatography is performed on a 2.1 gram column of 1% deactivated silica gel. The column, after transfer of the extract to the top of the column, is eluted using a 15:85 methylene chloride:hexane mixture. The solvent eluting from the column is collected and concentrated to 0.10 ml.

The discussion presented to this point, Sections 3.1.1 through 3.1.4, refers to the "Collection & Extraction of Tissue Sample" box in the tissue residue option flowchart, Figure 3.1. The sampling and analytical procedures, in most cases, will be performed by the discharger or contractor.

The above procedure is presented in detail for laboratory use in Appendix A.

3.1.5 Analyze via GC/MS

Gas chromatography/mass spectrometry (GC/MS) analyses are performed on the prepared tissue extracts using standard residue techniques. Prior to GC/MS analysis, an internal standard, d_{12}-chrysene, is added to the sample extract to calibrate the response of the mass spectrometer. Sample analyses are performed on a 30 m capillary column with a temperature program, e.g. 50-175°C at 10°C/min and then 175-275°C at 5°C/min, 275°C for 20 min. Mass spectral data are collected using full scan electron impact ionization mass spectrometry. After GC/MS analysis, the extract is saved for confirmation analysis.

After analysis of the sample extracts and standards, standard curves are calculated for the three surrogates; d_{10}-biphenyl, $^{13}C_6$-1,2,4,5-tetrachlorobenzene, and $^{13}C_6$-hexachlorobenzene; using an internal standard method. These curves are used to quantify the surrogates in the sample extracts. These quantifications are used to determine the percent recovery for

each of the surrogate chemicals. All other peak/components in the GC/MS data are quantified using the standard curve for the $^{13}C_6$-hexachlorobenzene.
The above procedure is presented in detail for laboratory use in Appendix A.

3.1.6 Library Searching using the Chemicals of Highest Concern Mass Spectral Library

All chromatographic peaks in the GC/MS data are compared with the Chemicals of Highest Concern (CHC) mass spectral library (see Table 3.1). Peaks with fits/matches of 70% and greater are considered tentatively identified. For each tentatively identified component, a list of the best mass spectral library identifications (up to a total of ten identifications) is reported along with the percent fit values, CAS number of the tentative identification, GC retention time, and the concentration for the GC/MS component. This report is identified as Report 1 in the tissue residue option flowchart, Figure 3.1.

The CHC mass spectral library can, in nearly all cases, be derived from the EPA/NIH/NBS mass spectral library using the software on the data system of the GC/MS system. The recommended 70% library searching fit criterion was chosen based upon best scientific judgement and is subject to modification by the regulatory authority.

Computer algorithms for identifying unknown mass spectra via library searching are often categorized as either forward or reverse searching. In general, reverse searching algorithms have demonstrated advantages for identifying unknown mass spectra when the unknown is not chemically pure [31]. With GC/MS analyses, mass spectral data can never be assumed to be pure and thus, in the procedure, the use of reverse searching algorithms is **required** when available. Unfortunately, some GC/MS systems do not have reverse searching algorithms. In these cases, library searching should be performed using the default algorithm provided by the manufacturer of the GC/MS system.

3.1.7 5 µg/kg Tissue Concentration Decision Point

If a GC/MS component is not identified with the CHC mass spectral library search (the no arrow in the flowchart), the chemicals present at concentrations below 5 µg/kg are dropped from further consideration. The concentration of 5 µg/kg is used as the cut-off for further investigation because 5 µg/kg is the concentration of the surrogate compounds added to the sample prior to extraction. The surrogate concentrations represent the minimum level at which adequate quantitation can occur. The amount of uncertainty associated with a value which lies below the surrogate concentrations increases greatly with decreasing concentration. The purpose of this cutoff level is to prioritize unknown/non-CHC peaks for further investigation.

GC/MS components with concentrations greater than 5 µg/kg which are not identified with CHC mass spectral library are then library searched with the EPA/NIH/NBS mass spectral library.

3.1.8 Library Searching using the EPA/NIH/NBS Mass Spectral Library

GC/MS components not identified using the CHC library search and with concentrations greater than 5 µg/kg are library searched against the EPA/NIH/NBS mass spectral database. Peaks with fits/matches of 70% and greater are considered tentatively identified. For each tentatively identified component, a list of the best mass spectral library identifications (up to a total of ten identifications) is reported along with the percent fit values, CAS number of the tentative identification, GC retention time, and the concentration for the GC/MS component. This report is identified as Report 2 in Figure 3.1.

For those components with fits/matches less than 70% but greater than 25%, the two best mass spectral library identifications along with the percent fit values, the CAS number of the tentative identifications, GC retention time, and the concentration are reported for each the GC/MS component. For GC/MS components with fits/matches less than 25%, the concentrations and GC retention times for these components should be reported and the components labeled as being "unknown". This report is identified as Report 3 in Figure 3.1. The tentative identifications listed in Report 3 are of less certain reliability and these results are provided for informational purposes only.

The EPA/NIH/NBS mass spectral library is available from the U.S. Government Printing Office (941 North Capitol St. N.E., Washington, D.C. 20401). In addition, most GC/MS manufacturers have this library available in a form suitable for their respective instruments.

The recommended 70% library searching fit criterion was chosen based upon best scientific judgement and is subject to modification by the regulatory authority. As with the CHC library searching process, the use of reverse searching algorithms is strongly suggested when available. In cases where reverse searching algorithms are not available, library searching should be performed using the default algorithm provided by the manufacturer of the GC/MS system.

3.1.9 Analytical Summary

With the generation of the three reports, Reports 1, 2, and 3, the procedures presented in Appendix A have been performed for the tissue assessment option. The discussion presented above in Section 3.1.1 through 3.1.8 outline this procedure and the

decision points for processing the GC/MS data collected on the tissue samples. Example data and reports are provided in Appendix J.

Reports 1, 2, and 3 as well as the QA/QC report for a tissue analysis will be sent to the regulatory authority. The regulatory authority will evaluate the data and decide which tentative identified chemicals will need confirmation by performing the tasks in the two subsequent decision rectangles labeled, "RfD, q1*, and/or Water Quality Standard?" and "(concentration x 4) > RTC".

3.1.10 RfD, q1*, and/or Water Quality Standard?

This decision rectangle in the tissue assessment option, Figure 3.1, simply asks the question, "Is there a Water Quality Standard or sufficient information (RfD, Q1*) to develop a RAC available for a tentative identification listed in Report 2?". The purpose of this decision is to identify the chemicals for which there is information to later develop NPDES limits. For each GC/MS component, up to 10 tentative identifications might be reported. For each of the tentative identifications, the above question must be answered. If any of the tentative identifications for a GC/MS component have a RfD, q1*, and/or Water Quality Standard available, the evaluation process for that component would proceed to the "(concentration x 4) > RTC" decision rectangle, the yes arrow in Figure 3.1. Further information concerning the Rfd, q1*, and water quality standards are presented in Chapter 4 of this guidance.

If none the tentative identifications have the required information for a single GC/MS component, the regulatory authority would issue a request for confirmation of that GC/MS component to the discharger (the no arrow leaving this decision rectangle in Figure 3.1).

This evaluation process is performed on all tentatively identified GC/MS components listed in Report 2. Furthermore, for each tentatively identified GC/MS component, each tentative identification must be evaluated.

3.1.11 (Tissue Concentration x 4) > RTC?

This decision rectangle in the tissue residue alternative, Figure 3.1, evaluates whether the measured concentration of a tentatively identified GC/MS component in the tissue sample is likely to be larger than the reference tissue concentration (RTC). This screening step compares, for tentatively identified chemicals, the residue concentration times 4 to the RTC. The factor of 4 was developed by using the statistical analysis for

reasonable potential as presented in the Technical Support Document [32]. The table below was derived by using the TSD approach with the only change here being the use of the population mean rather than the population extreme value as the target concentration. The table shows the maximum expected difference between one sample and the mean effluent concentration based on the expected effluent variability as measured by the coefficient of variation (CV):

	Percent Confidence	
CV	95%	99%
0.2	1.4	1.6
0.4	1.9	2.5
0.6	2.5	3.6
0.8	3.2	5.1
1.0	3.9	6.9

EPA's treatability database suggests that variability in many effluents can be characterized by a CV of 0.6 or less. At this CV and with 99% confidence, the maximum ratio of the effluent population mean to one sample is 3.6. At 95% confidence, the ratio is less (2.5). Therefore, the multiplier of 4 provides a reasonable way to estimate the mean concentration based on one sample for this screening comparison to the RTC.

For each GC/MS component, this comparison is performed for each of the tentative identifications which have Rfd, q1*, and/or water quality standards. If this comparison is true for any of the tentative identifications for a GC/MS component, a confirmation request is issued by the regulatory authority to the discharger for that component (the yes arrow leaving this decision point). If this comparison is false for all of the tentative identifications for a GC/MS component, no further action (the no arrow leaving this decision point) is required for this component.

All tentatively identified GC/MS components in Report 1 are evaluated at this decision point. For tentatively identified GC/MS components in Report 2, only those tentative identifications with RfD, q1*, and/or water quality standards information are evaluated.

The calculation of the reference tissue concentrations (RTC) is described in detail in Chapter 4 of this document. RTCs can be calculated using human dose information, Rfd and/or q1*, and fish consumption rates by humans.

3.1.12 Issue Confirmation Request

In the tissue option, confirmation requests by the regulatory authority can be issued from two different decision points, see Figure 3.1. In either case, the discharger is required to confirm the identity of the GC/MS component tentatively identified in the tissue extract. The confirmation process is discussed in further detail in Section 3.3. The

results of the confirmation process are reported back to the regulatory authority. These results include the confirmed identity of the GC/MS component and its concentration. Since confirmation may, for certain complex samples, involve considerable analytical chemistry work and the associated costs may be high, the requirement for confirmation was placed in the option logic sequence so that only the chemicals most significant concern would require confirmation.

3.1.13 Outputs From Tissue Residue Assessment

The outputs from the tissue assessment process are two lists of identified bioconcentratable chemicals. The first list consists of chemicals from Reports 1 and 2 which require reference concentration development (Chapter 4), waste load allocation (Chapter 5), and when necessary, permit limits (Chapter 6). The second list consists of chemicals which arises from Report 2 and do not have RfD's, q1*'s, and/or water quality standards. In addition to this, the actual GC/MS Chromatograms should be included in the reported information. Development of Rfd, q1* and/or water quality standards are required or requested to determine if current residue levels pose serious risk to human consumers of fish and shellfish. Monitoring of these chemicals in effluents is suggested in order to establish discharge information.

It must be noted that chemicals in both of these lists form residues in aquatic organisms. These chemicals would have been found in indigenous organisms from the receiving water under consideration.

3.1.14 Caveats for the Tissue Option

The tissue assessment option will not detect all bioconcentratable chemicals which form residues in aquatic organisms. The analytical procedures outlined above will only detect nonpolar organic chemicals which can be successfully analyzed using GC/MS. These procedures are fairly robust; however, they do have limitations. Some of these limitations include sensitivity of the analytical method, our ability to identify unknown GC/MS components, and the lack of RfD and q1* information.

3.2 Effluent Option

3.2.1 Sampling Considerations

For successful implementation of the effluent option, samples which are representative of the discharger's effluent are necessary. Analyses performed on unrepresentative samples can lead to permitting situations which are not protective for bioconcentratable chemicals.

Sampling should be performed when the facility operations are typical. Sampling during unusual facility operations, i.e, storm events, plant shutdowns, production and treatment facility changes, should be avoided. 24 hour composite samples are recommended and grab effluent samples should be avoided. Volume of effluent collected per sample should be at a minimum of 10 liters; 12 liters is preferable. Samples should be stored in the dark at 4°C and be extracted within 7 days after collection.

3.2.2 Extraction of Effluent Sample

The extraction process consists of spiking a 10 liter effluent sample with three surrogate chemicals. After thorough mixing, the effluent sample is extracted using liquid-liquid extraction with hexane three times. The extract is dried using anhydrous sodium sulfate and concentrated using a Kuderna-Danish concentrator to approximately 10 mls.

The apparatus for performing the extraction of 10 liters of effluent will be laboratory specific. Depending upon the available equipment, different bottles, vessels, shakers, tumblers, etc. will be used by different laboratories for the extraction process.

The surrogate chemicals, d_{10}-biphenyl, $^{13}C_6$-1,2,4,5-tetrachlorobenzene, and $^{13}C_6$-hexachlorobenzene, are added to the effluent at a 100 ng/l concentration prior to extraction of the sample. These chemicals are referred to as surrogates because their behavior mimics that of bioconcentratable chemicals in the analytical procedure. The surrogate chemicals are used for quality control and quantification in this analytical procedure.

3.2.3 Sample Cleanup

To remove biologically-derived and some easily metabolized organic chemicals commonly found in effluents, an acid clean-up of the effluent extract has been included in the analytical procedure. These materials, e.g., fatty acids, fatty acid esters, sterols, phthlates, and phenolic plant materials, if not removed, cause serious interferences in common chemical residue analysis procedures. This procedure consists of constructing a column containing (bottom to top) glass wool, silica gel (2 g), sodium sulfate (2 g), 70% sulfuric acid solution (5 ml) on Celite (10 g), and sodium sulfate (2 g). After placing the sample extract on top of the column, the column is eluted with hexane. The eluate from the column is collected and then, concentrated using a Kuderna-Danish concentrator to approximately 10 ml. The extract is further reduced to 0.5 ml using a gentle stream of clean air.

This procedure which successful removes these interfering materials from the extract will also remove bioconcentratable

chemicals which are unstable in acidic conditions. **Consequently, bioconcentratable chemicals which are unstable in acidic conditions will not be detected with the effluent assessment procedure.**

The acid treatment of the sample causes reactions like hydrolysis of esters, cleavage of ethers, additions to olefins which changes the biologically-derived materials to polar organic materials. These materials are removed from the sample extract by passing the extract though a silica gel column. This column retains the polar materials and allows the nonpolar materials to pass through the column. This commonly used acid clean-up procedure [33] yields sample extracts which predominantly contain the chemicals of interest (i.e., nonpolar organic chemicals). In addition, this treatment allows significantly lower detection limits for the method since substantially fewer components are in the sample extracts during the subsequent steps of the procedure which utilize instrumental analysis (e.g. HPLC, GC/MS).

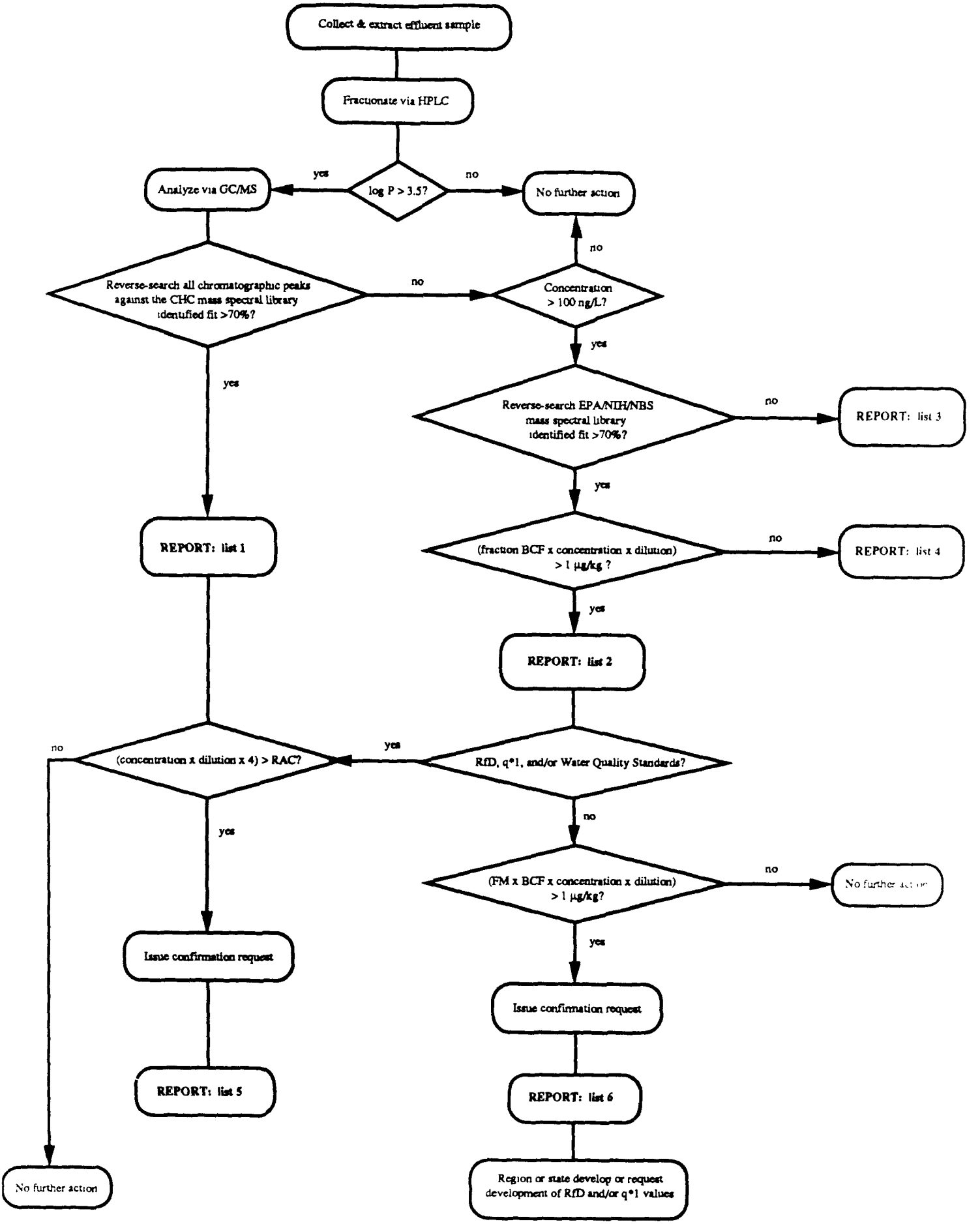
3.2.4 Fractionation via HPLC

The effluent extract after acid clean-up contains numerous chemicals with low potential to bioconcentrate. Since these chemicals are of lower concern, these chemicals are removed from the effluent extract using a well documented high performance liquid chromatography (HPLC) technique.

Chemicals with low potential for bioconcentration are defined in this guidance document as chemicals with log P values below 3.5. This bioconcentration threshold was derived by comparing the relative exposure for humans to bioconcentratable chemicals from the consumption of contaminated drinking water and fish. As the BCF increases above 100, the intake from fish will become proportionately greater than from water. This finding was derived using the average consumption amounts for drinking water of 2 liters/day, and for fish 20 g/day. EPA recognizes that this fish consumption rate is higher than that assumed in deriving §304(a) water quality criteria. This higher rate is used as a conservative approach to screen for toxicants which have the potential to be a problem. Pollutants screens for further analysis need not necessarily require further controls to meet water quality standards.

Therefore, the effluent assessment procedure in this guidance document was developed and optimized for organic chemicals with log P values of 3.5 and greater. This threshold corresponds to a BCF of approximately 100 on a 3% mean lipid content using the recommended equation for estimating a BCF from log P (Equation 1.3). This threshold value was selected to target those organic chemicals of greatest concern.

Figure 3.2
EFFLUENT ANALYSIS



To remove chemicals below the bioconcentration threshold, a relationship between log P and the retention time of chemicals on a reverse phase HPLC column is used to fractionate the effluent extract [34,35]. Fractionation of the effluent extract allows chemicals with log P values less than the bioconcentration threshold to be removed and allows the rest of the chemicals to be subdivided into 3 separate fractions. By subdividing the bioconcentratable portion of the effluent extract into sub-portions (fractions), an initial estimate for a chemical's BCF can be provided by using the average log P value of the fraction containing the chemical and the log P/log BCF relationship previously discussed.

The log P/retention time relationship is usually expressed in the following form [35-38]:

$$\log P = C + D * \log T_R$$

where C and D are constants derived for the HPLC system used and T_R is the corrected retention time for a chemical. By using the appropriate HPLC conditions (which are derived using the procedures specified in Appendix B), fractionation is performed by collecting the solvent eluting from the HPLC column during specified timed intervals after injection of the effluent extract onto the HPLC column.

Three fractions are collected from the HPLC column. These fractions have log P ranges of 3.5 to 4.5, 4.5 to 5.7, and 5.7 to 8.2. These log P ranges result in BCF value ranges of 91 to 560, 560 to 5000, and 5000 to 470,000 (3% lipid content) and have average BCF values of 230, 1,700, and 49,000, respectively.

The effluent extract (which now is divided into three fractions) after the HPLC fractionation, contains primarily those chemicals with high potential to bioconcentrate, i.e., chemicals with higher log P values. These fractions are prepared for GC/MS analysis by diluting the HPLC fractions with water and extracting the bioconcentratable chemicals using liquid-liquid extraction or solid phase extraction techniques. The fractions after extraction are reduced to 0.10 ml and are stored at -10°C until GC/MS analysis.

3.2.5 Analyze via GC/MS

Gas chromatography/mass spectrometry (GC/MS) analyses are performed on the three HPLC fractions using standard residue techniques. Prior to GC/MS analysis, an internal standard, d_{12}-chrysene, is added to each of the HPLC fractions to calibrate the response of the mass spectrometer. Sample analyses are performed on a 30 m capillary column with a temperature program, e.g. 50-175°C at 10°C/min and then 175-275°C at 5°C/min, 275°C for 20 min. Mass spectral data are collected using full scan electron impact ionization mass spectrometry. After GC/MS analysis, all of the extracts are saved for confirmation analysis.

After analysis of all three fractions and standards, standard curves are calculated for the three surrogates; d_{10}-biphenyl, $^{13}C_6$-1,2,4,5-tetrachlorobenzene, and $^{13}C_6$-hexachlorobenzene; using an internal standard method. These curves are used to quantify the surrogates in the sample extracts. These quantifications are used to determine the percent recovery for each of the surrogate chemicals.

The three surrogate chemicals were chosen so that each of the HPLC fractions will contain only one of the surrogates. These chemicals, d_{10}-biphenyl, $^{13}C_6$-1,2,4,5-tetrachlorobenzene, and $^{13}C_6$-hexachlorobenzene, will be in the first, second, and third fractions, respectively. If these chemicals are present in different fractions, the HPLC fractionation procedure was performed incorrectly or with improper HPLC conditions. Quality control procedures would require that corrective actions be taken and that the new effluent sample be extracted.

Quantification of the remaining GC/MS components are performed by using the responses of the surrogates in their respective fractions. For the first fraction, all of the GC/MS components are quantified using the standard curve for d_{10}-biphenyl. For the second fraction, all of the GC/MS components are quantified using the standard curve for $^{13}C_6$-1,2,4,5-tetrachlorobenzene. For the third fraction, all of the GC/MS components are quantified using the standard curve for $^{13}C_6$-hexachlorobenzene. This procedure is presented in detail for laboratory use in Appendix B.

3.2.6 Library Searching using the Chemicals of Highest Concern Mass Spectral Library

All chromatographic peaks in the GC/MS data are compared with the Chemicals of Highest Concern (CHC) mass spectral library (see Table 2.1). Peaks with fits/matches of 70% and greater are considered tentatively identified. For each tentatively identified component, a list of the best mass spectral library identifications (up to a total of ten identifications) is reported along with the percent fit values, CAS number of the tentative identification, HPLC fraction number, GC retention time, and the concentration for the GC/MS component. This report is identified as Report 1 in the Effluent option flowchart, Figure 3.2.

The CHC mass spectral library can, in nearly all cases, be derived from the EPA/NIH/NBS mass spectral library using the software on the data system of the GC/MS system. The recommended 70% library searching fit criterion was chosen based upon best scientific judgement and is subject to modification by the regulatory authority.

Computer algorithms for identifying unknown mass spectra via library searching are often categorized as either forward or

reverse searching. In general, reverse searching algorithms have demonstrated advantages for identifying unknown mass spectra when the unknown is not chemically pure [31]. With GC/MS analyses, mass spectral data can never be assumed to be pure and thus, in the procedure, the use of reverse searching algorithms is strongly recommended when available. Unfortunately, some GC/MS systems do not have reverse searching algorithms. In these cases, library searching should be performed using the default algorithm provided by the manufacturer of the GC/MS system.

3.2.7 100 ng/L Effluent Concentration Decision Point

If a GC/MS component is not identified with the CHC mass spectral library search (the no arrow in the flowchart), the chemicals present at concentrations below 100 ng/l are dropped from further consideration. The concentration of 100 ng/l is used as the cut-off for further investigation because 100 ng/l is the concentration of the surrogate compounds added to the sample prior to extraction. The surrogate concentrations represent the minimum level at which adequate quantitation can occur. The amount of uncertainty associated with a value which lies below the surrogate concentrations increases greatly with decreasing concentration. The purpose of this cutoff level is to prioritize unknown/non-CHC peaks for further investigation.

GC/MS components with concentrations greater than 100 ng/l which are not identified with CHC mass spectral library are then library searched with the EPA/NIH/NBS mass spectral library.

3.2.8 Library Searching using the EPA/NIH/NBS Mass Spectral Library

GC/MS components not identified using the CHC library search and with concentrations greater than 100 ng/l are library searched against the EPA/NIH/NBS mass spectral database. Peaks with fits/matches of 70% and greater are considered tentatively identified. These components are then evaluated in the "(fraction BCF x concentration x dilution) > 1 μg/kg" rectangle in Figure 3.2 (the yes arrow leaving the EPA/NIH/NBS mass spectral library search rectangle).

For those components with fits/matches less than 70% but greater than 25%, the two best mass spectral library identifications along with the percent fit values, the CAS number of the tentative identifications, HPLC fraction number, GC retention time, and the concentration are reported for each the GC/MS component. For GC/MS components with fits/matches less than 25%, the concentrations, HPLC fraction number, and GC retention times for these components should be reported and the components labeled as being "unknown". This report is identified as Report 3 in the effluent option flowchart, Figure 3.2. The tentative identifications listed in Report 3 are of less certain

reliability and these results are provided for informational purposes only.

The EPA/NIH/NBS mass spectral library is available from the U.S. Government Printing Office (941 North Capitol St. N.E., Washington, D.C. 20401). In addition, most GC/MS manufacturers have this library available in a form suitable for their respective instruments.

The recommended 70% library searching fit criterion was chosen based upon best scientific judgement and is subject to modification by the regulatory authority. As with the CHC library searching process, the use of reverse searching algorithms is strongly recommended when available. In cases where reverse searching algorithms are not available, library searching should be performed using the default algorithm provided by the manufacturer of the GC/MS system.

3.2.9 (Fraction BCF x Concentration x Dilution) > 1 µg/kg

For each of the tentatively identified components in the EPA/NIH/NBS library search rectangle, the product of the fraction BCF, effluent concentration, and effluent dilution is calculated and compared to a 1 µg/kg tissue residue concentration. For those GC/MS components with a product exceeding 1 ug/kg, a list of the best mass spectral library identifications (up to a total of ten identifications) is reported along with the percent fit values, CAS number of the tentative identifications, HPLC fraction number, GC retention time, and the concentration for the GC/MS component. This report is identified as Report 2 in Figure 3.2.

For those GC/MS components with a product less than 1 µg/kg, a list of the best mass spectral library identifications (up to a total of ten identifications) is reported along with the percent fit values, CAS number of the tentative identifications, HPLC fraction number, GC retention time, and the concentration for the GC/MS component. This report is identified as Report 4 in Figure 3.2.

This decision point removes chemicals which are present at concentrations too low to form residues in aquatic organisms which pose risks to human consumers. The 1 µg/kg cutoff for residue concentrations in aquatic organisms was selected by determining the reference tissue concentrations (RTCs) for all chemicals in the EPA's IRIS database with log P values of 3.5 and greater. Using a fish consumption rate of 6.5 g/day and a risk level of 10^{-6}, RTC values were calculated for 83 nonpolar organic chemicals (Appendix D) and these values are summarized in Figure 3.3.

Two chemicals, aldrin and alpha-hexachlorocyclohexane, have RTCs below 1 µg/kg and for both chemicals, production and use in the United States has been discontinued. Both of these chemicals

are included in the CHC mass spectral library and thus, GC/MS components tentatively identified as aldrin or alpha-hexachlorocyclohexane will be reported in Report 1. These components, those tentatively identified as aldrin and alpha-hexachlorocyclohexane, will never be evaluated in the "(fraction BCF x concentration x dilution) > 1 µg/kg".

Based upon the above considerations, i.e., no chemicals in current production have RTCs below 1 µg/kg, and that the tissue assessment option has detection limits approaching 1 µg/kg for tissue analyses, the 1 µg/kg cutoff was selected for the effluent analysis.

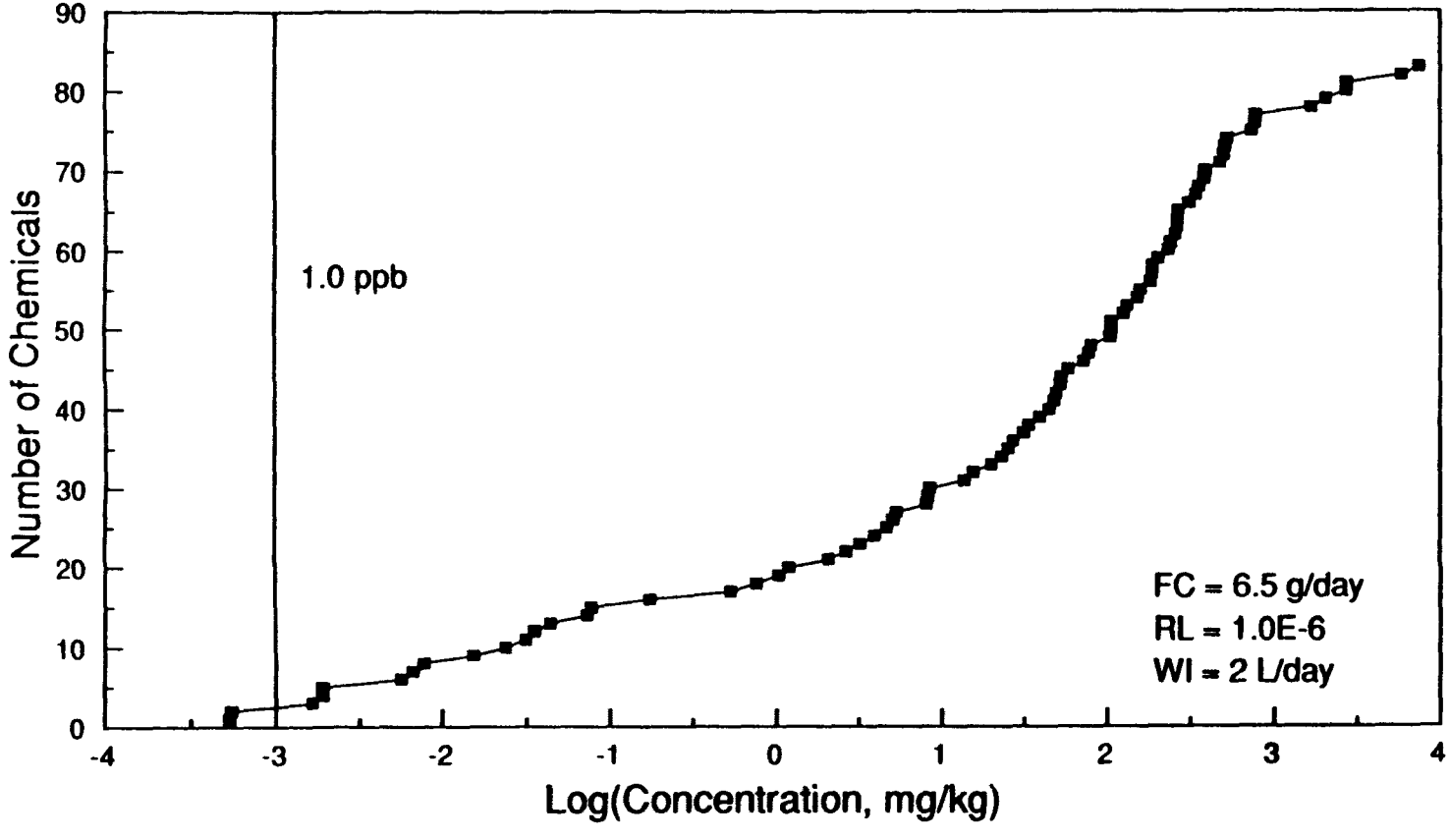
This evaluation process is performed using assumed fraction BCFs of 560, 5000, and 470000 for HPLC fractions 1, 2, and 3, respectively. These values correspond to the calculated BCF from the log P at the ending eluate collection time from the HPLC column for each HPLC fraction. The dilution factor must be specified by the regulatory authority for a specific discharger prior to the assessment, based on site specific factors, or alternatively could be conservatively estimated at 1.

3.2.10 Analytical Summary

With the generation of the four reports, Reports 1, 2, 3, and 4, the procedures presented in Appendix B have been performed for the effluent assessment option. The discussion presented above in Section 3.2.1 through 3.2.9 outline this procedure and the decision points for processing the GC/MS data collected on the HPLC effluent fractions. Example results and reports can be found in Appendix J.

Reports 1, 2, 3, and 4 as well as the QA/QC report for a effluent analysis will be sent to the regulatory authority. In addition to this, the actual GC/MS Chromatograms for each of the three fractions should be included in the reported information. The regulatory authority will evaluate the data and decide which tentatively identified chemicals will need confirmation by performing the tasks in the two decision rectangles in Figure 3.2 labeled, "RfD, q1*, and/or Water Quality Standard?" and "(concentration x dilution x 4) > RAC".

Figure 3.3 RTCs for IRIS Chemicals, log P > 3.5



3.2.11 RfD, q1*, and/or Water Quality Standard?

This decision rectangle in the effluent assessment option, Figure 3.2, simply asks the question, "Is there a Water Quality Standard available or sufficient information (RfD or q1*) to develop a RAC for a tentative identification reported in Report 2?". The purpose of this decision criterion is the same as for Section 3.1.10. For each GC/MS component, up to 10 tentative identifications might be reported. For each of the tentative identifications, the above question must be answered. If any of the tentative identifications for a GC/MS component have a RfD, q1*, and/or Water Quality Standard available, the evaluation process for that component would proceed to the "(concentration x dilution x 4) > RAC" decision rectangle, the yes arrow in Figure 3.2. Further information concerning the RfD, q1*, reference concentrations, and BCF are presented in Chapter 4 of this guidance.

If none the tentative identifications have the required information for a given GC/MS component (i.e. peak), the regulatory authority may evaluate the tentative identifications further by obtaining improved BCF (and FM) data for each tentative identification.

This evaluation process is performed on all tentatively identified GC/MS components listed in Report 2. Furthermore, for each tentatively identified GC/MS component, each tentative identification should be evaluated.

3.2.12 (FM x BCF X concentration x dilution) > 1 µg/kg

For those GC/MS components with no RfD, q1*, and/or water quality standards available for any of their tentative identifications (the no arrow leaving the "RfD, q1*, and/or Water Quality Standards?" decision point), BCFs for each tentative identification may be derived. With these values and the FM for the species of interest, the product of the FM, BCF, concentration, and dilution is determined and compared to a 1 µg/kg residue concentration. If the product of any of the tentative identifications for a GC/MS component are above the 1 µg/kg value, the regulatory authority will issue a request for confirmation of the identity of that component. If the product is below the 1 µg/kg value for all tentative identifications, no further action is taken on that component. This evaluation and decision is performed by the regulatory authority in order to focus on those compounds of greatest potential hazard and to reduce the potential for requiring confirmation for chemicals of lesser importance for this assessment.

3.2.13 (Concentration x dilution x 4) > RAC?

This decision rectangle in the effluent option, Figure 3.2, evaluates whether the measured concentration of a tentatively

identified GC/MS component in the effluent is likely to be larger than the reference ambient concentration (RAC). The RAC is the highest receiving water concentration for chemical that doesn't result in a residue which poses health risks to humans consuming fish and shell. This comparison is based on the same principles described in Section 3.1.11.

For each GC/MS component, this comparison is performed for each of the tentative identifications which have available Rfd, q1*, and/or water quality standards. If this comparison is true for any of the tentative identifications for a GC/MS component, a confirmation request is issued by the regulatory authority to the discharger for that component (the yes arrow leaving this decision point). If this comparison is false for all of the tentative identifications for a GC/MS component, no further action (no arrow leaving this decision point) is recommended for that component.

All tentatively identified GC/MS components in Report 1, CHC chemicals, are evaluated at this decision point. For tentatively identified GC/MS components in Report 2, only those tentative identifications with RfD, q1*, and/or water quality standards information available are evaluated.

The calculation of the reference ambient concentrations (RAC) is described in detail in Chapter 4 of this document. RACs are calculated using human dose information, Rfd and/or q1*, BCF, and fish consumption rates by humans.

3.2.14 Issue Confirmation Request

In the effluent option, confirmation requests by the regulatory authority can be issued from two different decision points, see Figure 3.2. In either case, the discharger is required to confirm the identity of the GC/MS component tentatively identified in the HPLC fractions. This confirmation process is discussed in further detail in Section 3.3. The results of the confirmation process are reported back to the regulatory authority. These results include the confirmed identity of the GC/MS component and its concentration. Confirmation of the identity of the chemicals is conducted at this point in the logic sequence in order to focus on those contaminants of greatest concern and to for complex samples reduce the potential number of chemicals requiring confirmation.

3.2.15 Outputs From Effluent Assessment

The outputs from the effluent assessment process are two lists of identified bioconcentratable chemicals. The first list consists of chemicals from Reports 1 and 2 which require reference concentration development (Chapter 4), wasteload allocation (Chapter 5), and when necessary, permit limits (Chapter 6). The second list consists of chemicals which also

arises from Report 2 and do not have RfD's, q1*'s, and/or water quality standards. Development of Rfd, q1* information for that chemical may be requested to determine if current residue levels pose serious risk to human consumers of fish and shellfish (the yes arrow leaving the "(FM x BCF x concentration x dilution) > 1 µg/kg" decision point). Monitoring of these chemicals in effluents is recommended in order to establish discharge information.

It must be noted that chemicals in both of these lists have high potential to form residues in aquatic organisms.

3.2.16 Caveats for the Effluent Option

The effluent assessment option will not detect all bioconcentratable chemicals which form residues in aquatic organisms. The analytical procedures outlined above will detect acid stable nonpolar organic chemicals with log P values of 3.5 and greater which can be successfully analyzed using GC/MS. These procedures are fairly robust; however, they do have limitations. Some of these limitations include interferences from hydrocarbons, sensitivity of the analytical method, the lack of reliably measured BCFs and/or BAFs, and the possible absence of RfD and q1* information for a given contaminant. (Note: hydrocarbon interference will only be a concern for facilities with a predominance of this type of chemical, such as refineries.)

3.3 Confirmation

3.3.1 General Considerations

When a discharger is issued a request for confirmation by the regulatory authority, the primary objective for this process is to attain an accurate identification of the GC/MS component. Determining the exact concentration of a chemical is of secondary concern for two reasons. First, the confirmation of the concentration of the chemical in the sample extract does not directly affect attaining a BCF value for that chemical. Second, the procedures for the evaluation have an acceptable recovery range of from 25-120%. This means that there is a very low likelihood of over estimating the actual concentration of a bioconcentratable contaminant in an effluent or tissue sample. In fact, it is more likely that the quantitation will be lower than the actual effluent concentration. It can be expected that some of the contaminant will be lost in the analytical procedures and not all of the contaminant will be recovered in the GC/MS quantification.

This potential underestimation is not a direct concern at this stage of the procedure since the concentration is not used for attaining the BCF value, the derivation of the RAC, nor developing control requirements such as NPDES limits. The limit

is unaffected by effluent or tissue concentration but rather dependent on the standard or reference concentration and the available dilution. Underestimation does not become a concern until that step of the procedure where the regulatory authority will determine the need for a permit limit by comparing the RAC with the receiving water concentration (RWC). The discussion of characterization of the effluent for human health effects in Chapter 6 describes how this potential underestimation may be addressed in that step of the procedure.

3.3.2 Confirmation Process

Confirmation is performed by obtaining relative retention times and mass spectral data for standards made from pure reference chemicals. When confirming a tentatively identified chemical, chemicals analyzed on the GC/MS should probably include the tentative identifications reported for the GC/MS component and perhaps other logical chemicals. The discharger should utilize any available facility specific information to assist in the confirmation since this information will often decrease the cost and time required for confirmation. Such data might include process information, chemical use, and/or MSDS information.

In some cases, none of the tentative identifications assigned to a GC/MS component will be the correct identification. Additional confirmation analyses beyond the initial tentative identifications and other logical choices will require increased efforts and costs, and it must be recognized that there is no single analytical approach that will work with all effluents and/or tissue extracts. The analytical methods for identifying such a chemical will in all likelihood be site specific and will depend upon the complexity of the sample, the knowledge of the processes which generate the discharger's effluent, and the complexity of the mass spectrum for the unknown. Some of the analytical approaches which may be utilized in conjunction with available information on the facility are: 1) concentrating the extract and reanalyzing the fraction on the GC/MS, 2) inventorying and evaluating the chemicals used and produced by the manufacturing process, 3) evaluating the reaction pathways in the manufacturing process for reaction by-products, 4) analyzing the extract using different GC conditions, GC columns, MS conditions, and/or MS systems, 5) manual interpretation of the mass spectrum for the unknown, and 6) performing more exotic MS analyses such as GC/MS/MS or GC/FTIR/MS with manual interpretation of the spectral data.

A chemical would be considered confirmed when its relative retention time and mass spectral data from the tissue or effluent extract match the GC/MS data for the standard made from the pure reference chemical. This procedure should be conducted by following the method described in EPA method 1625 [39]. Quantification of the confirmed chemical in the extract should

also be performed using EPA method 1625 with d_{12}-chrysene as the internal standard [39].

The discharger should report the results of the confirmation analyses including the confirmed "true" identification and quantification as well as the results for each of the other tentative identifications which were analyzed but found to be false identifications. Additional QA/QC information should also be reported as described in the EPA method utilized for the confirmation.

3.4 Sediment Assessment

3.4.1 General Considerations

In some receiving waters, sediments may be a significant source for bioconcentratable chemicals. In this section, an analytical procedure for identifying bioconcentratable chemicals in sediments is outlined. A flowchart outlining the sediment analytical procedure is given in Figure 3.4. A detailed procedure, in a form suitable for dispersement to contract laboratories, is provided in Appendix C. This procedure is combines various portions of the tissue and effluent analytical procedures in Appendices A and B to derive this methodology for sediments.

3.4.2 Sampling

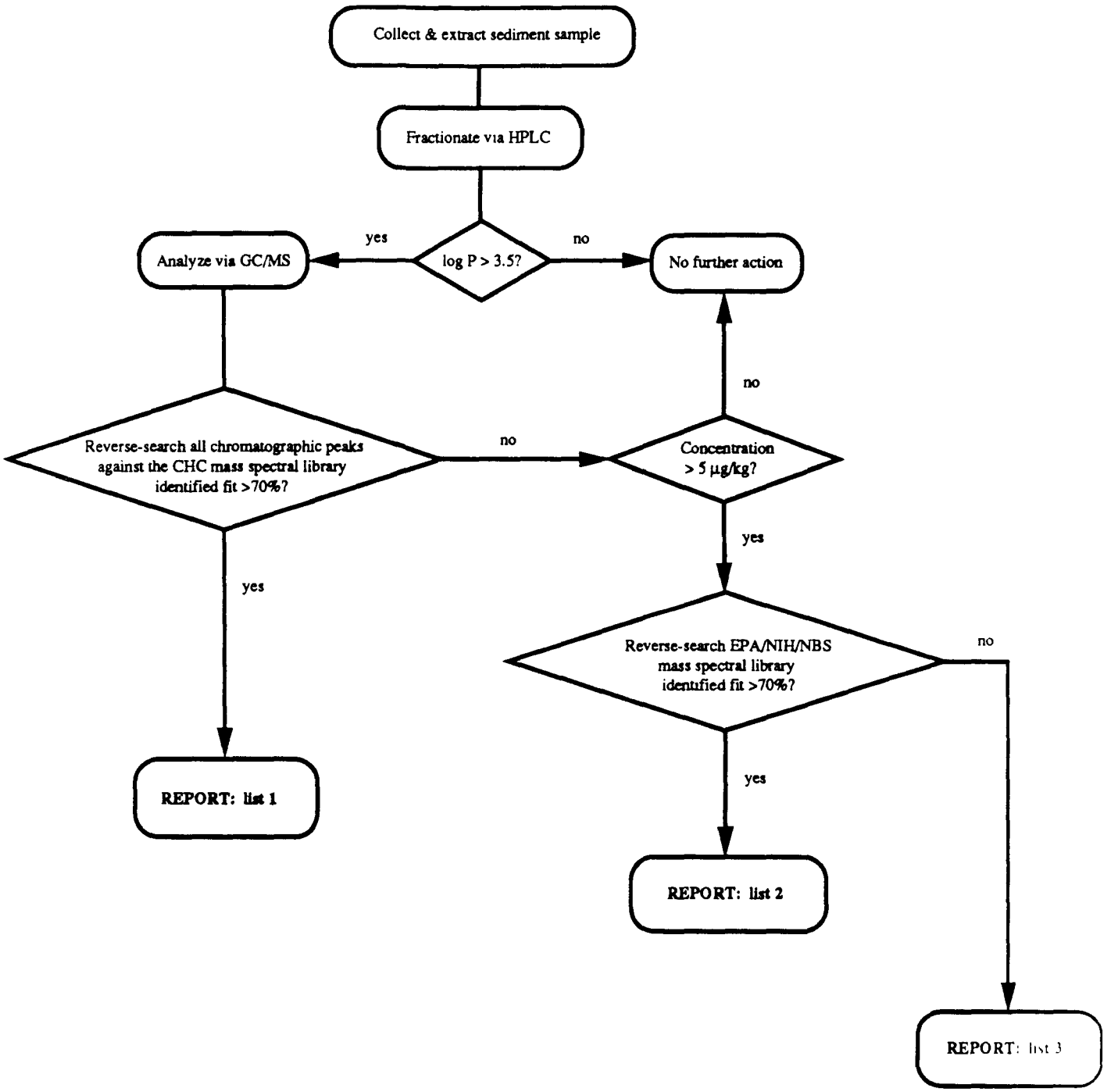
As with the tissue and effluent assessment options, collection of representative samples is important for accurate assessment of the bioconcentratable chemicals present in the sediments. Collection of representative samples will involve the following site-specific decisions:

When to Sample

In some receiving waters, sediments are the ultimate sink for bioconcentratable chemicals since sedimentation processes will gradually bury the sediments containing the residue forming chemicals. Sedimentation processes occur on a much slower time scale than the processes associated with the water column and biotic compartments of the receiving water. Consequently, sediment sampling can be performed almost anytime during the year and result in representative samples.

Figure 3.4

SEDIMENT ANALYSIS



In the absence of other evidence, sampling should be done after periods of normal or sub-normal dilution of the effluents. Sampling is not recommended during and after major storm events, i.e., floods and hurricanes, and possibly, during spring runoff since sediment resuspension during these events can dramatically alter the composition of the sediments. Generally, sampling once or possibly, twice a year will be sufficient for an initial assessment. Additional sampling, i.e., monthly or weekly, may be necessary for a more comprehensive sediment assessment.

How to Sample

Surface sediments, those in the benthic mixing zone, should be collected using a Ponar dredge, Eckman dredge and/or coring sampler. The coring sampler type is preferred since better control is provide so that surface sediments can be collected. Sediments with low organic carbon content, i.e., those with high sand and gravel content, should not be collected since organic bioconcentratable chemicals reside in the organic carbon fraction of the sediment. Collected sediments should be placed into clean solvent rinsed glass jars and sealed with aluminum foil or teflon lined lid. Samples should be stored in the dark at 4°C.

Where to Sample

The location of sampling is a site-specific decision. In general, samples should be collected in the depositional zone close to the outfall of interest. Collection of samples in the mixing zone of the effluent may not be representative since deposition of particulate matter with the bioconcentratable chemicals from that discharge may not occur there. Collection of samples too far from the outfall is not recommmended since dilution and degradation may diminish the residue concentrations in the sediments.

Sampling sites with well defined depositional zones and receiving water hydraulics may be assessed with one well placed site. As the complexity of the receiving water and deposition zones increase, additional sampling sites along a gradient may be necessary.

3.4.3 Sample Extraction

The extraction procedure consists of mixing 20 grams of ground, air dried, sediment with anhydrous sodium sulfate. Half of this mixture is placed into a Soxhlet extraction thimble and three surrogate chemicals are spiked onto the sediment in extraction thimble. The remaining sodium sulfate/sediment mixture is then placed into the extraction thimble and the extraction thimble is placed into a Soxhlet extractor body. The sediment is extracted using acetone for 4 hours, and then, Soxhlet extracted using 1:3 toluene:methanol for a minimum of 12 hours. The extract, acetone and toluene:methanol, is

concentrated using a Kuderna-Danish concentrator to approximately 10 mls. Percent moisture and organic carbon of the sediment are measured using standard techniques.

The surrogate chemicals, d_{10}-biphenyl, $^{13}C_6$-1,2,4,5-tetrachlorobenzene, and $^{13}C_6$-hexachlorobenzene, are added to the sediment at a 5 µg/kg concentration prior to extraction of the sample. These chemicals are referred to as surrogates because their behavior mimics that of bioconcentratable chemicals in the analytical procedure. The surrogate chemicals are used for quality control and quantification in this analytical procedure.

3.4.4 Sample Cleanup

The sediment extract is cleaned up using the procedures outlined in the effluent option, see Section 3.2.3. Elemental sulfur is removed from the sediment extract by passing the extract through a column containing activated copper filings. The extract after removal of the sulfur is concentrated to 0.5 mls.

3.4.5 HPLC and GC/MS Analysis

The sediment extract is fractionated using the procedures outlined in the effluent option, see Section 3.2.4. The three HPLC fractions are analyzed using the procedures outlined in the effluent option, see Section 3.2.5

3.4.6 Library Searching using CHC Mass Spectral Library

The GC/MS data for the HPLC fractions are library searched using the procedures outlined in the effluent option, see Section 3.2.6. Peaks with fits/matches of 70% and greater are considered tentatively identified. For each tentatively identified component, a list of the best mass spectral library identifications (up to a total of ten identifications) is reported along with the percent fit values, CAS number of the tentative identification, HPLC fraction number, GC retention time, and the concentration for the GC/MS component. This report is identified as Report 1 in the sediment analysis flowchart, Figure 2.3.

3.4.7 5 µg/kg Sediment Concentration Decision Point

For GC/MS components not tentatively identified in the CHC mass library search, those present at concentrations less than 5 µg/kg are dropped from further consideration. For further information. see the discussion in the tissue option, Section 3.1.7.

3.4.8 Library Searching using the EPA/NIH/NBS Mass Spectral Library

The GC/MS components not tentatively identified by the CHC mass spectral library search and having concentrations greater than 5 μg/kg are library searched against the EPA/NIH/NBS mass spectral library. Two reports are generated by this procedure, Reports 2 and 3 (see Figure 3.4). For further information, see the discussion in the tissue option section 3.1.8.

3.4.9 Analytical Summary

Three reports are generated by the sediment analytical procedure outlined above. These reports, Reports 1, 2, and 3, are identical in format to those created in the tissue option. The actual GC/MS chromatogram should also be submitted as part of the reporting requirements. The detailed analytical procedure is presented in Appendix C.

3.4.10 Caveats for the Sediment Analytical Procedure

The sediment analytical assessment procedure will not detect all bioconcentratable chemicals which form residues in aquatic organisms. The analytical procedures outlined above will detect acid stable nonpolar organic chemicals with log P values of 3.5 and greater which can be successfully analyzed using GC/MS. These procedures are fairly robust; however, they do have limitations. Some of these limitations include interferences from hydrocarbons, sensitivity of the analytical method, and our ability to identify unknown GC/MS components. (Note: hydrocarbon interference will only be a concern for facilities with a predominated with this type of chemical, such as refineries.)

3.5 Quality Assurance/Quality Control for Analytical Procedures

Data obtained as a result of the tissue, effluent, and sediment analysis procedures must pass their quality control (QC) requirements. In Appendices A, B, and C, these requirements are presented with the detailed procedure.

For all of the procedures, recoveries for the surrogates must be greater than 25% but not more than 120%. If this criteria is not met, the analytical data is not of sufficient quality and the analysis must be repeated.

For the effluent and sediment procedures which use HPLC fractionation, the HPLC fractions 1, 2, and 3 should contain the surrogates d_{10}-biphenyl, $^{13}C_6$-1,2,4,5-tetrachlorobenzene, and $^{13}C_6$-hexachlorobenzene, respectively. If surrogates are in the wrong HPLC fractions, the analytical data is not of sufficient quality and the analysis should be repeated.

QC information must also be provided for HPLC, GC, and MS performance as specified and this information must be evaluated. If the HPLC, GC, and MS performance criteria are not met, the analyses should also be repeated. For samples which do not meet the QC criteria, the discharger should be required to analyze a new sample. QC information should be included in the reported information to the regulatory authority.

CHAPTER 4

Reference Concentration Derivation

The assessment options described in the preceding chapter produce two list of identified bioconcentratable chemicals. The first list consists of chemicals from Reports 1 and 2 which require reference concentration development and the second list consists of those chemicals from Report 2 which lack RfD's, q1*'s, and/or water quality standards. The identity of these chemicals, from both lists, will have been confirmed.

This chapter presents procedures for developing reference concentrations if and when no State numeric water quality standard exists for a given contaminant to protect human health based on the consumption of fish. These procedures require knowledge of fish consumption rates, lipid content of the species consumed, human dose information (RfD and/or q1*). and the BCF (or BAF) for each bioconcentratable chemical.

The reference ambient concentrations (RACs) and reference tissue concentrations (RTCs) are used in the effluent and tissue assessment options respectively, to derive the first list of bioconcentratable chemicals discussed above. The RAC is also used in both the tissue and effluent options for waste allocation and permit development. The RACs and RTCs are derived to **protect human health** from contaminants in consumed fish only. RACs and RTCs are not derived to protect aquatic organisms from direct toxicity or human health from drinking water.

4.1 Establishing RACs for Bioconcentratable Contaminants

This section describes procedures to avoid unacceptable tissue residues in organisms used for human consumption. The procedures for setting levels of exposure or concentrations for human consumption are not a part of this document. A complete human health effects discussion is included in the (draft) "Guidelines and Methodology Used in the Preparation of Health Effects Assessment Chapters of the Consent Decree Water Documents," by EPA's Environmental Criteria and Assessment Office, Cincinnati (ECAO-Cin) [40].

The procedures contained in the above ECAO-Cin document are used in the development and updating of EPA water quality criteria and may be used in developing RACs for those pollutants lacking State or EPA human health criteria. Although the same procedures are used to develop criteria and RACs, only those values which are subjected to the regulatory process of Regional, State, and public comment can be considered "criteria." RACs may be applied as site-specific interpretation of narrative standards

and as a basis for permit limitations under 40 CFR 122.44(d)(1)(vi). Developing Reference Ambient Concentrations (RACs) using the guidelines established in this document does not absolve States from developing criteria under 303(c)(2)(B).

4.2 Human Exposure Considerations

The RAC value for protecting human health should include all exposures. These include consumption of water, consumption of fish, and combined consumption of both water and fish on a per-person per-day basis. Levels of actual human exposures from consuming contaminated fish vary depending upon a number of case-specific consumption factors. These factors include type of fish species consumed, type of fish tissue consumed, tissue lipid content, consumption rate and pattern, and food preparation practices. In addition, depending on the spatial variability in the fishery area, the behavior of the fish species, and the point of application of the RAC or criterion, the average exposure of landed fish may be only a small fraction of the expected exposure at the point of application of the criterion, or if an effluent attracts fish, it might be greater than the expected exposure. Another consumption factor is the percentage of fish consumed from the fishery area of concern, which could vary from 0% to 100%, depending on size, character and the value of the fishery.

With shellfish, such as oysters, snails, and mussels, the entire body tissue is commonly eaten, whereas with fish, only muscle tissue and roe are commonly eaten. This difference in the types of tissues consumed has implications for the amount of bioconcentratable contaminants likely to be ingested. Whole body shellfish consumption presumably means ingestion of the entire burden of bioconcentratable contaminants. However, with most fish, selective cleaning and removal of internal organs, and sometimes body fat as well, from edible tissues may result in removal of the lipid rich tissues that contain the majority of bioconcentratable contaminants.

This document focuses primarily on fish consumption and only incidentally on the drinking water exposure route. A complete human exposure evaluation for toxic pollutants of bioconcentration concern would not only encompass estimates of exposures due to fish consumption, but also exposure due to background concentrations and other exposure routes, including recreational contact, occupational, dietary intake from other than fish, inhalation of air, etc. However, other exposure route information should be considered to the extent it is available.

4.3 Fish Consumption Values

EPA's 1980 human health criteria assumed a human body weight of 70 kg and the consumption of 6.5 g/day of fish and shellfish. The national fish consumption value for freshwater and estuarine fish and shellfish, calculated by the US EPA based on data from a

1974 food survey, was 6.5 g/day with the 95th percentile for all seafood at 42 g/day [41]. The mean lipid content of fish tissue found to be consumed was 3.0% [42].

EPA recommends that the consumption values used in deriving RACs from the formulas in this chapter reflect the most current relevant and/or site-specific information available. For example, some States have adopted their own fish consumption estimates, ranging from 20 g/day in Wisconsin, Louisiana, Illinois and Arizona to 37 g/day in Delaware for salt water species [43].

Currently, four levels of fish consumption are provided in the EPA guidance manual "Assessing Human Health Risk from Chemically Contaminated Fish and Shellfish [44]" These are:

- 6.5 g/day to represent a low estimate of average consumption of fish and shellfish from estuarine and fresh waters by the entire U.S. population [45]. This fish consumption level is based on the average of both consumers and non-consumers of fish.

- 20 g/day to represent a high estimate of the average consumption of fish and shellfish from marine, estuarine, and fresh waters by the entire U.S. population [46]. This average also includes both consumers and non-consumers of fish.

- 165 g/day to represent average consumption of fish and shellfish from marine, estuarine, and fresh waters by the 99.9th percentile of the U.S. population consuming the most fish or seafood [47].

- 180 g/day to represent a "reasonable worst case" based on the assumption that some individuals would consume fish at a rate equal to the combined consumption of red meat, poultry, fish, and shellfish in the U.S. (EPA Risk Assessment Council assumption based on data from the USDA Nationwide Food Consumption Survey of 1977-1978; see Appendix H [44]).

EPA is currently updating the national estuarine and freshwater fish and shellfish consumption values to provide a range of recommended national consumption values. This range will include: 1) mean values appropriate to the population at large, and 2) values appropriate for those fishermen who consume a relatively large proportion of fish in their diets (maximally exposed individuals).

4.4 BCF Evaluation/Selection

In order to derive a reference concentration the regulatory authority will need to determine a BCF for each identified bioconcentratable compound using one of the following methods:

1) Use the estimated BCFs from the Quantitative Structure Activity Relationship (QSAR) [48] data base. BCF values in QSAR are based on 7.6% lipid content and may need to be normalized to 3% by multiplying by 0.395 (3%/7.6%).

2) For compounds with laboratory-derived and/or estimated log P values (laboratory-derived is preferred [49]), but unknown BCFs, use the following relationship to estimate the BCF at a 3% lipid content [22]:

$$\log BCF = 0.79 \log P - 0.40 - \log (7.6/3.0)$$

3) For compounds for which BCF or log P values cannot be attained using 1 or 2, use the average BCF for the fraction in which the compound was found. These averages were calculated by applying the equation above in 2) to the average log P in each of the fractions in the HPLC separation procedure described in Chapter 3.

Average BCF for Fraction

Fraction	(3% lipid content)
1	230
2	1,700
3	49,000

In situations where metabolism of the chemical is known or suspected to be of importance or where information on measured BCF values are desired, the following sources of information may be consulted:

1) US-EPA Ambient Water Quality Criteria documents issued in 1980 or later.
2) AQUIRE on-line data base.
3) Published scientific literature.
4) Reports issued by US-EPA or other sources.
5) Unpublished data which meets minimum ASTM test requirements.

For BCFs selected using 2 through 5 the the minimum test requirements as specified in Section 2.6 must be met. When more than one BCF value meets the minimum data requirement, the geometric mean of those BCFs, after normalization for lipid content, should be used. In order to calculate the geometric mean BCF value, the acceptable BCF values should be normalized to 3.0% lipid content using the following relationship:

$$\text{BCF at 3\% lipid content} = (\text{BCF @ L\% lipid content}) \times (3.0/L)$$

4.5 Bioaccumulation Considerations: Food Chain Multiplier Selection

In this document, bioaccumulation considerations are incorporated in the calculation of the RTCs and RACs by use of the food chain multiplier (FM) term along with the BCF value. In Table 4.1, FM values derived from the work of Thomann [17,18] are listed according to log P value and trophic level of the organism. **Trophic level 4 organisms are generally the most desirable species for sport fishing and therefore, FMs for trophic level 4 are recommended for use in the equations for calculating RTCs and RACs.** In those situations where only lower trophic level organisms are found, e.g., oyster beds, a FM for a lower trophic level might be used in calculating the RTCs and RACs.

Experimentally measured BAFs reported in the literature may be used when available (this may be most desirable for those chemicals with log P values above 6.5). To use experimentally measured BAFs in calculating the RAC or RTC, the "(FM x BCF)" term, is replaced by the BAF in the equations in Sections 4.7-4.10. Relatively few BAFs have been measured accurately and reported, and their application to sites other than the specific ecosystem where they were developed is problematic and subject to uncertainty. The BAF may have to be corrected for trophic level if the measured BAF is for a lower trophic species and the species of interest is a higher predatory species. Of course, the option is also available to develop BAFs experimentally, but this will be extremely resource intensive if done on a chemical and site specific basis with all the necessary experimental and quality controls.

4.6 Updating Criteria and Generating RACs and RTCs Using IRIS

EPA recommends that the process of updating criteria and generating RACs and RTCs use the most current risk information. The Integrated Risk Information System (IRIS) is an electronic on line data base of the EPA that provides chemical-specific risk information on the relationship between chemical exposure and estimated human health effects. The health assessment information contained in IRIS, except as specifically noted, has been reviewed and agreed upon by an interdisciplinary group of scientists representing various Program Offices within the Agency and represent an Agency-wide consensus. Risk assessment information and values are updated on a monthly basis and are approved for Agency-wide use [50].

IRIS is intended to make risk assessment information readily available to those individuals who must perform risk assessments and also to increase consistency among risk assessment/risk management decisions. IRIS is available to Federal and some State and local environmental agencies through the EPA's

Table 4.1
Estimated Food Chain Multipliers (FMs)

Log P	Trophic Levels 2	3	4
3.5	1.0	1.0	1.0
3.6	1.0	1.0	1.0
3.7	1.0	1.0	1.0
3.8	1.0	1.0	1.0
3.9	1.0	1.0	1.0
4.0	1.1	1.0	1.0
4.1	1.1	1.1	1.1
4.2	1.1	1.1	1.1
4.3	1.1	1.1	1.1
4.4	1.2	1.1	1.1
4.5	1.2	1.2	1.2
4.6	1.2	1.3	1.3
4.7	1.3	1.4	1.4
4.8	1.4	1.5	1.6
4.9	1.5	1.8	2.0
5.0	1.6	2.1	2.6
5.1	1.7	2.5	3.2
5.2	1.9	3.0	4.3
5.3	2.2	3.7	5.8
5.4	2.4	4.6	8.0
5.5	2.8	5.9	11.4
5.6	3.3	7.5	16.2
5.7	3.9	9.8	23.2
5.8	4.6	12.7	33.3
5.9	5.6	16.5	47.2
6.0	6.8	21.4	66.5
6.1	8.2	25.2	75.2
6.2	10.1	29.4	84.1
6.3	12.5	34.1	91.8
6.4	15.4	39.3	98.4
6.5	19.2*	44.9*	103.8*
≥6.5	19.2*	44.9*	103.8*

* These recommended FMs are conservative estimates, FMs for log P values greater than 6.5 may range from the values given to as low as 0.1 for contaminants with very low bioavailability.

electronic MAIL system and is also available to the public through the Public Health Network (PHN) and TOXNET. Since IRIS is designed to be a publicly available database, interested parties may submit studies or documents for consideration by the appropriate interdisciplinary review group for chemicals currently on IRIS or scheduled for review. Information regarding the submission of studies of chemicals may be obtained from the IRIS Information Submission Desk. In addition to chemical specific summaries of hazard and dose-response assessments, IRIS contains a series of sections identified by service codes which serve as a user's guide as well as provide background documentation on methodology. Additional information is available from IRIS USERS SUPPORT: 513/FTS 684-7254.

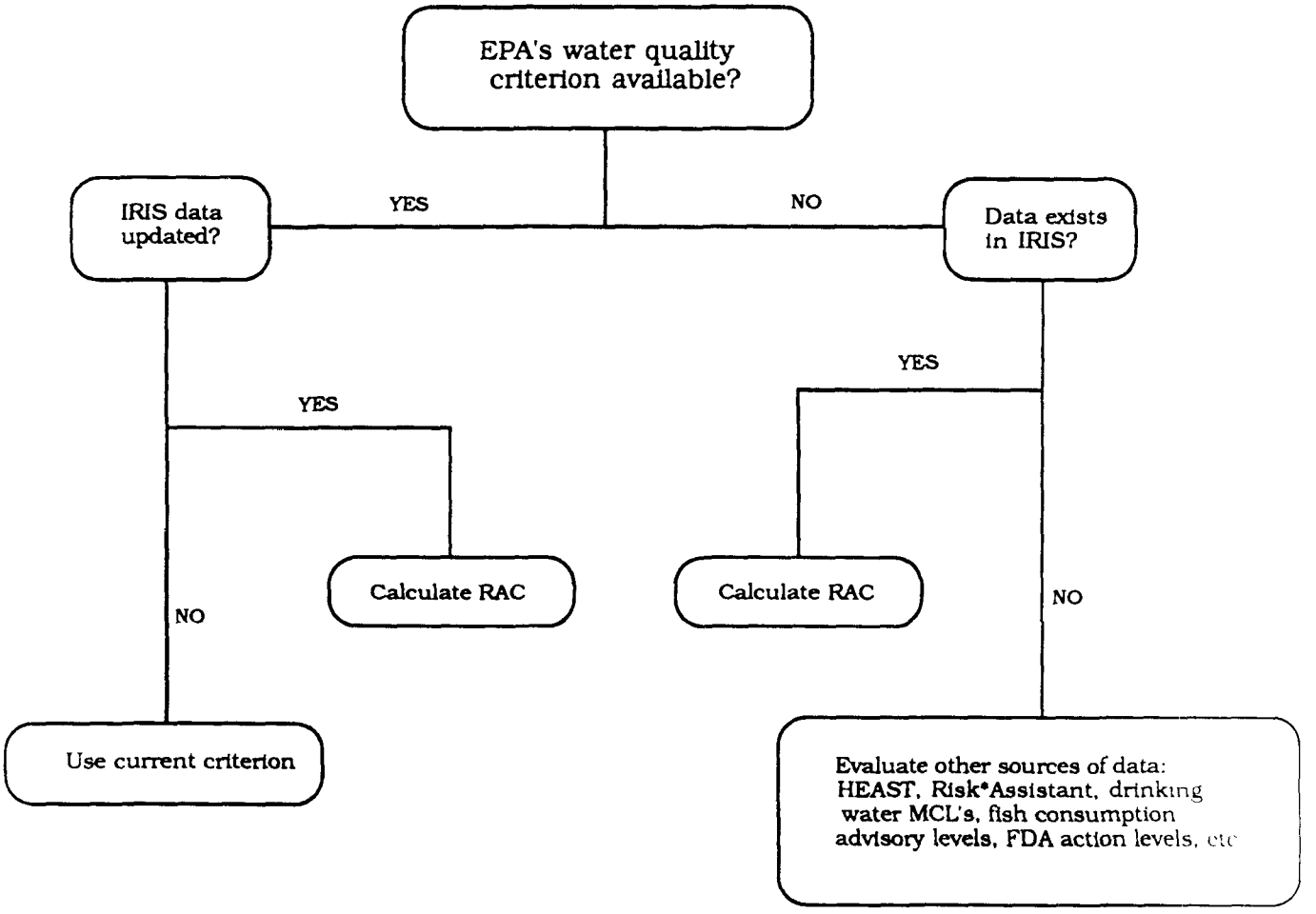
IRIS contains two types of quantitative risks values: Reference Dose (RfD) and the Carcinogenic potency estimate or slope factor. The RfD (formerly known as the acceptable daily intake or ADI) is the human health hazard assessment for non-carcinogenic (target organ) effects. The carcinogenic potency estimate (formerly known as q1*) represents the cancer causing potential resulting from lifetime exposure to a substance. The RfD or the oral carcinogenic potency estimate are used in the derivation of an RAC. Appendix E contains additional information on the derivation of RfDs and q1*s.

EPA periodically updates risk assessment information including RfDs, cancer potency estimates, and related information on contaminant effects, and reports the current information on IRIS. A list of the BCF, RfD and carcinogenic potency estimates values current at the time of publication of this document is included in Appendix D. The inclusion of this list in this document is intended for use in initial screening only. Since IRIS contains the Agency's most recent quantitative risk assessment values, current IRIS values should always be used in developing new RACs. This also applies to updating the 1980 EPA human health criteria. The procedure for deriving an updated human health water quality criterion would require inserting the current Rfd or Carcinogenic potency estimate on IRIS into the appropriate equation in Sections 4.7 - 4.10. In the absence of a promulgated State numeric standard, the RAC may be calculated using these formulas. **Appendix D <u>should not be used</u> to revise any criteria or derive RACs without checking IRIS for the most current values.**

Figure 4.1 shows the procedure for updating a criterion or deriving a RAC using IRIS data. **If a chemical has both carcinogenic and non-carcinogenic effects; i.e., both a cancer potency estimate and RfD, the carcinogen RAC formula in Section 4.8 should be used as it will typically result in the more stringent RAC of the two.**

Figure 4.1

Procedure for Revising an EPA Human Health Criterion
or Developing a Reference Ambient Concentration



4.7 Calculating RACs for Non-Carcinogens

The RfD is an estimate of the daily exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of causing deleterious effects during a lifetime. The RfD is expressed in units of mg toxicant/kg human body weight/day.

RfDs are derived from the "no observed adverse effect level"(NOAEL) or in the absence of a NOAEL the "lowest observed adverse effect level" (LOAEL) identified from chronic or subchronic human epidemiology studies with clearly defined exposure levels or from experimental animal studies. LOAEL and NOAEL refer to animal and human toxicology and are therefore distinct from the aquatic toxicity terms "no observed effect concentration (NOEC) and the "lowest observed effect concentration" (LOEC). Uncertainty factors are then applied to the NOAEL or LOAEL to account for uncertainties in the data associated with variability among individuals, extrapolation from nonhuman test species to humans, data on other than long-term exposures and the use of a LOAEL [44]. An additional uncertainty factor may be applied to account for significant weakness or gaps in the database.

The RfD is a threshold below which adverse effects are unlikely to occur. While exposures above the RfD increase the probability of adverse effects, they do not produce a certainty of adverse effects. Similarly, while exposure at or below the RfD reduces the probability, it does not guarantee the absence of effects in all persons. The RfDs contained in IRIS are values that represent EPA's consensus judgement. These values may have uncertainty spanning perhaps an order of magnitude.

For non-carcinogenic effects, an updated criterion or an RAC can be derived using the following equation:

Equation 4.1:

$$C \text{ or } RAC \text{ (mg/l)} = \frac{(RfD \times WT) - (DT + IN) \times WT}{WI + [FC \times L \times (FM \times BCF)]}$$

where:

C	=	updated water quality criterion (mg/l)
RAC	=	reference ambient concentration (mg/l)
RfD	=	reference dose (mg toxicant/kg human body weight/day)
WT	=	weight of an average human adult [70 kg]
DT	=	dietary exposure [other than fish] (mg toxicant/kg human body weight/day)
IN	=	inhalation exposure (mg toxicant/kg human body weight/day)
WI	=	average human adult water intake [2 liters/day]

FC = daily fish consumption (kg fish/day)
L = ratio of lipid fraction of fish tissue consumed to 3%
FM = food chain multiplier [from Table 4.1]
BCF = bioconcentration factor (mg toxicant/kg fish divided by mg toxicant/L water) for fish with 3% lipid

If the receiving water body is not used as a drinking water source, the factor WI can be deleted. Where dietary and/or inhalation exposure values are unknown, these factors may be deleted from the above calculation. For identified non-carcinogenic chemicals without known RfDs, State or EPA procedures can be used to estimate the RfD (see Appendix E).

4.8 Calculating RACs for Carcinogens

Any human health criterion for a carcinogen is based on at least three inter-related considerations: potency, exposure, and risk characterization. States may make their own judgments on each of these factors within reasonable scientific bounds, but documentation to support their judgments should be clear.

Maximum protection of human health from the potential effects of exposure to carcinogens via contaminated fish would require an RAC of zero. The zero level is based upon the assumption of non-threshold effects (i.e., no safe level exists below which any increase in exposure does not result in an increase in the risk of cancer) for carcinogens. However, because the zero level may never be attainable, a numerical estimate of risk (in $\mu g/l$) which corresponds to a given level of risk for a population of a specified size is selected instead. A cancer risk level is defined as the number of new cancers that may result in a population of specified size due to an increase in exposure (i.e., 10^{-5} risk level = 1 additional cancer in a population of 100,000). Cancer risk is calculated by multiplying the experimentally derived cancer potency estimate by the concentration of the chemical in the fish and the average daily human consumption of contaminated fish. The risk for a specified population (i.e. 100,000 people or 10^{-5}) is then calculated by dividing the risk level by the specific cancer risk. EPA's ambient water quality criteria documents provide risk levels ranging from 10^{-5} to 10^{-7} as examples.

When the cancer potency estimate, or slope factor (formerly known as the q1*), is derived using animal studies, high dose exposures are extrapolated to low dose concentrations and adjusted to a lifetime exposure period through the use of a linearized multistage model. The model calculates the upper 95 percent confidence limit of the slope of a straight line which the model postulates to occur at low doses. When based on human (epidemiological) data, the slope factor is based on the observed increase in cancer risk, and is not extrapolated. For deriving

RACs for carcinogens, the oral cancer potency estimates or slope factors from IRIS are used.

It is important to note that cancer potency factors may overestimate actual risk. Such potency estimates are subject to great uncertainty due to two primary factors: 1) adequacy of the cancer data base (i.e., human vs. animal data) and 2) limited information regarding the mechanism of cancer causation. The actual risk may be much lower, perhaps as low as zero, particularly for those chemicals for which human carcinogenicity information is lacking. Risk levels of 10^{-5}, 10^{-6}, and 10^{-7} are often used by States as minimal risk levels in interpreting their standards. EPA considers risks to be additive, i.e., the risk from individual chemicals is not necessarily the overall risk from exposure to water. For example an individual risk level of 10^{-6} may yiel a higher overall risk level if multiple carcinogenic chemicals are present.

For carcinogenic effects, the RAC can be determined by using the following equation:

Equation 4.2:

$$C \text{ or } RAC \text{ (mg/l)} = \frac{(RL \times WT)}{q1^* \ [WI + FC \times L \times (FM \times BCF)]}$$

where:

C	=	updated water quality criterion (mg/l)
RAC	=	reference ambient concentration (mg/l)
RL	=	risk level (10^{-X})
WT	=	weight of an average human adult [70 kg]
q1*	=	carcinogenic potency factor (kg day/mg)
WI	=	average human adult water intake [2 liters/day]
FC	=	daily fish consumption (kg fish/day)
L	=	ratio of lipid fraction of fish tissue consumed to 3%
FM	=	food chain multiplier (from Table 4.1)
BCF	=	bioconcentration factor (mg toxicant/ kg fish divided by mg toxicant/L water) for fish with 3% lipid

If the receiving water body is not used as a drinking water source, the factor WI can be deleted. For identified carcinogenic chemicals without known cancer potency estimate values, extrapolation procedures can be used to estimate the cancer potency (see Appendix E).

4.9 Calculating RTCs for Non-Carcinogens

The following formula may be used to calculate a RTC for a non-carcinogens. Readers may also consult EPA's "Assessing Human Health Risks from Chemically Contaminated Fish and Shellfish" [44].

The basic equations for deriving RTCs uses the same variables as in Equations 4.1 where the BCF is normalized at 3.0% lipid:

Equation 4.3: $$RTC\ (mg/kg) = \frac{(RFD \times WT) - (DT + IN) \times WT}{WI\ /(BCF \times FM \times L) + FC}$$

The above equation should be corrected for site specific lipid content and bioaccumulation factors where data are available.

4.10 Calculating RTCs for Carcinogens

The following formula may be used to calculate a RTC for a carcinogens. Again, the basic equations for deriving RTCs use the same variables as in Equations 4.2 where BCF is normalized at 3.0% lipid:

Equation 4.4: $$RTC\ (mg/kg) = \frac{RL \times WT}{ql^* [WI/(BCF \times FM \times L) + FC]}$$

The above equation should also be corrected for site specific lipid content and bioaccumulation factors where data are available.

4.11 Calculating RTCs from State Numeric Water Quality Standards

When a State numeric water quality standard is available for a chemical, a RTC may be needed for the evaluation of tissue residue assessment results. In these cases an RTC can be calculated from the numeric standard by using the BCF relationship. In equation form:

Equation 4.5:

$$RTC\ (mg/kg) = (BCF \times FM \times L) \times (State\ numeric\ standard)$$

The above equation should be corrected for site specific lipid content (L) and bioaccumulation factors (BCF x FM) where data are available.

4.12 Deriving Quantitative Risk Assessment in the Absence of IRIS Values

The RfDs or cancer potency factors provide the existing quantitative risk assessments for developing RACs. However, as indicated above, effluents and tissues may contain identified for which no risk value is currently available on IRIS. When IRIS data are unavailable, quantitative risk level information may be determined according to a State's own procedures. Some States have established procedures whereby risk factors can be developed based upon extrapolation of acute and/or chronic animal data to

concentrations of exposure protective of fish consumption by humans (See Appendix H). Where no State procedure exists, risk assessment values may be based upon extrapolation from mammalian or other data using the IRIS documentation in Appendix E or information available through other EPA risk data bases such as HEAST or Risk*Assistant (Appendix H). Also, where no other information or procedure exists, drinking water maximum contaminant levels (MCLs) or FDA Action Levels may be used as guidance in developing numerical estimates.

As a general matter, some of the assumptions made in deriving FDA action levels for fish and shellfish (as well as FDA levels of concern and tolerance levels) are inappropriate for use in regulating water quality [44]. In particular, FDA exposure assumptions, in accordance with its legislative mandate, reflect expected consumption by buyers of fish in interstate commerce. FDA generally assumes, for example, that contaminated fish would not constitute a high proportion of such a consumer's diet. In contrast, to adequately protect the user, EPA and States must consider the individual who frequently fishes at the site being regulated or who regularly eats fish from the area. Thus, without a separate analysis and determination that at a particular site the FDA level of concern sufficiently meets the Clean Water Act's objective, the FDA level of concern is not an appropriate basis for a water quality criterion or RAC.

CHAPTER 5

Exposure Assessment and Wasteload Allocations

The purpose of this chapter is to provide procedures for developing wasteload allocations (WLAs) for bioconcentratable contaminants. Procedures for deriving bioconcentration-based WLAs differ somewhat from derivation of aquatic toxicity-based WLAs because bioconcentration-based WLAs involve human health protection and thus, can involve different exposure considerations. Exposure considerations and design conditions for control of bioconcentratable pollutants involve longer time frames than for pollutants causing acute or chronic aquatic because the total lifetime exposure to humans must be considered.

At this step in the control of bioconcentratable pollutants, chemicals of concern have been identified, and RACs have been developed or State water quality standards identified. Where State numeric criteria for the protection of human health from bioconcentration in consumed fish are not available, an RAC should be developed, in accordance with 40 CFR 122.44(d)(1)(vi) using the procedures described by the State for interpretation of the narrative standards or if none exist, the procedures described in Chapter 4 should be used. The next step is to calculate the WLA to determine if permit limits are necessary.

The WLA condition is that which attains the RAC at the critical design conditions. In discharge situations where nonpoint source contributions are significant, controls of point sources alone may be insufficient to attain State water quality standards. Critical conditions at alternate higher flow conditions may be necessary in these instances. After developing a human health based WLA for a given chemical, the two WLAs for protection of aquatic life should be compared with the human health WLA, and the most stringent of these should be used in permit limit development. The permit limit is the effluent concentration and/or mass limitation that attains the WLA for the required exposure periods. Where the human health WLA is determined to be most stringent, the regulatory authority should follow the procedures in Chapter 6 to apply this WLA in permit limit development. The reader is referred to the TSD for a more detailed discussion of exposure assessment and wasteload allocations. The discussion in this document is intended to provide a brief introduction to this subject, focusing on those aspects of direct concern for the control of bioconcentratable pollutants.

5.1 Total Maximum Daily Loads

The development of a wasteload allocation is based on the existing total maximum daily load (TMDL) for the receiving water. A TMDL is the sum of the individual wasteload allocations (WLAs) for point sources and the load allocations (LAs) for nonpoint sources of pollution and natural background sources, tributaries, or adjacent segments. A TMDL is the amount of a pollutant from point sources and nonpoint and natural background sources, including a margin of safety, that may be discharged to a water quality-limited waterbody without exceeding the applicable water quality criterion. WLAs represent that portion of a TMDL that is established to limit the amount of pollutants from existing and future point sources so that surface water quality is protected at all flow conditions.

The TMDL process uses water quality analyses to predict water quality conditions and pollutant concentrations. Limits on wastewater pollutant loads are set and controls on nonpoint source loadings are established so that predicted receiving water concentrations do not exceed water quality criteria or RACs. TMDLs and WLAs/LAs should be established at levels necessary to attain and maintain the applicable narrative and numerical water quality standard (WQS) with seasonal variations and a margin of safety that takes into account any lack of knowledge concerning the relationship between point and nonpoint source loadings and water quality. Determination of WLAs and TMDLs should take into account critical conditions for stream flow, loading, and water quality parameters. WLAs and Permit limitations should be issued based on TMDLs where available. A more detailed discussion of TMDLs can be found in the Technical Support Document [32].

5.2 Mixing Zones

It is not always necessary to meet all water quality criteria within the discharge pipe to protect the integrity of the waterbody as a whole. Sometimes it is appropriate to allow for ambient concentrations above the criteria in small areas near outfalls. These areas are called mixing zones. Since these areas of impact, if disproportionally large, could potentially adversely impact the productivity of the waterbody, and have unanticipated ecological consequences, they should be carefully evaluated and appropriately limited in size. As understanding of pollutant impacts on ecological systems evolves, there may be cases identified where no mixing zone is appropriate.

To ensure mixing zones do not impair the integrity of the waterbody, it should be determined that the mixing zone will not cause lethality to passing organisms and that, considering likely pathways of exposure, there are no significant human health

risks. One means to achieve these objectives is to limit the size of the area affected by the mixing zones.

In the general case, where a State has both acute and chronic aquatic life criteria, as well as human health criteria, independently established mixing zone specifications may apply to each of the three types of criteria. The acute mixing zone may be sized to prevent lethality to passing organisms, the chronic mixing zone sized to protect the ecology of the waterbody as a whole, and the health criteria mixing zone sized to prevent significant human risks. The acute mixing zone need not be smaller than the other two. For any particular pollutant from any particular discharge, the magnitude, duration, frequency, and mixing zone associated with each of the three types of criteria will determine which one most limits the allowable discharge.

Mixing zone allowances will increase the mass loadings of the pollutant to the waterbody, and decrease treatment requirements. They adversely impact immobile species, such as benthic communities, in the immediate vicinity of the outfall. Because of these and other factors, mixing zones must be applied carefully, so as not to impede progress toward the Clean Water Act goals of maintaining and improving water quality. EPA recommendations for allowances for mixing zones, and appropriate cautions about their use, are contained in this section.

The Clean Water Act allows mixing zones at the discretion of the State. **EPA recommends that States have a definitive statement in their standards on whether or not mixing zones are allowed.** Where mixing zones provisions are part of the State standards, the State should describe the procedures for defining mixing zones.

In all cases, the size of mixing zone and the area within certain concentration isopleths should be evaluated for their effect on the overall biological integrity of the waterbody. If the total area affected by elevated concentrations within all mixing zones combined is small compared to the total area of a waterbody (such as a river segment), then mixing zones are likely to have little effect on the integrity of the waterbody as a whole, provided that they do not impinge on unique or critical habitats. EPA has developed a multi-step procedure for evaluating the overall acceptability of mixing zones [32].

For protection of human health, the presence of mixing zones should not result in significant health risks, when evaluated using reasonable assumptions about exposure pathways. Thus, where drinking water contaminants are a concern, mixing zones should not encroach on drinking water intakes. Where fish tissue residues are a concern (either because of measured or predicted

residues), mixing zones should not be projected to result in significant health risks to average consumers of fish and shellfish, after considering exposure duration of the affected aquatic organisms in the mixing zone, and the patterns of fisheries use in the area.

While fish tissue contamination tends to be a far-field problem affecting entire waterbodies rather than a narrow-scale problem confined to mixing zones, restricting or eliminating mixing zones for bioaccumulative pollutants may be appropriate under conditions such as the following: (A) Mixing zones should be restricted such that they do not encroach on areas often used for fish harvesting particularly of stationary species such as shellfish. (B) Mixing zones might be denied where such denial is used as a device to compensate for uncertainties in the protectiveness of the water quality criteria or uncertainties in the assimilative capacity of the waterbody.

5.3 Point of Application of the Criteria

The point at which the human health criteria or RACs are to be met in the receiving water may be fixed by existing State standards or may be determined by considerations for managing individual and aggregate risks. The several possibilities include the following:

- Where State standards allow no mixing zone and no spatial averaging, the criterion would be met at the end of the pipe.

- Where State standards specify that the criterion must be met at the edge of the mixing zone, the criterion would be applied at that point.

- Where State standards allow considerations of spatial averaging, the criterion may be met as an average within a specified area.

- Where State standards apply to aggregate risk, the criterion for each specific chemical must be reduced such that the sum of the exposures does not exceed the maximum level permissible under the standards.

5.4 Determining the Wasteload Allocation

If a State does not allow a mixing zone, then the criterion, or RAC, is applied at the end-of-pipe and no wasteload allocation (WLA) determination is necessary unless a facility can achieve compliance with water quality standards using a diffuser. In

some instances, a "complete mix" situation will exist, especially in an effluent-dominated scenario. In those situations, the criterion or RAC is applied after the dilution determination using the appropriate critical flow condition (as described in Sections 5.6.1 and 5.6.2). For the purpose of this document, WLAs are only necessary or required where instream dilution is allowed for bioconcentratable pollutants.

Where dilution is allowed, a WLA should be calculated to achieve the RAC selected above [29,51]. The human health WLA should be compared with the acute and chronic aquatic life WLAs for a given chemical and the most stringent WLA selected for use to determine the permit limit.

5.4.1 Steady State Dilution Models

For the purpose of the following discussion, use of simple, steady-state dilution models is assumed. However, these models may be inappropriate for certain situations where sediments serve as a sink for bioconcentratable pollutants, where intermittent nonpoint sources contributions are significant, and where additional factors need to be considered. In some cases, fate and transport models, where sufficient input data and information are available, are useful tools for accounting for an array of variables which may have an impact on the fate of bioconcentratable pollutants in the environment.

In simple situations, the WLA is determined from the ambient criterion and the dilution flow of the receiving water. In more complicated situations (e.g., where mixing is not rapid or where lakes or estuaries are involved) a spatial averaging scale must be chosen. Selection of the spatial scale must be consistent with the State water quality standards requirements which include reasonable assumptions about the behavior of aquatic organisms and the target human population.

In some cases, it may be necessary to apply the RAC within this spatial averaging scale mixing zone, if it is reasonable to assume that (a) the bioconcentrating aquatic organisms have little mobility, thus spending most of their time within the mixing zone, and (b) the target human population consumes a lifetime exposure to pollutants in fish from the mixing zone. The lifetime exposure consists of the sum of all fish tissue concentrations consumed over a 70 year time period. It includes infrequent high concentrations as well as more frequent lower concentrations.

5.4.2 Dynamic Models for Wasteload Allocations

Steady-state modeling considers only a single condition; the effluent flow and loading are assumed to be constant for the design condition. The impact of variability in receiving water flow on the exceedence of the criteria is implicitly included in the critical design conditions; effluents are considered constant. Dynamic modeling techniques explicitly predict the effects of receiving water and effluent flow and concentration variability. The three dynamic modeling techniques recommended by EPA for wasteload allocations are continuous simulation, Monte Carlo simulation, and log-normal probability modeling. These methods calculate a probability distribution for receiving water concentrations rather than a single, worst-case concentration based on critical conditions.

The dynamic modeling techniques have an additional advantage over steady-state modeling in that they determine the entire effluent concentration frequency distribution required to produce the desired frequency of criteria compliance. Maximum and monthly average permit limits can be obtained directly from this distribution. For the purposes of this discussion only the Monte Carlo simulation, dynamic modeling technique will be described. The other methods use slightly different means to obtain a similar result.

5.4.3 Monte Carlo Simulation Models

Monte Carlo simulation combines probabilistic and deterministic analyses since it uses a fate and transport mathematical model with statistically described inputs. The probability distributions of effluent flow, effluent concentration, and other model input must be defined using the appropriate duration for comparison to the criteria. If 90-day average receiving water concentrations must be predicted for RAC comparisons, probability distributions of 90 day model input data are needed for Monte Carlo simulation. If 70 year average concentrations must be predicted for RAC comparisons, the probability distributions of 70 year average input data are required. The computer selects input values from these distributions using a random generating function. The fate and transport model is repetitively run for a large number of randomly selected input data sets. The result is a simulated sequence of receiving water concentrations. These concentrations do not follow the temporal sequence but they can be ranked in order of magnitude and used to form a frequency distribution. Monte Carlo analyses can be used with steady-state or continuous simulation models [52].

The approach for calculating the allowable effluent concentration distribution using Monte Carlo simulation is the same as that for continuous simulation models. The advantages of Monte Carlo simulation are the following:

- it can predict the frequency and duration of toxicant concentrations in a receiving water;
- it can be used with steady-state or continuous simulation models that include fate processes for specific pollutants;
- it can be used with steady-state or continuous simulation models that include transport processes for rivers, lakes, and estuaries;
- it can be used with steady-state or continuous simulation models that are designed for single or multiple pollutant source analyses;
- it does not require time series data;
- it does not require model input data to follow a specific statistical distribution or function; and
- it can incorporate the cross-correlation and interaction of time-varying flow and pollutant discharges if the analysis is developed separately for each season and the results are combined.

The primary disadvantage of Monte Carlo simulation is that it requires the availability of data for effluent variability and receiving water flows.

5.5 Averaging Periods for WLAs

The duration of exposure assumed in deriving the reference ambient concentration (RAC), should affect the selection of the design conditions and may affect the point of application of a criterion. This duration of exposure or averaging period underlying the derivation of the water quality criterion or RAC is the time period required to obtain an adverse effect or an unacceptable risk from marginally exceeding the RAC or criterion. The duration should have a logical connection with the design flow. Often, however, the flow averaging period only approximates the criterion averaging period, as for example when the 7Q10 is coupled with the 4-day average aquatic life criterion.

The duration of the averaging period for determining the WLA should be consistent with the assumptions used to derive the criterion or RAC being applied. For example, the human health criteria for carcinogens are based on the assumed risks associated with a lifetime exposure which can consist of a series of short term exposures or a continuous exposure over a 70-year period.

The human health criteria for non-carcinogens is pollutant specific; health effects could develop from one short-term exposure for some compounds. The criteria are based on various exposure periods ranging from a few days to many years depending upon the pollutant.

Therefore, the averaging period underlying the RAC is 70 years for carcinogens, but may be pollutant specific or unknown for noncarcinogens. The duration of exposure assumed in deriving criteria for non-carcinogens is more complicated due to a wide variety of endpoints; some developmental (and thus age-specific and perhaps sex-specific), some lifetime as with carcinogens, and some, such as organoleptic effects, not age-related at all.

5.6 Dilution Design Conditions for Freshwater Receiving Waters

The appropriate dilution design conditions to use in calculating a WLA depend on the averaging period of the exposure. **For averaging periods greater than one year, as is the case for carcinogens, the harmonic mean flow should be used as the critical design flow. For averaging periods of one month to one year, the 30-day flow with an appropriate recurrence interval (e.g. five or ten years) should be used; for averaging periods less than one month, the 7Q10 as the design flow should be used.**

5.6.1 Carcinogens

In well mixed situations, the receiving water concentration is determined by the pollutant load, and the combined receiving water plus effluent flow, such that:

Equation 5.1 $$C = W/Q$$

where C is the instream concentration, W is the effluent load, and Q is the combined effluent and river flow.

The design flow for calculating average receiving water concentrations over a several-year period is selected as the flow that results in the average dilution obtained over that period. Since the dilution obtained by a given flow is proportional to

the reciprocal of the flow (the instream concentration is equal to the discharge load divided by stream flow), a simple mean of the daily flows does not characterize the average dilution obtained. The harmonic mean flow, is the flow at which average dilution is obtained, and this flow should be used as the design flow for multiple-year averaging periods as is the case for carcinogens.

The recommendation of the mean flow is the best estimator of the lifetime exposure to carcinogenic pollutants. The adverse impact of carcinogenic pollutants is estimated in terms of receptors' (human) lifetime intakes. To be within the acceptable level of a lifetime exposure of any carcinogen, such intakes should not exceed the RAC during "the lifetime" of the receptor. A lifetime for carcinogenic pollutants is defined by EPA as 70 years or approximately 365 (days/year) x 70 years. In estimating the lifetime exposure, EPA assumes that people will catch and consume fish at random times spaced over a year. Consumption over 70 years of random fishing can be best estimated by calculating the exposure of fish to the average concentration in the receiving water which in turn is best calculated by using the harmonic mean flow. Once fish tissue concentrations approach an equilibrium with the average receiving water concentrations, variations in the tissue concentrations will reflect variations in the receiving water, but the averages will not change.

The exposure to carcinogenic pollutants is numerically expressed as:

Equation 5.2 $$C_{LT} = (C_1 + C_2 + --- + C_n) / n$$

where C_{LT} is the averaged lifetime exposure, C is the yearly exposure to fish tissue, and n is (365 days/year) x 70 years.

Based on an assumed constant daily load from a treatment facility, the fully mixed in-stream concentration will go up or down inversely with the ups and downs of receiving water flows. Therefore, in-stream concentration is inversely proportional to, the streamflow downstream of the discharge:

Equation 5.3 $$1/Q_{LT} = (1/Q_1 + 1/Q_2 + --- + 1/Q_n) / n$$

The inverse of Equation 5.3 is the definition of the harmonic mean:

Equation 5.4 $$Q_{hm} = n / (1/Q_1 + 1/Q_2 + --- + 1/Q_n)$$

Equation 5.5: $$Q_{hm} = n / \sum_{i=1}^{i=n} (1/Q_i)$$

where n = the number of recorded flows, and Q is the observed streamflow plus effluent flow on day i. A five-year minimum period of record is recommended to assess the average flow.

The harmonic mean flow represents the long-term average based flow of a stream minimizing the rainfall-induced spikes of high flow which can skew the annual arithmetic average flow. This average base flow is the flow which aquatic life is subjected to for the majority of the year and is more appropriate for relating to bioconcentration. The difference between the arithmetic and harmonic mean flow is greatest in rivers characterized by long periods of a low base flow followed by short periods of high flows.

With bioaccumulative pollutants, there may be some concern that use of a long term harmonic mean flow may undercalculate the actual lifetime exposure if humans consume an annual amount of fish in a short period (i.e. warm weather months amenable to recreation) or for pollutants which cannot be depurated or metabolized quickly. However, there is a distinct possibility in some instances that people fish only during the periods when a river is at a low flow condition. An example of this is in western streams where low flows occur during the summer months. In these instances, use of the harmonic mean flow based on annual flows can undercalculate the exposure. The exposure can be adjusted to reflect this in two ways. First, the harmonic mean flow can be recalculated based on the flows from only the months most likely for fishing. Actual data is necessary to do this because the relationships shown below only hold for annual data. Second, the RAC can be adjusted to reflect the fish consumption during the fishing months.

In order to establish a maximum allowable discharge load, one would set the average receiving water concentration to the level established by risk-based exposure analysis (the RAC) and multiply it by the harmonic mean flow. Thus from this perspective, the harmonic mean flow serves as a design flow for performing waste load allocations for carcinogens.

EPA recommends that the harmonic mean flow be calculated directly from the historical flow record if possible. In the absence of adequate data (5 years or more), the harmonic mean flow, Q_{hm}, may be estimated by any of several methods described in Appendix F, if flows are approximately log-normally distributed. One method that assumes a log normal distribution of flows relates the harmonic mean to the geometric and arithmetic mean flows:

$$Q_{hm} = \frac{Q_{gm}^{2}}{Q_{am}}$$

where Q_{gm} is the geometric mean flow, and Q_{am} is the arithmetic mean flow. Kurtosis and skewness coefficients of the log transformed data should be computed before using harmonic mean flows (which assumes log normality of the distribution).

Normally, if there are at least 5 years of data to compute these coefficients, then it would be more appropriate to compute harmonic mean flow directly using HHDFLOW below. However, in some instances there may only be summaries of the statistical parameters in USGS flow records. In cases where at least 5 years of flow data are not available and a 7Q10 has been estimated, EPA recommends the use of multiplication factors as described below to estimate the Q_{hm}.

The two software packages which are available for computation of harmonic mean flow are: WQAB FLOW (a description on how to use this software is in Appendix F) and HHDFLOW. The HHDFLOW program can be used regardless if data are log-normally distributed or not. HHDFLOW has been incorporated into the WQAB system as a computational tool within the existing DFLOW software package (see the TSD [32] for more information on these exposure assessment tools).

In order to develop some quantitative sense of how a long-term harmonic mean flow of any stream compares with its 7-day 10-year low flow, EPA's Assessment and Watershed Protection Division and the Risk Reduction Engineering Laboratory at Cincinnati, Ohio analyzed flow records of 60 streams. These are the same stream flow records which had been analyzed for stream design flow condition for aquatic life protection criteria as listed in Book VI, Design Conditions: Chapter 1, Stream Design Flow for Steady-State Modeling [53]. Based on the long-term harmonic flow and 7-day 10-year low flow estimates for these sixty streams, the long-term harmonic mean flows of all 60 streams were equal to or greater than 2 times the 7Q10 low flow. Fifty-four of the streams' harmonic mean flows were equal to or greater than 2.5 times their 7Q10 low flows. Finally, 40 of the 60 streams' harmonic mean flows were equal to or greater than 3.5 times the 7Q10.

Based on the above observations, permit authorities may choose a multiplication factor of 3 x 7Q10 to estimate stream design flow for human health protection for carcinogenic pollutants, except for regulated streams where HHDFLOW should be used instead. This provides an upper bound estimate of the harmonic mean flow based on the data from the 60 streams referenced above. For those States with 7Q2 design flow, the 7Q2 is generally 2 times greater than 7Q10.

Alternatively, in situations where long-term daily flow data do not exist but estimates of common flow statistics are available from limited data, for example in ungauged discharge sites, the following equation may be used to estimate harmonic mean flow [54]:

$$Q_{hm} = [1.194 * (Q_{am})^{0.473}] * [(7Q10)^{0.552}]$$

The 7Q10 low flow and annual average flow (Q_{am}) for most parts of the U.S. are readily available. The 7Q10 and Q_{am} can be estimated using the USGS computer program, FLOSTAT.

5.6.2 Non-carcinogens

The choice of return interval represents a level-of-protection consideration inherent in the risk management decision to be made by the regulatory authority. If a short-term duration of exposure is chosen (i.e., 90 days or less), design flows may be appropriately based on critical low flow conditions. In that the effects from non-carcinogens can be associated with short term exposures, the Technical Support Document [32] recommends the use of the design flow 30Q5. However, in the comparisons of flows for smaller rivers (i.e., low flow 50 cfs) the 30Q5 flow was only 1.1 times that of the 7Q10. For larger rivers (i.e., low flow of 600 cfs) the factor was 1.4 times. Therefore, multiplication of these factors by 7Q10 can be used as an approximation.

5.7 Lake, Marine and Estuarine Receiving Waters

Estuarine and lake areas in particular are often characterized by long retention times, low potential for quick initial mixing, and concentrated numbers of dischargers. For these reasons, pollutants tend to reside within these areas rather than become quickly washed out. Impacts from individual dischargers can overlap. Therefore, cumulative impacts should be considered, and a model to incorporate such impacts may need to be used. The following discussion summarizes the characteristics of marine and estuarine discharges and presents a few of the different types of approaches available for developing WLAs for lake, marine and estuarine discharges.

5.7.1 Lake, Marine and Estuarine Mixing

The first stage of mixing that occurs as a wastewater is discharged to the lake, marine and estuarine environment is caused by the properties of the discharge itself. The models which describe discharge-induced mixing are termed nearfield

models. Discharge-induced mixing is caused by two influences: jets and plumes. Jets are caused by discharge velocity, as the difference in velocity between the discharge and the ambient environment tends to entrain ambient water into the discharge and cause dilution. Plumes are caused by effluents which are buoyant with respect to the ambient environment. Effluents discharged to the environment are typically less dense than the ambient water and tend to rise upon discharge; this movement also serves to entrain ambient water and cause dilution. Often times, a discharge will contain both buoyancy and momentum. This situation is termed a buoyant jet, and the two types of mixing work in concert to dilute the effluent.

As the distance from the outfall increases, mixing caused by ambient turbulence in the receiving water becomes important. The models which describe ambient induced mixing are typically referred to as farfield models. The point where ambient-induced mixing dominates discharge-induced mixing is a function of the ambient turbulence and the discharge's buoyancy and momentum. This point is not easily defined. Ambient mixing in marine and estuarine systems can be caused by many factors; tidal currents, Coriolis effects, freshwater inflow and wind-driven currents are a few examples.

Generally, for the development of WLAs for bioconcentratable contaminants discharged to estuarine and marine receiving waters, the use of steady-state, nearfield models for mixing zone applications, is recommended. The inputs to the nearfield mixing zone models depend primarily on discharge characteristics and can be realistically assumed to remain constant over time. Also, the response time of the waterbody to changes in inputs is also relatively fast. Consequently, steady-state models are appropriate for use in assessing nearfield mixing zone issues. Cumulative impacts, however, depend upon ambient conditions and may require an extension to steady-state modeling called tidal averaging (see TSD [32] for more information on this subject).

Water quality models described in this section can be applied either deterministically or stochastically to assess compliance under these conditions. A deterministic model application predicts a single environmental response to a single set of model inputs. Stochastic model application, such as Monte Carlo Simulation (Section 5.4.3), predicts the entire distribution of water quality conditions which can occur in response to the expected range of environmental and loading conditions.

5.7.2 Approaches for Marine and Estuarine Nearfield Models

The following discussion describes the approaches which may be taken to determine the bioconcentratable pollutant concentrations at the edge of the mixing zone. Four approaches are addressed:

1. Desktop calculations
2. UPLUME model
3. UMERGE model
4. COREMIX model

1. Desktop Calculations

The most direct recommended approach for predicting pollutant concentrations at the edge of a mixing zone consists of an equation which can be solved using a hand calculator. The desktop equation recommended for use considers dilution caused by discharge momentum only. It may be appropriately applied to cases where the effluent is neutrally buoyant in the ambient water, such as freshwater outfalls. Effluents discharged into estuarine and coastal waters tend to be positively buoyant, and are rarely completely neutrally buoyant. The equation, taken from the Technical Support Document for Water Quality-based Toxics Control [32] is:

$$S = 0.3 \frac{x}{d}$$

where: S = average dilution (dimensionless)
x = distance from outlet to edge of mixing zone
d = diameter of outlet

The equation provides an estimate of mixing zone dilution only, since it assumes that ambient-induced mixing is zero. Ambient-induced mixing begins to dominate jet-induced mixing as the distance from the outfall increases. Consequently, predictions using this equation are most appropriate for predicting dilution close to the outfall. Predictions become increasingly conservative as distance from the outlet to the edge of the mixing zone increases, and ambient-induced mixing becomes more important.

2. UPLUME Model

EPA supports a series of nearfield mixing models designed to describe the dilution of effluents discharged to marine environments which are described in detail in the TSD [32]. UPLUME, considers a buoyant plume issuing at an arbitrary angle

into a stagnant environment. UPLUME is designed to assess a single port discharge, although the model will predict dilution for multiple port discharges up to the point where adjacent plumes merge. UPLUME is recommended for use for single port discharges only. The model UMERGE discussed subsequently is recommended for multi-port diffusers.

UPLUME will predict average dilution in the effluent plume at increasing distances from the discharge. These dilution calculations can then be used to determine allowable effluent concentrations. Calculations continue until the plume stops rising and its trajectory becomes horizontal. This endpoint will be caused by either: 1) the density of the plume becoming equal to the density of the ambient environment, or 2) the plume reaching the water surface. UPLUME can be applied to either stratified or non-stratified environments.

3. UMERGE Model

UMERGE extends upon UPLUME's capabilities in two ways. First, UMERGE accounts for merging among adjacent plumes and is therefore appropriately applied to multi-port diffusers. Second, UMERGE allows specification of ambient current velocities at various depths in the water column. Similar to UPLUME, UMERGE will provide calculations of average dilution within the plume at various depths until the plume vertical velocity becomes zero.

4. COREMIX Model

EPA has supported development of an expert system for the analysis of submerged discharges, entitled CORMIX (EPA, 1989). The user supplies CORMIX with information about the discharge and ambient environment. CORMIX then provides information regarding the dilution and geometric configuration of the plume in the ambient water. CORMIX also explicitly considers regulatory mixing zone dimensions and requirements. CORMIX contains two key elements. The first is a rigorous analytical scheme that classifies any given discharge/environment situation into one of several categories with distinct hydrodynamic features. The second element is a collection of predictive models appropriate for different classification schemes. CORMIX automatically selects and applies the model framework appropriate for the discharge/environmental conditions supplied by the user, providing dilution and mixing zone compliance information.

In summary, for the four approaches for determining WLAs for marine and estuarine discharges, the desktop method is used to assess dilution of neutrally buoyant jet discharges, UPLUME for single-port buoyant jets, and UMERGE for multi-port buoyant jets. The COREMIX model can be used for all applications.

5.8 Exposure from Contaminants in Sediments

Sediments may act as sinks for bioconcentratable pollutants discharged from effluents or from other sources. Bioaccumulative organic pollutants will adsorb to particulates in an effluent or receiving water, the particulates may later be deposited as sediments. Dissolved organic compounds may also partition from the water column to the particulates forming a sediment deposit. High log P chemicals (above log P of 6.5) are known to have a greater tendency to concentrate in sediments. Sediments also act as sources of bioconcentratable and bioaccumulative organic chemicals to aquatic life. The chemicals adsorbed to sediments can partition from the sediments back into the water column and the sediments themselves may be ingested by benthic organisms and fish species, both of which may contribute food chain effects and bioaccumulation.

The assessment of sediment samples for bioconcentratable contaminants, described in Chapter 3, can determine the presence, identity, and concentrations of these pollutants. Since sediments can accumulate these chemicals over relatively long periods of time the bioaccumulative chemicals may be present in greater concentrations in sediment than in a given effluent sample. In some cases, this may facilitate detection of contaminants which are present in an effluent or other sources at very low concentrations or which are only released periodically. For assessments of point source discharges, the results of the sediment assessment may be used to specify chemical specific tests of effluent samples from a given discharger in order to confirm the presence and concentrations of the bioconcentratable chemicals in the effluent.

The relationship between bioconcentratable chemicals and sediments is, in many ways, parallel to that between these chemicals and biota. That is, sediments provide a chemical environment that is energetically favorable for hydrophobic chemicals relative to water. It is for this reason that sediments are a potentially a major reservoir for hydrophobic chemicals. The organic carbon fraction of the sediment acts like the lipid fraction of the biota: it is the phase into which the chemicals partition. It is commonly observed that the concentration of hydrophobic chemicals in sediment dwelling organisms are in the same order of magnitude as the surrounding sediment. This is the result of the similar concentrations of lipid in organisms and organic carbon in sediments. Hence the sediments provide another environmental compartment in which bioconcentratable chemicals can be found.

When an effluent containing bioconcentratable chemicals is discharged into a receiving water, a fraction of the chemical is adsorbed by the organic carbon fraction of the suspended particles. The fate of the sorbed fraction of the chemical is determined by the fate of these particles. During quiescent periods a portion of these particles settle thereby transporting the chemical to the bottom sediment and removing it from the water column. However, during more turbulent periods, periods of high flows for example, particles from the sediment can be scoured into the overlying water, thereby providing a source of bioconcentratable chemicals. The chemical that remains in the sediment can also affect the overlying water concentration via another mechanism. Chemicals sorbed to sediment particles can desorb into the interstitial water of the sediment (surface sediments are typically 90% water and only 10% particles by weight), Depending on the concentration in the overlying water, chemical can either diffuse into or out of the sediment interstitial water.

As a consequence of these mechanisms, the sediments of a body of water receiving a discharge of bioconcentratable chemical can accumulate a large mass of the chemical. However, the quantity of chemical stored is not equivalent to the removal rate of the chemical. If the net burial rate of sediments is large, then a fraction of the sorbed chemical can be lost to the deep sediments by burial. However, in most flowing waters, the sediments act as a temporary storage, accumulating chemicals during quiescent periods and releasing chemicals during turbulent periods. Hence, when computing the exposure concentration for fish, it is imprudent to assume that a significant rate of loss of chemical occurs via sedimentation and burial. A detailed mass balance analysis of the suspended solids and the chemical of concern is required to substantiate this loss. Any net release of a chemical from the sediments would need to be accounted for in the LA portion of the TMDL. **In the absence of such an analysis, EPA recommends that the TMDL consider no significant loss of chemical to the sediments.** That is, the chemical is assumed to behave as a conservative substance in the water column and the receiving water concentration is computed using only the effluent chemical concentration and the dilution that occurs, as was described above.

CHAPTER 6

Permit Limits for Control of Bioconcentratable Pollutants

Once the pollutants of concern, the Reference Ambient Concentration or State water quality criteria for those pollutants, and the wasteload allocation have been determined, as were described in Chapters 3, 4 and 5 respectively, the next step is to characterize the effluent for human health effects. The purpose of effluent characterization is to determine whether or not the discharge of the identified bioconcentratable pollutants causes, has the reasonable potential to cause, or contributes to an adverse impact to human health.

The effects of the discharge of bioconcentratable pollutants can be characterized by utilizing the measured effluent concentrations of those pollutants of concern to determine the expected exposure concentrations in the receiving water and then by comparing this exposure concentration to the criteria or RAC for that pollutant. The receiving water concentration (RWC) is the calculated exposure concentration of a pollutant in the receiving water at the critical flow condition and after mixing. An appropriate effluent flow based on the exposure period should be used in this calculation. Chapter 5 provides a detailed discussion of the appropriate critical flow conditions and mixing zone considerations to be used in developing wasteload allocations (WLAs). The same basic components described in that discussion for wasteload allocations are used for the purposes of calculating the RWC for effluent characterization. In brief, the recommendations for RWCs are to use the harmonic mean flow for carcinogenic pollutants and the 30Q5 for non-carcinogenic pollutants.

6.1 Basis for Effluent Characterization for Human Health

For individual pollutants, the potential for adverse impact in the receiving water is minimized where the RWC is less than the RAC, and becomes maximized where the RWC exceeds the RAC (the term, RAC is used throughout this section interchangeably with EPA human health criteria). **Therefore, to prevent adverse impacts, the RWC of the pollutant (based on allowable dilution for the discharge and pollutant) must be less than the applicable criterion.** Protection of human health will be achieved where the RWC is less than the EPA criterion or the RAC:

$RWC \leq RAC$ (RAC is equivalent to human health criterion)

Where no mixing zone is allowed, the RAC would be applied at the end-of-pipe:

Effluent Concentration $\leq$ RAC

The water quality analyst will use the same basic components in the above-described relationship (i.e., critical receiving water flows, ambient criteria values, measures of effluent quality) for both effluent characterization and wasteload allocation development albeit from different perspectives. In the case of effluent characterization, the objective is to project receiving water concentrations based upon existing effluent quality to determine whether or not an excursion above ambient criteria occurs, or has the reasonable potential to occur. In developing wasteload allocations on the other hand, the objective is to fix the RWC at the desired criteria level and determine an allowable effluent loading which will not cause excursions above the criteria.

Recommendations for projecting the RWC are described within this chapter. Chapter 5, Exposure Assessment and Wasteload Allocation, provides recommendations for determining allowable effluent loadings to achieve established ambient criteria and for calculating wasteload allocations for establishing permit limits. The procedures described within Chapter 5 can also be used to calculate the dilution for the purpose of effluent characterization. The Permit Limits section of this chapter describes the actual calculation of permit limits after effluent characterization and loadings and wasteload allocations are complete.

6.1.1 Recommended Approach for Effluent Characterization

The following four step approach for effluent characterization is adapted from the recommendations provided in the Technical Support Document for Water Quality-based Toxics Control [32]. The objective of this approach is to determine whether or not permit limits need to be developed based upon the results of the effluent bioconcentration evaluation.

Step 1 - Identify the Pollutants of Concern

The pollutants which are of concern are identified in report no. 4 of the analytical procedures described in Chapter 2.

Step 2 - Determine the Basis for Establishing RACs for the Pollutants of Concern

If a State has a numeric water quality criterion for bioconcentration for the pollutant of concern, this should be

used to characterize the effluent and develop permit limits (40 CFR 122.44(d)(1)(iii). If a State has not adopted a numeric water quality criterion for the pollutant of concern, then one of three options for using the narrative criterion may be used (40 CFR 122.44(d)(1)(vi)) to determine whether a discharge causes, has the reasonable potential to cause, or contributes to an excursion above a narrative standard due to an individual pollutant. Although the provisions of 40 CFR 122.44(d)(1)(vi) are presented in the regulation in the context of permit limit development, these same considerations may be applied in characterizing effluents in order to determine whether limits are necessary.

Option A allows the regulatory authority to establish limitations using a "calculated numeric water quality criterion" which the regulatory authority demonstrates will attain and maintain applicable water quality standards. This option allows the regulatory authority to use any criterion that protects aquatic life and human health. This option would also allow the use of site specific factors, including local human consumption rates of aquatic foods, the State's determination of an appropriate risk level, and any current data that may be available to calculate an RAC.

Option B allows the regulatory authority to establish effluent limits using EPA's Water Quality Criteria guidance documents, if EPA has published a criteria document for the pollutant (supplemented, where necessary, by other relevant information, such as recent information from IRIS).

Option C may be used to develop limits based on an indicator parameter under limited circumstances. An example of an indicator parameter is total toxic organics (TTO); effluent limits on TTO are useful where an effluent contains organic compounds. However, use of this option must be justified to show that controls on one pollutant control one or more other pollutants. Where such data are available, this option may be used provided several conditions are met. (See 40 CFR 122.44(d)(1)(vi)(C)). Using this option presents complications. When trying to determine whether or not a pollutant of concern has a reasonable potential to cause an excursion above the narrative standard, development of specific information on the pollutant of concern and comparison with an RAC (using option A or option B) will normally be necessary.

Step 3 - Dilution Determination

Once a basis for comparison has been established, the margin between projected RWC and the RAC after any allowable mixing may be compared. If the Water Qualtiy Standards so allow the water quality analyst will apply the appropriate mixing zone considerations for human health, which are distinctive from those generally used for aquatic life (see Chapter 4 for a discussion of mixing zones). These comparisons will lead to one of the three outcomes discussed below.

There are two options for comparing the RWC to RAC, both based on the margin between measured chemical concentration and the RAC. The first level is to use simple fate models based on a dilution analysis and comparison with the RAC. The second level of analysis is to use more complex fate models, including dynamic models. These may be applied to lakes, rivers, estuaries, and coastal systems using a desktop calculator or microcomputer [32].

Step 4 - Decision Criteria for Permit Limit Development

After this dilution analysis has been performed, the projected RWC is compared to the RAC for each pollutant of concern. This step should be performed using the highest concentration in each data set for a pollutant. The three possible outcomes discussed above in step 3 are:

1. Excursion above the RAC.

2. Potential for an excursion above the RAC.

3. No potential excursion above the RAC.

If these evaluations project excursions or the reasonable potential to cause or contribute to excursions above the RAC, then a permit limit is required (40 CFR 122.44(d)(1)(ii)). The statistical approach described in Section 6.1.2 or an analogous approach developed by a regulatory authority can be used to determine the reasonable potential. Effluents that are shown not to cause or have a reasonable potential to cause or contribute to an excursion above an RAC should be re-evaluated at permit reissuance.

Where these test results do not show a reasonable potential but indicate a basis for concern after consideration of other facility specific factors discussed Chapters 1 and 3, or if there were inadequate information to make a decision, the permit should contain chemical testing requirements and a reopener clause. This clause would require reopening of the permit and establishment of a limit based upon any test results which show chemical concentrations at levels which cause or have a

reasonable potential to cause or contribute to an excursion above the RAC.

6.1.2 Determination of "Reasonable Potential" for Excursions Above RACs

The procedure below or a similar method required by a permitting authority, can be used to determine if a reasonable potential to exceed an RAC exists using the results of the effluent bioconcentration evaluation. A permittee does not have reasonable potential to exceed a Water Quality Standard or RAC if it can be demonstrated with high confidence that the mean of the log-normally distributed effluent concentrations is below the RAC at the specified flow condition. If the permittee cannot make this demonstration, then there is a reasonable potential to exceed the receiving water standard, and the discharge must be limited.

This recommended procedure consists of the following 5 steps:

Step 1 — Determine the number of total observations ("n") for a particular set of effluent data (concentrations or TUs), and determine the highest value from that data set.

Step 2: — Determine the coefficient of variation for the data set (actual or estimated) and probability basis (95% or 99%).

Step 3 — Determine the appropriate ratio from Table 6-1 or 6-2.

Step 4 — Multiply the highest value from a data set by the value from Table 6-1 or 6-2. Use this value with the appropriate dilution to project a maximum RWC.

Step 5 — Compare the projected maximum RWC to the RAC. Reasonable potential occurs when the projected RWC is greater than the RAC.

This approach combines knowledge of effluent variability as estimated by a coefficient of variation with the uncertainty due to a limited number of data to project an estimated mean concentration for the effluent. The estimated mean concentration is calculated from the log-normal distribution of effluent concentrations at a high confidence level. The projected effluent concentration after consideration of dilution can then be compared to an appropriate water quality criterion to determine the potential for exceeding that criterion and the need

for an effluent limit. This procedure is discussed in more detail in the Technical Support Document with the difference that the mean effluent concentration is estimated rather than an upper bound concentration [32].

6.2 The Wasteload Allocation as Basis for Permit Limit Derivation

A WLA value must be applied in a regulatory context by translation into daily maximum and monthly average permit limits. Compliance monitoring associated with permit limitations allows the regulatory authority to determine if the permit limitations are violated and if the wasteload allocation is being achieved.

WLAs for the protection of human health are typically based upon a single criterion, and, in contrast to WLAs for protection of aquatic organisms, do not specify two levels of protection for both acute and chronic effects. The human health WLAs provide a level of protection which in a broad sense can be considered a long term, or chronic, exposure. This is appropriate for the protection from bioconcentration effects on human health exposure durations of up to 70 years. As part of the water quality-based approach to the control of toxics, the human health WLA should be compared with the acute and chronic aquatic life WLAs for a given chemical, and the most stringent WLA must be selected for use to determine the permit limit. This comparison should be made for each chemical specific water quality-based permit limit which is developed.

Steady-state analyses or models used to develop these wasteload allocations assume that the effluent is constant, and therefore, that the WLA value will never be exceeded. This presents a problem in deriving permit limits because permit limits must address effluent variability. The proper application of a steady-state WLA depends on the exposure period for the criteria or RAC for the pollutant which is to be limited. The impact associated with bioconcentratable contaminants is time dependent as reflected in the averaging periods for the RACs for carcinogens and non-carcinogens. Because of these factors the WLA outputs have specific implications for the subsequent permit limit development process.

6.3 Procedures for Developing Limits

Since compliance with permit limitations is by regulation determined on a daily and monthly basis, it is necessary to set permit limitations expressed in these contexts that meet a given

Table 6-1
Ratio of Maximum Sample to Mean for the 95th Percent Confidence Interval

	Effluent Coefficient of Variation																			
N	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0
1	1.2	1.4	1.6	1.9	2.2	2.5	2.8	3.2	3.6	3.9	4.3	4.7	5.1	5.5	6.0	6.4	6.8	7.2	7.6	8.1
2	1.1	1.2	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0	2.0	2.1	2.2	2.3	2.4	2.4	2.5	2.6	2.6
3	1.0	1.1	1.1	1.2	1.2	1.2	1.3	1.3	1.3	1.4	1.4	1.4	1.4	1.5	1.5	1.5	1.5	1.6	1.6	1.6
4	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
5	1.0	1.0	1.0	1.0	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
6	1.0	0.9	0.9	0.9	0.9	0.9	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.7	0.7	0.7	0.7	0.7	0.7
7	1.0	0.9	0.9	0.9	0.8	0.8	0.8	0.8	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.6	0.6	0.6	0.6	0.6
8	1.0	0.9	0.9	0.8	0.8	0.8	0.7	0.7	0.7	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.5	0.5
9	0.9	0.9	0.8	0.8	0.8	0.7	0.7	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.5	0.5	0.5	0.5	0.5	0.5
10	0.9	0.9	0.8	0.8	0.7	0.7	0.7	0.6	0.6	0.6	0.6	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.4	0.4
11	0.9	0.9	0.8	0.8	0.7	0.7	0.6	0.6	0.6	0.6	0.5	0.5	0.5	0.5	0.5	0.4	0.4	0.4	0.4	0.4
12	0.9	0.9	0.8	0.7	0.7	0.7	0.6	0.6	0.6	0.5	0.5	0.5	0.5	0.5	0.4	0.4	0.4	0.4	0.4	0.4
13	0.9	0.8	0.8	0.7	0.7	0.6	0.6	0.6	0.5	0.5	0.5	0.5	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
14	0.9	0.8	0.8	0.7	0.7	0.6	0.6	0.5	0.5	0.5	0.5	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.3	0.3
15	0.9	0.8	0.8	0.7	0.7	0.6	0.6	0.5	0.5	0.5	0.4	0.4	0.4	0.4	0.4	0.4	0.3	0.3	0.3	0.3
16	0.9	0.8	0.8	0.7	0.6	0.6	0.5	0.5	0.5	0.5	0.4	0.4	0.4	0.4	0.4	0.3	0.3	0.3	0.3	0.3
17	0.9	0.8	0.7	0.7	0.6	0.6	0.5	0.5	0.5	0.4	0.4	0.4	0.4	0.4	0.3	0.3	0.3	0.3	0.3	0.3
18	0.9	0.8	0.7	0.7	0.6	0.6	0.5	0.5	0.5	0.4	0.4	0.4	0.4	0.4	0.3	0.3	0.3	0.3	0.3	0.3
19	0.9	0.8	0.7	0.7	0.6	0.6	0.5	0.5	0.4	0.4	0.4	0.4	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3
20	0.9	0.8	0.7	0.7	0.6	0.5	0.5	0.5	0.4	0.4	0.4	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3

Table 6-2
Ratio of Maximum Sample to Mean for the 99th Percent Confidence Interval

	Effluent Coefficient of Variation																			
N	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0
1	1.3	1.6	2.0	2.5	3.0	3.6	4.3	5.1	6.0	6.9	7.9	9.0	10.1	11.3	12.5	13.7	15.0	16.4	17.7	19.1
2	1.1	1.3	1.5	1.6	1.8	2.0	2.2	2.5	2.7	2.9	3.1	3.4	3.6	3.8	4.0	4.2	4.5	4.7	4.9	5.1
3	1.1	1.2	1.3	1.4	1.5	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.4	2.5	2.6	2.6	2.7
4	1.0	1.1	1.2	1.2	1.3	1.3	1.4	1.4	1.4	1.5	1.5	1.6	1.6	1.6	1.7	1.7	1.7	1.8	1.8	1.8
5	1.0	1.1	1.1	1.1	1.1	1.2	1.2	1.2	1.2	1.2	1.3	1.3	1.3	1.3	1.3	1.3	1.4	1.4	1.4	1.4
6	1.0	1.0	1.0	1.0	1.0	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	0.9	0.9	0.9
8	1.0	1.0	1.0	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.8	0.8	0.8	0.8	0.8	0.8	0.8
9	1.0	1.0	0.9	0.9	0.9	0.9	0.9	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.7	0.7	0.7	0.7
10	1.0	0.9	0.9	0.9	0.9	0.8	0.8	0.8	0.8	0.8	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
11	1.0	0.9	0.9	0.9	0.8	0.8	0.8	0.8	0.7	0.7	0.7	0.7	0.7	0.7	0.6	0.6	0.6	0.6	0.6	0.6
12	1.0	0.9	0.9	0.8	0.8	0.8	0.7	0.7	0.7	0.7	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
13	0.9	0.9	0.9	0.8	0.8	0.7	0.7	0.7	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.5	0.5	0.5	0.5
14	0.9	0.9	0.8	0.8	0.8	0.7	0.7	0.7	0.6	0.6	0.6	0.6	0.6	0.5	0.5	0.5	0.5	0.5	0.5	0.5
15	0.9	0.9	0.8	0.8	0.7	0.7	0.7	0.6	0.6	0.6	0.6	0.6	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.4
16	0.9	0.9	0.8	0.8	0.7	0.7	0.7	0.6	0.6	0.6	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.4	0.4	0.4
17	0.9	0.9	0.8	0.8	0.7	0.7	0.6	0.6	0.6	0.6	0.5	0.5	0.5	0.5	0.5	0.4	0.4	0.4	0.4	0.4
18	0.9	0.9	0.8	0.7	0.7	0.7	0.6	0.6	0.6	0.5	0.5	0.5	0.5	0.5	0.4	0.4	0.4	0.4	0.4	0.4
19	0.9	0.9	0.8	0.7	0.7	0.6	0.6	0.6	0.5	0.5	0.5	0.5	0.5	0.4	0.4	0.4	0.4	0.4	0.4	0.4
20	0.9	0.8	0.8	0.7	0.7	0.6	0.6	0.6	0.5	0.5	0.5	0.5	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4

WLA every month. The statistical procedures for permit limit derivation in the TSD are designed to accomplish this for aquatic life protection where the use of shorter term averaging periods is consistent with two number aquatic life criteria [32].

However, if the TSD procedures were directly used for setting permit limits on bioconcentratable pollutants, both maximum daily and average monthly permit limits could exceed the wasteload allocation necessary to meet the criterion [32]. These permit limits would assure that the long term average effluent discharge would comply with the human health derived WLA only if the assessment of the effluent variability was precise. With bioconcentratable pollutants where exposure duration ranges up to 70 years, EPA believes that effluent variability cannot be reliably estimated from existing data for exposure periods of over 30 days. If the effluent variability was over-estimated when establishing the permit limits, then a facility could be discharging in compliance with the permit limits but would be exceeding the wasteload allocation for human health protection. This approach is clearly unacceptable.

This problem does not arise when using the TSD statistical procedure for setting permit limits for protecting against aquatic toxicity [32]. In this case, the monthly average and daily maximum permit limits are more closely related to the four day average and one hour maximum used as exposure periods for the criteria. Any imprecision in assessing effluent variability would therefore not have as great an effect on the permit limits.

EPA recommends that for setting water quality-based permit limitations for human health protection the average monthly limit must be set equal to the wasteload allocation, and the maximum daily permit limit must be calculated based on effluent variability and the number of samples per month (see Table 6.3). This approach ensures that the water quality standards will be met over the long term regardless of the uncertainties in estimating effluent variability and provides a defensible method for calculating a maximum daily permit limit.

6.4 Water Quality-based Permit Limit Derivation for Human Health Protection

The recommended approach for setting water quality-based limitations for human health protection with statistical procedures is as follows:

- Set the monthly average limit equal to the WLA.

- Calculate the daily maximum limit based on effluent variability and the number of samples per month using the multipliers provided in Table 6.3.

This approach ensures that the State numerical water quality standard (or RAC in the absence of a numerical standard) will be met over the long term and provides a defensible method for calculating a maximum daily permit limit. Appendix G contains example permit language for requiring the effluent bioconcentration assessment and for the incorporation of permit limits for bioconcentratable pollutants.

6.5 Detection Limits for Compliance Monitoring

A commonly encountered problem is the expression of calculated limits for specific chemicals where the concentration of the limit is below the analytical detection level for the pollutant of concern. This is particularly true for pollutants which are toxic in extremely low concentrations (e.g., dioxin). The recommended approach for these situations is to include, in Part I of the permit, the appropriate permit limitation derived from the water quality model and the wasteload allocation for the parameter of concern, regardless of the proximity of the limit to the analytical detection level.

However, the limit should also contain an accompanying notation indicating the specific analytical method which should be used for purposes of compliance monitoring. The note should indicate that any sample which is analyzed in accordance with the specified method and found to be below the detection level will be deemed to be in compliance with the permit limit, unless other monitoring information (as discussed below) indicates a problem.

The detection level for the analytical method cited in the permit should be clearly defined and quantified. **For most NPDES permitting situations, EPA recommends that the detection level be defined in the permit as the minimum level** (i.e., the level at which the entire analytical system gives recognizable mass spectra and acceptable calibration points). The minimum level is developed based on inter-laboratory analyses of the analyte in the matrix of concern (i.e., wastewater effluents). The minimum level should not be confused with the method detection level, which is based on a single laboratory analysis of the analyte in a given matrix, usually reagent water.

Where water quality-based limitations below analytical detection levels are placed in permits, it is recommended that permit special conditions also be included in the permit to help ensure that the limitations are being met and that excursion above water quality standards are not occurring. Examples of

such special conditions include: fish tissue collection and analysis; limitations and/or monitoring requirements on internal waste streams; and limitations and/or monitoring for surrogate parameters.

To summarize, where the final calculated limitation will be below the current level of detection the permit should contain:

a) The calculated water quality-based permit limitation for the pollutant.

b) A statement in the permit that the minimum level is the threshold for compliance/non-compliance determinations.

c) Requirements for additional monitoring.

6.6 Compliance Monitoring

Monitoring to determine the effectiveness of and compliance with permit limits for bioconcentratable pollutants is essential. Once the bioconcentratable chemical is identified and limited, a chemical-specific monitoring frequency of once a week is recommended. Monthly monitoring is the recommended absolute minimum. These frequencies are recommended because of the need to precisely measure the discharge to assure that short intense discharges do not lead to long term ambient problems. Since the bioconcentratable pollutants are very persistent in the environment, any discharged mass will tend to reside for long periods. The permit writer will need to determine a representative monitoring program which assures that spike loadings are addressed. If monitoring costs are of a concern due to the size of the facility, the permit writer may consider compositing over multiple days as a way to balance the need for more precise monitoring against the costs.

The effluent limit should also contain an accompanying notation indicating the specific analytical method which should be used for purposes of compliance monitoring. This is particularly important for those pollutants for which there are no promulgated analytical methods under 40 CFR 136. In these instances, the permit writer may need to consult with EPA chemists to specify an appropriate method that provides sufficient resolution at the calculated permit limit.

Additional annual effluent analysis according to the procedures in Appendix B is recommended for selected dischargers due to the potentially hazardous nature and persistence of bioconcentratable compounds. Where the contaminants in a discharge can be expected to change due to process changes, etc., repeating the effluent analysis is also recommended.

Table 6.3

Multipliers for Calculating Maximum Daily Permit Limits from Average Monthly Permit Limits

To obtain the maximum daily permit limit for a bioconcentratable pollutant, multiply the average monthly permit limit (the wasteload allocation) by the appropriate value in the following table.

Each value in the table is the ratio of the maximum daily permit limit, MDL, to the average monthly permit limit, AML, as calculated by the following relationship derived from step 4 of the statistically-based permit limit calculation procedure in the Technical Support Document for Water Quality-based Toxics Control [32].

$$\frac{MDL}{AML} = \frac{\exp\left[z_m\sigma - 0.5\sigma^2\right]}{\exp\left[z_a\sigma_n - 0.5\sigma_n^2\right]}$$

where $\sigma_n^2 = \ln(CV^2/n + 1)$
$\sigma^2 = \ln(CV^2 + 1)$
CV = the coefficient of variation of the effluent concentration
n = the number of samples per month
z_m = the percentile exceedance probability for the maximum daily limit
z_a = the percentile exceedance probability for the average monthly limit

	Ratio between maximum daily and average monthly permit limits									
	Maximum = 99th percentile Average = 95th percentile					Maximum = 99th percentile Average = 99th percentile				
CV	n=1	n=2	n=4	n=8	n=30	n=1	n=2	n=4	n=8	n=30
0.1	1.07	1.12	1.16	1.18	1.22	1.00	1.07	1.12	1.16	1.20
0.2	1.14	1.25	1.33	1.39	1.46	1.00	1.13	1.24	1.32	1.43
0.3	1.22	1.37	1.50	1.60	1.74	1.00	1.19	1.36	1.49	1.67
0.4	1.30	1.50	1.67	1.82	2.02	1.00	1.24	1.46	1.66	1.92
0.5	1.38	1.62	1.84	2.04	2.32	1.00	1.28	1.56	1.81	2.18
0.6	1.46	1.73	2.01	2.25	2.62	1.00	1.31	1.64	1.95	2.43
0.7	1.54	1.84	2.16	2.45	2.91	1.00	1.34	1.71	2.08	2.67
0.8	1.61	1.94	2.29	2.64	3.19	1.00	1.35	1.76	2.19	2.89
0.9	1.69	2.03	2.41	2.81	3.45	1.00	1.36	1.80	2.27	3.09
1.0	1.76	2.11	2.52	2.96	3.70	1.00	1.37	1.83	2.34	3.27
1.1	1.83	2.18	2.62	3.09	3.93	1.00	1.37	1.84	2.39	3.43
1.2	1.90	2.25	2.70	3.20	4.13	1.00	1.36	1.85	2.43	3.56
1.3	1.97	2.31	2.77	3.30	4.31	1.00	1.36	1.85	2.45	3.68
1.4	2.03	2.37	2.83	3.39	4.47	1.00	1.35	1.84	2.46	3.77
1.5	2.09	2.42	2.89	3.46	4.62	1.00	1.34	1.83	2.46	3.84
1.6	2.15	2.47	2.93	3.52	4.74	1.00	1.33	1.82	2.46	3.90
1.7	2.21	2.52	2.98	3.57	4.85	1.00	1.32	1.80	2.45	3.94
1.8	2.27	2.56	3.01	3.61	4.94	1.00	1.31	1.78	2.43	3.97
1.9	2.32	2.60	3.05	3.65	5.02	1.00	1.30	1.76	2.41	3.99
2.0	2.37	2.64	3.07	3.67	5.09	1.00	1.29	1.74	2.38	4.00

6.7 Tissue Residue Monitoring

Discharger monitoring may also include periodic (e.g., annual for those dischargers with chemicals on the CHC List in their effluent) analysis of aquatic organisms exposed to the effluent in the receiving water. This is particularly important for those pollutants where the calculated permit limit is below the analytical minimum level. **Tissue residue monitoring should also be repeated periodically to double check receiving waters where facilities have previously conducted the assessment procedures described in this document.** The regulatory authority may perform this periodic assessment or require the permittee to repeat the tissue residue analysis described in Appendix A. Where only the specific chemicals identified by the effluent bioconcentration option are of concern, chemical specific analyses of the tissue for those pollutants may be required in the permit. Tissue concentrations found in the exposed fish should then be compared with RTCs derived from the equations in Chapter 4. Where fish tissue concentrations exceed an RTC, analysis of the effluent for concentrations exceeding the RAC should be made to evaluate the need for revised limits in a modified or reissued permit.

6.8 Data Generation Mechanisms

Two mechanisms for data generation are available to the regulatory authority: 1) a Section 308 letter, or 2) the Special Conditions section of the permit. With either option, data generation may be required at the same time various effluent parameters are limited. For example, the permit writer could both set requirements for additional effluent bioconcentration analysis or tissue residue monitoring and, at the same time, set limits for human health in the permit. The data requirements recommended for both the effluent bioconcentration analysis and for tissue analysis are discussed below along with the options available to the regulatory authority after receiving the data generated. The results of either of these data generation analyses should subsequently result in modification under 40 CFR § 122.62(a)(2) or reissuance of the NPDES permit if the data generated show the presence of bioconcentratable contaminants which may exceed a State standard or RAC.

Option 1: The Section 308 letter

> Section 308 of the Clean Water Act authorizes EPA and the States to impose monitoring requirements on any point source discharge so long as the data generation conforms to the criteria of reasonableness. Specifically, biological monitoring (including tissue residue monitoring) is listed in Section 308.

Example language is provided in Appendix G. The example provided requires effluent bioconcentration evaluation (the procedures listed are not necessarily recommended, but are only examples).

Option 2: NPDES Permit Special Conditions

Permits can be issued with data generation requirements described in the Special Conditions section of the permit itself. The special conditions are written to augment the limits imposed on other parameters. Testing procedures require permittees to generate data on their effluents so that the permit writer can determine if additional permit limits should be imposed. An example of a bioconcentration analysis monitoring requirement that could be placed in the Special Conditions section of an NPDES permit is provided in Appendix G.

These two possible mechanisms for requiring a discharger to conduct an effluent bioconcentration evaluation also apply for requiring additional follow up analyses or confirmation if indicated by the results of the initial assessment options. The appropriate mechanism to be used for a given facility will be determined by the timing for requiring this assessment as discussed above. The regulatory mechanisms which may be used to require this assessment are:

- Via a Section 308 letter prior to permit issuance or after permit issuance; if needed limits may be developed for the permit at reissuance or the permit may be reopened.

- Via permit requirement placed in the special conditions section of the permit; if needed limits may be placed in reopened permit or in permit at next reissuance.

An example Section 308 letter and example permit language for requiring additional analyses or confirmation based on the results of the initial screening assessment are provided in Appendix G. Following notification of the requirement for this additional analyses, the selected discharger will repeat the assessment option or conduct confirmation procedures as is described in Chapter 3 and Appendix A and B. Appendix G also contains example permit language for requiring fish tissue and sediment evaluations for bioaccumulative pollutants. The specific laboratory procedures for fish tissues and for sediments are contained in Appendices A and C of this document.

APPENDIX A

LABORATORY PROCEDURES FOR DETERMINING BIOCONCENTRATABLE CHEMICALS IN TISSUE SAMPLES

Appendix A

Fish and Shellfish Tissue Analysis Procedure - Revision 1.0

1. Scope and Application

This method provides procedures for preparing fish and shellfish samples for GC/MS analysis using Soxhlet extraction, gel permeation chromatography, and silica gel chromatography, and procedures for identifying and quantifying bioconcentratable chemicals in the prepared samples with GC/MS. This method is applicable to organic chemicals which can be chromatographed using gas chromatography and detected using mass spectrometry.

2. Summary of Method

20 grams of a ground fish or shellfish sample is mixed with anhydrous sodium sulfate, spiked with a surrogate standard mixture, and then Soxhlet extracted using methylene chloride/hexane for a minimum of 12 hours. Gel Permeation Chromatography (GPC) followed up with silica gel chromatography are performed to remove lipids and cholesterol-like materials from the extract, respectively. Extract is then concentrated to 0.10 ml and is spiked with an internal standard. The extract is analyzed using capillary column gas chromatography with full scan electron impact ionization mass spectrometry (GC/MS). After GC/MS analysis, the extract is saved for confirmation analysis and the peaks in the GC/MS data are identified and quantitated. Also included is a procedure for measuring lipid content of the fish or shellfish sample.

The three surrogate compounds, added to each sample before extraction, are d_{10}-biphenyl, $^{13}C_6$-1,2,4,5-tetrachlorobenzene, and $^{13}C_6$-hexachlorobenzene and the internal standard is d_{12}-chrysene. Standard curves are calculated using an internal standard method for each surrogate and subsequently, percent recovery for each surrogate is determined. All other GC/MS components are quantified using the response factor of $^{13}C_6$-hexachlorobenzene with d_{12}-chrysene as the internal standard.

All chromatographic peaks are reverse-searched against (compared with) the Chemicals of Highest Concern (CHC) mass spectral library (see Table A-1). Those GC/MS components with fits of 70% and greater are considered tentatively identified. For each tentatively identified component, a list of the best mass spectral library identifications (up to a total of ten identifications) is reported along with the percent fit values, CAS number of each tentative identification, GC retention time,

and the concentration for the GC/MS component. This report is called Report 1.

For those GC/MS components not identified with the CHC search with tissue concentrations greater than or equal to 5 ug/kg, these components are reversed-searched against the EPA/NIH/NBS mass spectral library. Those GC/MS components with fits of 70% and greater are considered tentatively identified. For each tentatively identified component, a list of the best mass spectral library identifications (up to a total of ten identifications) is reported along with the percent fit values, CAS number of each tentative identification, GC retention time, and the concentration for the GC/MS component. This report is called Report 2.

For those components with fits/matches less than 70% but greater than 25%, the two best mass spectral library identifications along with the percent fit values, the CAS number of each tentative identification, GC retention time, and the concentration are reported for each the GC/MS component. For GC/MS components with fits/matches less than 25%, the concentrations and GC retention times for these components are reported and the components labeled as being "unknown". This report is identified as Report 3.

This procedure yields four reports which will be sent to the regulatory authority. These reports are:

1) Report 1, components tentatively identified using the CHC mass spectral library.

2) Report 2, components tentatively identified with tissue concentrations greater than or equal to 5 ng/g using the EPA/NIH/NBS mass spectral library.

3) Report 3, components with tissue concentrations greater than or equal to 5 ng/g, and fits less than 70% using the EPA/NIH/NBS mass spectral library.

4) QA/QC Report, recoveries of the three surrogate chemicals in the sample and blank, percent lipid content of the tissue, GC/MS chromatograms for the sample and blank, GPC performace data, silica gel performance data, GC/MS performace data, and precision data.

3. Definitions

3.1 Bioconcentration Factor (BCF). Ratio of the concentration in the tissue of the organism to that in water for an individual chemical. In equation form,

$$BCF = C_F/C_W$$

where C_F and C_W are the concentrations in the tissue and aqueous phase.

3.2 Surrogate Compound. A pure compound added to a sample before extraction.

3.3 Internal Standard. A pure compound added to a sample extract prior to GC/MS analysis.

3.4 CHC Mass Spectral Library. This library is a subset of the EPA/NIH/NBS Mass Spectral Library which contains the chemicals in Table A-1.

3.5 EPA/NIH/NBS Mass Spectral Library. A library of reference mass spectra published by National Bureau of Standards, U.S. Government Printing Office, Washington, D.C.

3.6 Procedural Blank. A sample analysis performed in the laboratory with no sample tissue that is treated as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples.

3.7 Laboratory Duplicate. Two sample aliquots taken in the analytical laboratory and analyzed separately with identical procedures.

4. Interferences

4.1 Interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing equipment. Procedural blanks are analyzed routinely to demonstrate that these materials are free of interferences under the analytical conditions used for samples.

4.2 To minimize interferences, glassware (including sample bottles) should be meticulously cleaned. As soon as possible after use, rinse glassware with the last solvent used. Then wash with detergent in hot water and rinse with tap water followed by distilled water. Drain dry and heat in a muffle furnace at 450°C for a few hours. After cooling, store glassware inverted or covered with aluminum foil. Before using, rinse each

piece with an appropriate solvent. Volumetric glassware should not be heated in a muffle furnace.

5. Safety

5.1 The toxicity or carcinogenicity of each chemical used in this method has not been precisely defined. Therefore, each should be treated as a potential health hazard, and exposure should be reduced to the lowest feasible concentration. Each laboratory is responsible for safely disposing materials and for maintaining awareness of OSHA regulations regarding safe handling of the chemicals used in this method. A reference file of material data handling sheets should be made available to all personnel involved in analyses. Additional information on laboratory safety is available [11.1,11.2,11.3].

5.2 The following method analytes have been classified as known or suspected human or mammalian carcinogens: d_{10}-biphenyl, $^{13}C_6$-1,2,4,5-tetrachlorobenzene, d_{12}-chrysene, and $^{13}C_6$-hexachlorobenzene. Primary standards of these compounds should be prepared in a hood. A toxic gas respirator should be worn when the analyst handles solutions containing high concentrations of these compounds.

6. Apparatus and Equipment (All specification are suggested. Catalog numbers are included for illustration only.)

6.1 Glassware

6.1.1 Soxhlet extractors -- 200 ml capacity, 500 ml flask, coarse fritted glass Soxhlet extraction thimble.

6.1.2 Chromatography Column -- glass column approximately 190 mm long X 9 mm ID with Teflon stopcock.

6.1.3 Concentrator Tube -- 10 mL graduated Kuderna-Danish design with ground-glass stopper.

6.1.4 Evaporative Flask -- 500 mL Kuderna-Danish design that is attached to concentrator tube with springs.

6.1.5 Snyder Column -- three-ball macro Kuderna-Danish design.

6.1.6 200 μL autosampler microvials and/or microvial inserts.

6.2 Gel Permeation Chromatography (GPC) System.

The GPC system must be capable of injecting large sample volumes, e.g., 1 to 10 mL, UV detection at 254 nm, pumping capacity of 3 to 10 ml/min, and capacity to collect fractions.

6.3 GPC Column

2.5 x 50 cm glass column packed with neutral, porous styrene-divinylbenzene copolymer beads with molecular weight exclusion limit of 2000, e.g. Bio-Beads SX-3. Note, a larger diameter column might be desirable, see 9.2.

6.4 GC/MS System

6.4.1 The GC must be capable of temperature programming, splitless or on-column injection, and have a designed capillary column injector.

6.4.2 The MS must be capable of full scan mass spectral analysis using electron ionization at a electron energy of 70 ev. The required MS scan rate should be ≥0.5 s and ≤1.5 s.

6.4.3 An interfaced data system (DS) is required to acquire, store, reduce, and output mass spectral data. The DS must be capable of performing typical mass spectral data manipulations; i.e., creating and plotting total and selected ion current profiles, integrating chromatographic peak areas, perform quantifications using an internal standard method, etc.

6.4.4 The data system must be capable of library searching detected chromatographic peaks.

6.4.5 The DS must have the latest release of the EPA/NIH/NBS mass spectral library. This library is available for most GC/MS systems from their manufacture.

6.4.6 The DS must have a mass spectral library consisting of the chemicals in Table A-1. This library, the CHC library, is a subset of the EPA/NIH/NBS mass spectral library. This library

can be constructed on most GC/MS systems without running GC/MS analyses on standard solutions!

6.6 GC Column

A 30 m X 0.32 mm or 30 m X 0.25 mm ID fused silica capillary column coated/bonded with a 0.25 μm or thicker film crosslinked 5% phenyl methyl or methyl silicone.

6.7 Miscellaneous Equipment

6.7.1 Microsyringes -- various standard sizes.

6.7.2 Boiling chips -- approximately 10/40 mesh. Heat at 400°C for 30 min. or extract with methylene chloride in a Soxhlet apparatus.

6.7.3 Water bath -- heated, with concentric ring cover, capable of temperature control within ±2°C.

6.7.4 Analytical balance -- capable of accurately weighing to 0.0001 g.

6.7.5 Beakers -- 250 ml.

6.7.6 Disposable aluminum weighing pans

6.7.8 Desiccator

6.7.9 Soxhlet extractor heating mantle

7. Reagents and Consumable Materials

7.1 Solvents. High purity, distilled-in-glass hexane and methylene chloride. For precise injections with splitless injectors and capillary columns, all samples and standards should be contained in the same solvent. Effects of minor variations in solvent composition (i.e., small percentage of another solvent remaining in hexane extracts) are minimized with the use of internal standards. (External standard calibration is not acceptable).

7.2 Sodium Sulfate. ACS, granular, anhydrous. Purify by heating at 400°C for 4 h in a shallow tray.

7.3 Silica Gel. 60-200 Mesh, Soxhlet extracted with hexane/methylene chloride (1:1).

7.4 Glass Wool. Purify by heating at 400°C for 24 h.

7.5 Nitrogen Gas. High purity, dry.

7.6 GPC Performance Solution. 5 mg/ml Dacthal, 0.2 mg/ml pyrene, and 4 mg/ml di-2-ethylhexylphthalate in methylene chloride.

7.7 Silica-Gel Performance Solution. 2 mg/ml dieldrin and 10 mg/ml cholesterol in hexane.

7.8 Internal Standard Spiking Solution. 200 ppm solution (in hexane) containing d_{12}-chrysene.

7.9 Surrogate Standard Spiking Solution. 1 ppm solution (acetone) containing d_{10}-biphenyl, $^{13}C_6$-1,2,4,5-tetrachlorobenzene, and $^{13}C_6$-hexachlorobenzene.

7.10 MS Performance Check Solution. 10 ppm solution (in hexane) containing decafluorotriphenyl-phosphine (DFTPP).

7.11 GC/MS Calibration Solutions. Five hexane solutions are required. These solutions contain constant concentrations of the internal standard, d_{12}-chrysene and varying concentrations of the surrogates. Composition and approximate concentrations are given in Table A-2.

7.12 GC Performance Solution. 10 ppm solution (in hexane) containing β-BHC, -BHC, d_{12}-chrysene, and endrin ketone or a 10 ppm solution (in hexane) containing anthracene, phenanthrene, benz[a]anthracene, and chrysene.

8. Calibration

8.1 GPC Chromatography Conditions

Chromatography Conditions -- Fill solvent reservoir with HPLC grade methylene chloride, set the flow rate to 5.0 mL/min, and set the UV detector at 254 nm.

8.2 GPC Performance Criteria

8.2.1 Inject 350 μL of the GPC performance solution and record GPC chromatogram.

8.2.2 Baseline resolution should exist among Dacthal, di-2-ethylhexylphthalate and pyrene.

8.2.3 If conditions stated in 8.2.2 are not met, modify chromatography conditions or replace the chromatography column. Repeat 8.2.2 until its

conditions are met before performing GPC on samples.

8.3 GPC Fraction Time (Collection Time) Identification.

8.3.1 Inject 350 µL of the GPC performance solution and record the retention times (RTs) of Dacthal, di-2-ethylhexylphthalate, and pyrene. Calculate the starting and ending times for collection of the eluate from the GPC column. The starting time is equal to the average of the retention times of di-2-ethylhexylphthalate and Dacthal. The ending time is equal to 1.7 times the average of the retention times of pyrene and di-2-ethylhexylphthalate.

8.3.2 RT reproducibility -- For each compound in the GPC performance solution, the absolute RTs should not vary by more than ±0.40 minutes from one analysis to the next.

8.4 Silica Gel Chromatography Conditions

Clean silica gel activated at 225°C for 18 hours is allowed to come to room temperature. 100 grams of silica gel and 1 ml of clean water are placed into a clean sealable container. The container is sealed, shaken to disperse the water, and then, allowed equilibrate for 18 hours. After equilibration, a silica gel column is prepared for use.

8.5 Silica Gel Chromatography Criteria

8.5.1. A silica gel column is prepared and 1 ml of the silica gel performance solution is placed on to the top of the column. The column is eluted with methylene chloride/hexane (15%, v:v, 60 mL). The eluant, analyzed by GC/MS or flame ionization detector/gas chromatography (FID/GC), must not contain more than 10% of the cholesterol while at least 90% of the dieldrin must be recovered.

8.5.2 If conditions in 8.5.1 are not met, modify silica gel chromatography conditions to obtain desired separation. These conditions must be met before performing silica gel chromatography on samples.

8.6 GC/MS Conditions.

8.6.1 Recommended gas chromatography conditions

Column Type: DB-5
Film Thickness: 0.25 μm
Column Dimensions: 30 m X 0.32 mm or 30 m X 0.25 mm
Helium Linear Velocity:
30 cm/sec @ 250°C

Temperature Program:
Inject 50°C, hold 4 mins.,
increase to 175°C at 10°C/min,
increase to 275°C at 5°C/m,
hold at 275°C for 20 mins.

Injection Volume: 1 or 2 μL

8.6.2 Recommended acquisition conditions for mass spectrometer.

Mass Range: 45-545 m/z
Total Cycle Time per Scan: 0.5 ≤ cycle time ≤ 1.5 seconds

8.6.3 Mass Spectrometer Calibration

8.6.3.1 Calibrate and tune MS with standards and procedures prescribed by the manufacturer.

8.6.3.2 Inject 1 μL or 2 μL aliquot of MS performance check solution. If spectrum does not meet criteria for DFTPP (Table A-3); return to 8.6.3.1 and recalibrate/tune MS.

8.7 GC/MS Performance Criteria

8.7.1 GC Performance -- Inject 1.0 μL of the GC performance solution. Baseline separation between β-BHC and -BHC and between endrin ketone and d_{12}-chrysene should exist. Alternatively, anthracene and phenanthrene should be separated by baseline and benz[a]anthracene and chrysene should be separated by a valley whose height is less than 25% of the average peak height of these two compounds.

8.7.2 MS Sensitivity -- Inject 1.0 μL of the 0.5 ppm GC/MS calibration solution. Using the total ion chromatogram, a signal to noise ratio of greater than 3 should be observed for each surrogate.

8.7.3 MS Calibration -- Inject 1.0 μL of the 0.5 ppm GC/MS calibration solution. For d_{12}-chrysene,

abundance of m/z 241 relative to that of m/z 240 should be >15% and <25%.

8.7.4 GC Stability -- Perform multiple GC/MS analyses on the same GC/MS calibration solution. RTs should not vary by more than $\pm t$ seconds. Calculate the value of $\pm t$ with the equation, $\pm t = (RT)^{1/3}$, where RT is the observed average RT (in seconds).

8.8 Response Factor Calculation for MS

8.8.1 Inject 1.0 μL of each GC/MS calibration solution and acquire GC/MS data.

8.8.2 Calculate response factors (RF) for each surrogate relative to d_{12}-chrysene

$$RF = A_s Q_c / A_c Q_s$$

where A_s = integrated total ion abundance for the surrogate
A_c = integrated total ion abundance for the internal standard, d_{12}-chrysene
Q_s = injected quantity of surrogate
Q_c = injected quantity of d_{12}-chrysene

8.8.3 RF Reproducibility -- For each surrogate, calculate the mean RF. When the relative standard deviation (RSD) exceeds 30%, analyze additional aliquots of GC/MS calibration solutions to obtain acceptable RSD for the RF, or take action to improve GC/MS performance.

8.9 Continuing Calibration Check

8.9.1 GPC

8.9.1.1 With the following procedures, verify GPC column performance at the beginning and end of each 12 h period during which analyses are performed.

8.9.1.2 Demonstrate acceptable performance for criteria described in Section 8.2.3.

8.9.1.3 Demonstrate acceptable performance for criteria described in Section 8.3.2.

8.9.2 GC/MS

8.9.2.1 With the following procedures, verify initial calibration at beginning and end of each 12 h period during which analyses are performed.

8.9.2.2 Inject 1 or 2 μL aliquot of MS performance check solution. Ensure acceptable MS calibration and performance.

8.9.2.3 Demonstrate acceptable performance for 8.7.

8.9.2.4 Determine the area for d_{12}-chrysene has not changed by more than 30% from most recent analyses of the GC/MS calibration solutions.

8.9.2.5 For an acceptable continuing calibration check, the measured RF for each surrogate must be within 30% of the mean value calculated during initial calibration.

8.9.3 Silica Gel

8.9.3.1 With the following procedure, verify silica gel chromatography performance for each lot of material and/or every 2 months, which ever comes first.

8.9.3.2 Demonstrate acceptable performance for criteria described in Section 8.5

8.9.4 Remedial Actions

Remedial actions must be taken if criteria are not met; possible remedies are:

8.9.4.1 Check and adjust operating conditions.

8.9.4.2 Clean or replace injector liner on GC.

8.9.4.3 Flush column with solvent according to manufacturers instructions.

8.9.4.4 Break off a short portion (approximately 0.33 m) of the GC column; check column performance by analysis of performance check solution for GC.

8.9.4.5 Replace column; performance of all initial calibration procedures then required.

8.9.4.6 Adjust MS for greater or lesser resolution.

8.9.4.7 Calibrate MS mass scale.

8.9.4.8 Prepare and analyze new concentration calibration/performance solutions.

8.9.4.9 Prepare new concentration calibration curve(s).

9. Procedures

9.1 Extraction

9.1.1 Ground fish or shellfish tissue (20 g) is blended with enough anhydrous sodium sulfate (100 to 140 g) in a 250 ml beaker to completely dry the sample.

9.1.2 Place two-thirds of the mixture into a coarse fritted glass Soxhlet extraction thimble and then add 0.100 mL of the surrogate solution. The remaining sample is added to the thimble and the extraction thimble is placed into a clean Soxhlet extractor body.

9.1.3 300 ml of methylene chloride/hexane (50:50 v:v) and one or two boiling chips are placed into the Soxhlet extractor flask. The flask along with Soxhlet extractor body are placed on to the extraction rack.

9.1.4 The heaters on the extraction rack are turned on and the sample is extracted for at least 12 hours.

9.1.5 After allowing the extractor to cool, quantitatively transfer the extract to a 500 mL Kuderna-Danish (KD) apparatus fitted with a 10 ml lower tube.

9.1.6 Add one or two clean boiling chips to the flask and attach a 3-ball Snyder column to the KD apparatus. Concentrate the extract on a steam bath to a volume of approximately 8 ml.

9.1.7 Place a weighing pan in a 105±5°C oven for 15 minutes, let cool for 15 minutes in desiccator, and then weigh to 5 places.

9.1.8 Quantitatively transfer extract to 10 ml volumetric with hexane and adjust volume to 10 ml.

9.1.9 Take tared weighing pan from desiccator and place 1.0 ml of the extract into the weighing pan.

9.1.10 Let solvent evaporate from weighing pan, place pan into 105±5°C oven for 15 minutes, let cool in desiccator, and reweigh to 5 places.

9.1.11 Using a gentle stream of dry air or nitrogen concentrate remaining sample, i.e., the remaining 9 ml, to approximately 1.0 ml.

9.1.12 Transfer extract to vial suitable for use with GPC system using methylene chloride. Cap vial and store in freezer.

9.2 GPC Cleanup

9.2.1 Remove sample from freezer and allow it to warm to ambient temperature. Dilute extract with methylene chloride to volume needed for GPC analysis, e.g. 2 ml. The size of the injection loop on the GPC systems vary according to manufacture. Analyst judgement/experience with their GPC system is required to determine this volume.

9.2.2 Using the GPC conditions determined previously (see Section 9.1), inject all of the sample into the GPC column. Note, the capacity of the GPC column (described in 6.3) is approximately 0.5 g of lipid. If the total amount of lipid exceeds 0.5 g, steps 9.2.2 (injection) and 9.2.3 (collection of purified extract) should be repeated using the appropriate injection volumes so that all of the extract has been fractionated on the GPC. Analyst judgement and experience are required for this procedure.

9.2.3 At the starting time determined in 8.3.1, a clean flask under the waste tube from the UV detector and collect the column eluate. Collect eluate from the UV detector until the ending time determined in 8.3.1.

9.2.4 Quantitatively transfer all of the collected eluate for a sample to a 500 ml KD with a 10 ml lower tube.

9.2.5 Add one or two clean boiling chips to the KD and attach a 3 ball Snyder column to Kuderna-Danish apparatus. Add approximately 30 ml of hexane to KD. Concentrate the extract on a steam bath until volume of the extract is less than 8 mL. Allow

Kuderna-Danish apparatus to cool and detach the lower tube, rinsing the joint with hexane into the sample.

9.2.6 Use a nitrogen stream and a warm (<40°C) water bath to concentrate the extract to a volume of 1.0 ml.

9.2.7 Quantitatively transfer sample to vial using hexane. Cap vial and store in freezer.

9.3 Silica Gel Chromatography

9.3.1 Remove extract from freezer and allow it to come to room temperature.

9.3.2 Adjust extract volume to 0.5 mL.

9.3.2 Prepare silica gel column using 19 cm X 9 mm ID glass column containing from bottom to top, glass wool, 0.5 cm of sodium sulfate, 2.1 g of deactivated silica gel (see 8.4), and 0.5 cm sodium sulfate. Wash column with 50 ml of hexane.

9.3.3 Quantitatively transfer extract to column using hexane.

9.3.4 Using elution conditions determine in 8.5, elute column and collect eluate in receiving container.

9.3.5 Use a nitrogen stream and a warm (<40°C) water bath to concentrate the eluate to a volume of 0.50 mL.

9.3.6 Quantitatively transfer sample to GC microvial and concentrate to 0.100 ml. Cap vial and store in freezer.

9.4 GC/MS Analysis

9.4.1 Remove sample from freezer and allow it to warm to ambient temperature. Adjust volume of sample to 100 μL and then spike sample with 5 μL of the internal standard spiking solution, 1000 ng of d_{12}-chrysene. Mix sample after spiking sample with d_{12}-chrysene.

9.4.2 Inject a 1 μL or 2 μL aliquot of blank or sample extract into GC operated under conditions used to produce acceptable results during calibration.

9.4.3 Acquire MS data using full scan conditions (see Section 8.6).

9.4.4 Recap samples and store extracts in freezer. Note, autosampler crimp caps after being punctured by the syringe used for sample injection must be replaced.

9.5 Percent Lipid Calculation

9.5.1 Calculate difference between the before and after oven drying of the tared weighing pan. This difference is the residue weight.

9.5.2 Calculate percent lipid with the following formula:

Percent lipid =

$$= \frac{\text{Residue weight} * 100}{0.10 * \text{mass of tissue extracted}}$$

9.6 GC/MS Data Analysis

Data analysis for this analytical procedure can be divided into three tasks, 1) quantification of surrogates and other GC/MS components, 2) library searching with the CHC mass spectral library, and 3) library searching with the EPA/NIH/NBS mass spectral library.

9.6.1 Quantification of Surrogates and Other GC/MS Components

9.6.1.1 Surrogate and Internal Standard Identification

Identify surrogates and internal standards by comparison of their mass spectrum (after background subtraction) to reference spectrums in the user-created data base. The GC retention time of the sample component should be within 10 sec of the time observed for that same compound when a calibration solution was analyzed. In general, all ions that are present above 10% relative abundance in the mass spectrum of the standard should be present in the mass spectrum of the sample component and should agree within absolute 20%. For example, if an ion has a relative abundance of 30% in the standard spectrum, its abundance in the sample spectrum should be in the range of 10 to 50%. Some ions, particularly the molecular ion, are of special importance, and should be evaluated even if they are below 10% relative abundance.

9.6.1.2 Peak Integration

Use the GC/MS peak detection and integration software to obtain areas for all chromatographic components from the total ion chromatogram with a signal to noise of 3 and greater. (Note, the solvent front need not be examined.) Verify that each surrogate was integrated. If not, use a more sensitive peak detection and integration settings to obtain peak areas for the surrogates.

9.6.1.3 Calculate Surrogate Concentrations

$$C_x = (A_x \cdot Q_{IS}) / (A_{IS} \cdot RF \cdot M)$$

Where C_x = Concentration of surrogate

A_x = Area of surrogate in total ion chromatogram

Q_{IS} = Quantity of internal standard added to extract before GC/MS analysis

A_{IS} = Area of internal standard in total ion chromatogram added to the extract before GC/MS analysis

RF = Mean response factor of surrogate from initial calibration analysis and/or from GC/MS calibration solutions run with sample analyses.

M = Mass of extracted tissue, i.e., 20 g.

Alternatively, use the GC/MS system software or other available proven software to compute the concentrations of surrogate using linear, second, or third order regression or using piecewise calibration curves.

9.6.1.4 Calculate Surrogate Recoveries

$$\text{Surrogate Recovery} = (C_x \bullet 100)/SSC$$

Where C_x = Concentration of surrogate
SSC = Surrogate spiking concentration, i.e., 5 ng/g

9.6.1.5 Calculate concentrations of all chromatographic components

$$C_x = (A_x \bullet Q_{IS} \bullet 100)/(A_{IS} \bullet RF \bullet M \bullet REC)$$

Where C_x = Concentration of chromatographic component

A_x = Area of chromatographic component in total ion chromatogram

Q_{IS} = Quantity of internal standard added to extract before GC/MS analysis

A_{IS} = Area of internal standard in total ion chromatogram

M = Mass of extracted tissue, i.e. 20 g

RF = Mean response factor of surrogate from initial calibration analyses and/or from GC/MS calibration solutions, run with sample analyses. Use RF for $^{13}C_6$-hexachlorobenzene.

REC = Surrogate recovery of $^{13}C_6$-hexachlorobenzene.

Alternatively, use the GC/MS system software or other available proven software to compute the concentration of all chromatographic components using linear, second, or third order regression or using piecewise calibration curves. These calculations must use the response curve for the $^{13}C_6$-hexachlorobenzene surrogate and the reported concentrations must be corrected for recovery of the $^{13}C_6$-hexachlorobenzene surrogate.

9.6.2 Library searching with the CHC mass spectral library

9.6.2.1 Algorithm Selection

A reverse searching algorithm is required when available. If GC/MS system does not have a reverse searching algorithm, library searching should be performed using the default algorithm supplied by the manufacture of the instrument.

9.6.2.2 Searching

All chromatographic components detected in 9.6.1.2 (Peak Integration) are searched against the CHC mass spectral library. Those GC/MS components with fits of 70% and greater are considered tentatively identified. For each tentatively identified component, a list of the best mass spectral library identifications (up to a total of ten identifications) is reported along with the percent fit values, CAS number of each tentative identification, GC retention time, and the concentration for the GC/MS component. This report is called Report 1.

9.6.3 Library Searching with EPA/NIH/NBS Mass Spectral Library.

9.6.3.1 Algorithm Selection

A reverse searching algorithm is required when available. If GC/MS system does not have a reverse searching algorithm, library searching should be performed using the default algorithm supplied by the manufacture of the instrument.

9.6.3.2 Eliminating of Compounds Below 5 ng/g

All unidentified components from the CHC library search with concentrations less than 5 ng/g are eliminated from further data processing. This elimination can be performed by comparing peak areas or heights of each chromatographic peak to the peak area or height of the $^{13}C_6$-hexachlorobenzene surrogate. This elimination may also be performed by comparing the recovery corrected concentration of each chromatographic component to 5 ng/g.

9.6.3.3 Searching

The remaining components are searched against the EPA/NIH/NBS mass spectral library. Those GC/MS components with fits of 70% and greater are considered tentatively identified. For each tentatively identified component, a list of the best mass spectral library identifications (up to a total of ten identifications) is reported along with the percent fit values, CAS number of each tentative identification, GC retention time, and

the concentration for the GC/MS component. This report is called Report 2.

For those components with fits/matches less than 70% but greater than 25%, the two best mass spectral library identifications along with the percent fit values, the CAS number of each tentative identification, GC retention time, and the concentration are reported for each the GC/MS component. For GC/MS components with fits/matches less than 25%, the concentrations and GC retention times for these components are reported and the components labeled as being "unknown". This report is identified as Report 3.

9.6.4 Elimination of chromatographic components common to the spiked blank and tissue GC/MS data.

Chromatographic components with retention times within ten seconds between the spiked blank and tissue and with the same mass spectrums should be removed from the analysis.

9.7 Reporting of Data

9.7.1 Report 1: CHC mass spectral identifications.

For each chromatographic component tentatively identified using the CHC search (fits greater than or equal to 70%), a list of the best mass spectral library identifications (up to a total of ten identifications) is reported along with the percent fit values, CAS number of each tentative identification, GC retention time, and the concentration for the GC/MS component.

9.7.2 Report 2: EPA/NIH/NBS mass spectral tentative identifications (fits $\geq$ 70% and $\geq$ 5 ug/kg).

GC/MS components tentatively identified in the EPA/NIH/NBS mass spectral search. For each tentatively identified component, a list of the best mass spectral library identifications (up to a total of ten identifications) is reported along with the percent fit values, CAS number of each tentative identification, GC retention time, and the concentration for the GC/MS component.

9.7.3 Report 3: EPA/NIH/NBS mass spectral tentative identifications (fits < 70% and $\geq$ 5 ug/kg).

GC/MS components not tentatively identified in the EPA/NIH/NBS mass spectral search. For those components with fits/matches less than 70% but greater than 25%, the two best mass spectral library identifications along with the percent fit values, the CAS number of each tentative identification, GC retention time, and the concentration are reported for each the GC/MS component. For GC/MS components with fits/matches less than 25%, the concentrations and GC retention times for these components are reported and the components labeled as being "unknown".

9.7.4 QA/QC Report

9.7.4.1 Percent lipid content of the sample.

9.7.4.2 Recoveries.

For d_{10}-biphenyl, $^{13}C_6$-1,2,4,5-tetrachlorobenzene, and $^{13}C_6$-hexachlorobenzene, recoveries will be reported.

9.7.4.3 GC/MS Chromatograms.

For the sample and its corresponding blank, total ion chromatograms must be provided.

9.7.4.4 QA/QC.

Data demonstrating GPC Resolution, see Section 8.2.2
Data demonstrating silica gel performance, see Section 8.5.
Data demonstrating GC resolution, see Section 8.7.1
Data demonstrating MS sensitivity, see Section 8.7.2
Data demonstrating MS calibration, see Section 8.7.3
Data demonstrating DFTPP performance, see Section 8.6.3.2
Data demonstrating precision, see Section 10.4

10. Quality Control

10.1 Recoveries of Surrogates. Method:

$$\% \text{ Recovery} = \frac{\text{measured surrogate amount}}{\text{spiked surrogate amount}} \times 100$$

Quality Assurance Requirement

25% < % recovery < 120%

Quality Control Action. If percent recovery is out of range, re-extract and re-analyze sample.

10.2 Calibration Stability

10.2.1 Continuing Calibration Checks

Quality Assurance. See Sections 8.9

Quality Control Action. Re-extract and re-analyze samples. For corrective actions see Section 8.9.

10.2.2 GPC Stability.

Quality Assurance. Immediately before and after fractionation solution must be analyzed. See Section 8.3 for stability requirements.

Quality Control Action. If RT stability requirements are not met, re-extract and re-analyze the sample.

10.2.3 GC/MS Stability

Quality Control. During the GC/MS analysis run, a GC/MS calibration solution should be analyzed twice; once at the beginning and once at the end of the GC/MS analysis sample sequence. See Sections 8.7 and 8.9 for stability requirements.

Quality Control Action. If RT and/or MS sensitivity stability requirements are not met, repeat GC/MS analysis on samples after correction of instrumental problems.

10.3 Blanks

10.3.1 Procedural Blanks

Quality Control. One procedural blank should be performed with every set of tissue samples analyzed.

An acceptable procedural blank:

a) Meets Section 10.1 requirements.

b) Contains no compound with elution characteristics and mass spectral features that would interfere with identification and quantification of the surrogates.
c) Contains few chromatographic peaks in the GC/MS total ion chromatograms.

Quality Control Action. Locate and eliminate the source of contamination. Re-extract and re-analyze the entire batch of sample.

10.4.2 Unspiked Procedural Blanks

Quality Control. One procedural blank not spiked with the surrogate spiking solution should be performed with every 20 tissue samples.

An acceptable unspiked blank. The sample should not contain detectable amounts of the surrogates.

Quality Control Action. Locate and eliminate source of contamination, re-extract and re-analyze entire batch of sample.

10.4 Precision

Quality Control. A duplicate set of tissue samples will be analyzed with every set of 10 samples. To measure the precision, the relative percent difference between the lab duplicate will be determined for each surrogate.

$$\text{Relative \% difference} = \frac{\text{surrogate amount duplicate 1} - \text{surrogate amount duplicate 2}}{\text{Average surrogate amount}} \times 100$$

The relative percent difference should be less than 150%.

Quality Control Action. If relative percent difference is out-range for any of three surrogates, re-extract and re-analyze the sample.

10.5 Sample Sets.

Sample sets are defined as a group of samples that are carried through the analytical procedure at the same time. Each sample set will include a minimum of two QC samples, one of them being a spiked blank. QC samples include spiked blanks, unspiked blanks, and replicates.

A set will normally contain 10 samples plus the required QC samples.

10.6 GC/MS Analysis Sets.

A GC/MS analysis set is defined as a group of prepared samples (tissues and QC samples) and GC/MS calibration solutions analyzed during one GC/MS run. A set will normally contain 12 prepared samples, all five GC/MS calibration solutions, and 1 or 2 duplicates of the GC/MS calibration solutions. The duplicate GC/MS calibration solutions will be analyzed in the beginning and at the end of GC/MS sample sequence.

11. Appendix A References.

11.1 "Carcinogens -- Working with Carcinogens", Department of Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, August 1977.

11.2 "OSHA Safety and Health Standards, General Industry", 29 CFR 1910, Occupational Safety and Health Administration, OSHA 2206, Revised January 1976.

11.3 "Safety in Academic Chemistry Laboratories", American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.

TABLE A-1

CHEMICALS OF HIGHEST CONCERN LIST

CAS number	chemical name
50-29-3	p,p'-dichlorodiphenyltrichloroethane (DDT)
57-74-9	chlordane
58-89-9	hexachlorocyclohexane (lindane)
60-57-1	dieldrin
70-30-4	hexachlorophene
72-54-8	p,p'-dichlorodiphenyldichloroethane (DDD)
72-55-9	p,p'-dichlorodiphenyldichloroethylene (DDE)
76-44-8	heptachlor
91-94-1	3,3'-dichlorobenzidine
95-94-3	1,2,4,5-tetrachlorobenzene
101-61-1	4,4'-methylene bis(N,N'-dimethyl) aniline
115-32-2	dicofol
117-81-7	bis(2-ethylhexyl)phthalate (BEHP)
118-74-1	hexachlorobenzene
309-00-2	aldrin
319-84-6	alpha-hexachlorocyclohexane (alpha-HCH)
319-85-7	beta-hexachlorocyclohexane (beta-HCH)
608-73-1	technical-hexachlorocyclohexane (t-HCH)
608-93-5	pentachlorobenzene
924-16-3	N-nitroso-di-n-butylamine
1024-57-3	heptachlor epoxide
1746-01-6	dioxin (2,3,7,8-TCDD)
2104-64-5	ethylp-nitrophenylphenylphosphorothioate(EPN)
2385-85-5	mirex
8001-35-2	toxaphene
39515-41-8	danitol
11096-82-5	polychlorinated biphenyl 1260
11097-69-1	polychlorinated biphenyl 1254
11104-28-2	polychlorinated biphenyl 1221
11141-16-5	polychlorinated biphenyl 1232
12672-29-6	polychlorinated biphenyl 1248
12674-11-2	polychlorinated biphenyl 1016
53469-21-9	polychlorinated biphenyl 1242

Table A-2. GC/MS Calibration Solutions, Concentrations of Surrogates and Internal Standards.

$^{13}C_6$-1,2,4,5-tetrachlorobenzene ppm	d_{10}-biphenyl ppm	d_{12}-chrysene ppm	$^{13}C_6$-hexachlorobenzene ppm
0.5	0.5	10	0.5
1	1	10	1
10	10	10	10
50	50	10	50
100	100	10	100

Table A-3. DFTPP Ion Abundance Criteria.

m/z	Criteria
51	10-80% of the base peak
68	≤2% of m/z 69
70	≤2% of m/z 69
127	10-80% of the base peak
197	≤2% of m/z 198
198	base peak or >50% of 442
199	5-9% of m/z 198
275	10-60% of the base peak
365	>1% of base peak
441	present and <mass 433
442	base peak or >50% of m/z 198
443	15-24% of m/z 442

APPENDIX B

LABORATORY PROCEDURES FOR DETERMINING BIOCONCENTRATABLE CHEMICALS IN AQUEOUS SAMPLES

Appendix B

Effluent Analysis Procedure - Revision 1.0

1. Scope and Application

This method provides procedures for fractionating effluents using HPLC and procedures for identification and quantification of bioconcentratable chemicals in these fractions using GC/MS. This method is applicable to organic chemicals which are stable under acidic conditions, are hexane extractable, and can be chromatographed using gas chromatography.

2. Summary of Method

A 10 L undiluted effluent sample is spiked with three surrogate chemicals, i.e., d_{10}-biphenyl, $^{13}C_6$-1,2,4,5-tetrachlorobenzene, and $^{13}C_6$-hexachlorobenzene, and extracted with hexane. The hexane extract is subsequently cleaned up using sulfuric acid, and concentrated to a volume of 0.50 mL. The extract is chromatographed using reverse phase HPLC, and three fractions are collected. These fractions have nominal BCF ranges of approximately 91 to 560, 560 to 5,000, and 5,000 to 470,000. The fractions are extracted and concentrated to 0.10 mL, and are subsequently spiked with the internal standard, d_{12}-chrysene. The fractions are analyzed using capillary gas chromatography with full scan electron impact ionization mass spectrometry (GC/MS). After GC/MS analysis, the fractions are saved for confirmation analysis and the peaks in the GC/MS data are identified and quantitated.

Standard curves are calculated using an internal standard method for each surrogate and subsequently, percent recovery for each surrogate is determined. All other GC/MS components are quantified using the response factor appropriate for each HPLC fraction: d_{10}-biphenyl/d_{12}-chrysene for the first fraction, $^{13}C_6$-1,2,4,5-tetrachlorobenzene/d_{12}-chrysene for the second fraction, and $^{13}C_6$-hexachlorobenzene/d_{12}-chrysene for the third fraction.

For each HPLC fraction, all chromatographic peaks are reverse-searched against (compared with) the Chemicals of Highest Concern (CHC) mass spectral library (see Table B-1). Those chemicals with fits of 70% and greater are considered tentatively identified. For each tentatively identified component, a list of the best mass spectral library identifications (up to a total of ten identifications) is reported along with the percent fit values, CAS number of each tentative identification, HPLC

fraction number, GC retention time, and the concentration for the GC/MS component. This report is called Report 1.

For those GC/MS components not identified with the CHC search with effluent concentrations greater than or equal to 100 ng/l, these components are reversed-searched against the EPA/NIH/NBS mass spectral library. Those chemicals with fits of 70% and greater are considered tentatively identified and these components are, then, further evaluated.

This evaluation process consists of calculating a predicted tissue concentration (the product of fraction BCF, effluent concentration, and effluent dilution) and subsequently, comparing this product to 1 ug/kg. For those GC/MS components with fits of 70% and greater and a product $\geq$ 1 ug/kg, a list of the best mass spectral library identifications (up to a total of ten identifications) is reported along with the percent fit values, CAS number of each tentative identification, HPLC fraction number, GC retention time, and the concentration for the GC/MS component. This report is called Report 2.

For those GC/MS components with fits of 70% and greater and a product less than 1 ug/kg, a list of the best mass spectral library identifications (up to a total of ten identifications) is reported along with the percent fit values, CAS number of each tentative identification, HPLC fraction number, HPLC fraction number, GC retention time, and the concentration for the GC/MS component. This report is identified as Report 4

For those components with fits less than 70% and greater than 25% and present at concentrations of greater than or equal to 100 ng/l, the two best mass spectral library identifications along with the percent fit values, the CAS number of each tentative identification, GC retention time, and the concentration are reported for each the GC/MS component. For GC/MS components with fits less than 25%, the concentrations, HPLC fraction numbers, and GC retention times for these components are reported and the components labeled as being "unknown". This report is identified as Report 3.

This procedure yields five reports which will be sent to the regulatory authority. These reports are:

1) Report 1, components tentatively identified using the CHC mass spectral library.

2) Report 2, components tentatively identified with effluent concentrations greater than or equal to 100 ng/l and a prediction tissue concentration $\geq$ 1 ug/kg using the EPA/NIH/NBS mass spectral library.

3) Report 3, components with effluent concentrations greater than or equal to 100 ng/l, and fits less than 70% using the EPA/NIH/NBS mass spectral library.

4) Report 4, components tentatively identified with effluent concentrations greater than or equal to 100 ng/l and a predicted tissue concentration < 1 ug/kg using the EPA/NIH/NBS mass spectral library.

4) QA/QC Report, recoveries of the three surrogate chemicals in the six sample and blank HPLC fractions, GC/MS chromatograms for the sample and blank, HPLC performance data, GC/MS performance data, and precision data.

3. Definitions

3.1 Bioconcentration Factor (BCF). Ratio of the concentration in the tissue of the organism to that in water for an individual chemical. In equation form,

$$BCF = C_F/C_W$$

where C_F and C_W are the concentrations in the tissue and aqueous phase.

3.2 Surrogate Compound. A pure compound added to a sample before extraction.

3.3 Internal Standard. A pure compound added to a sample extract prior to GC/MS analysis.

3.4 CHC Mass Spectral Library. This library is a subset of the EPA/NIH/NBS Mass Spectral Library which contains the chemicals in Table B-1.

3.5 EPA/NIH/NBS Mass Spectral Library. A library of reference mass spectra published by National Bureau of Standards, U.S. Government Printing Office, Washington, D.C.

3.6 Laboratory Reagent Blank. An aliquot of reagent water that is treated as a sample.

4. Interferences

4.1 Interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing equipment. Laboratory reagent blanks (LRBs) are analyzed routinely to demonstrate that these

materials are free of interferences under the analytical conditions used for samples.

4.2 To minimize interferences, glassware (including sample bottles) should be meticulously cleaned. As soon as possible after use, rinse glassware with the last solvent used. Then wash with detergent in hot water and rinse with tap water followed by distilled water. Drain dry and heat in a muffle furnace at 450°C for a few hours. After cooling, store glassware inverted or covered with aluminum foil. Before using, rinse each piece with an appropriate solvent. Volumetric glassware should not be heated in a muffle furnace.

5. Safety

5.1 The toxicity or carcinogenicity of each chemical used in this method has not been precisely defined. Therefore, each should be treated as a potential health hazard, and exposure should be reduced to the lowest feasible concentration. Each laboratory is responsible for safely disposing materials and for maintaining awareness of OSHA regulations regarding safe handling of the chemicals used in this method. A reference file of material data handling sheets should be made available to all personnel involved in analyses. Additional information on laboratory safety is available [12.1,12.2,12.3].

5.2 The following method analytes have been classified as known or suspected human or mammalian carcinogens: d_{10}-biphenyl, $^{13}C_6$-1,2,4,5-tetrachlorobenzene, d_{12}-chrysene, and $^{13}C_6$-hexachlorobenzene. Primary standards of these compounds should be prepared in a hood. A toxic gas respirator should be worn when the analyst handles solutions containing high concentrations of these compounds.

6. Apparatus and Equipment (All specification are suggested. Catalog numbers are included for illustration only.)

6.1 Sampling Equipment

6.1.1 Water Sample Bottles -- Meticulously cleaned (Section 4.2) glass bottles fitted with a Teflon, aluminum foil, or polypropylene lined screw cap. A 10 L aliquot of sample is required for one analysis and thus, water sampling bottles could consist of one 10 L, two 5 L, or three 1 gallon bottles. (Bottles in which high purity solvents were received can be used as

sample bottles without additional cleaning if they have been handled carefully to avoid contamination during and after use of original contents.)

6.2 Glassware

6.2.1 Extraction Glassware -- six 2 L separatory funnels, one 10 L bottle, two 5 L bottles, six 2 L volumetrics, one 5 gallon carboy, or three 1 gallon solvent bottles.

6.2.2 Chromatography Column -- glass column approximately 400 mm long X 19 mm ID with Teflon stopcock and 300 mL reservoir.

6.2.3 Concentrator Tube -- 10 mL graduated Kuderna-Danish design with ground-glass stopper.

6.2.4 Evaporative Flask -- 500 mL Kuderna-Danish design that is attached to concentrator tube with springs.

6.2.5 Snyder Column -- three-ball macro Kuderna-Danish design.

6.2.6 200 µL autosampler microvials and/or microvial inserts.

6.3 High Performance Liquid Chromatography System.

6.3.1 The HPLC must be capable of solvent programming, be capable of injecting large sample volumes, i.e., 175 ul, and be capable of UV detection at 200 nm.

6.3.2 Stop watch/timing device (required if 6.3.3 not available) for manual collection of HPLC fractions.

6.3.3 Fraction collector (optional) if used, should be capable of taking time window fractions from HPLC.

6.4 HPLC Column

A 25 cm X 4.6 mm i.d., 5 micron C_{18} column.

6.5 GC/MS System

6.5.1 The GC must be capable of temperature programming, splitless or on-column injection, and have a designed capillary column injector.

6.5.2 The MS must be capable of full scan mass spectral analysis using electron ionization at a electron energy of 70 ev. The required MS scan rate should be $\geq$0.5 s and $\leq$1.5 s.

6.5.3 An interfaced data system (DS) is required to acquire, store, reduce, and output mass spectral data. The DS must be capable of performing typical mass spectral data manipulations; i.e., creating and plotting total and selected ion current profiles, integrating chromatographic peak areas, perform quantifications using an internal standard method, etc.

6.5.4 The data system must be capable of library searching detected chromatographic peaks.

6.5.5 The DS must have the latest release of the EPA/NIH/NBS mass spectral library. This library is available for most GC/MS systems from their manufacture.

6.5.6 The DS must have a mass spectral library consisting of the chemicals in Table B-1. This library, the CHC library, is a subset of the EPA/NIH/NBS mass spectral library. This library can be constructed on most GC/MS systems <u>without</u> running GC/MS analyses on standard solutions!

6.6 GC Column

A 30 m X 0.32 mm or 30 m X 0.25 mm ID fused silica capillary column coated/bonded with a 0.25 μm or thicker film crosslinked 5% phenyl methyl or methyl silicone.

6.7 Miscellaneous Equipment

6.7.1 Volumetric flasks -- 2 mL, 5 mL, 10 mL, 25 mL, 50 mL, 100 mL, 200 mL, and 1 L with ground glass stoppers.

6.7.2 Microsyringes -- various standard sizes.

6.7.3 Boiling chips -- approximately 10/40 mesh. Heat at 400^{o}C for 30 min. or extract with methylene chloride in a Soxhlet apparatus.

6.7.4 Water bath -- heated, with concentric ring cover, capable of temperature control within ±2°C.

6.7.5 Analytical balance -- capable of accurately weighing to 0.0001 g.

6.7.6 Mixing device -- Magnetic stir plates and stir bars, stirrers, tumbler, or separatory funnel shake (optional).

7. Reagents and Consumable Materials

7.1 Solvents. High purity, distilled-in-glass hexane and methylene chloride. For precise injections with splitless injectors and capillary columns, all samples and standards should be contained in the same solvent. Effects of minor variations in solvent composition (i.e., small percentage of another solvent remaining in hexane extracts) are minimized with the use of internal standards. (External standard calibration is not acceptable).

7.2 Sodium Sulfate. ACS, granular, anhydrous. Purify by heating at 400°C for 4 h in a shallow tray.

7.3 Sulfuric Acid. AR Select, Mallinckrodt or equivalent quality.

7.4 Celite 545. Fisher Scientific.

7.5 Silica Gel. 60-200 Mesh, Soxhlet extracted with hexane/methylene chloride (1:1), dried, stored at 110°C for 48 h before use.

7.6 Glass Wool. Purify by heating at 400°C for 24 h.

7.7 Solid Phase Extraction Columns. 1 mL, 3 mL low density (LD), 6 mL high capacity (HC), J.T. Baker.

7.8 Internal Standard Spiking Solution. 200 ppm solution (in hexane) containing d_{12}-chrysene.

7.9 Nitrogen Gas. High purity, dry.

7.10 Surrogate Standard Spiking Solution. 1 ppm solution (acetone) containing d_{10}-biphenyl, $^{13}C_6$-1,2,4,5-tetrachlorobenzene, and $^{13}C_6$-hexachlorobenzene.

7.11 HPLC Calibration Solution. 10 ppm solution (in hexane) containing benzene, bromobenzene, biphenyl, bibenzyl, p,p'-DDE, and 2,2',4,5,5'-pentachlorobiphenyl.

7.12 HPLC Fractionation Standard. 10 ppm solution (in hexane) containing 1,4-dichlorobenzene, 1,3-diethylbenzene, p,p'-DDE, and decachlorobiphenyl.

7.13 MS Performance Check Solution. 10 ppm solution (in hexane) containing decafluorotriphenyl-phosphine (DFTPP).

7.14 GC/MS Calibration Solutions. Five hexane solutions are required. These solutions contain constant concentrations of the internal standard, d_{12}-chrysene and varying concentrations of the surrogates. Composition and approximate concentrations are given in Table B-2.

7.15 GC Performance Solution. 10 ppm solution (in hexane) containing β-BHC, -BHC, d_{12}-chrysene, and endrin ketone, or a 10 ppm solution (in hexane) containing anthracene, phenanthrene, benz[a]anthracene, and chrysene.

7.16 HPLC Performance Solution. 10 ppm solution (in hexane) containing biphenyl, 1,3-diethylbenzene, and bibenzyl.

8. Sample Collection, Preservation and Storage

8.1 Water Samples

8.1.1 Samples must be collected in clean (Section 4.2) glass containers.

8.1.2 Samples must be iced or refrigerated at 4°C from time of collection until extraction. If samples will not be extracted within 72 h after collection, use either sodium hydroxide or sulfuric acid to adjust sample pH to within a range of 5 to 9. Record the volume of acid or base used.

8.1.3 Samples should be extracted within 7 days after collection and analyzed within 40 days after extraction.

9. Calibration

9.1 HPLC Chromatography Conditions

9.1.1 Initial HPLC Chromatography Conditions -- Fill solvent reservoirs with HPLC grade water and

acetonitrile, set the flow rate to 1.0 mL/min, and set the UV detector at 200 nm. Solvent conditions should be isocratic for 1.0 minutes initially with 80% acetonitrile/20% water, linearly programmed to 100% acetonitrile at a rate of 1.5%/min., and then, isocratic for 30 minutes with 100% acetonitrile. A 10 minute recycle time should be used between runs with the initial isocratic solvent conditions.

9.1.2 Inject 10 μL of the HPLC calibration solution. RTs should be within 20% of those shown in Table B-3. If RTs are not within 20%, adjust either or both the gradient program and/or flow rates to obtain the desired RTs.

9.2 HPLC Performance Criteria

9.2.1 RT -- log P conditions

9.2.1.1 Using finalized HPLC conditions (see 9.1.2), inject 10 μL of the HPLC calibration solution and record RTs of each compound.

9.2.1.2 Using finalized HPLC conditions (see 9.1.2), inject 10 μL of acetone and record its RT.

9.2.1.3 Determine corrected RTs (i.e., RT of each compound minus the RT of acetone).

9.2.1.4 Perform a regression analysis using the log P values in Table B-3 and the corrected RTs. An equation of the form: $\log P = A * \log t_c + B$ where A and B are regression coefficients and t_c is the corrected RT, should be used.

9.2.1.5 The correlation coefficient for the regression, r^2, should be greater than 0.95. If this condition is not met, HPLC chromatography conditions are not correct. Return to 9.1.2 and determine improved conditions.

9.2.2 Resolution

9.2.2.1 Using the finalized HPLC conditions (see 9.1.2), inject 10 μL of the HPLC performance solution. Baseline separation

between biphenyl, 1,3-diethylbenzene, and bibenzyl should exist.

9.2.2.2 If baseline separation between all three chemicals does not exist, either replace the chromatography column and return to 9.1 or return to 9.1.2 and determine improved conditions.

9.3 HPLC Fraction Time (Collection Time) Identification.

9.3.1 Inject 10 µL of the HPLC fractionation standard using the finalized chromatographic conditions and record RTs of 1,4-dichlorobenzene, 1,3-diethylbenzene, p,p'-DDE, and decachlorobiphenyl. These times are used for fraction collection from the HPLC.

9.3.2 RT reproducibility -- For each compound in the HPLC fractionation standard, the absolute RTs should not vary by more than ±0.10 minutes from one analysis to the next.

9.4 GC/MS Conditions.

9.4.1 Recommended gas chromatography conditions

Column Type:	DB-5
Film Thickness:	0.25 µm
Column Dimensions:	30 m X 0.32 mm or 30 m X 0.25 mm
Helium Linear Velocity:	30 cm/sec @ 250°C
Temperature Program:	Inject 50°C, hold 4 mins., increase to 175°C at 10°C/min, increase to 275°C at 5°C/m, hold at 275°C for 20 mins.
Injection Volume:	1 or 2 µL

9.4.2 Recommended acquisition conditions for mass spectrometer.

Mass Range:	45-545 m/z
Total Cycle Time per Scan:	0.5 ≤ cycle time ≤ 1.5 seconds

9.4.3 Mass Spectrometer Calibration

9.4.3.1 Calibrate and tune MS with standards and procedures prescribed by the manufacturer.

9.4.3.2 Inject 1 μL or 2 μL aliquot of MS performance check solution. If spectrum does not meet criteria for DFTPP (Table B-4); return to 9.4.3.1 and recalibrate/tune MS.

9.5 GC/MS Performance Criteria

9.5.1 GC Performance -- Inject 1.0 μL of the GC performance solution. Baseline separation between β-BHC and -BHC and between endrin ketone and d_{12}-chrysene should exist. Alternatively, anthracene and phenanthrene should be separated by baseline and benz[a]anthracene and chrysene should be separated by a valley whose height is less than 25% of the average peak height of these two components.

9.5.2 MS Sensitivity -- Inject 1.0 μL of the 0.5 ppm GC/MS calibration solution. Using the total ion chromatogram, a signal to noise ratio of greater than 3 should be observed for each surrogate.

9.5.3 MS Calibration -- Inject 1.0 μL of the 0.50 ppm GC/MS calibration solution. For d_{12}-chrysene, abundance of m/z 241 relative to that of m/z 240 should be >15% and <25%.

9.5.4 GC Stability -- Perform multiple GC/MS analyses on the same GC/MS calibration solution. RTs should not vary by more than $\underline{t}$ seconds. Calculate the value of $\underline{t}$ with the equation, $\underline{t} = (RT)^{1/3}$, where RT is the observed average RT (in seconds).

9.6 Response Factor Calculation for MS

9.6.1 Inject 1.0 μL of each GC/MS calibration solution and acquire GC/MS data.

9.6.2 Calculate response factors (RF) for each surrogate relative to d_{12}-chrysene

$$RF = A_s Q_c / A_c Q_s$$

where A_s = integrated total ion abundance for the surrogate
A_c = integrated total ion abundance for the internal standard, d_{12}-chrysene
Q_s = injected quantity of surrogate
Q_c = injected quantity of d_{12}-chrysene

9.6.3 RF Reproducibility -- For each surrogate, calculate the mean RF. When the relative standard deviation (RSD) exceeds 30%, analyze additional aliquots of GC/MS calibration solutions to obtain acceptable RSD for the RF, or take action to improve GC/MS performance.

9.7 Continuing Calibration Check

9.7.1 HPLC

9.7.1.1 With the following procedures, verify HPLC column performance at the beginning and end of each 12 h period during which analyses are performed.

9.7.1.2 Demonstrate acceptable performance for criteria described in Section 9.2.2.

9.7.1.3 Demonstrate acceptable performance for criteria described in Section 9.3.2.

9.7.2 GC/MS

9.7.2.1 With the following procedures, verify initial calibration at beginning and end of each 12 h period during which analyses are performed.

9.7.2.2 Inject 1 or 2 μL aliquot of MS performance check solution. Ensure acceptable MS calibration and performance.

9.7.2.3 Demonstrate acceptable performance for 9.5.

9.7.2.4 Determine the area for chrysene-d_{12} has not changed by more than 30% from most recent analyses of the GC/MS calibration solutions.

9.7.2.5 For an acceptable continuing calibration check, the measured RF for each surrogate must be within 30% of the mean value calculated during initial calibration.

9.7.3 Remedial Actions

Remedial actions must be taken if criteria are not met; possible remedies are:

9.7.3.1 Check and adjust instrumental operating conditions.

9.7.3.2 Clean or replace injector liner on GC.

9.7.3.3 Flush column with solvent according to manufacturers instructions.

9.7.3.4 Break off a short portion (approximately 0.33 m) of the column; check column performance by analysis of performance check solution for GC.

9.7.3.5 Replace column; performance of all initial calibration procedures then required.

9.7.3.6 Adjust MS for greater or lesser resolution.

9.7.3.7 Calibrate MS mass scale.

9.7.3.8 Prepare and analyze new concentration calibration/performance check solution.

9.7.3.9 Prepare new concentration calibration curve(s).

10. Procedures

10.1 Extraction (Semi-automated)

10.1.1 Mix effluent. Place 10 L of effluent into extraction bottle(s). Depending upon your laboratory setup, one 10 L bottle, two 5 L bottles, or five 2 L bottles will be required.

10.1.2 Add 1 mL of surrogate solution to the effluent. This 1 mL volume of solution must be equally divided among all bottles. Seal bottles and mix effluent for 15 minutes.

10.1.3 Open bottles and add 60 mL of hexane per liter of effluent to each extraction bottle. Seal bottle(s).

10.1.4 Place bottles onto mixing device, i.e., shaker, tumbler, or stirring apparatus. Shake, tumble, or mix the sample for a minimum of 30 minutes. Longer mixing times might be required for your apparatus to ensure that quantitative extraction is obtained.

10.1.5 Remove sample containers from mixing device. Allow hexane layer to separate from water phase for 10-15 minutes. If emulsions are greater than one third of the solvent layer, use mechanical techniques to complete the phase separation. Optimum technique depends upon sample, but may include sonication, centrifugation, filtration through glass wool, or physical methods. Collect the hexane in a clean flask.

10.1.6 Repeat steps 10.1.3-5 two additional times and combine hexane extraction solvent.

10.1.7 Dry extract by passing it through a drying column containing about 10 cm of anhydrous sodium sulfate. Collect the dried extract in a Kuderna-Danish concentrator flask. Rinse flask which contained the solvent extract with 20-30 ml of hexane and add it to the column to complete the quantitative transfer. Optional, see 10.1.9.

10.1.8 Add one or two clean boiling chips to the flask and attach a three ball Snyder column to Kuderna-Danish apparatus. Concentrate the extract on a steam bath until volume of the extract is less than 8 mL. Allow Kuderna-Danish apparatus to cool and detach the lower tube, rinsing the joint with hexane into the sample. Optional, see 10.1.9.

10.1.9 Note, steps 10.1.7, sample drying, and 10.1.8, sample concentration, are optional. The hexane extracts resulting from 10.1.6 may be taken directly to 10.2, sample cleanup. If 10.1.7 and 10.1.8 are not performed, flasks for collecting and concentrating the eluate from the acid/Celite columns must be larger than what is written in 10.2 to accommodate the increased volume of hexane.

10.2 Sample Cleanup

10.2.1 Construct column containing (bottom to top) glass wool, silica gel (2 g), sodium sulfate (2 g), 70% sulfuric acid solution (5 ml) on Celite (10 g), and sodium sulfate (2 g). The column is washed with 100 mL of hexane is not allowed to go dry.

10.2.2 Quantitatively transfer the hexane extract from 10.1.7 to the column using hexane solvent. Collect eluate into a 500 mL Kuderna-Danish evaporation flask with a 10 mL lower tube.

10.2.3 Wash the acid/Celite column with 100 mL hexane and collect the eluate in the same Kuderna-Danish flask.

10.2.4 Add one or two clean boiling chips to the flask and attach a three ball Snyder column to Kuderna-Danish apparatus. Concentrate the extract on a steam bath until volume of the extract is less than 8 mL. Allow Kuderna-Danish apparatus to cool and detach the lower tube, rinsing the joint with hexane into the sample.

10.2.5 Use a nitrogen stream and a warm (<40°C) water bath to concentrate the extract to a volume of 0.50 mL.

10.2.6 Quantitatively transfer extract to a HPLC autosampler vial. Cap and store extract in freezer.

10.3 Fractionation of Effluent Extract

10.3.1 Remove sample from freezer and allow it to warm to ambient temperature. Uncap the vial and allow the sample to evaporate naturally to the volume of 500 µL. Recap the vial and mix well.

10.3.2 Using the HPLC conditions determined previously (see Section 9.1), inject 175 µL of the sample into the HPLC column.

10.3.3 At the RT of 1,4-dichlorobenzene (determined in Section 9.3), place a clean flask under the waste tube from the UV detector and collect the column eluate. Label this fraction as Fraction 1.

10.3.4 At the RT of 1,3-diethylbenzene, place a clean flask under the waste tube from the UV detector and collect the column eluate. Label this fraction as Fraction 2.

10.3.5 At the RT of p,p'-DDE, place a clean flask under the waste tube from the UV detector and collect the column eluate. Collection should continue until the RT of decachlorobiphenyl. Label this fraction as Fraction 3.

10.3.6 Repeat steps 10.3.2 to 10.3.5 two more times so that all the sample has been injected onto the HPLC column. Combine the like fractions into the flasks.

10.4 Concentration of Effluent Extracts (Liquid/Liquid Extraction)

10.4.1 Quantitatively transfer each HPLC fraction into its own extraction bottle, e.g., separatory funnels, bottles, etc. Dilute fractions a minimum of 10 fold with HPLC grade water. Seal bottle and mix diluted fractions thoroughly.

10.4.2 Open bottles and add 60 mL of hexane to each diluted fraction. Seal bottles.

10.4.3 Place bottles onto mixing device, i.e., shaker, tumbler, or stirring apparatus. Shake, tumble, or mix the sample for a minimum of 30 minutes. Alternatively, separatory funnel extraction by hand with periodic venting may be used; shake for minimum of 2 minutes

10.4.4 Remove bottles containing the diluted fractions from mixing device. Allow hexane layer to separate from water phase to 10-15 minutes. Collect the hexane in a clean flask. Each HPLC fraction must have its own flask.

10.4.5 Repeat steps 10.4.2-4 two additional times and combine the hexane from these extractions with the hexane from the first extraction.

10.4.6 Dry each extract by passing it through a drying column containing about 10 cm of anhydrous sodium sulfate. Collect the dried extract in a Kuderna-Danish concentrator flask. Rinse flask which contained the solvent extract with 20-30 mL of hexane and add it to the column to complete the quantitative transfer.

10.4.7 Add one or two clean boiling chips to the flask and attach a three ball Snyder column to Kuderna-Danish apparatus. Concentrate the extract on a steam bath until volume of the extract is less than 8 mL. Allow Kuderna-Danish apparatus to cool and detach the lower tube, rinsing the joint with hexane into the sample. Three extracts for each sample will results, i.e., fractions 1, 2, and 3.

10.4.8 Using a stream of dry clean air, evaporate extracts to approximately 1 mL.

10.4.9 Quantitatively transfer and concentrate extracts for each fraction to 100 μL in microvials. Cap and store in freezer.

10.5 Concentration of Effluent Extracts (C_{18} SPE)

10.5.1 Dilute fractions a minimum of 10 fold with HPLC grade water and mix diluted fractions thoroughly.

10.5.2 Activate three C_{18} solid phase extraction (SPE) columns by passing methanol and then HPLC grade water through the column as specified by the manufacture. The size of the column required is dependent upon the volume of diluted HPLC fractions; use manufacturer's recommendations. In general, 1 mL, 3 mL LD, and 3 mL columns will be needed.

10.5.3 Pass diluted extracts through the C_{18} SPE columns. Do not exceed maximum flowrates recommended by manufacture.

10.5.4 Remove excess water for the column. This can be done using a slight positive pressure of clean air or nitrogen gas, a slight vacuum, or (spun out using) a centrifuge. Do not let the columns go dry.

10.5.5 Elute C_{18} SPE columns with three 500 μL aliquots of methylene chloride and then one 500 μL aliquot of methylene chloride.

10.5.6 Dry extracts by passing C_{18} SPE eluent through micro Na_2SO_4 columns. Micro drying columns may be prepared by placing 1 cm of Na_2SO_4 into an empty 1 mL SPE column and passing approximately 2 ml of hexane through the column.

10.5.7 Concentrate eluate from drying column to approximately 100 μL and quantitatively transfer concentrate to microvials. Cap and store in freezer. Three extracts for each sample will be obtained.

10.6 GC/MS Analysis

10.6.1 Remove sample from freezer and allow it to warm to ambient temperature. Adjust volume of sample to 100 μL and then spike sample with 5 μL of the internal standard spiking solution, 1000 ng of d_{12}-

chrysene. Mix sample after spiking sample with d_{12}-chrysene.

10.6.2 Inject a 1 μL or 2 μL aliquot of blank or sample extract into GC operated under conditions used to produce acceptable results during calibration.

10.6.3 Acquire MS data using full scan conditions (see Section 9.4).

10.6.4 Recap samples and store extracts in freezer. Note, autosampler crimp caps after being punctured by the syringe used for sample injection must be replaced.

10.7 GC/MS Data Analysis

Data analysis for this analytical procedure can be divided into three tasks, 1) quantification of unknowns and surrogates, 2) library searching with the CHC mass spectral library, and 3) library searching with the EPA/NIH/NBS mass spectral library. This process is performed on the sample and blank extracts.

10.7.1 Quantification of Surrogates and Other GC/MS Components

10.7.1.1 Surrogate and Internal Standard Identification

Identify surrogates and internal standards by comparison of their mass spectrum (after background subtraction) to reference spectrums in the user-created data base. The GC retention time of the sample component should be within 10 sec of the time observed for that same compound when a calibration solution was analyzed. In general, all ions that are present above 10% relative abundance in the mass spectrum of the standard should be present in the mass spectrum of the sample component and should agree within absolute 20%. For example, if an ion has a relative abundance of 30% in the standard spectrum, its abundance in the sample spectrum should be in the range of 10 to 50%. Some ions, particularly the molecular ion, are of special importance, and should be evaluated even if they are below 10% relative abundance.

10.7.1.2 Peak Integration

For each fraction, use the GC/MS peak detection and integration software to obtain areas for all

chromatographic components from the total ion chromatogram with a signal to noise of 3 and greater. (Note, the solvent front need not be examined.) Verify that the surrogate in each fraction was integrated. If surrogate was not integrated use more sensitive peak detection and integration settings to obtain peak areas for the surrogates.

10.7.1.3 Calculate Surrogate Concentrations

$$C_x = (A_x \cdot Q_{IS}) / (A_{IS} \cdot RF \cdot V)$$

Where C_x = Concentration of surrogate

A_x = Area of surrogate in total ion chromatogram

Q_{IS} = Quantity of internal standard added to extract before GC/MS analysis

A_{IS} = Area of internal standard in total ion chromatogram added to extract before GC/MS analysis

RF = Mean response factor of surrogate from initial calibration analysis and/or from GC/MS calibration solutions run with sample analyses.

V = Volume of extracted water, 10 L.

Alternatively, use the GC/MS system software or other available proven software to complete the concentrations of surrogate using linear, second, or third order regression or using piecewise calibration curves.

10.7.1.4 Calculate Surrogate Recoveries

$$\text{Surrogate Recovery} = (C_x \bullet 100)/SSC$$

Where C_x = Concentration of surrogate
SSC = Surrogate spiking concentration, i.e., 100 ng/L

10.7.1.5 Calculate concentrations of all chromatographic components

$$C_x = (A_x \bullet Q_{IS} \bullet 100)/(A_{IS} \bullet RF \bullet V \bullet REC)$$

Where C_x = Concentration of chromatographic component

A_x = Area of chromatographic component in total ion chromatogram

Q_{IS} = Quantity of internal standard added to extract before GC/MS analysis

A_{IS} = Area of internal standard in total ion chromatogram

V = Volume of extracted water, i.e. 10 L

RF = Mean response factor of surrogate from initial calibration analyses and/or from GC/MS calibration solutions, run with sample analyses. For fraction #1, use RF for d_{10}-biphenyl. For fraction #2, use RF for $^{13}C_6$-1,2,4,5-tetrachlorobenzene. For fraction #3, use RF for $^{13}C_6$-hexachlorobenzene.

REC = Surrogate recovery calculated in Section 10.4.1.4. For fraction #1, use recovery of d_{10}-biphenyl. For fraction #2, use recovery of $^{13}C_6$-1,2,4,5-tetrachlorobenzene. For fraction #3, use recovery of $^{13}C_6$-hexachlorobenzene.

Alternatively, use the GC/MS system software or other available proven software to compute the concentration of all chromatographic components using linear, second, or third order regression or using piecewise calibration curves. These calculations must use the response curve for the surrogate appropriate for the fraction of interest and the reported concentrations must be corrected for recovery of the surrogate. For fraction #1, use response curve and recovery of d_{10}-biphenyl. For fraction #2, use response curve and recovery of $^{13}C_6$-1,2,4,5-tetrachlorobenzene. For fraction #3, use response curve and recovery of $^{13}C_6$-hexachlorobenzenes.

10.7.2 Library searching with the CHC mass spectral library

10.7.2.1 Algorithm Selection

A reverse searching algorithm is required when available. If GC/MS system does not have a reverse searching algorithm, library searching should be performed using the default algorithm supplied by the manufacture of the instrument.

10.7.2.2 Searching

All chromatographic components detected in 10.7.1.2 (Peak Integration) are searched against the CHC mass spectral library. Those chemicals with fits of 70% and greater are considered tentatively identified. For each tentatively identified component, a list of the best mass spectral library identifications (up to a total of ten identifications) is reported along with the percent fit values, CAS number of each tentative identification, HPLC fraction number, GC retention time, and the concentration for the GC/MS component. This report is called Report 1.

10.7.3 Library Searching with EPA/NIH/NBS Mass Spectral Library.

10.7.3.1 Algorithm Selection

A reverse searching algorithm is required when available. If GC/MS system does not have a reverse searching algorithm, library searching should be performed using the default algorithm supplied by the manufacture of the instrument.

10.7.3.2 Eliminating of Compounds Below 100 ng/L

All unidentified components from the CHC library search with concentrations less than 100 ng/L are eliminated from further data processing. This elimination can be performed by comparing peak areas or heights of each chromatographic peak to the peak area or height of the surrogate appropriate for that fraction. This elimination may also be performed by comparing the <u>recovery corrected concentration</u> of each chromatographic component to 100 ng/L.

10.7.3.3 *Searching*

For those GC/MS components not identified with the CHC search with effluent concentrations greater than or equal to 100 ng/l, these components are reversed-searched against the *EPA/NIH/NBS mass* spectral library. Those chemicals with fits of 70% and greater are considered tentatively identified. These components are then further evaluated, see 10.7.3.4.

For those components with fits/matches less than 70% but greater than 25%, the two best mass spectral library identifications along with the percent fit values, the CAS number of each tentative identification, HPLC fraction number, GC retention time, and the concentration are reported for each the GC/MS component. For GC/MS components with fits/matches less than 25%, the concentration, HPLC fraction number and GC retention time for each component is reported and the components labeled as being "unknown". This report is identified as Report 3.

10.7.3.4 Segregating GC/MS Components Tentatively Identified Components Using the EPA/NIH/NBS Mass Spectral Library Search.

For HPLC fractions 1, 2, and 3, predicted tissue concentrations are calculated for the effluent. Predicted tissue concentrations are determined by calculating the product of fraction BCF, effluent concentration, and effluent dilution. For fractions 1, 2, and 3, fraction BCF values of 560, 5000, and 470000 are used. The effluent dilution value is provided by the regulatory authority and the effluent concentration calculated in section 10.7.1.3.

For those GC/MS components with fits of 70% and greater and a predicted tissue concentration $\geq$ 1 ug/kg, a list of the best mass spectral library identifications (up to a total of ten identifications) is reported along with the percent fit values, CAS number of each tentative identification, HPLC fraction number, GC retention time, and the concentration for the GC/MS component. This report is called Report 2.

For those GC/MS components with fits of 70% and greater and a predicted tissue concentration less

than 1 ug/kg, a list of the best mass spectral library identifications (up to a total of ten identifications) is reported along with the percent fit values, CAS number of each tentative identification, HPLC fraction number, GC retention time, and the concentration for the GC/MS component. This report is identified as Report 4.

10.7.4 Elimination of chromatographic components common to the spiked blank and effluent GC/MS data.

Chromatographic components with retention times within ten seconds between the spiked blank and effluent and with the same mass spectrums should be removed from the analysis.

10.8 Reporting of Data

10.8.1 Report 1: CHC mass spectral identifications.

For each chromatographic component tentatively identified using the CHC search (fits greater to and greater than 70%), a list of the best mass spectral library identifications (up to a total of ten identifications) is reported along with the percent fit values, CAS number of each tentative identification, HPLC fraction number, GC retention time, and the concentration for the GC/MS component.

10.8.2 Report 2: EPA/NIH/NBS mass spectral tentative identifications (fits $\geq$ 70%, $\geq$ 100 ng/l, and predicted tissue concentration $\geq$ 1 ug/kg).

GC/MS components tentatively identified in the EPA/NIH/NBS mass spectral search and a predicted tissue concentration $\geq$ 1 ug/kg. For each tentatively identified component, a list of the best mass spectral library identifications (up to a total of ten identifications) is reported along with the percent fit values, CAS number of each tentative identification, HPLC fraction number, GC retention time, and the concentration for the GC/MS component.

10.8.3 Report 3: EPA/NIH/NBS mass spectral tentative identifications (fits < 70% and $\geq$ 100 ng/l).

GC/MS components not tentatively identified in the EPA/NIH/NBS mass spectral search. For those components with fits less than 70% and greater than

25%, the two best mass spectral library identifications along with the percent fit values, the CAS number of each tentative identification, HPLC fraction number, GC retention time, and the concentration are reported for each the GC/MS component. For GC/MS components with fits/matches less than 25%, the concentration, HPLC fraction number, and GC retention time for each component is reported and the components labeled as being "unknown".

10.8.4 Report 4: EPA/NIH/NBS mass spectral tentative identifications (fits ≥ 70%, ≥ 100 ng/l, and predicted tissue concentration < 1 ug/kg).

GC/MS components tentatively identified in the EPA/NIH/NBS mass spectral search and a predicted tissue concentration < 1 ug/kg. For each tentatively identified component, a list of the best mass spectral library identifications (up to a total of ten identifications) is reported along with the percent fit values, CAS number of each tentative identification, HPLC fraction number, GC retention time, and the concentration for the GC/MS component.

10.8.5 QA/QC Report

10.8.5.1 Recoveries.

For d_{10}-biphenyl, $^{13}C_6$-1,2,4,5-tetrachlorobenzene, and $^{13}C_6$-hexachlorobenzene, recoveries are reported for each sample and blank HPLC fraction.

10.8.5.2 GC/MS Chromatograms.

For the sample and its corresponding blank, total ion chromatograms must be provided for each sample and blank HPLC fraction.

10.8.5.4 QA/QC.

Chromatogram demonstrating HPLC resolution, see Section 9.2.2.1
Data demonstrating GC resolution, see Section 9.5.1
Data demonstrating MS sensitivity, see Section 9.5.2
Data demonstrating MS calibration, see Section 9.5.3

Data demonstrating DFTPP performance, see Section 9.4.3.2
Data demonstrating precision, see see Section 11.5.

11. Quality Control

11.1 Recoveries of Surrogates. Method:

$$\% \text{ Recovery} = \frac{\text{measured surrogate amount}}{\text{spiked surrogate amount}} \times 100$$

Quality Assurance Requirement

$$25\% < \% \text{ recovery} < 120\%$$

Quality Control Action. If percent recovery is out of range, re-extract and re-analyze sample.

11.2 Surrogate Fraction Location.

Quality Assurance Requirement

Fraction 1	should contain d_{10}-biphenyl and should not contain $^{13}C_6$-1,2,4,5-tetrachlorobenzene or $^{13}C_6$-hexachlorobenzene
Fraction 2	should contain $^{13}C_6$-1,2,4,5-tetrachlorobenzene and should not contain d_{10}-biphenyl or $^{13}C_6$-hexachlorobenzene
Fraction 3	should contain $^{13}C_6$-hexachlorobenzene and should not contain $^{13}C_6$-1,2,4,5-tetrachlorobenzene or d_{10}-biphenyl

Quality Control Action. If surrogate is in wrong fraction, take corrective action for improper HPLC conditions. Re-extract and re-analyze the sample.

11.3 Calibration Stability

11.3.1 Continuing Calibration Checks

Quality Assurance. See Sections 9.7

Quality Control Action. Re-extract and re-analyze samples. For corrective actions see Section 9.7.

11.3.2 HPLC Stability.

Quality Assurance. Immediately before and after fractionation solution must be analyzed. See Section 9.3 for stability requirements.

Quality Control Action. If RT stability requirements are not met, re-extract and re-analyze the sample.

11.3.3 GC/MS Stability

Quality Assurance. During the GC/MS analysis run, a GC/MS calibration solution should be analyzed twice; once at the beginning and once at the end of the GC/MS analysis sample sequence. See Sections 9.5 and 9.7 for stability requirements.

Quality Control Action. If RT and/or MS sensitivity stability requirements are not met, repeat GC/MS analysis on samples after correction of instrumental problems.

11.4 Blanks

11.4.1 Spiked Blanks

Quality Control. A 10 L (preferred) or a 2 L sample of reagent water is analyzed using the analytical procedure (Section 10). The sample is spiked with the surrogates using the same amount of compound, i.e., 1000 ngs, as the effluent samples.

One spiked blank should be performed with every set of effluent samples analyzed.

An acceptable spiked blank:

a) Meets Sections 11.1 and 11.2 requirements.
b) Contains no compound with elution characteristics and mass spectral features that would interfere with identification and quantification of the surrogates.
c) Contains few chromatographic peaks in the GC/MS total ion chromatograms for each fraction.

Quality Control Action. Locate and eliminate the source of contamination. Re-extract and re-analyze the entire batch of sample.

11.4.2 Unspiked Blanks

Quality Control. A 10 L (preferred) or a 2 L sample of reagent water is analyzed using the analytical procedure (Section 10). The sample is NOT spiked with the surrogates! It is spiked with the internal standards. One unspiked blank should be performed with every 20 effluent samples.

An acceptable unspiked blank. The sample should not contain detectable amounts of the surrogates.

Quality Control Action. Locate and eliminate source of contamination, re-extract and re-analyze entire batch of sample.

11.5 Precision

Quality Control. A duplicate set of effluent samples will be analyzed with every set of 10 samples. To measure the precision, the relative percent difference between the lab duplicate will be determined for each surrogate.

$$\text{Relative \% difference} = \frac{\text{surrogate amount duplicate 1} - \text{surrogate amount duplicate 2}}{\text{Average surrogate amount}} \times 100$$

The relative percent difference should be less than 150%.

Quality Control Action. If relative percent difference is out-range for any of three surrogates, re-extract and re-analyze the sample.

11.6 Sample Sets.

Sample sets are defined as a group of samples that are carried through the analytical procedure at the same time. Each sample set will include a minimum of two QC samples, one of them being a spiked blank. QC samples include spiked blanks, unspiked blanks, and replicates.

A set will normally contain 10 samples plus the required QC samples.

11.7 GC/MS Analysis Sets.

A GC/MS analysis set is defined as a group of prepared samples (effluents and QC samples) and GC/MS calibration

solutions analyzed during one GC/MS run. A set will normally contain 12 prepared samples, all five GC/MS calibration solutions, and 1 or 2 duplicates of the GC/MS calibration solutions. The duplicate GC/MS calibration solutions will be analyzed in the beginning and at the end of GC/MS sample sequence.

12. Appendix B References.

12.1 "Carcinogens -- Working with Carcinogens", Department of Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, August 1977.

12.2 "OSHA Safety and Health Standards, General Industry", 29 CFR 1910, Occupational Safety and Health Administration, OSHA 2206, Revised January 1976.

12.3 "Safety in Academic Chemistry Laboratories", American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.

12.4 Robert A. Hughes, Gilman D. Veith, and G. Fred Lee, "Gas Chromatographic Analysis of Toxaphene in Natural Waters, Fish and Lake Sediments."

TABLE B-1

CHEMICALS OF HIGHEST CONCERN LIST

CAS number	chemical name
50-29-3	p,p'-dichlorodiphenyltrichloroethane (DDT)
57-74-9	chlordane
58-89-9	hexachlorocyclohexane (lindane)
60-57-1	dieldrin
70-30-4	hexachlorophene
72-54-8	p,p'-dichlorodiphenyldichloroethane (DDD)
72-55-9	p,p'-dichlorodiphenyldichloroethylene (DDE)
76-44-8	heptachlor
91-94-1	3,3'-dichlorobenzidine
95-94-3	1,2,4,5-tetrachlorobenzene
101-61-1	4,4'-methylene bis(N,N'-dimethyl) aniline
115-32-2	dicofol
117-81-7	bis(2-ethylhexyl)phthalate (BEHP)
118-74-1	hexachlorobenzene
309-00-2	aldrin
319-84-6	alpha-hexachlorocyclohexane (alpha-HCH)
319-85-7	beta-hexachlorocyclohexane (beta-HCH)
608-73-1	technical-hexachlorocyclohexane (t-HCH)
608-93-5	pentachlorobenzene
924-16-3	N-nitroso-di-n-butylamine
1024-57-3	heptachlor epoxide
1746-01-6	dioxin (2,3,7,8-TCDD)
2104-64-5	ethylp-nitrophenylphenylphosphorothioate(EPN)
2385-85-5	mirex
8001-35-2	toxaphene
39515-41-8	danitol
11096-82-5	polychlorinated biphenyl 1260
11097-69-1	polychlorinated biphenyl 1254
11104-28-2	polychlorinated biphenyl 1221
11141-16-5	polychlorinated biphenyl 1232
12672-29-6	polychlorinated biphenyl 1248
12674-11-2	polychlorinated biphenyl 1016
53469-21-9	polychlorinated biphenyl 1242

Table B-2. GC/MS Calibration Solutions, Concentrations of Surrogates and Internal Standards.

$^{13}C_6$-1,2,4,5-tetrachlorobenzene ppm	d_{10}-biphenyl ppm	d_{12}-chrysene ppm	$^{13}C_6$-hexachlorobenzene ppm
0.5	0.5	10	0.5
1	1	10	1
10	10	10	10
50	50	10	50
100	100	10	100

Table B-3. Suggested Retention Times (RTs) for Compounds in the HPLC Calibration Solution.

Chemical	Retention Time	Log P
Benzene	4.3	2.13
Bromobenzene	5.5	2.99
Biphenyl	6.8	3.76
Bibenzyl	8.1	4.81
p,p'-DDE	13.4	5.69
2,2',4,5,5'-Pentachlorobiphenyl	15.1	6.11

Table B-4. DFTPP Ion Abundance Criteria.

m/z	Criteria
51	10-80% of the base peak
68	≤2% of m/z 69
70	≤2% of m/z 69
127	10-80% of the base peak
197	≤2% of m/z 198
198	base peak or >50% of 442
199	5-9% of m/z 198
275	10-60% of the base peak
365	>1% of base peak
441	present and <mass 433
442	base peak or >50% of m/z 198
443	15-24% of m/z 442

APPENDIX C

LABORATORY PROCEDURES FOR DETERMINING BIOCONCENTRATABLE CHEMICALS IN SEDIMENT SAMPLES

Appendix C

Sediment Analysis Procedure - Revision 1.0

1. Scope and Application

This method provides procedures for sample preparation using reverse phase HPLC and procedures for identification and quantification of bioconcentratable chemicals using GC/MS for sediments. This method is applicable to organic chemicals which are stable under acidic conditions and can be chromatographed using gas chromatography.

2. Summary of Method

20 grams of ground, air dried, sediment is mixed with anhydrous sodium sulfate, spiked with a surrogate standard mixture, Soxhlet extracted using acetone for 4 hours, and then, Soxhlet extracted using 1:3 toluene:methanol for a minimum of 12 hours. The extract is subsequently cleaned up using copper and sulfuric acid, and then, concentrated to a volume of 0.50 mL. The extract is chromatographed using reverse phase HPLC, and three fractions are collected. These fractions have nominal BCF ranges of approximately 91 to 560, 560 to 5,000, and 5,000 to 470,000. The fractions are extracted and concentrated to 0.10 mL, and are subsequently spiked with the internal standard. The fractions are analyzed using capillary gas chromatography with full scan electron impact ionization mass spectrometry (GC/MS). After GC/MS analysis, the fractions are saved for confirmation analysis and the peaks in the GC/MS data are identified and quantified. Also included is a procedure for determining the percent moisture of the sediment. However, a method for determining the organic carbon content of the sediment not provided even though reporting of this parameter is required.

The three surrogate compounds, added to each sample before extraction, are d_{10}-biphenyl, $^{13}C_6$-1,2,4,5-tetrachlorobenzene, and $^{13}C_6$-hexachlorobenzene and the internal standard is d_{12}-chrysene. Standard curves are calculated using an internal standard method for each surrogate and subsequently, percent recovery for each surrogate is determined. All other GC/MS components are quantified using the response factor appropriate for each HPLC fraction: d_{10}-biphenyl/d_{12}-chrysene for the first fraction, $^{13}C_6$-1,2,4,5-tetrachlorobenzene/d_{12}-chrysene for the second fraction, and $^{13}C_6$-hexachlorobenzene/d_{12}-chrysene for the third fraction.

For each HPLC fraction, all chromatographic peaks are reverse-searched against (compared with) the Chemicals of Highest

Concern (CHC) mass spectral library (see Table J-1). Those GC/MS components with fits of 70% and greater are considered tentatively identified. For each tentatively identified component, a list of the best mass spectral library identifications (up to a total of ten identifications) is reported along with the percent fit values, CAS number of each tentative identification, HPLC fraction number, GC retention time, and the concentration for the GC/MS component. This report is called Report 1.

For those GC/MS components not identified with the CHC search with sediment concentrations greater than or equal to 5 ug/kg, these components are reversed-searched against the EPA/NIH/NBS mass spectral library. Those GC/MS components with fits of 70% and greater are considered tentatively identified. For each tentatively identified component, a list of the best mass spectral library identifications (up to a total of ten identifications) is reported along with the percent fit values, CAS number of each tentative identifications, HPLC fraction number, GC retention time, and the concentration for the GC/MS component. This report is called Report 2.

For those components with fits/matches less than 70% and greater than 25%, the two best mass spectral library identifications along with the percent fit values, the CAS number of each tentative identification, HPLC fraction number, GC retention time, and the concentration are reported for each the GC/MS component. For GC/MS components with fits/matches less than 25%, the concentrations and GC retention times for these components are reported and the components labeled as being "unknown". This report is identified as Report 3.

This procedure yields four reports which will be sent to the regulatory authority. These reports are:

1) Report 1, components tentatively identified using the CHC mass spectral library.

2) Report 2, components tentatively identified with sediment concentrations greater than or equal to 5 ng/g using the EPA/NIH/NBS mass spectral library.

3) Report 3, components with sediment concentrations greater than or equal to 5 ng/g and fits less than 70% using the EPA/NIH/NBS mass spectral library.

4) QA/QC Report, recoveries of the three surrogate chemicals in the six sample and blank fractions, percent organic carbon, percent moisture, GC/MS chromatograms for the sample and blank, HPLC performace data, GC/MS performace data, and precision data.

3. Definitions

3.1 Bioconcentration Factor (BCF). Ratio of the concentration in the tissue of the organism to that in water for an individual chemical. In equation form,

$$BCF = C_F/C_W$$

where C_F and C_W are the concentrations in the tissue and aqueous phase.

3.2 Surrogate Compound. A pure compound added to a sample before extraction.

3.3 Internal Standard. A pure compound added to a sample extract prior to GC/MS analysis.

3.4 CHC Mass Spectral Library. This library is a subset of the EPA/NIH/NBS Mass Spectral Library which contains the chemicals in Table C-1.

3.5 EPA/NIH/NBS Mass Spectral Library. A library of reference mass spectra published by National Bureau of Standards, U.S. Government Printing Office, Washington, D.C.

3.6 Procedural Blank. A sample analysis performed in the laboratory with no sediment sample that is treated as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples.

3.7 Laboratory Duplicate. Two sample aliquots taken in the analytical laboratory and analyzed separately with identical procedures.

4. Interferences

4.1 Interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing equipment. Procedural blanks are analyzed routinely to demonstrate that these materials are free of interferences under the analytical conditions used for samples.

4.2 To minimize interferences, glassware (including sample bottles) should be meticulously cleaned. As soon as possible after use, rinse glassware with the last solvent used. Then wash with detergent in hot water and rinse with tap water followed by distilled water. Drain dry and heat in a muffle furnace at 450°C for a

few hours. After cooling, store glassware inverted or covered with aluminum foil. Before using, rinse each piece with an appropriate solvent. Volumetric glassware should not be heated in a muffle furnace.

5. Safety

5.1 The toxicity or carcinogenicity of each chemical used in this method has not been precisely defined. Therefore, each should be treated as a potential health hazard, and exposure should be reduced to the lowest feasible concentration. Each laboratory is responsible for safely disposing materials and for maintaining awareness of OSHA regulations regarding safe handling of the chemicals used in this method. A reference file of material data handling sheets should be made available to all personnel involved in analyses. Additional information on laboratory safety is available [11.1,11.2,11.3].

5.2 The following method analytes have been classified as known or suspected human or mammalian carcinogens: d_{10}-biphenyl, $^{13}C_6$-1,2,4,5-tetrachlorobenzene, d_{12}-chrysene, and $^{13}C_6$-hexachlorobenzene. Primary standards of these compounds should be prepared in a hood. A toxic gas respirator should be worn when the analyst handles solutions containing high concentrations of these compounds.

6. Apparatus and Equipment (All specification are suggested. Catalog numbers are included for illustration only.)

6.1 Glassware

6.1.1 Soxhlet extractors -- 200 ml capacity, 500 ml flask, coarse fritted glass Soxhlet extraction thimble.

6.1.2 Concentrator Tube -- 10 mL graduated Kuderna-Danish design with ground-glass stopper.

6.1.3 Evaporative Flask -- 500 mL Kuderna-Danish design that is attached to concentrator tube with springs.

6.1.4 Snyder Column -- three-ball macro Kuderna-Danish design.

6.1.5 200 µL autosampler microvials and/or microvial inserts.

6.1.6 Chromatography Column -- glass column approximately 400 mm long X 19 mm ID with Teflon stopcock and 300 mL reservoir.

6.2 High Performance Liquid Chromatography System.

6.2.1 The HPLC must be capable of solvent programming, be capable of injecting large sample volumes, i.e., 1 mL and greater, and be capable of UV detection at 200 mm.

6.2.2 Stop watch/timing device (required if 6.2.3 not available) for manual collection of HPLC fractions.

6.2.3 Fraction collector (optional) if used, should be capable of taking time window fractions from HPLC.

6.3 HPLC Column

A 25 cm X 4.6 mm i.d., 5 micron C_{18} column.

6.4 GC/MS System

6.4.1 The GC must be capable of temperature programming, splitless or on-column injection, and have a designed capillary column injector.

6.4.2 The MS must be capable of full scan mass spectral analysis using electron ionization at a electron energy of 70 ev. The required MS scan rate should be $\geq$0.5 s and $\leq$1.5 s.

6.4.3 An interfaced data system (DS) is required to acquire, store, reduce, and output mass spectral data. The DS must be capable of performing typical mass spectral data manipulations; i.e., creating and plotting total and selected ion current profiles, integrating chromatographic peak areas, perform quantifications using an internal standard method, etc.

6.4.4 The data system must be capable of library searching detected chromatographic peaks.

6.4.5 The DS must have the latest release of the EPA/NIH/NBS mass spectral library. This library is available for most GC/MS systems from their manufacture.

6.4.6 The DS must have a mass spectral library consisting of the chemicals in Table C-1. This library, the CHC library, is a subset of the EPA/NIH/NBS mass spectral library. This library can be constructed on most GC/MS systems without running GC/MS analyses on standard solutions!

6.6 GC Column

A 30 m X 0.32 mm or 30 m X 0.25 mm ID fused silica capillary column coated/bonded with a 0.25 μm or thicker film crosslinked 5% phenyl methyl or methyl silicone.

6.7 Miscellaneous Equipment

6.7.1 Microsyringes -- various standard sizes.

6.7.2 Boiling chips -- approximately 10/40 mesh. Heat at 400°C for 30 min. or extract with methylene chloride in a Soxhlet apparatus.

6.7.3 Water bath -- heated, with concentric ring cover, capable of temperature control within ±2°C.

6.7.4 Analytical balance -- capable of accurately weighing to 0.0001 g.

6.7.5 Beakers -- 250 ml.

6.7.6 Disposable aluminum weighing pans

6.7.8 Desiccator

6.7.9 Soxhlet extractor heating mantle

6.7.10 Mortar and pestle

6.7.11 Spatulas

6.7.12 Drying oven

6.7.13 Mixing device -- Magnetic stir plates and stir bars, stirrers, tumbler, or separatory funnel shake (optional).

6.7.14 Extraction flasks -- separatory funnels: 250, 500, 1000 ml; flasks: 100, 250, 500, and 1000 ml volumetrics; etc.

6.7.15 Disposable Pasteur pipets

7. Reagents and Consumable Materials

7.1 Solvents. High purity, distilled-in-glass acetone, hexane, toluene, and methanol. For precise injections with splitless injectors and capillary columns, all samples and standards should be contained in the same solvent. Effects of minor variations in solvent composition (i.e., small percentage of another solvent remaining in hexane extracts) are minimized with the use of internal standards. (External standard calibration is not acceptable).

7.2 Sodium Sulfate. ACS, granular, anhydrous. Purify by heating at 400°C for 4 h in a shallow tray.

7.3 Silica Gel. 60-200 Mesh, Soxhlet extracted with hexane/methylene chloride (1:1), dried stored at 110°C for 48 h before use.

7.4 Glass Wool. Purify by heating at 400°C for 24 h.

7.5 Nitrogen Gas. High purity, dry.

7.6 Sulfuric Acid. AR Select, Mallinckrodt or equivalent quality.

7.7 Celite 545. Fisher Scientific.

7.8 Solid Phase Extraction Columns. 1 mL, 3 mL low density (LD), 6 mL high capacity (HC), J.T. Baker.

7.9 HPLC Calibration Solution. 10 ppm solution (in hexane) containing benzene, bromobenzene, biphenyl, bibenzyl, p,p'-DDE, and 2,2',4,5,5'-pentachlorobiphenyl.

7.10 HPLC Fractionation Standard. 10 ppm solution (in hexane) containing 1,4-dichlorobenzene, 1,3-diethylbenzene, p,p'-DDE, and decachlorobiphenyl.

7.11 HPLC Performance Solution. 10 ppm solution (in hexane) containing biphenyl, 1,3-diethylbenzene, and bibenzyl.

7.12 Internal Standard Spiking Solution. 200 ppm solution (in hexane) containing d_{12}-chrysene.

7.13 Surrogate Standard Spiking Solution. 1 ppm solution (acetone) containing d_{10}-biphenyl, $^{13}C_6$-1,2,4,5-tetrachlorobenzene, and $^{13}C_6$-hexachlorobenzene.

7.14 MS Performance Check Solution. 10 ppm solution (in hexane) containing decafluorotriphenyl-phosphine (DFTPP).

7.15 GC/MS Calibration Solutions. Five hexane solutions are required. These solutions contain constant concentrations of the internal standard, d_{12}-chrysene and varying concentrations of the surrogates. Composition and approximate concentrations are given in Table C-2.

7.16 GC Performance Solution. 10 ppm solution (in hexane) containing β-BHC, -BHC, d_{12}-chrysene, and endrin ketone or a 10 ppm solution (in hexane) containing anthracene, phenanthrene, benz[a]anthracene, and chrysene.

8. Calibration

8.1 HPLC Chromatography Conditions

8.1.1 Initial HPLC Chromatography Conditions -- Fill solvent reservoirs with HPLC grade water and acetonitrile, set the flow rate to 1.0 mL/min, and set the UV detector at 200 nm. Solvent conditions should be isocratic for 1.0 minutes initially with 80% acetonitrile/20% water, linearly programmed to 100% acetonitrile at a rate of 1.5%/min., and then, isocratic for 30 minutes with 100% acetonitrile. A 10 minute recycle time should be used between runs with the initial isocratic solvent conditions.

8.1.2 Inject 10 μL of the HPLC calibration solution. RTs should be within 20% of those shown in Table C-3. If RTs are not within 20%, adjust either or both the gradient program and/or flow rates to obtain the desired RTs.

8.2 HPLC Performance Criteria

8.2.1 RT -- log P conditions

8.2.1.1 Using finalized HPLC conditions (see 8.1.2), inject 10 μL of the HPLC calibration solution and record RTs of each compound.

8.2.1.2 Using finalized HPLC conditions (see 8.1.2), inject 10 μL of acetone and record its retention time (RT).

8.2.1.3 Determine corrected RTs (i.e., RT of each compound minus the RT of acetone).

8.2.1.4 Perform a regression analysis using the log P values in Table C-3 and the corrected RTs. An equation of the form: $\log P = A * \log t_c + B$ where A and B are regression coefficients and t_c is the corrected RT, should be used.

8.2.1.5 The correlation coefficient for the regression, r^2, should be greater than 0.95. If this condition is not met, HPLC chromatography conditions are not correct. Return to 8.1.2 and determine improved conditions.

8.2.2 Resolution

8.2.2.1 Using the finalized HPLC conditions (see 8.1.2), inject 10 μL of the HPLC performance solution. Baseline separation between biphenyl, 1,3-diethylbenzene, and bibenzyl should exist.

8.2.2.2 If baseline separation between all three chemicals does not exist, either replace the chromatography column and return to 8.1 or return to 8.1.2 and determine improved conditions.

8.3 HPLC Fraction Time (Collection Time) Identification.

8.3.1 Inject 10 μL of the HPLC fractionation standard using the finalized chromatographic conditions and record RTs of 1,4-dichlorobenzene, 1,3-diethylbenzene, p,p'-DDE, and decachlorobiphenyl. These times are used for fraction collection from the HPLC.

8.3.2 RT reproducibility -- For each compound in the HPLC fractionation standard, the absolute RTs should not vary by more than ±0.10 minutes from one analysis to the next.

8.4 GC/MS Conditions.

8.4.1 Recommended gas chromatography conditions

Column Type:	DB-5
Film Thickness:	0.25 μm

Column Dimensions: 30 m X 0.32 mm or 30 m X 0.25 mm
Helium Linear Velocity: 30 cm/sec @ 250°C
Temperature Program:
Inject 50°C, hold 4 mins.,
increase to 175°C at 10°C/min,
increase to 275°C at 5°C/m,
hold at 275°C for 20 mins.
Injection Volume: 1 or 2 μL

8.4.2 Recommended acquisition conditions for mass spectrometer.

Mass Range: 45-545 m/z
Total Cycle Time per Scan: 0.5 ≤ cycle time ≤ 1.5 seconds

8.4.3 Mass Spectrometer Calibration

8.4.3.1 Calibrate and tune MS with standards and procedures prescribed by the manufacturer.

8.4.3.2 Inject 1 μL or 2 μL aliquot of MS performance check solution. If spectrum does not meet criteria for DFTPP (Table C-4); return to 8.4.3.1 and recalibrate/tune MS.

8.5 GC/MS Performance Criteria

8.5.1 GC Performance -- Inject 1.0 μL of the GC performance solution. Baseline separation between β-BHC and -BHC and between endrin ketone and d_{12}-chrysene should exist. Alternatively, anthracene and phenanthrene should be separated by baseline and benz[a]anthracene and chrysene should be separated by a valley whose height is less than 25% of the average peak height of these two compounds.

8.5.2 MS Sensitivity -- Inject 1.0 μL of the 0.5 ppm GC/MS calibration solution. Using the total ion chromatogram, a signal to noise ratio of greater than 3 should be observed for each surrogate.

8.5.3 MS Calibration -- Inject 1.0 μL of the 0.5 ppm GC/MS calibration solution. For d_{12}-chrysene, abundance of m/z 241 relative to that of m/z 240 should be >15% and <25%.

8.5.4 GC Stability -- Perform multiple GC/MS analyses on the same GC/MS calibration solution. RTs should not vary by more than $\underline{t}$ seconds. Calculate the value of $\underline{t}$ with the equation, $\underline{t} = (RT)^{1/3}$, where RT is the observed average RT (in seconds).

8.6 Response Factor Calculation for MS

8.6.1 Inject 1.0 μL of each GC/MS calibration solution and acquire GC/MS data.

8.6.2 Calculate response factors (RF) for each surrogate relative to d_{12}-chrysene

$$RF = A_s Q_c / A_c Q_s$$

where A_s = integrated total ion abundance for the surrogate
A_c = integrated total ion abundance for the internal standard, d_{12}-chrysene
Q_s = injected quantity of surrogate
Q_c = injected quantity of d_{12}-chrysene

8.6.3 RF Reproducibility -- For each surrogate, calculate the mean RF. When the relative standard deviation (RSD) exceeds 30%, analyze additional aliquots of GC/MS calibration solutions to obtain acceptable RSD for the RF, or take action to improve GC/MS performance.

8.7 Continuing Calibration Check

8.7.1 HPLC

8.7.1.1 With the following procedures, verify HPLC column performance at the beginning and end of each 12 h period during which analyses are performed.

8.7.1.2 Demonstrate acceptable performance for criteria described in Section 8.2.2.

8.7.1.3 Demonstrate acceptable performance for criteria described in Section 8.3.2.

8.7.2 GC/MS

8.7.2.1 With the following procedures, verify initial calibration at beginning and end of each 12 h period during which analyses are performed.

8.7.2.2 Inject 1 or 2 μL aliquot of MS performance check solution. Ensure acceptable MS calibration and performance.

8.7.2.3 Demonstrate acceptable performance for 8.5.

8.7.2.4 Determine the area for chrysene-d_{12} has not changed by more than 30% from most recent analyses of the GC/MS calibration solutions.

8.7.2.5 For an acceptable continuing calibration check, the measured RF for each surrogate must be within 30% of the mean value calculated during initial calibration.

8.7.3 Remedial Actions

Remedial actions must be taken if criteria are not met; possible remedies are:

8.7.3.1 Check and adjust operating conditions.

8.7.3.2 Clean or replace injector liner on GC.

8.7.3.3 Flush column with solvent according to manufacturers instructions.

8.7.3.4 Break off a short portion (approximately 0.33 m) of the GC column; check column performance by analysis of performance check solution for GC.

8.7.3.5 Replace column; performance of all initial calibration procedures then required.

8.7.3.6 Adjust MS for greater or lesser resolution.

8.7.3.7 Calibrate MS mass scale.

8.7.3.8 Prepare and analyze new concentration calibration/performance solutions.

8.7.3.9 Prepare new concentration calibration curve(s).

9. Procedures

9.1 Sample Preparation for Extraction

9.1.1 Homogenize wet sediment sample.

9.1.2 Place enough wet sediment on solvent rinsed aluminum foil to obtain approximately 25 g of sample after drying.

9.1.3 Place aluminum foil with sample in hood and allow to air dry.

9.1.4 Grind air dried sample in mortar and pestle until uniform and stored the ground material in solvent rinsed jar and cover with a foil lined lid.

9.2 Percent Moisture

9.2.1 Place a weighing pan in a 105±5°C oven for 15 minutes, let cool for 15 minutes in desiccator, and then weigh to 5 places.

9.2.2 At the same time as 9.1.2 is performed, place 1 or 2 grams of wet sediment into tared weighing pan.

9.2.3 Measure weight of tared weighing pan with wet sediment to 5 places.

9.2.4 Place tared weighing pan with wet sample in an oven at 105±5°C and dry sample for a minimum of 12 hours.

9.2.5 Let weighing pan cool in desiccator, and reweigh to 5 places.

9.2.6 Repeat 9.2.4 and 9.2.5 until a constant weight is obtained, i.e., ±1.0%. Note, if a forced-draft oven is used, 10 h usually is considered sufficient. If a convection oven is used, samples should be dried for a least 24 h.

9.2.7 Calculate percent moisture with the following equation:

= (Wet weight - Dry weight)*100/(Wet weight)

9.3 Percent Organic Carbon

9.3.1 Measure organic carbon content of ground sediment.

9.3.2 Calculated percent organic carbon of the sediment.

9.4 Extraction of Dried Sediment

9.4.1 Place 20 g of ground sediment into a 250 ml beaker. Add 20 g of coarse sodium sulfate and mix.

9.4.2 Place 1/4" of silica gel into a coarse fritted glass Soxhlet extraction thimble.

9.4.3 Add 30 g of sodium sulfate to the thimble

9.4.4 Place two-thirds of the mixture into a coarse fritted glass Soxhlet extraction thimble and then add 0.100 mL of the surrogate solution. The remaining sample is added to the thimble.

9.4.5 Add 30 g of sodium sulfate to extraction thimble and then a layer of glass wool

9.4.6 Place the extraction thimble into a clean Soxhlet extractor body.

9.4.7 Add 200 mL of acetone and one or two boiling chips to Soxhlet extraction flask.

9.4.8 Rinse 250 ml beaker with acetone 3 times and pour acetone into Soxhlet body.

9.4.9 Assemble Soxhlet extractor and place onto extraction rack. Turn heaters on and extract sediment for 4 hours.

9.4.10 Turn off heaters and after cooling, quantitatively transfer sample to a Kuderna-Danish concentrator. Cap Kuderna-Danish concentrator.

9.4.11 200 ml of toluene:methanol (1:3) solution and one or two boiling chips are placed into the emptied Soxhlet extractor flask. The flask along with Soxhlet extractor body are placed on to the extraction rack.

9.4.12 The heaters on the extraction rack are turned on and the sample is extracted for at least 12 hours.

9.4.13 Turn off extraction rack and allow toluene:methanol extract to cool.

9.4.14 Add one or two clean boiling chips and attach a 3-ball Snyder column to the KD apparatus. Concentrate acetone extract on a steam bath to a volume of approximately 20 ml.

9.4.15 After allowing toluene:methanol to cool, quantitatively transfer this extract to the KD with the concentrated acetone extract.

9.4.16 Add one or two clean boiling chips and attach a 3-ball Snyder column to the KD apparatus. Concentrate the extract on a steam bath to a volume of approximately 8 ml.

9.4.17 Transfer extract to vial suitable for storage with hexane. Cap vial and store in freezer.

9.5 Sample Cleanup

9.5.1 Construct column containing (bottom to top) glass wool, silica gel (2 g), sodium sulfate (2 g), 70% sulfuric acid solution (5 ml) on Celite (10 g), and sodium sulfate (2 g). The column is washed with 100 mL of hexane is not allowed to go dry.

9.5.2 Quantitatively transfer the hexane extract from 9.4.17 to the column using hexane solvent. Collect eluate into a 500 mL Kuderna-Danish evaporation flask with a 10 mL lower tube.

9.5.3 Wash the acid/Celite column with 100 mL hexane and collect the eluate in the same Kuderna-Danish flask.

9.5.4 Add one or two clean boiling chips to the flask and attach a three ball Snyder column to the Kuderna-Danish apparatus. Concentrate the extract on a steam bath until volume of the extract is less than 8 mL. Allow Kuderna-Danish apparatus to cool and detach the lower tube, rinsing the joint with hexane into the sample.

9.5.5 Use a nitrogen stream and a warm (<40°C) water bath to concentrate the extract to a volume of 2.0 mL.

9.5.6 Cap extract and store in freezer.

9.6 Removal of Sulfur

9.6.1 A column consisting of a disposable Pasteur pipet packed from bottom to top with glass wool, 5 cm of activated copper filings, and glass wool is made and the column is washed sequentially with 20 mL of acetone, 20 mL toluene, and 2X 20 mL hexane.

9.6.2 Remove extract from freezer and allow it to come to room temperature. Quantitatively transfer the extract to the column using hexane and collect eluate.

9.6.3 Wash column with three 10 ml aliquots of hexane or methylene chloride and collect eluate in the same receiving vessel.

9.6.4 Use a nitrogen stream and a warm water bath to concentrate the extract to a volume of 0.3 mL.

9.6.5 Quantitatively transfer extract to a HPLC autosampler vial. Cap vial and store vial in freezer.

9.7 Fractionation of Sediment Extract

9.7.1 Remove sample from freezer and allow it to warm to ambient temperature. Uncap the vial and allow the sample to evaporate naturally to the volume of 500 μL. Mix extract and cap vial.

9.7.2 Using the HPLC conditions determined previously (see Section 8.2), inject 175 μL of the sample into the HPLC column.

9.7.3 At the RT of 1,4-dichlorobenzene (determined in Section 8.3), place a clean flask under the waste tube from the UV detector and collect the column eluate. Label this fraction as Fraction 1.

9.7.4 At the RT of 1,3-diethylbenzene, place a clean flask under the waste tube from the UV detector and collect the column eluate. Label this fraction as Fraction 2.

9.7.5 At the RT of p,p'-DDE, place a clean flask under the waste tube from the UV detector and collect the column eluate. Collection should continue until the RT of decachlorobiphenyl. Label this fraction as Fraction 3.

9.7.6 Repeat steps 9.7.2 to 9.7.5 two more times so that all the sample has been injected onto the HPLC column. Combine the like fractions into the same flasks.

9.8 Concentration of Sediment Extracts (Liquid/Liquid Extraction)

9.8.1 Quantitatively transfer each HPLC fraction into its own extraction bottle, e.g., separatory funnels, bottles, etc. Dilute fractions a minimum of 10 fold with HPLC grade water. Seal bottle and mix diluted fractions thoroughly.

9.8.2 Open bottles and add 60 mL of hexane to each diluted fraction. Seal bottles.

9.8.3 Place bottles onto mixing device, i.e., shaker, tumbler, or stirring apparatus. Shake, tumble, or mix the sample for a minimum of 30 minutes. (Note, longer extraction times might be necessary to obtain proper extraction with your apparatus.) Alternatively, separatory funnel extraction by hand with periodic venting may be used; shake for minimum of 2 minutes

9.8.4 Remove bottles containing the diluted fractions from mixing device. Allow hexane layer to separate from water phase to 10-15 minutes. Collect the hexane in a clean flask. Each HPLC fraction must have its own flask.

9.8.5 Repeat steps 9.8.2-4 two additional times and combine the hexane from these extractions with the hexane from the first extraction.

9.8.6 Dry each extract by passing it through a drying column containing about 10 cm of anhydrous sodium sulfate. Collect the dried extract in a Kuderna-Danish concentrator flask. Rinse flask which contained the solvent extract with 20-30 mL of hexane and add it to the column to complete the quantitative transfer.

9.8.7 Add one or two clean boiling chips to the flask and attach a three ball Snyder column to Kuderna-Danish apparatus. Concentrate the extract on a steam bath until volume of the extract is less than 8 mL. Allow Kuderna-Danish apparatus to cool and detach the lower tube, rinsing the joint with hexane into the sample. Three extracts for each sample will results, i.e., fractions 1, 2, and 3.

9.8.8 Using a stream of dry clean air, evaporate extracts to approximately 1 mL.

9.8.9 Quantitatively transfer and concentrate extracts for each fraction to 100 μL in microvials. Cap and store in freezer.

9.9 Concentration of Sediment Extracts (C_{18} SPE)

9.9.1 Dilute fractions a minimum of 10 fold with HPLC grade water and mix diluted fractions thoroughly.

9.9.2 Activate three C_{18} solid phase extraction (SPE) columns by passing methanol and then HPLC grade water through the column as specified by the manufacture. The size of the column required is dependent upon the volume of diluted HPLC fractions; use manufacturer's recommendations. In general, 1 mL, 3 mL LD, and 3 mL columns will be needed.

9.9.3 Pass diluted extracts through the C_{18} SPE columns. Do not exceed maximum flowrates recommended by manufacture.

9.9.4 Remove excess water for the column. This can be done using a slight positive pressure of clean air or nitrogen gas, a slight vacuum, or (spun out using) a centrifuge. Do not let the columns go dry.

9.9.5 Elute C_{18} SPE columns with three 500 µL aliquots of methylene chloride and then one 500 µL aliquot of hexane.

9.9.6 Dry extracts by passing C_{18} SPE eluent through micro Na_2SO_4 columns. Micro drying columns may be prepared by placing 1 cm of Na_2SO_4 into an empty 1 mL SPE column and passing approximately 2 ml of hexane through the column.

9.9.7 Concentrate eluate from drying column to approximately 100 µL and quantitatively transfer concentrate to microvials. Cap and store in freezer. Three extracts for each sample will be obtained.

9.10 GC/MS Analysis

9.10.1 Remove sample from freezer and allow it to warm to ambient temperature. Adjust volume of sample to 100 µL and then spike sample with 5 µL of the internal standard spiking solution, 1000 ng of d_{12}-chrysene. Mix sample after spiking sample with d_{12}-chrysene.

9.10.2 Inject a 1 μL or 2 μL aliquot of blank or sample extract into GC operated under conditions used to produce acceptable results during calibration.

9.10.3 Acquire MS data using full scan conditions (see Section 8.5).

9.10.4 Recap samples and store extracts in freezer. Note, autosampler crimp caps after being punctured by the syringe used for sample injection must be replaced.

9.11 GC/MS Data Analysis

Data analysis for this analytical procedure can be divided into three tasks, 1) quantification of surrogates and other GC/MS components, 2) library searching with the CHC mass spectral library, and 3) library searching with the EPA/NIH/NBS mass spectral library. These procedures are performed on all HPLC fractions for the the sample and blank.

9.11.1 Quantification of Surrogates and Other GC/MS Components

9.11.1.1 Surrogate and Internal Standard Identification

Identify surrogates and internal standards by comparison of their mass spectrum (after background subtraction) to reference spectrums in the user-created data base. The GC retention time of the sample component should be within 10 sec of the time observed for that same compound when a calibration solution was analyzed. In general, all ions that are present above 10% relative abundance in the mass spectrum of the standard should be present in the mass spectrum of the sample component and should agree within absolute 20%. For example, if an ion has a relative abundance of 30% in the standard spectrum, its abundance in the sample spectrum should be in the range of 10 to 50%. Some ions, particularly the molecular ion, are of special importance, and should be evaluated even if they are below 10% relative abundance.

9.11.1.2 Peak Integration

For each fraction, use the GC/MS peak detection and integration software to obtain areas for all chromatographic components from the total ion chromatogram with a signal to noise of 3 and

greater. (Note, the solvent front need not be examined.) Verify that the surrogate in each fraction was integrated. If surrogate was not integrated use more sensitive peak detection and integration settings to obtain peak areas for the surrogates.

9.11.1.3 Calculate Surrogate Concentrations

$$C_x = (A_x \cdot Q_{IS}) / (A_{IS} \cdot RF \cdot M)$$

Where C_x = Concentration of surrogate

A_x = Area of surrogate in total ion chromatogram

Q_{IS} = Quantity of internal standard added to extract before GC/MS analysis

A_{IS} = Area of internal standard in total ion chromatogram added to extract before GC/MS analysis

RF = Mean response factor of surrogate from initial calibration analysis and/or from GC/MS calibration solutions run with sample analyses. For fraction #1, use RF for d_{10}-biphenyl. For fraction #2, use RF for $^{13}C_6$-1,2,4,5-tetrachlorobenzene. For fraction #3, use RF for $^{13}C_6$-hexachlorobenzene.

M = Mass of extracted sediment, i.e., 20 g.

Alternatively, use the GC/MS system software or other available proven software to compute the concentration of the surrogates using linear, second, or third order regression or using piecewise calibration curves.

9.11.1.4 Calculate Surrogate Recoveries

$$\text{Surrogate Recovery} = (C_x \bullet 100)/SSC$$

Where C_x = Concentration of surrogate

SSC = Surrogate spiking concentration, i.e., 5 ng/g

9.11.1.5 Calculate concentrations of all chromatographic components

$$C_x = (A_x \bullet Q_{IS} \bullet 100)/(A_{IS} \bullet RF \bullet M \bullet REC)$$

Where C_x = Concentration of chromatographic component

A_x = Area of chromatographic component in total ion chromatogram

Q_{IS} = Quantity of internal standard added to extract before GC/MS analysis

A_{IS} = Area of internal standard in total ion chromatogram

M = Mass of extracted sediment, i.e. 20 g

RF = Mean response factor of surrogate from initial calibration analyses and/or from GC/MS calibration solutions, run with sample analyses. For fraction #1, use RF for d_{10}-biphenyl. For fraction #2, use RF for $^{13}C_6$-1,2,4,5-tetrachlorobenzene. For fraction #3, use RF for $^{13}C_6$-hexachlorobenzene.

REC = Surrogate recovery calculated in Section 9.11.1.4. For fraction #1, use recovery of d_{10}-biphenyl. For fraction #2, use recovery of $^{13}C_6$-1,2,4,5-tetrachlorobenzene. For fraction #3, use recovery of $^{13}C_6$-hexachlorobenzene.

Alternatively, use the GC/MS system software or other available proven software to compute the concentration of all chromatographic components using linear, second, or third order regression or using piecewise calibration curves. These

calculations must use the response curve for the surrogate appropriate for the fraction of interest and the reported concentrations must be corrected for recovery of the surrogate. For fraction #1, use response curve and recovery of d_{10}-biphenyl. For fraction #2, use response curve and recovery of $^{13}C_6$-1,2,4,5-tetrachlorobenzene. For fraction #3, use response curve and recovery of $^{13}C_6$-hexachlorobenzene.

9.11.2 Library searching with the CHC mass spectral library

9.11.2.1 Algorithm Selection

A reverse searching algorithm is required when available. If GC/MS system does not have a reverse searching algorithm, library searching should be performed using the default algorithm supplied by the manufacture of the instrument.

9.11.2.2 Searching

All chromatographic components detected in 9.11.1.2 (Peak Integration) are searched against the CHC mass spectral library. Those GC/MS components with of 70% and greater are considered tentatively identified. For each tentatively identified component, a list of the best mass spectral library identifications (up to a total of ten identifications) is reported along with the percent fit values, CAS number of each tentative identification, HPLC fraction number, GC retention time, and the concentration for the GC/MS component. This report is called Report 1.

9.11.3 Library Searching with EPA/NIH/NBS Mass Spectral Library.

9.11.3.1 Algorithm Selection

A reverse searching algorithm is required when available. If GC/MS system does not have a reverse searching algorithm, library searching should be performed using the default algorithm supplied by the manufacture of the instrument.

9.11.3.2 Eliminating of Compounds Below 5 ng/g

All unidentified components from the CHC library search with concentrations less than 5 ng/g are

eliminated from further data processing. This elimination can be performed by comparing peak areas or heights of each chromatographic peak to the peak area or height of the surrogate appropriate for that fraction. This elimination may also be performed by comparing the recovery corrected concentration of each chromatographic component to 5 ng/g.

9.11.3.3 Searching

The remaining components are searched against the EPA/NIH/NBS mass spectral library. Those GC/MS components with fits of 70% and greater are considered tentatively identified. For each tentatively identified component, a list of the best mass spectral library identifications (up to a total of ten identifications) is reported along with the percent fit values, CAS number of each tentative identification, HPLC fraction number, GC retention time, and the concentration for the GC/MS component. This report is called Report 2.

For those components with fits/matches less than 70% and greater than 25%, the two best mass spectral library identifications along with the percent fit values, the CAS number of each tentative identification, HPLC fraction number, GC retention time, and the concentration are reported for each the GC/MS component. For GC/MS components with fits/matches less than 25%, the concentration, HPLC fraction number, and GC retention time for each component is reported and the components labeled as being "unknown". This report is identified as Report 3.

9.11.4 Elimination of chromatographic components common to the spiked blank and sediment GC/MS data.

Chromatographic components with retention times within ten seconds between the spiked blank and sediment and with the same mass spectrums should be removed from the analysis.

9.12 Reporting of Data

9.12.1 Report 1: CHC mass spectral identifications.

For each chromatographic component tentatively identified using the CHC search (fits greater than or equal to 70%), a list of the best mass spectral

library identifications (up to a total of ten identifications) is reported along with the percent fit values, CAS number of each tentative identification, HPLC fraction number, GC retention time, and the concentration for the GC/MS component.

9.12.2 Report 2: EPA/NIH/NBS mass spectral tentative identifications (fits ≥ 70% and ≥ 5 ug/kg).

GC/MS components tentatively identified in the EPA/NIH/NBS mass spectral search. For each tentatively identified component, a list of the best mass spectral library identifications (up to a total of ten identifications) is reported along with the percent fit values, CAS number of each tentative identification, HPLC fraction number, GC retention time, and the concentration for the GC/MS component.

9.12.3 Report 3: EPA/NIH/NBS mass spectral tentative identifications (fits < 70% and ≥ 5 ug/kg).

GC/MS components not tentatively identified in the EPA/NIH/NBS mass spectral search. For those components with fits/matches less than 70% but greater than 25%, the two best mass spectral library identifications along with the percent fit values, the CAS number of each tentative identification, HPLC fraction number, GC retention time, and the concentration are reported for each the GC/MS component. For GC/MS components with fits/matches less than 25%, the concentration, HPLC fraction number, and GC retention time for each component is reported and the components labeled as being "unknown".

9.12.4 QA/QC Report

9.7.4.1 Percent moisture of the sample.

9.7.4.2 Percent organic carbon content of the sediment.

9.7.4.3 Recoveries.

For d_{10}-biphenyl, $^{13}C_6$-1,2,4,5-tetrachlorobenzene, and $^{13}C_6$-hexachlorobenzene, recoveries are reported for each sample and blank HPLC fraction.

9.7.4.4 GC/MS Chromatograms.

For the sample and its corresponding blank, total ion chromatograms must be provided for each sample and blank HPLC fraction.

9.7.4.5 QA/QC.

Chromatogram demonstrating HPLC resolution, see Section 8.2.2
Data demonstrating GC resolution, see Section 8.5.1
Data demonstrating MS sensitivity, see Section 8.5.2
Data demonstrating MS calibration, see Section 8.5.3
Data demonstrating DFTPP performance, see Section 8.4.3.2
Data demonstrating precision, see Section 10.5

10. Quality Control

10.1 Recoveries of Surrogates. Method:

$$\% \text{ Recovery} = \frac{\text{measured surrogate amount}}{\text{spiked surrogate amount}} \times 100$$

Quality Assurance Requirement

$$25\% < \% \text{ recovery} < 120\%$$

Quality Control Action. If percent recovery is out of range, re-extract and re-analyze sample.

10.2 Surrogate Fraction Location.

Quality Assurance Requirement

Fraction 1	should contain d_{10}-biphenyl and should not contain $^{13}C_6$-1,2,4,5-tetrachlorobenzene or $^{13}C_6$- hexachlorobenzene
Fraction 2	should contain $^{13}C_6$-1,2,4,5-tetrachlorobenzene and should not contain d_{10}-biphenyl or $^{13}C_6$-hexachlorobenzene
Fraction 3	should contain $^{13}C_6$-hexachlorobenzene and should not contain $^{13}C_6$-1,2,4,5-tetrachlorobenzene or d_{10}-biphenyl

Quality Control Action. If surrogate is in wrong fraction, take corrective action for improper HPLC conditions. Re-extract and re-analyze the sample.

10.3 Calibration Stability

10.3.1 Continuing Calibration Checks

Quality Assurance. See Sections 8.7

Quality Control Action. Re-extract and re-analyze samples. For corrective actions see Section 8.7.

10.3.2 HPLC Stability.

Quality Assurance. Immediately before and after fractionation solution must be analyzed. See Section 8.7 for stability requirements.

Quality Control Action. If RT stability requirements are not met, re-extract and re-analyze the sample.

10.3.3 GC/MS Stability

Quality Assurance. During the GC/MS analysis run, a GC/MS calibration solution should be analyzed twice; once at the beginning and once at the end of the GC/MS analysis sample sequence. See Sections 8.5 and 8.7 for stability requirements.

Quality Control Action. If RT and/or MS sensitivity stability requirements are not met, repeat GC/MS analysis on samples after correction of instrumental problems.

10.4 Blanks

10.4.1 Procedural Blanks

Quality Control. One procedural blank should be performed with every set of sediment samples analyzed.

An acceptable procedural blank:

a) Meets Section 10.1 requirements.
b) Contains no compound with elution characteristics and mass spectral features that would interfere with identification and quantification of the surrogates.

c) Contains few chromatographic peaks in the GC/MS total ion chromatograms for each fraction.

Quality Control Action. Locate and eliminate the source of contamination. Re-extract and re-analyze the entire batch of sample.

10.4.2 Unspiked Procedural Blanks

Quality Control. One procedural blank not spiked with the surrogate spiking solution should be performed with every 20 sediment samples.

An acceptable unspiked blank. The sample should not contain detectable amounts of the surrogates.

Quality Control Action. Locate and eliminate source of contamination, re-extract and re-analyze entire batch of sample.

10.5 Precision

Quality Control. A duplicate set of sediment samples will be analyzed with every set of 10 samples. To measure the precision, the relative percent difference between the lab duplicate will be determined for each surrogate.

$$\text{Relative \% difference} = \frac{\text{surrogate amount duplicate 1} - \text{surrogate amount duplicate 2}}{\text{Average surrogate amount}} \times 100$$

The relative percent difference should be less than 150%.

Quality Control Action. If relative percent difference is out-range for any of three surrogates, re-extract and re-analyze the sample.

10.6 Sample Sets.

Sample sets are defined as a group of samples that are carried through the analytical procedure at the same time. Each sample set will include a minimum of two QC samples, one of them being a spiked blank. QC samples include spiked blanks, unspiked blanks, and replicates.

A set will normally contain 10 samples plus the required QC samples.

10.7 GC/MS Analysis Sets.

A GC/MS analysis set is defined as a group of prepared samples (sediments and QC samples) and GC/MS calibration solutions analyzed during one GC/MS run. A set will normally contain 12 prepared samples, all five GC/MS calibration solutions, and 1 or 2 duplicates of the GC/MS calibration solutions. The duplicate GC/MS calibration solutions will be analyzed in the beginning and at the end of GC/MS sample sequence.

11. Appendix C References.

11.1 "Carcinogens -- Working with Carcinogens", Department of Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, August 1977.

11.2 "OSHA Safety and Health Standards, General Industry", 29 CFR 1910, Occupational Safety and Health Administration, OSHA 2206, Revised January 1976.

11.3 "Safety in Academic Chemistry Laboratories", American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.

TABLE C-1

CHEMICALS OF HIGHEST CONCERN LIST

CAS number	chemical name
50-29-3	p,p'-dichlorodiphenyltrichloroethane (DDT)
57-74-9	chlordane
58-89-9	hexachlorocyclohexane (lindane)
60-57-1	dieldrin
70-30-4	hexachlorophene
72-54-8	p,p'-dichlorodiphenyldichloroethane (DDD)
72-55-9	p,p'-dichlorodiphenyldichloroethylene (DDE)
76-44-8	heptachlor
91-94-1	3,3'-dichlorobenzidine
95-94-3	1,2,4,5-tetrachlorobenzene
101-61-1	4,4'-methylene bis(N,N'-dimethyl) aniline
115-32-2	dicofol
117-81-7	bis(2-ethylhexyl)phthalate (BEHP)
118-74-1	hexachlorobenzene
309-00-2	aldrin
319-84-6	alpha-hexachlorocyclohexane (alpha-HCH)
319-85-7	beta-hexachlorocyclohexane (beta-HCH)
608-73-1	technical-hexachlorocyclohexane (t-HCH)
608-93-5	pentachlorobenzene
924-16-3	N-nitroso-di-n-butylamine
1024-57-3	heptachlor epoxide
1746-01-6	dioxin (2,3,7,8-TCDD)
2104-64-5	ethylp-nitrophenylphenylphosphorothioate(EPN)
2385-85-5	mirex
8001-35-2	toxaphene
39515-41-8	danitol
11096-82-5	polychlorinated biphenyl 1260
11097-69-1	polychlorinated biphenyl 1254
11104-28-2	polychlorinated biphenyl 1221
11141-16-5	polychlorinated biphenyl 1232
12672-29-6	polychlorinated biphenyl 1248
12674-11-2	polychlorinated biphenyl 1016
53469-21-9	polychlorinated biphenyl 1242

Table C-2. GC/MS Calibration Solutions, Concentrations of Surrogates and Internal Standards.

$^{13}C_6$-1,2,4,5-tetrachlorobenzene ppm	d_{10}-biphenyl ppm	d_{12}-chrysene ppm	$^{13}C_6$-hexachlorobenzene ppm
0.5	0.5	10	0.5
1	1	10	1
10	10	10	10
50	50	10	50
100	100	10	100

Table C-3. Suggested Retention Times (RTs) for Compounds in the HPLC Calibration Solution.

Chemical	Retention Time	Log P
Benzene	4.3	2.13
Bromobenzene	5.5	2.99
Biphenyl	6.8	3.76
Bibenzyl	8.1	4.81
p,p'-DDE	13.4	5.69
2,2',4,5,5'-Pentachlorobiphenyl	15.1	6.11

Table C-4. DFTPP Ion Abundance Criteria.

m/z	Criteria
51	10-80% of the base peak
68	≤2% of m/z 69
70	≤2% of m/z 69
127	10-80% of the base peak
197	≤2% of m/z 198
198	base peak or >50% of 442
199	5-9% of m/z 198
275	10-60% of the base peak
365	>1% of base peak
441	present and <mass 433
442	base peak or >50% of m/z 198
443	15-24% of m/z 442

APPENDIX D

CHEMICALS AVAILABLE IN IRIS

USE ONLY FOR SCREENING Values from IRIS 9/1/90
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CAS number	Chemical Name	RfD mg/kg/day	q1* /mg/kg/day	Estimated BCF 3% Lipid	RAC (mg/l) RL: 10E-6 0.0065 kg/day Consumption	RAC (mg/l) RL: 10E-6 0.020 kg/day Consumption
50000	Formaldehyde	0.2	**			
50293	p,p'-DDT	0.0005		40000	0.0001	0.00004
50328	Benzo(a)pyrene	**	**	10960		
51285	2,4-Dinitrophenol	0.002		5.2	4	1
55185	N-Nitrosodiethylamine	**	150	0.40	0.0002	0.00006
56235	Carbon tetrachloride	0.0007	0.13	29.72	0.003	0.0009
56359	Tributyltin oxide	0.00003				
56382	Parathion	**	**	87.6		
57125	Cyanide, free	0.02				
57249	Strychnine	0.0003		0.4	8	3
57749	Chlordane	0.00006	1.3	3804	0.000002	0.0000007
58899	gamma-Hexachlorocyclohexane	0.0003		146.8	0.00002	0.000007
58902	2,3,4,6-Tetrachlorophenol	0.03		416	0.8	0.3
60297	Ethyl ether	0.2		0.77	3000	900
60515	Dimethoate	0.0002		0.4	6	2
60571	Dieldrin	0.00005	16	32.04	0.00002	0.000007
62384	Phenylmercuric acetate	0.00008		0.58	2	0.5
62533	Aniline	**	0.0057	0.84	2	0.7
62737	Dichlorvos	0.0008	0.29	0.4	0.1	0.3
62759	N Nitrosodimethylamine	**	51	0.4	0.0005	0.0002
63252	Carbaryl	0.1		12.2	90	30
64186	Formic acid	2				
65850	Benzoic acid	4		4.92	9000	3000
67561	Methanol	0.5		0.4	14000	4000
67641	Acetone	0.1	**	0.4		
67663	Chloroform	0.01		5.56	20	6
67721	Hexachloroethane	0.001	0.014	700	0.001	0.0004
70304	Hexachlorophene	0.0003		40000	0.00008	0.00002
71363	n Butanol	0.1		0.71	2000	500

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71432	Benzene	**	0.029	7.84	0.05	0.01
71556	1,1,1-Trichloroethane	0.09		14.52	70	20
72208	Endrin	0.0003	**	32.04	0.1	0.03
72435	Methoxychlor	0.005	**	2564	0.02	0.007
72548	p,p'-DDD	**	0.24	12840	0.000004	0.000001
72559	p,p'-DDE	**	0.34	40000	0.0000008	0.0000002
74839	Bromomethane	0.0014		1.13	10	4
74908	Hydrogen cyanide	0.02				
75058	Acetonitrile	0.006		0.4	200	50
75070	Acetaldehyde	**	**	0.4		
75092	Dichloromethane	0.06	0.0075	1.54	0.9	0.3
75150	Carbon disulfide	0.1				
75252	Bromoform	0.02	0.0079	11.92	0.1	0.04
75274	Bromodichloromethane	0.02		7.16	30	10
75354	1,1-Dichloroethylene	0.009	0.6	7.44	0.002	0.0008
75694	Trichloromonofluoromethane	0.3		13.36	200	80
75718	Dichlorodifluoromethane	0.2		6	400	100
75876	Chloral	0.002		3.23	7	2
75990	Dalapon, sodium salt	0.03		2.31	100	50
76131	CFC-113	30		63.2	5000	2000
76448	Heptachlor	0.0005	4.5	692	0.000004	0.000001
77474	Hexachlorocyclopentadiene	0.007	**	744	0.1	0.03
77781	Dimethyl sulfate	**	**	0.4		
78002	Tetraethyl lead	0.0000001				
78488	Merphos oxide	0.00003				
78591	Isophorone	0.2	0.0041	9	0.3	0.09
78831	Isobutyl alcohol	0.3		0.56	6000	2000
78864	2-Chlorobutane	**	**	15.5		
78933	Methyl ethyl ketone	0.05	**	0.4	1000	400

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79005	1,1,2-Trichloroethane	0.004	0.057	6.64	0.03	0.009
79016	Trichloroethylene	**	0.011	9.84	0.1	0.03
79061	Acrylamide	0.0002	4.5	0.4	0.006	0.002
79107	Acrylic acid	0.08		0.4	2000	700
79221	Methyl chlorocarbonate	**				
79345	1,1,2,2-Tetrachloroethane	**	0.20	19.52	0.003	0.0009
80057	Bishpenol A.	0.05		166.8	3	1
81812	Warfarin	0.0003		4.76	0.7	0.2
82688	Pentachloronitrobenzene	0.003		1160	0.03	0.009
83794	Rotenone	0.004		91.2	0.5	0.2
84662	Diethyl phthalate	0.8	**	17.2	500	200
84720	Ethylphthalyl ethylglycolate	3		7.92	4000	1000
84742	Dibutyl phthalate	0.1	**	808	1	0.4
85007	Diquat	0.0022				
85449	Phthalic anhydride	2				
85687	Butyl benzyl phthalate	0.2	**	1120	2	0.6
85701	Butylphthalyl butylglycolate	1		372.4	30	9
86306	N-Nitrosodiphenylamine	**	0.0049	50	0.04	0.01
87683	Hexachlorobutadiene	0.002	0.078	397.2	0.0004	0.0001
87821	Hexabromobenzene	0.002		4560	0.005	0.002
87865	Pentachlorophenol	0.03		1568	0.2	0.07
88062	2,4,6-Trichlorophenol	**	**	106		
88857	Dinoseb	0.001		239.2	0.05	0.01
91941	3,3'-dichlorobenzidine	**	0.45	117	0.0002	0.00007
92524	1,1-Biphenyl	0.05		243.2	2	0.7
92875	Benzidine	0.002	230	2.8	0.00002	0.000005
93652	MCPP	0.003		48	0.7	0.2
93721	2,4,5-TP	0.008	**	143.2	0.6	0.2
93765	2,4,5-Trichlorophenoxyacetic acid	0.01		81.6	1	0.4

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94746	MCPA	0.0005		27.32	0.2	0.06
94757	2,4-Dichlorophenoxyacetic acid	0.01		20.96	5	2
94815	MCPB	0.01		50.4	2	0.7
94826	4-(2,4-Dichlorophenoxybutyric acid	0.008		38.56	2	0.7
95487	o-Cresol	0.05	**	7.6	70	20
95498	o-Chlorotoluene	0.02		88	2	0.8
95501	1,2-Dichlorobenzene	0.09	**	103	9	3
95578	2-Chlorophenol	0.005		8.8	6	2
95658	3,4-Dimethylphenol	0.001		24.72	0.4	0.1
95943	1,2,4,5-Tetrachlorobenzene	0.0003		1404	0.002	0.0007
95954	2,4,5-Trichlorophenol	0.1		176.4	6	2
96184	1,2,3-Trichloropropane	0.006		5.84	10	4
98011	Furfural	0.003		0.54	60	20
98077	Benzotrichloride	**	**	283		
98828	Cumene	0.04		138	3	1
98862	Acetophenone	0.1		2.82	400	100
98953	Nitrobenzene	0.0005		4.92	1	0.4
99354	1,3,5-Trinitrobenzene	0.00005		1.93	0.3	0.09
99650	m-Dinitrobenzene	0.0001		3.08	0.4	0.1
100414	Ethylbenzene	0.1	**	66.8	20	5
100425	Styrene	0.2		29.24	80	20
100447	Benzyl chloride	**	0.17	21.5	0.003	0.001
100527	Benzaldehyde	0.1		2.42	500	100
101213	Chlorpropham (CIPC)	0.2		96.4	20	7
101553	p-Bromodiphenyl ether	**	**	2179		
101611	44'Methylenebis(NN'dimethyl)aniline	**	0.046	888	0.0003	0.00009
103231	Di-(2-ethylhexyl)adipate	0.7		40000	0.2	0.06
103333	Azobenzene	**	0.11	175.2	0.0006	0.0002
105602	Caprolactam	0.5		0.4	10000	4000

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106376	1,4-Dibromobenzene	0.01		181.2	0.6	0.2
106445	p-Cresol	0.05	**	7.6	70	20
106478	p-Chloroaniline	0.004		5.32	8	2
106898	Epichlorohydrin	0.002	0.0099	0.4	3	0.9
106934	1,2-Dibromoethane	**	85	3.76	0.00003	0.00001
106990	1,3-Butadiene	**		5.08		
107028	Acrolein	**	**	0.4		
107051	Allyl chloride	**	**	2.20		
107062	1,2-Dichloroethane	**	910	2.26	0.000005	0.000002
107131	Acrylonitrile	**	0.54	0.4	0.05	0.02
107186	Allyl alcohol	0.005		0.4	100	40
107211	Ethylene glycol	2		0.4	60000	20000
107302	Chloromethyl methyl ether	**	**	0.4		
108101	Methyl isobutyl ketone	0.05		1.38	400	100
108316	Maleic anhydride	0.1				
108394	m-Cresol	0.05	**	7.6	70	20
108452	m-Phenylenediamine	0.006		0.4	200	50
108883	Toluene	0.2	**	25.52	80	30
108907	Chlorobenzene	0.02	**	28	8	3
108918	Cyclohexylamine	0.2		1.92	1000	400
108941	Cyclohexanone	5		0.69	80000	30000
108952	Phenol	0.6		2.33	3000	900
109693	1-Chlorobutane	**	**	15.5		
110009	Furan	0.001		1.75	6	2
110543	n-Hexane	**	**	179		
110861	Pyridine	0.001		0.53	20	7
111444	Bis(chloroethyl)ether	**	1.1	0.98	0.01	0.003
114261	Baygon	0.004		2.81	20	5
115297	Endosulfan	0.00005				

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115322	Dicofol	**	0.44	9680	0.000003	0.0000008
116063	Aldicarb	**	**	1.21		
117817	Bis(2-ethylhexyl)phthalate	0.02	0.014	40000	0.00002	0.000006
118741	Hexachlorobenzene	0.0008		18800	0.0005	0.0001
118967	2,4,6-Trinitrotoluene	0.0005		2.27	2	0.8
120127	Anthracene	0.3	**	550	6	2
120616	Dimethyl terephthalate	0.1		11.56	100	30
120821	1,2,4-Trichlorobenzene	**	**	383.6		
120832	2,4-Dichlorophenol	0.003		42	0.8	0.3
121142	2,4/2,6-Dinitrotoluene mixture	**	0.68	5.96	0.002	0.0009
121697	N-N-Dimethylaniline	0.002		11.16	2	0.6
121755	Malathion	0.02		3.83	60	20
121824	RDX	0.003	0.11			
122349	Simazine	0.002	**	15.36	1	0.5
122394	Diphenylamine	0.025		115.2	2	0.8
122429	Propham	0.02		18	10	4
122667	1,2-Diphenylhydrazine	**	0.80	35.36	0.0004	0.0001
123331	Maleic hydrazide	0.5		0.4	10000	4000
123911	1,4-Dioxane	**	0.011	0.4	3	0.8
124403	Dimethylamine	**	**	0.4		
124481	Dibromochloromethane	0.02		9.24	20	8
126987	Methacrylonitrile	0.0001		0.42	3	0.8
127184	Tetrachloroethylene	0.01		38.72	3	0.9
129000	Pyrene	0.03	**	1280	0.3	0.08
131113	Dimethyl phthalate	**	**	2.51		
131895	4,6-Dinitro-o-cyclohexyl phenol	0.002		800	0.03	0.009
133062	Captan	0.013				
133073	Folpet	0.1	0.0035			
133904	Chloramben	0.015		8.24	20	6

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137268	Thiram	0.005				
139402	Propazine	0.02		47.2	5	1
141662	Bidrin	0.0001		0.4	3	0.9
141786	Ethyl acetate	0.9		0.54	20000	6000
143339	Sodium cyanide	0.04				
145733	Endothall	0.02		0.4	600	200
148185	Sodium diethyldithiocarbamate	0.03				
150505	Merphos	0.00003				
151508	Potassium cyanide	0.05				
156605	trans-1,2-Dichloroethylene	0.02		2.5	90	30
206440	Fluoranthene	0.04	**	1280	0.3	0.1
298000	Methyl parathion	0.00025		25.48	0.1	0.03
298044	Disulfoton	0.00004		60	0.007	0.002
300765	Naled	0.002		65.9	0.3	0.1
302012	Hydrazine/Hydrazine sulfate	**	3.0			
309002	Aldrin	0.00003	17	1638	0.0000004	0.0000001
319846	alpha-Hexachlorocyclohexane	**	6.3	146.8	0.00001	0.000004
319857	beta-Hexachlorocyclohexane	**	1.8	146.8	0.00004	0.00001
319868	delta-Hexachlorocyclohexane	**	**	146.8		
330541	Diuron	0.002		23.56	0.9	0.3
330552	Linuron	0.002	**	51.6	0.4	0.1
460195	Cyanogen	0.04				
504245	4-Aminopyridine	**	**	0.4		
506616	Potassium silver cyanide	0.2				
506649	Silver cyanide	0.1				
506683	Cyanogen bromide	0.09				
506774	Chlorine cyanide	0.05				
507200	t-Butylchloride	**	**	12.2		
510156	Chlorobenzilate	0.02		434	0.5	0.2

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541731	1,3-Dichlorobenzene	**	**	103		
542621	Barium cyanide	0.07				
542756	1,3-Dichloropropene	0.0003	**	2.94	1	0.4
542881	Bis(chloromethyl)ether	**	220	1.05	0.00005	0.00002
544923	Copper cyanide	0.005				
556887	Nitroguanidine	0.1				
556887	Nitroguanidine	0.1	**			
557211	Zinc cyanide	0.05				
563122	Ethion	0.0005		191	0.03	0.009
563688	Thallium acetate	0.00009	**			
576261	2,6-Dimethylphenol	0.0006		24.72	0.3	0.08
592018	Calcium Cyanide	0.04				
598776	1,1,2-Trichloropropane	0.005		17.4	3	1
608731	tech-Hexachlorocyclohexane	0.003	1.8	146.8	0.00004	0.00001
608935	Pentachlorobenzene	0.0008		5120	0.002	0.0005
615543	1,2,4-Tribromobenzene	0.005		524	0.1	0.03
621647	N-Nitrosodi-N-propylamine	**	7.0	1.85	0.0009	0.0003
630104	Selenourea	0.005				
630206	1,1,1,2-Tetrachloroethane	0.03		39.72	8	3
709988	Propanil	0.005		105.2	0.5	0.2
732116	Phosmet	0.02		15.2	10	4
759944	S-Ethyl dipropylthiocarbamate	0.025		68.4	4	1
765344	Glycidyaldehyde	0.0004				
834128	Ametryn	0.009		40.4	2	0.8
886500	Terbutryn	0.001		83.6	0.1	0.04
924163	N-Nitroso-di-n-butylamine	**	5.4	12.68	0.0002	0.00005
930552	N-Nitrosopyrrolidine	**	2.1	0.4	0.01	0.004
944229	Fonofos	0.002		193.6	0.1	0.04
950378	Methidathion	0.001				

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957517	Diphenamid	0.03		10.72	30	10
961115	Tetrachlorovinphos	0.03		64.8	5	2
1024573	Heptachlor epoxide	0.000013	9.1	13.72	0.00009	0.00003
1071836	Glyphosate	0.1	**			
1116547	N-Nitrosodiethanolamine	**	2.8	0.4	0.01	0.003
1163195	Decabromodiphenyl ether	0.01	**	40000	0.003	0.0009
1314325	Thallic oxide	**	**			
1314621	Vanadium pentoxide	0.009				
1314847	Zinc phosphide	0.0003				
1330207	Xylenes	2	**			
1332214	Asbestos	**	**			
1336363	Polychlorinated biphenyls	**	7.7			
1445756	Diisopropyl methyl phosphonate	0.08		0.4	2000	700
1563662	Carbofuran	0.005		14.32	4	1
1582098	Trifluralin	0.0075	0.0077	1784	0.0008	0.0003
1596845	Alar	0.15		0.4	4000	1000
1610180	Prometon	0.015		40.8	4	1
1646884	Aldicarb sulfone	0.0003		0.4	8	3
1689845	Bromoxynil	0.02		36.36	6	2
1689992	Bromoxynil octanoate	0.02		5800	0.04	0.01
1861321	Dacthal	0.5		327.6	20	5
1861401	Benefin	0.3		1784	2	0.6
1897456	Chlorothalonil	0.015		178.4	0.9	0.3
1910425	Paraquat	0.0045				
1912249	Atrazine	0.005	**	26.96	2	0.6
1918009	Dicamba	0.03		14.08	20	7
1918021	Picloram	0.07		11.56	70	20
1918167	Propachlor	0.013		16.72	9	3
1929777	Vernam	0.001		179.2	0.06	0.02

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CAS number	Chemical Name	RfD mg/kg/day	q1* /mg/kg/day	Estimated BCF 3% Lipid	RAC (mg/l) RL: 10E-6 0.0065 kg/day Consumption	RAC (mg/l) RL: 10E-6 0.020 kg/day Consumption
1929824	Nitrapyrin	0.0015		79.2	0.2	0.07
2008415	Butylate	0.1		292	4	1
2050477	p,p'-Dibromodiphenyl ether	**	**	11882		
2104645	EPN	0.00001		648	0.0002	0.00005
2164172	Fluometuron	0.013		10.08	10	5
2212671	Molinate	0.002		35.28	0.6	0.2
2303175	Triallate	0.013		388	0.4	0.1
2310170	Phosalone	0.0025				
2312358	Propargite	0.02				
2385855	Mirex	0.000002		752	0.00003	0.000009
2425061	Captafol	0.002				
2439103	Dodine	0.004				
2691410	Octahydro-1,3,5,7-tetranitro-1,3,5,	0.05				
2921882	Chlorpyrifos	0.003		800	0.04	0.01
3337711	Asulam	0.05		0.4	1000	500
3689245	Tetraethyldithiopyrophosphate	0.0005				
5234684	Carboxin	0.1		3.37	300	100
5902512	Terbacil	0.013		5.4	30	8
6108107	epsilon-Hexachlorocyclohexane	**	**	146.8		
6533739	Thallium carbonate	0.00008	**			
7287196	Prometryn	0.004		71.2	0.6	0.2
7439921	Lead and compounds	**	**			
7439965	Manganese	0.1	**			
7439976	Mercury, (inorganic)	**				
7440020	Nickel, soluble salts	0.02	**			
7440144	Radium 226 and 228	**	**			
7440144	Radium 228 (and 226)	**	2.6E-5/pCi/L			
7440224	Silver	0.003				
7440360	Antimony	0.0004				

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7440382	Arsenic, inorganic	**	**			
7440393	Barium	0.07				
7440417	Beryllium	0.005	4.3			
7440428	Boron (Boron and Borates only)	0.09				
7440439	Cadmium	**	**			
7440473	Chromium(VI)	0.005	**			
7440508	Copper	**	**			
7440611	Uranium, natural	**	**			
7446186	Thallium(I) sulfate	0.00008	**			
7723140	White phosphorus	0.00002	**			
7773060	Ammonium sulfamate	0.25				
7782414	Fluorine (soluble fluoride)	0.06				
7783008	Selenious acid	0.003				
7783064	Hydrogen sulfide	0.003				
7791120	Thallium chloride	0.00008	**			
7803512	Phosphine	0.0003				
8001352	Toxaphene	**	1.1	414	0.00002	0.000008
8001589	Creosote	**	**			
8007452	Coke oven emissions	**				
8065483	Demeton	0.00004				
10102439	Nitric oxide	0.1				
10102440	Nitrogen dioxide	1				
10102451	Thallium nitrate	0.00009	**			
10265926	Methamidophos	0.00005		0.4	1	0.4
10453868	Resmethrin	0.03		11900	0.03	0.009
10595956	N-Nitroso-N-methylethylamine	**	22	0.4	0.001	0.0004
12035722	Nickel subsulfide	**	**			
12039520	Thallium selenite	0.00009	**			
12122677	Zineb	0.05				

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12427382	Maneb	0.005				
13463393	Nickel carbonyl	**	**			
13593038	Quinalphos	0.0005		42.8	0.1	0.04
13684634	Phenmedipham	0.25		124	20	7
14797558	Nitrate	**				
14797650	Nitrite	0.1				
14859677	Radon 222	**	1.8E-6/pCi/L			
15299997	Napropamide	0.1		156	7	2
15972608	Alachlor	0.01		226	0.5	0.2
16065831	Chromium(III)	1				
16672870	Ethephon	0.005				
16752775	Methomyl	0.025				
17804352	Benomyl	0.05				
19044883	Oryzalin	**		31.1		
19408743	Hexachlorodibenzo-p-dioxin mixture	**	6200			
19666309	Oxadiazon	0.005				
20859738	Aluminum Phosphide	0.0004				
21087649	Metribuzin	0.025				
21725462	Cyanazine	0.002		3.64	6	2
22224926	Fenamiphos	0.00025		21.5	0.1	0.04
22967926	Methyl mercury	0.0003				
23135220	Oxamyl	0.025				
23564058	Thiophanate-methyl	0.08		2.92	300	100
23950585	Pronamide	0.075		33.92	20	8
24307264	Mepiquat chloride	0.03				
25057890	Bentazon	0.0025				
25329355	Pentachlorocyclopentadiene	**	**			
26628228	Sodium azide	0.004				
27314132	Norflurazon	0.04				

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28249776	Thiobencarb	0.01		71.6	2	0.5
29232937	Pirimiphos-methyl	0.01		66.7	2	0.5
30560191	Acephate	**	0.0087	0.4	3	1
32534819	Pentabromodiphenyl ether	0.002	**			
32536520	Octabromodiphenyl ether	0.003	**			
33089611	Amitraz	0.0025				
33820530	Isopropalin	0.015		5520	0.03	0.01
34014181	Tebuthiuron	0.07		0.4	2000	600
35367385	Diflubenzuron	0.02		44.8	5	2
35554440	Imazalil	0.013		19.84	7	2
36483600	Hexabromodiphenyl ether	**	**			
36734197	Iprodione (Rovral)	0.04				
39148248	Fosetyl-al	3	**			
39515418	Danitol	0.0005		5170	0.001	0.0003
39638329	Bis(2-chloroisopropyl) ether	0.04		21.9	20	6
40088479	Tetrabromodiphenyl ether	**	**			
40487421	Pendimethalin (Prowl)	0.04		3416	0.1	0.04
41851507	Chlorocyclopentadiene	**	**			
42874033	Oxyfluorfen	0.003		11040	0.003	0.001
43121433	Bayleton	0.03		39.28	8	3
43222486	Difenzoquat	0.08				
49690940	Tribromodiphenyl ether	**	**			
50471448	Vinclozolin	0.025				
51218452	Metolachlor	0.1	**	252.4	4	1
51235042	Hexazinone	0.033		348.4	1	0.3
51630581	Pydrin	0.025		35200	0.008	0.002
52315078	Cypermethrin	0.01		10320	0.01	0.003
52645531	Permethrin	0.05		40000	0.01	0.004
55285148	Carbosulfan	0.01				

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55290647	Dimethipin	0.02	**			
57837191	Metalaxyl	0.06		24.1	30	9
58138082	Tridiphane	0.003				
59756604	Fluridone	0.08		1856	0.5	0.2
60207901	Propiconazole	0.013				
60568050	Furmecyclox	**	0.030			
62476599	Sodium acifluorfen	0.013				
63936561	Nonabromodiphenyl ether	**	**			
64902723	Chlorsulfuron	0.05		19.0	30	9
65195553	Avermectin B1	0.004				
66215278	Cyromazine	0.0075		0.54	200	50
66332965	Flutolanil	0.06				
66841256	Tralomethrin	0.0075		40000	0.002	0.0007
67485294	Amdro	0.0003				
67747095	Prochloraz	0.009	0.15			
68085858	Cyhalothrin/Karate	0.005		10700	0.005	0.002
68359375	Baythroid	0.025		40000	0.007	0.002
69409945	Fluvalinate	0.01		40000	0.003	0.0009
69806402	Haloxyfop-methyl	0.00005				
72128020	Fomesafen	**	0.19			
72128020	Fomesafen	**	0.19			
74051802	Sethoxydim	0.09				
74115245	Apollo	0.013				
74223646	Ally	0.25				
76578148	Assure	0.009				
76738620	Paclobutrazol	0.013				
77182822	Glufosinate-ammonium	0.0004				
77323843	Trichlorocyclopentadiene	**	**			
77323854	Tetrachlorocyclopentadiene	**	**			

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CAS number	Chemical Name	RfD mg/kg/day	q1* /mg/kg/day	Estimated BCF 3% Lipid	RAC (mg/l) RL: 10E-6 0.0065 kg/day Consumption	RAC (mg/l) RL: 10E-6 0.020 kg/day Consumption
77501634	Lactofen	0.002				
78587050	Savey	0.025				
79277273	Harmony	0.013				
81335377	Imazaquin	0.25				
81335775	Pursuit	0.25	**			
82558507	Isoxaben	0.05				
82657043	Biphenthrin	0.015		40000	0.004	0.001
83055996	Londax	0.2				
85509199	NuStar	0.0007				
88671890	Systhane	0.025				
90982324	Chlorimuron-ethyl	0.02				
101200480	Express	0.008				

Appendix E

IRIS Values:

1) Reference Dose Description and Use in Health Risk Assessments

and

2) EPA Approach for Assessing the Risk Associated with Exposure to Environmental Carcinogens

REFERENCE DOSE (RfD): DESCRIPTION AND USE IN HEALTH RISK ASSESSMENTS

PRINCIPAL AUTHOR:

Donald Barnes, Ph. D. (OPTS)

RfD WORK GROUP:

Donald Barnes, Ph.D. (OPTS)
Judith Bellin, Ph.D. (OSWER)
Christopher IDeRosa, Ph.D. (ORD)
Michael Dourson, Ph.D. (ORD)*
Reto Engler, Ph.D. (OPTS)
Linda Erdreich, Ph.D (ORD)
Theodore Farber, Ph.D. (OPTS)
Penny Fenner-Crisp, Ph.D. (O0W)
Elaine Francis, Ph.D. (OPTS)
George Ghali, Ph.D. (OPTS)
Richard Hill, M.D., Ph.D. (OPTS)
Stephanie Irene, Ph.D (OPTS)
William Marcus, Ph.D. (OW)
David Patrick, P.E., B.S. (OAR)
Susan Perlin, Ph.D. (OPPE)
Peter Preuss, Ph. D. (ORD)*
Aggie Revesz, 8.5. (OPTS)
Reva Rubenstein, Ph.D. (O5WER)
Jerry Stara, D.V.M., Ph. D (ORD)
Jeanette Wiltse, Ph.D (OPTS)
Larry Zaragosa, Ph.D. (OAR)

*Co-Chair

I. INTRODUCTION

This concept paper describes the U.S. Environmental Protection Agency's principal approach to and rationale for assessing risks for health effects other than cancer and gene mutations from chronic chemical Exposure. By outlining principles and concepts that guide EPA risk assessment for such systemic"' effects, the report complements the new risk assessment guidelines, which describe the Agency's approach to risk assessment in other areas (carcinogenicity, mutagenicity, developmental toxicity, exposure, and chemical mixtures). See the IRIS glossary for a description and citation of each guideline.

A. Background

Chemicals that give rise to toxic end points other than cancer and gene mutations are often referred to as "systemic toxicants" because of their effects on the function of various organ systems. It should be noted, however, that chemicals which cause cancer and gene mutations also commonly evoke other toxic effects (systemic toxicity). Generally, based on our understanding of homeostatic and adaptive mechanisms, systemic toxicity is treated as if there is an identifiable exposure threshold (both for the individual and for the population) below which effects are not observable. This characteristic distinguishes systemic end points from carcinogenic and mutagenic end points, which are often treated as nonthreshold processes.

Systemic effects have traditionally been evaluated in terms of concepts such as "acceptable daily intake" and "margin of safety." The scientific community has identified certain limits on some of these approaches, and these limits have been borne out in EPA's experience. Nonetheless, EPA is called upon to apply these concepts in making and explaining decisions about the significance for human health of certain chemicals in the environment.

To meet these needs, the RfD Work Group has drawn on traditional concepts, as well as on recommendations in the I 983 National Academy of Sciences (NAS) report on risk assessment, to more fully articulate the use of noncancer, nonmutagenic experimental data in reaching decisions on the significance of exposures to chemicals. In the process, the Agency has coined new terminology to clarify and distinguish between aspects of risk assessment and risk management. EPA has tested and implemented these innovations in developing consistent information for several recent regulatory needs, for instance under RCRA.

B. Overview

This Appendix consists of four parts in addition to this introduction. In Section II, much of the traditional information on assessing risks of systemic toxicity is presented, with the focus on the concepts of "acceptable daily intake (ADI)" and "safety factor (SF)." Issues associated with these approaches are identified and discussed. In Section III, the Agency's approach to assessing the risks of systemic toxicity is presentecl in the context of the NAS scheme of risk assessment and risk management in regulatory decision-making. This approach includes recasting earlier ADI and SF concepts into the less value-laden terms "referencedose (RfD)" and "uncertainty factor (UF)." A new term, "margin of exposure," as utilized in the EPA regulatory context, is introduced to avoid some 'of the issues associated with the traditional approach.

Section IV examines how these new concepts can be applied in reaching risk management decisions, while Section V briefly mentions some of the additional approaches the Agency is using and exploring to address this issue. Section VI provides a sample RfD calculation.

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* In this document the term systemic refers to an effect other than carcinogenicity or mutagenicity induced by toxic chemical

** In this Appendix, the ratio of the NOAEL to the estimated exposure (often referred to as "margin of safety") is referred to as the "margin of exposure (MOE)" in order to avoid confusion with the original use of the term "margin of safety" in pharmacology (i.e., the ratio of the toxic dose to the theraputic dose) and to avoid the use of the value-laden term "safety '

II. TRADITIONAL APPROACH TO ASSESSING SYSTEMIC (NONCARCINOGENIC) TOXICITY

The Agency's approach to assessing the risks associated with systemic toxicity is different from that for the risks associated with carcinogenicity. This is because different mechanisms of action are thought to be involved in the two cases. In the case of carcinogens, the Agency assumes that a small number of molecular eiiients can evoke changes in a single cell that can lead to uncontrolled cellular proliferation. This mechanism for carcinogenesis is referred to as "nonthreshold," since there is essentially no level of exposure for such a chemical that does not pose a small, but finite, probability of generating a carcinogenic response. In the case of systemic toxicity, organic homeostatic, compensating, and adaptive mechanisms exist that must be overcome before the toxic end point is manifested. For example, there could be a large number of cells performing the same or similar function whose population must be significantly depleted before the effect is seen.

The threshold concept is important in the regulatory context. The individual threshold hypothesis holds that a range of exposures from zero to some finite value can be tolerated by the organism with essentially no chance of expression of the toxic effect. Further, it is often prudent to focus on the - most sensitive members of the population; therefore, regulatory efforts are generally made to keep exposures below the population threshold, which is defined as the lowest of the thresholds of the individuals within a population.

A. The Traditional Approach

In many cases, risk decisions on systemic toxicity have been made by the Agency using the concept of the "acceptable daily intake (ADI)." This quantity is derived by dividing the appropriate "no-observed-adverse-effect level (NOAEL)" by a "safety factor (SF)" as follows:*

$$\text{ADI (human dose)} = \text{NOAEL (experimental dose)} \div \text{SF} \qquad (1)$$

The ADI is often viewed as the amount of a chemical to which one can be exposed on a daily basis over an extended period of time (usually a lifetime) without suffering a deleterious effect. Often, the ADI has been used as a tool in reaching risk management decisions; e.g., establishing allowable levels of contaminants in foodstuffs and water.

Once the critical study demonstrating the toxic effect of concern has been identified, the selection of the NOAEL derives from an essentially objective, scientific examination of the data available on the chemical in question.

Generally, the SF consists of multiples of 10, each factor representing a specific area of uncertainty inherent in the available data. For example, an SF may be developed by taking into account the expected differences in responsiveness between humans and animals in prolonged exposure studies; i.e., a 10- fold factor. In addition, a second factor of 10 may be introduced to account for variability among individuals within the human population. For many chemicals, the resultant SF of 100 has been judged to be appropriate. For other chemicals, with a less complete data base (e.g., those for which only the results of chronic studies are available), an additional factor of 10 (leading to an SF of 1,000) might be judged to be more appropriate. On the other hand, for some chemicals, based on well-characterized responses in sensitive humans (e.g., effect of fluoride on human teeth), an SF as small as 1 might be selected.

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* A NOAFL is an experimentally determined dose at which there was no statistically or biologically significant indication of the toxic effect of concern. In an experiment with several NOAELs, the regulatory focus is normally on the highest one, leading to the common usage of the term NOAEL as the highest experimentally determined dose without statistical or adverse biological effect. In some treatments, the NOAEL for the critical toxic effect is simply referred to as the NOEL. This latter term, however, invites ambiguity in that there may be observable effects which are of toxic- significance, i e they are not "adverse." In order to be explicit, this Appendix uses the term NOAEL and it refers to the highest NOAEL in an experiment. Further, in cases in which a NOAEL has not been demonstrated experimentally, the formulation calls for use of the "lowest-observed-adverse-effectlevel (LOAEL)". In order to focus on the major concepts, however, we will use NOAEL as a general example.

While the original selection of SFs appears to have been rather arbitrary (Lehman and Fitzhugh, 1954)*, subsequent analysis of data as reviewed by Dourson and Stara (1983) lends theoretical (and in some instances experimental) support for their selection. Further, some scientists, but not all, within the EPA interpret the absence of widespread effects in the exposed human populations as evidence of the adequacy of the SFs traditionally employed.

B. Some Difficulties in Utilizing the Traditional Approach

1. Scientific Issues

While the traditional approach has performed well over the years and the Agency has sought to be consistent in its application, observers have identified scientific shortcomings of the approach. Examples include the following:

- By focusing on the NOAEL, information on the shape of the dose-response curve is ignored. Such data could be important in estimating levels of concern for public safety.

- As scientific knowledge is increased and the correlation of precursor effects (e.g., enzyme induction) with frank toxicity becomes known, questions about the selection of the appropriate "adverse effect" arise.

- Guidelines have not been developed to take into account the fact that some studies have used larger numbers of animals and, hence, are generally more reliable than other studies.

These and other "generic issues" are not susceptible to immediate resolution, because the data base needed is not yet sufficiently developed or analyzed. Therefore, these issues are beyond the scope of this Appendix. However, the Agency has established a work group to consider them.

2. Management-related Issues

a. The use of the term "safety factor"

The term "safety factor" suggests, perhaps inadvertently, the notion of absolute safety, i.e., absence of risk. While there is a conceptual basis for believing in the existence of a threshold and "absolute safety" associated with certain chemicals, in the majority of cases a firm experimental basis for this notion does not exist.

b. The implication that any exposure in excess of the ADI is "unacceptable" and that any exposure less than the ADI is "acceptable" or "safe"

In practice, the ADI is viewed by many as an "acceptable" level of exposure, and, by inference, any exposure greater than the ADI is seen as "unacceptable." This strict demarcation between what is "acceptable" and what is "unacceptable" is contrary to the views of most toxicologists, who typically interpret the ADI as a relatively crude estimate of a level of chronic exposure not likely to result in adverse effects to humans. The ADI is generally viewed as a "soft" estimate, whose bounds of uncertainty can span an order of magnitude. That is, within reasonable limits, while exposures somewhat higher than the ADI are associated with increased probability of adverse effects, that probability is not a certainty. Similarly, while the ADI is seen as a level at which the probability of adverse effects is low, the absence of risk to all people cannot be assured at this level.

c. Possible limitations imposed on risk management decisions

Awareness of the "softness" of the ADI estimate (see b. above) argues for careful case-by-case consideration of the implications of the toxicological analysis as it applies to any particular situation. To the degree that ADIs generated by the traditional approach are the determining factors in risk

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* Lehman, A. J. and Fitzhugh, O.G. (1954) Association of Food Drug Officials. USQ Bulletin 18:33-35.

management decisions, they can take a significance beyond that intended by the toxicologist or merited by the underlying scientific support.

Further, in administering risk/benefit or cost/benefit statutes, the risk manager is required to consider factors other than risk (e.g., estimated exposures compared to the ADI) in reaching a decision. The AIDI is only one factor in a management decision and should not prevent the risk manager from weighing the full range of factors.

d. Development of different ADIs by different programs

In addition to occasionally selecting different critical toxic effects, Agency scientists have reflected their best scientific judgments in the final ADI by adopting factors different from the standard factors listed in Table A-I. *For example, if the toxic end point for a chemical in experimental animals is the same as that which has* been established for a related chemical in humans at similar doses, one could argue for an SF of less than the traditional 100. On the other hand, if the total toxicologic data base is incomplete, one could argue that an additional SF should be included, both as a matter of prudent public policy and as an incentive to others to generate the appropriate data.

Such practices, as employed by a number of scientists in different programs, exercising their best scientific judgment, have in many cases resulted in different ADIs for the same chemical. The fact that different ADs were generated (e.g., by adopting different SFs) can be a source of considerable confusion when the ADIs are applied in risk management decision making (see c. above). For example, although they generally agree on the experimental data base for 2,3,7,8-TCDD, regulatory agencies within the United States and around the world have generated different ADIs by selecting different "safety factors"; specifically, 1000, 500, 250, and 100. These different ADIs have been used to justify different regulatory decisions. The existence of different ADIs need not imply that any of them is more "wrong"—or "right"—than the rest. It is more nearly a reflection of the honest difference in scientific judgment.

These differences, which may reflect differences in the interpretation of the scientific data, can also be characterized as differences in the management of the risk. As a result, scientists may be inappropriately impugned, and/or perfectly justifiable risk management decisions may be tainted by charges of "tampering with the science." This unfortunate state of affairs arises, at least in part, from treating the ADI as an absolute measure of safety.

III. EPA ASSESSMENT OF RISKS ASSOCIATED WITH SYSTEMIC TOXICITY

In 1983, the National Academy of Sciences published a report* which discusses the conceptual framework within which regulatory decisions on toxic chemicals are made; see Figure C-1. The determination of the presence of risk and its potential magnitude is made during the risk assessment process, which consists of hazard identification, dose-response assessment, exposure assessment, and risk characterization. Having been apprised by the risk assessor that a potential risk exists, the risk manager answers the question: *"What, if anything, are we going to do about it?"*

A. Hazard Identification

1. Evidence

a. Type of effect

Exposure to a given chemical, depending on the dose employed, may result in a variety of toxic effects. These may range from gross effects, such as death, to more subtle biochemical, physiologic, or pathologic changes. The risk assessor considers each of the toxic end points from all studies evaluated in assessing the risk posed by a chemical, although primary attention usually is given to the effect exhibiting the lowest NOAEL, often referred to as the critical effect. For chemicals with a limited data base, there may be a need for more toxicity testing.

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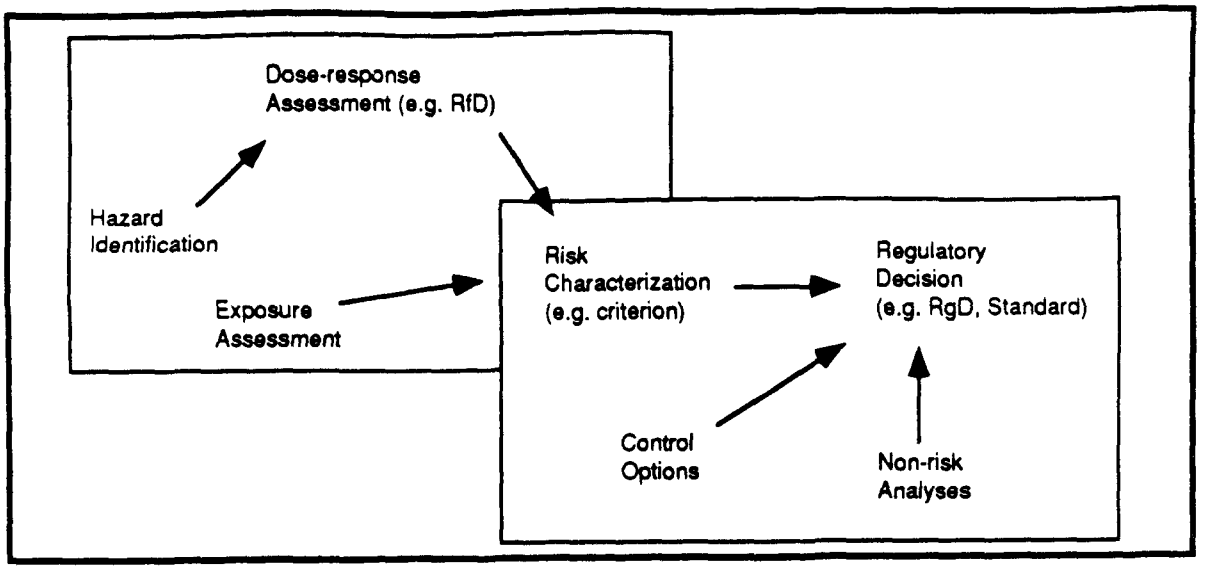
* NAS Risk Assessment in the Federal Goverment: Managing the Process (NAS Press, 1983).

FIGURE C-1



b. Principal studies

Principal studies are those that contribute most significantly to the qualitative assessment of whether or not a particular chemical is potentially a systemic toxicant in humans. In addition, they may be used in the quantitative dose-response assessment phase of the risk assessment. These studies are of two types:

(1) Human studies

Human data are often useful in qualitatively establishing the presence of an adverse effect in exposed human populations. Further, when there is information on the exposure level associated with an appropriate end point, epidemiologic studies can also provide the basis for a quantitative dose-response assessment. Use of these latter data avoids the necessity of extrapolating from animals to humans, and therefore, human studies, when available, are given first priority, with animal toxicity studies serving to complement them.

In epidemiologic studies, confounding factors that are recognized can be controlled and measured, within limits. Case reports and acute exposures resulting in severe effects provide support for the choice of critical toxic effect, but they are often of limited utility in establishing a quantitative relationship between environmental exposures and anticipated effects. Available human studies on ingestion are usually of this nature. Cohort studies and clinical studies may contain exposure-response information that can be used in estimating effect levels, but the method of establishing exposure must be evaluated for validity and applicability.

(2) Animal studies

Usually, the data base on a given chemical lacks appropriate information on effects in humans. In such cases, the principal studies are drawn from experiments conducted on non-human mammals, most often the rat, mouse, rabbit, guinea pig, hamster, dog, or monkey.

c. Supporting studies

Supporting studies include information from a wide variety of sources. For example, metabolic and other pharmacokinetic studies can provide insights into the mechanism of action of a particular compound. By comparing the metabolism of the compound exhibiting the toxic effect in the animal with the metabolism found in humans, some light may be cast on the potential for the toxic manifestation in humans or for estimating the equitoxic dose in humans.

Similarly, in vitro studies can provide insights into the compound's potential for biological activity, although

a definite connection to the human experience cannot be drawn. Under certain circumstances, consideration of structure-activity relationships between the chemical under test and the effects of structurally related agents can provide a clue to the biological activity of the former.

At the present time, these data are supportive, not definitive, in assessing risk. However, there i focused activity aimed at developing more reliable in vitro tests to minimize the need for live-animal testing. Similarly, there is increased emphasis on generating mechanism-of-action and pharmacokinetic information as a means of increasing the fundamental understanding of toxic processes in humans and nonhumans. It is expected that in the future these considerations will play a larger role in our determination of toxicity of chemicals.

d. Route of exposure

The Agency often approaches the investigation of a chemical with a particular route of exposure in mind; e.g., an oral exposure for a driving water contaminant or a residue in food. Although the route of exposure is oral in both cases, specific considerations may differ. for example, the bioavailability of the chemical administered in food may differ from that when administered in water or inhaled. Usually, the toxicologic data base on the compound does not include detailed testing on all possible routes of administration.

In general, it is the Agency's view that the poiential for toxicity manifested by one route of exposure is relevant to any other route of exposure, unless convincing evidence exists to the contrary Consideration is always given to potential differences in absorption or metabolism resulting from different routes of exposure, and whenever appropriate data (e.g., comparative metabolism studies) are available, the quantitative impacts of these differences on the risk assessment are fully delineated.

e. Length of exposure

The Agency is concerned about the potential toxic effects in humans associated with all possible exposures to chemicals. The magnitude, frequency, and duration of exposure may vary considerably in different situations. Animal studies are conducted using a variety of exposure durations (e.g., acute, subchronic, and chronic) and schedules (e.g., single, intermittent, or continuous dosing). Information from all of these studies is useful in the hazard identification phase of risk assessment. For example, overt neurological problems identified in high-dose acute studies tend to reinforce the observation of subtle neurological changes seen in a low-dose chronic study. Special concern exists for low-dose, chronic exposures, however, since such exposures can elicit effects absent in higher-dose, shorter exposures, through mechanisms such as accumulation of toxicants in the organisms.

f. Quality of the study

Evaluation of individual studies in humans and animals requires the consideration of several factors associated with a study's hypothesis, design, execution, and interpretation. An ideal study addresses a clearly delineated hypothesis, follows a carefully prescribed protocol, and includes sufficient subsequent analysis to support its conclusions convincingly.

In evaluating the results from such studies, consideration is given to many other factors, including chemical characterization of the compound(s) under study, the type of test species, similarities and differences between the test species and humans (e.g., chemical absorption and metabolism), the number of individuals in the study groups, the number of study groups, the spacing and choice of dose levels tested, the types of observations and methods of analysis, the nature of pathologic changes, the alteration in metabolic responses, the sex and age of test animals, and the route and duration of exposure.

2. Weight-of-Evidence Determination

As the culmination of the hazard identification step, a discussion of the weight-of-evidence summarizes the highlights of the information gleaned from the entire range of principal and supporting studies. Emphasis in the analysis is given to examining the results from different studies to determine the extent to which a

consistent, plausible picture of toxicity emerges. For example, the following factors add to the weight of the evidence that the chemical poses a hazard to humans: similar results in replicated animal studies by different investigators; similar effects across sex, strain, species, and route of exposure; clear evidence of a dose-response relationship; a plausible relation between data on metabolism, postulated mechanism-of-action, and the effect of concern; similar toxicity exhibited by structurally related compounds; and some link between the chemical and evidence of the effect of concern in humans. The greater the weight-of-evidence, the greater one's confidence in the conclusions drawn.

B. Dose-Response Assessment

1. Concepts and Problems

Empirical observation generally reveals that as the dosage of a toxicant is increased, the toxic response (in terms of severity and/or incidence of effect) also increases. This dose-response relationship is well-founded in the theory and practice of toxicology and pharmacology. Such behavior is observed in the following instances: in quantal responses in which the proportion of responding individuals in a population increases with dose; in graded responses, in which the severity of the toxic response within an individual increases with dose; and in continuous responses, in which changes in a biological parameter (e.g., body or organ weight) vary with dose.

However, in evaluating a dose-response relationship, certain difficulties arise. For example, one must decide on the critical end point to measure as the "response." One must also decide on the correct measure of "dose." In addition to the interspecies extrapolation aspects of the question of the appropriate units for dose, the more fundamental question of administered dose versus absorbed dose versus target organ dose should be considered. These questions are the subject of much current research.

2. Selection of the Critical Data

a. Critical study

Often animal data are selected as the governing information for quantitative risk assessments, since available human data are generally insufficient for this purpose. These animal studies typically reflect situations in which exposure to the toxicant has been carefully controlled and the problems of heterogeneity of the exposed population and concurrent exposures to other toxicants have been minimized. In evaluating animal data, a series of professional judgments are made that involve, among others, consideration of the scientific quality of the studies. Presented with data from several animal studies, the risk assessor first seeks to identify the animal model that is most relevant to humans, based on the most defensible biological rationale, for instance using comparative pharmacokinetic data. In the absence of a clearly most relevant species, however, the most sensitive species (i.e., the species showing a toxic effect at the lowest admininistered dose) is adopted as a matter of scientific policy at EPA, since no assurance exists that humans are not innately more sensitive than any species tested. This selection process is made more difficult if animal tests have been conducted using different routes of exposure, particularly if the routes are different from those involved in the human situation under investigation.

In any event, the use of data from carefully controlled studies of genetically homogeneous animals inescapably confronts the risk assessor with the problems of extrapolaing between species and the need to account for human heterogeneity and concurrent human exposures to other chemicals, which may modify the human risk.

While there is usually a lack of well-controlled cohort studies that investigate non-cancer end points and human exposure to chemicals of interest, in some cases human data may be selected as the Tcritical data (e.g., in cases of cholinesterase inhibition). Risk assessments based on human data have the advantage of avoiding the problems inherent in interspecies extrapolation. In many instances, use of such studies, as is the case with the animal investigations, involves extrapolation from relatively high doses (such as those found in occupational settings) to the low doses found in the environmental situations to which the general population is more likely to be exposed. In some cases, a well-designed and well-conducted epidemiologic

study that shows no association between known exposures and toxicity can be used to directly project an RfD (as has been done in the case of fluoride).

b. Critical data

In the simplest terms, an experimental exposure level is selected from the critical study that represents the highest level tested in which "no adverse effect" was demonstrated. This "no-observed-adverse-effect level" (NOAEL) is the key datum gleaned from the study of the dose-response relationship and, traditionally, is the primary basis for the scientific evaluation of the risk posed to humans by systemic toxicants. This approach is based on the assumption that if the critical toxic effect is prevented, then all toxic effects are prevented.

More formally, the NOAEL is defined in this discussion as the highest experimental dose of a chemical at which there is no statistically or biologically significant increase in frequency or severity of an adverse effect between individuals in an exposed group and those in its appropriate control. (See also discussion in the footnote on page A-4). As noted above, there may be sound professional differences of opinion in judging whether or not a particular response is adverse. In addition, the NOAEL is a function of the size of the population under study. Studies with a small number of subjects are less likely to detect low-dose effects than studies using larger numbers of subjects. Also, if the interval between doses in an experiment is large, it is possible that the experimentally determined NOAEL is lower than that which would be observed in a study using intervening doses.

c. Critical end point

A chemical may elicit more than one toxic effect (end point), even in one test animal, or in tests of the same or different duration (acute, subchronic, and chronic exposure studies). In general, NOAELs for these effects will differ. The critical end point used in the dose-response assessment is the one at the lowest NOAEL.

3. Reference Dose (RfD)

In response to many of the problems associated with AIDIs and SFs, which were outlined in Section II, the concept of the "reference dose (RfID)" and "uncertainty factor (UF)" is recommended. The RfID is a benchmark dose operationally derived from the NOAEL by consistent application of generally order of magnitude uncertainty factors (UFs) that reflect various types of data used to estimate RfDs (for example, a valid chronic human NOAEL normally is divided by an UF of 10) and an additional modifying factor (MF), which is based on a professional judgment of the entire data base of the chemical.* See Table A-I.

The RfD is determined by use of the following equation:

$$RfD = NOAEL/(UF \times MF) \qquad (2)$$

which is the functional equivalent of Eq. (1). In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfD is appropriately expressed in units of mg/kg-bw/day.

The RfD is useful a reference point for gauging the potential effects of other doses. Usually, doses that are less than the RfID are not likely to be associated with any health risks, and are therefore less likely to be of regulatory concern. However, as the frequency of exposures exceeding the RfO increases, and as the size of the excess increases, the probability increases that adverse effects may be observed in a human population. Nonetheless, a clear conclusion cannot categorically drawn that all doses below the RfD are "acceptable" and that all doses in excess of the RfD are "unacceptable."

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* "Uncertainty factor" is the new description applied to the term "safety factor" see Page A-4). This new name is more descriptive in that these factors represent scientific uncertainties, and avoids the risk management connotation of "safety" The "modifying factor" can range from greater than zero to 10, and reflects qualitative professional judgements regarding scientific uncertainties not covered under the standard UF, such as the completeness of the overall data base and the number of animals in the study.

TABLE A-I.
GUIDELINES FOR THE USE OF UNCERTAINTY FACTORS IN DERIVING REFERENCE DOSE (RfD)

Standard Uncertainty Factors (UFs)

Use a 10-fold factor when extrapolating from valid experimental results from studies using prolonged exposure to average healthy humans. This factor is intended to account for the variation in sensitivity among the members of the human population. [1 OH]

Use an additional 10-fold factor when extrapolating from valid results of long-term studies on experimental animals when results of studies of human exposure are not available or are inadequate. This factor is intended to account for the uncertainty in extrapolating animal data to the case of humans. [10A]

Use an additional 10-fold factor when extrapolating from less than chronic results on experimental animals when there are no useful long-term human data. This factor is intended to account for the uncertainty in extrapolating from less than chronic NOAELs to chronic NOAELs. [10S]

Use an additional 10-fold factor when deriving a RfD from a LOAEL, instead of a NOAEL. This factor is intended to account for the uncertainty in extrapolating from LOAELs to NOAELs. [10L]

Modifying Factor (MF)

Use professional judgment to determine another uncertainty factor (MF) which is greater than zero and less than or equal to 10. The magnitude of the MF depends upon the professional assessment of scientific uncertainties of the study and database not explicitly treated above; e.g., the completeness of the overall data base and the number of species tested. The default value for the MF is 1.

SOURCE: Adapted from Dourson, M.L.; and Stara, J.F. (1983)
Regulatary Toxicology and Pharmacology 3:224-238.

(This is a consequence of the inability of either the traditional or the RfD approach to completely address the question of dose-response extrapolation.)

The Agency is attempting to standardize its approach to determining RfDs. The RfD Work Group has developed a systematic approach to summarizing its evaluations, conclusions, and reservations regarding RfDs in a "cover sheet" of a few pages in length. The cover sheet includes a statement on the confidence the evaluators have in the stability of the RfD: high, medium, or low. High confidence indicates that the RfD is unlikely to change in the future because there is consistency among the toxic responses observed in different sexes, species, study designs, or in dose-response relationships, or the reasons for differences, if any, are well understood. Often, high confidence is given to RfDs that are based on human data for the exposure route of concern, because in such cases the problems of interspecies extrapolation are avoided. Low confidence indicates that the RfD may be especially vulnerable to change if additional chronic toxicity data are published on the chemical, because the data supporting the estimation of the RfD are of limited quality and/or quantity.

C. Exposure Assesment

The third step in the risk assessment process focuses on exposure issues. For a full discussion of exposure assessment, the reader is referred to EPA's recently published guidelines on the subject ($1 Federal Register 34042-340S4, Sept. 24, 1986). There is no substantive difference in the conceptual approach to exposure assessment in the case of systemic toxicants and of carcinogens.

In brief, the exposure assessment includes consideration of the populations exposed and the magnitude, frequency, duration and routes of exposure, as well as evaluation of the nature of the exposed populations.

D. Risk Characterization

Risk characterization is the final step in the risk assessment process and the first step in the risk management process. Its purpose is to present to the risk manager a synopsis and synthesis of all the data that contribute to a conclusion on the risk, including:

- The qualitative ("weight-of-evidence") conclusions about the likelihood that the chemical may pose a hazard to human health.
- A discussion of dose-response and how this information, through the use of particular uncertainty and modifying factors, was used to determine the RfD.
- Data such as the shapes and slopes of the dose-response curves for the various toxic end points toxicodynamics (absorption and metabolism), structure-activity correlations, and the nature and severity of the observed effect. These data should be clearly discussed by the risk assessor, since they may influence the final decision of the risk manager (see below).
- The estimates of exposure, the nature of the exposure, and the number and types of people exposed, together with a discussion of the uncertainties involved.
- A discussion of the sources of uncertainty, major assumptions, areas of scientific judgment, and, to the extent possible, estimates of the uncertainties embodied in the assessment.

In the risk characterization process, comparison is made between the RfD and the estimated (calculated or measured) exposure dose (EED), which should consider exposure by all sources and routes of exposure. The risk assessment should contain a discussion of the assumptions underlying the estimation of the RfD (nature of the critical end point, nature of other toxic end points, degree of confidence in the data base, etc.), and the degree of conservatism in its derivation. The assumptions used to derive the EED should also be discussed. If the EED is less than the RfD, the need for regulatory concern is likely to be small.

An alternative measure that may be useful to some risk managers is the "margin of exposure (MOE)" (see footnote on p. A-3), which is the magnitude by which the NOAEL of the critical toxic effect exceeds the estimated exposure dose (EED), where both are expressed in the same units:

$$\text{MOE} = \text{NOAEL (experimental dose)}/\text{EED (human dose)} \qquad (3)$$

In parallel to the statements above on EED and RfD, the risk assessment should contain a discussion of the assumptions underlying the estimates of the RfD and the degree of possible conservatism of the UF and MF. It can be noted that when the MOE is equal to or greater than UF x MF, the need for regulatory concern is likely to be small.

Section VI contains an example of the use of the concepts of NOAEL, UF, MF, RfD, and MOE.

IV. APPLICATION IN RISK MANAGEMENT

Once the risk characterization is completed, the focus turns to risk management. In reaching decisions, the risk manager must consider a number of risk factors, nonrisk factors, and regulatory options that influence the final judgment. It is generally useful to the risk manager to have information regarding the contribution to the RfD from various environmental media. Such information can provide insights that are helpful in choosing among available control options. However, in cases in which site-specific criteria are being considered, local exposures through various media can often be determined more accurately than exposure estimates based upon generic approaches. In such cases, the exposure assessor's role is particularly important. For instance, at a given site, consumption of fish may clearly dominate the local exposure routes, while, on a national basis, fish consumption may play a minor role compared to ingestion of treated crops.

RfDs should be apportioned by route of exposure. Where specific exposure analysis can be made, such apportionment is readily performed. If exposure information is not available, assumptions must be made concerning the relative contributions from different routes of exposure. At present, different EPA offices use assumptions that differ to some degree. These assumptions are being reviewed by an Agency risk assessment group.

As illustrated in Figure A-1, the risk manager utilizes the results of risk characterization, other technological factors, and nontechnical social and economic considerations in reaching a regulatory decision. Some of these factors include efficiency,
timeliness, equity, administrative simplicity, consistency, public acceptability, technological feasibility, and legislative mandate.

Because of the way these risk management factors may impact different cases, consistent--but not necessarily identical--risk management decisions must be made on a case-by-case basis. For example, the Clean Water Act calls for decisions with "an ample margin of safety"; the Federal Insecticide, Fungicide and Rodenticide Act (FIF~) calls for "an ample margin of safety," taking benefits into account; and the Safe Drinking Water Act (SDWA) calls for standards that protect the public 'to the extent feasible."Consequently, it is entirely possible and appropriate that a chemical with a specific RfD may be regulated under different statutes and situations through the use of different "regulatory doses (RgDs)".

Expressed in general terms, after carefully considering the various risk and nonrisk factors, regulatory options, and statutory mandates in a given case (i), the risk manager decides upon the appropriate statutory alternatives to arrive at an "ample" or "adequate" margin of exposure [MOE(i)], thereby establishing the regulatory dose, RgD(i) (e.g., a tolerance under FIFRA or a maximum contaminant level under SDWA), applicable to that case:

$$RgD(i) = NOAEL/MOE(i) \qquad (4)$$

Note that, for the same chemical (with a single RfD), the risk manager(s) can develop different regulatory doses for different situations that may involve different exposures, available control options, alternative chemicals, benefits, and statutory mandates. Also note that comparing the RfD to a particular RgD(i) is equivalent to comparing the MOE(i) with the UF x MF:

$$RfD/RgD(i) = MOE(i)/UF \times MF \qquad (5)$$

In assessing the significance of a case in which the RgD is greater (or less) than the RfD, the risk manager should carefully consider the case-specific data laid out by the risk assessors, as discussed in in Section III. D. 4. In some cases this may require additional explanation and insight from the risk assessor. In any event, the risk manager has the responsibility to clearly articulate the reasoning leading to the final RgD decision.

V. OTHER DIRECTIONS

While the Agency is in the process of systematizing the approach outlined in this Appendix, risk assessment research for systemic toxicity is also being conducted along entirely separate lines. For example, the Office of Air Quality Planning and Standards is using probabilistic risk assessment procedures for criteria pollutants. This procedure characterizes the population at risk, and the likelihood of various effects occurring, through the use of available scientific literature and elicitation of expert judgment concerning dose-response relationships. The dose-response information is combined with exposure analysis modeling to generate population risk estimates for alternative standards. These procedures present the decisionmaker with ranges of risk estimates, and explicitly consider the uncertainties associated with both the toxicity atid exposure information. The Office of Policy, Planning, and Evaluation is investigating similar procedures in order to balance health risk and cost. In addition, scientists in the Office of Research and Development have initiated a series of studies that should lead to future improvements in risk estimation. First, they are investigating the use of extrapolation models as well as the statistical variability of the NOAEL and underlying IJFs as means of estimating RfDs. Second, they are exploring procedures for less-than-lifetime health risk

assessment. Finally, they are working on ranking the severity of toxic effects as a way to further refine EPA's health risk assessments. While these procedures are promising, they cannot be expected at this time to serve as a foundation of a generalized health risk assessment for systemic toxicity in the Agency.

VI. HYPOTHETICAL, SIMPLIFIED EXAMPLE OF DETERMINING AND USING RfD

Suppose the Agency had a sound clay subchronic gavage study in rats with the following data:

A. Experimental Results

Dose (mg/kg-day)	Observation	Effect Level
0	Control - no adverse effects observed	-
1	No statistical or biological significant differences between treated and control animals	NOEL
5	2% decrease* in body weight gain (not considered to be of biological significance)	NOAEL
	Increased ratio of liver weight to body weight	
	Histopathology indistinguishable from controls Elevated liver enzyme levels	
25	20% decrease* in body weight gain	LOAEL
	Increased* ratio of liver weight to body weight	
	Enlarged, fatty liver with vacuole formation	
	Increased* liver enzyme levels	

* = Statistically significant compared to controls.

B. Analysis

1. Determination of the Reference Dose (RfD)

a. From the NOAEL

UF= 10H x 10A x 105 = 1000

MF = 0.8, a subjective adjustment based on the fact that the experiment involved an astonishing 250 animals per dose group

Therefore UF x MF = 800, so that

RfD = NOAEL/(UF x MF) = 5 mg/kg-day/800 = 0.006 mg/kg-day

b. From the LOAEL (i.e, if a NOAEL is not available)

If 25 mg/kg-day had been the lowest dose tested,

UF = 10H x 10A x 105 x 10L = 10,000

MF = 0.8

Therefore UF x MF = 8,000, so that

RfD = LOAEL/(UF x MF) = 25 (mg/kg-day)/8000 = .003 mg/kg-day)

2. Risk Characterization Considerations

Suppose the estimated exposure dose (EED) for humans exposed to the chemical under the proposed use pattern were .01 mg/kg-day; i.e.,

EED>RfD

Viewed alternatively, the MOE is:

MOE = NOAEL/EED = 5 mg/kg-day /0.01 mg/kg-day = 500

Because the EED exceeds the RfD (and the MOE is less than the UF x MF), the risk manager will need to look carefully at the data set, the assumptions for both the RfD and the exposure estimates, and the comments of the risk assessors. In addition, the risk manager will need to weigh the benefits associated with the case, and other nonrisk factors, in reaching a decision on the regulatory dose (RgD).

EPA APPROACH FOR ASSESSING THE RISK ASSOCIATED WITH EXPOSURE TO ENVIRONMENTAL CARCINOGENS

PRINCIPAL AUTHOR:

Robert E. McGaughy, Ph.D. (ORD)

CARCINOGEN RISK ASSESSMENT VERIFICATION ENDEAVOR (CRAVE) WORK GROUP:

Larry D. Anderson, Ph.D. (OW)
Diane D. Beal, Ph.D. (OPTS)
Judith Bellin, Ph.D. (ORD)
Chao W. Chen, Ph.D. (ORD)*
Ila Coty, Ph.D. (ORD)
Theodore Farber, Ph.D. (ORD)
William Farland, Ph.D. (ORD)
Herman J. Gibb, B.D.,M.P.H.(ORD)
Richard Hill, M.D., Ph.D. (OPTS)
Elizabeth H. Margosches, Ph.D (OPTS)
Robert E. McGaughy, Ph.D. (ORD)
Stephen Nesnow, Ph.D. (ORD)
Peter Preuss, Ph.D. (ORD)*
John A. Quest, Ph.D.(OPPE)
Reva Rubenstein, Ph.D. (OSWER)
Rita Schoeny, Ph.D. (ORD)*
Dot G. Wellington, Ph.D. (OPPE)

* Co-Chair

I. INTRODUCTION

In the analysis of data regarding the potential human carcinogenicity of chemicals, the Agency uses the approach described in the document entitled Guidelines for Carcinogen Risk Assessment (51 FR 33992-34003, Sept. 24, 1986). This approach had its origins in the 1976 Interim Guidelines for Health Risk and Economic Impact Assessments of Suspected Carcinogens (41 FR 21402-21405), which describes the conceptual basis of carcinogen risk assessment. The approach is consistent with the broad scientific principles of carcinogen risk assessment developed by the Office of Science and Technology Policy (OSTP) (50 FR 10372-10442), and the EPA guidelines quote the OSTP principles extensively. Detailed applications of the procedures currently used by the Agency are described in two documents: (1) Health Assessment Document for Epichlorohydrin, p. 7-32 to 7-48 (EPA 600/8-83-032F, December, 1984); and (2) Off Assessment of Health Risk of Garment Workers and Certain Home Residents from Exposure to Formaldehyde, Appendix 4 (April, 1986).

The Agency approach follows the general format of the National Academy of Sciences (NAS) description of the risk assessment process (see Risk Assessment in the Federal Government: Managing the Process [NAS Press, 1983]). In that report, the four elements of the risk assessment process are defined as follows:

(1) Hazard identification, in which a determination is made of whether human exposure to the agent in question has the potential to increase the incidence of cancer.

(2) Dose-response assessment, in which a quantitative relationship is derived between the dose, or more generally the human exposure, and the probability of induction of a carcinogenic effect.

(3) Exposure assessment, in which an evaluation is made of the human exposure to the agent. Exposure assessments identify the exposed population, describe its composition and size, and present the type, magnitude, frequency, and duration of exposure.

(4) Risk characterization, in which the exposure and dose-response assessments are combined to produce a quantitative risk estimate, and in which the strengths and weaknesses, major assumptions, judgments, and estimates of uncertainties are discussed.

The carcinogen summary sheets included in the IRIS system are designed to supply concise information about the hazard identification and dose-response assessment steps in this overall process. In order to use this information, individuals who wish to estimate geographic site-specific risks must be able to do an exposure evaluation based on the information available, and must be able to combine the first three elements into a comprehensive risk characterization which can support regulatory decision. The risk assessment process is an activity independent of the process of formulating regulatory control options being considered and independent of economic and political factors influencing the regulatory process. The Agency recognizes the distinction between these regulatory concerns (referred to as "risk management considerations" in the 1983 NAS report) and the risk assessment process.

II. ELEMENTS OF CARCINOGEN RISK ASSESSMENT

A. Hazard Identification

The purpose of this evaluation is to arrive at some conclusions as to whether or not the agent poses a carcinogenic hazard in exposed populations. The main types of evidence bearing on this question are: (1) human studies of the association between cancer incidence and exposure; and (2) long-term animal studies under controlled laboratory conditions. Other evidence such as short-term tests for genotoxicity, metabolic and pharmacokinetic properties, toxicological effects other than cancer, structure-activity relationships, and physical/chemical properties of the agent, is ancillary to the primary evidence.

The question of the likelihood that the agent is a human carcinogen is answered by considering all of the available information relevant to carcinogenicity, by judging the quality of the studies available, by attempting

to reconcile any differences found between studies an coming to an overall evaluation. This process is termed the weight-of-evidence approach, and the results are expressed in terms of an EPA stratification system for the weight of this evidence. The system, which is a modification of the approach taken by the International Agency for Research on Cancer (IARC),* classifies the likelihood that the agent is a human carcinogen into the following five categories:

Group	Description
A	Human Carcinogen
B1 or B2	Probable Human Carcinogen
B1	indicates that limited human data are available
B2	indicates sufficient evidence in animals and inadequate or no evidence in humans
C	Possible Human Carcinogen
D	Not Classifiable as to human carcinogenicity
E	Evidence of Non-Carcinogenicity for Humans

In making this classification for an agent, a two-stage procedure is followed. In the first stage, a provisional classification is made based on the degree of human and animal evidence. The degree of evidence is characterized separately for both human studies and animal studies as sufficient, limited, inadequate, no data, or evidence of no effect. The guidelines broadly define the meaning of these terms, which are basically the same as the IARC definitions. In the second stage, EPA scientists adjust these provisional classifications upwards or downwards, based on the supporting evidence of carcinogenicity described earlier, using judgments about the degree of adjustment warranted in each case. For further description of the role of supporting evidence, see the EPA Guidelines.

B. Dose-Response

The purpose of the dose-response assessment is to define the relationship between the dose of an agent and the likelihood of a carcinogenic effect, on the assumption that the agent is a human carcinogen. After the dose-response assessment is made, it is combined with the exposure evaluation to yield a numerical estimate of risk. Numerical estimates can be presented in one or more of the following four ways: 1) unit risk, 2) the concentration corresponding to a given level of risk, 3) individual, and 4) population risk. The summary sheets include only unit risk and risk-related air and water concentrations. The numerical risk estimation activity is not dependent on the likelihood of human carcinogenicity, as categorized in the hazard identification process. Instead, it is an independent piece of information which is to be combined with the hazard identification in making regulatory decisions.

As the Guidelines observe, dose-response assessment "usually entails an extrapolation from the generally high doses administered to experimental animals or exposures noted in epidemiologic studies to the exposure levels expected from human contact with the agent in the environment; it also includes considerations of the validity of these extrapolations." Extrapolation is ordinarily carried out first by fitting a mathematical model to the observed data and then by extending either the model or a bound on the risks it predicts in the observed range down toward risks expected at low exposure.

The main elements of a dose-response assessment are: (1) the selection of the appropriate data sets to use; (2) the derivation of estimates at low doses from experimental data at high doses using an extrapolation model; and (3) the choice of an equivalent human dose corresponding to the animal dose used.

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* IARC. (1982) IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Supplement 4 Lyon, France.

1. Choice of Data Sets

In choosing the appropriate data sets to use, the main principles are as follows:

(a) Human data are preferable to animal data, provided that quality is adequate.

(b) Data from a species which responds biologically most like humans (with respect to factors such as metabolism, physiology, and pharmacokinetics) are used. When no clear choice is possible on this basis, data corresponding to the most sensitive animal species/strain/sex combination are given the greatest emphasis.

(c) The route of administration which is the route of human exposure is used. When this is not possible, the route differences are noted as a source of uncertainty.

(d) When the incidence of tumors is significantly elevated at more than one site by the agent, risk estimates are made by determining the number of animals with one or more of these tumor sites.

(e) Benign tumors are generally combined with malignant tumors, unless the benigh tumors are not considered to have potential to progress to the associated malignancies of the same histogenic origin. See Guidelines for Combining Neoplasms for Evaluation of Rodent Carcinogenesis Studies (1986). McConnell, E.E., Solleveld, H.A., Swenberg, J.A., Boorman, G.A. JNCI 86:283-289.

2. Choice of Extrapolation Model

Since risk at low exposure levels cannot be measured directly either by animal experiments or by epidemiologic studies, a number of mathematical models and procedures have been developed to extrapolate from high to low dose. Different extrapolation methods may give reasonable fit to the observed data but may lead to large differences in the projected risk at low doses. In keeping with the Guidelines and the OSTP principles, the choice of low-dose extrapolation methocl is governed by consistency with current understanding of the mechanism of carcinogenesis and not solely on goodness-of-fit to the observed tumor data. When data are limited, and when uncertainty exists regarding the mechanisms of carcinogenic action, the OSTP principles suggest that models or procedures which incorporate low-dose linearity are preferred when compatible with the limited information available. The Guidelines recommend that the linearized multistage procedure be employed in the absence of adequate information to the contrary.

The first step of the linearized multistage procedure, abbreviated by LM on the summary sheets, calls for the fitting of a multistage model to the data. This is an exponential model approaching 100% risk at high doses with a shape at low doses described by a polynomial function. When the polynomial is of first degree, the model is equivalent to a one-hit or linear model, so called because at low doses it produces an approximately linear relationship between dose and cancer risk.

The second step of the procedure estimates an upper bound for risk by incorporating an appropriate linear term into the statistical bound for the polynomial. At sufficiently small exposures, any higher-order terms in the polynomial will contribute negligibly, and the graph of the upper bound will look like a straight line. The slope of this line is called the slope factor on the summary sheets. Since the slope at higher exposures could be different than at 1ow exposures r some chemicals, this slope factor is generally not valid when the exposures are sufficiently high. In the summary sheets the exposure corresponding to a risk of 1/100 is arbitrarily chosen as sufficiently high that the slope factor and the unit risks derived from it should not be used.

Other models that could be used-are the Weibull (W), Probit (P), Logit (LO) one-hit (OH), and gamma multihit (GM) models. These models are defined in the IRIS Glossary. Except for the one-hit model, these models all tend to give the characteristic S-shapes of many biological experiments, with varying curvature and tail length. Their upper bounds tend to parallel the curvature of the models themselves unless a procedure has been devised to provide otherwise, as is the case for the linearized multistage procedure. The slope factor designated on the summary sheets for these models is the slope of the straight line from

the upper bound at zero dose to the dose producing an upper bound of 1%.

Two alternative approaches have been used for dealing with the spontaneous background rate of tumor occurrence in risk estimation. Both approaches are summarized by a slope factor.

One approach defines "added risk" as the difference between the total response rate under an exposure condition and the background incidence in the absence of exposure. The corresponding equation is AR = P(d) - P(O). The other approach, called "extra risk", can be described as the "added risk" applied to that portion of the population that did not show background tumors. The corresponding equation is ER = [P(d) - P(0)]/[1 -P(O)]. "Extra risk" is the most commonly used approach, but the alternative approach, "added risk", is being explored by the Agency for its utility in certain circumstances and has been used in several cases. When the background response is sizable, "extra risk" is larger than "added risk", and when the background is small, both types of risk are essentially equal.

3. Determination of Human Equivalent Doses

The human dose that is equivalent to the dose in an animal study is calculated using the assumption that different species are equally sensitive to the effects of a toxin if they absorb the same dose per unit body surface area. This assumption is made only in the absence of specific information relevant to equivalent dose for that agent. Since surface area is approximately proportional to the two-thirds power of body weight, the equivalent dose is milligrams per (body weight raised to the two-thirds power) per day. It follows that if the animal dose is expressed in units of mg/kg'day, the equivalent human dose, in the same units, is smaller than the animal dose by a factor equal to the cube root of the ratio of human weight to animal weight. Since the Agency generally assumes a human weight of 70 kilograms, this factor becomes 13 for mice with a weight of 30 grams, and 5.8 for rats with a weight of 350 grams. In the calculation of human equivalent doses, the actual animal weight in the bioassay is used whenever that information is available; otherwise, standard species weights are used.

In using animal inhalation experiments to estimate lifetime human risks for partially-soluble vapott or gases, the air concentration is generally considered to be the equivalent d~ between species based on equivalent exposure times; i.e., a lifetime exposure to a 1-ppm concentration in humans is assumed to produce the same effect as a lifetime exposure to a l-ppm concentration in animals. In the inhalation of particulates or completely-absorbed gases, the amount absorbed per unit of body surface area is considered to be the equivalent dose between species.

In order to evaluate human risks for both air and water contamination when only one route has been tested in animals, additional assumptions with corresponding additional uncertainties must be introduced. For this reason, the summary sheets specify the route of exposure that was used for the calculation of air and drinking water unit risks.

4. Summary of Dose-Response Parameters

Quantitative risk estimates have several uses, and the expression of the results should tailored to each use. For comparing the carcinogenic characteristics of several agents, the cancer risk per unit absorbed dose is a useful parameter. It could be expressed on a weight basis (e.g., milligrams of the substance absorbed per kilogram body weight per day, mg/kg/day) or on a molar basis (e.g., m moles/kg/day). The low-dose slope factor described on page B-3 is used for this purpose in the IRIS summary.

For determining the concentrations of air or water at certain designated levels of lifetime risk, the ratio of that level of risk/unit risk for water or air is calculated. For example, if the water unit risk is 0.4 E-4 per ug/L, the water concentration corresponding to an upper bound of E-5/(0.4 E-4)=0.25 ug/L.

For evaluating risks to environmental agents, the concentrations of the agent in the medium where human contact occurs is the measure of exposure used. Therefore, the appropriate measure of dose response is risk per concentration unit, with standardized conventions of exposure durations and of intake of each medium being understood. These measures are called the unit risk for air and the unit risk for drinking

water. The standardized duration assumption is understood to be continuous lifetime exposure. The concentration units for air and drinking water are usually micrograms per cubic meter (ug/cu m) of air and micrograms per liter (ug/L) of water, respectively. For food, the agents are usually identified in specific foods (e.g., fish or corn) which constitute characteristic fractions of the daily diet, so the amount of the agents consumed per day in all food known or expected to contain residues of the agent is the most appropriate measure of exposure. For this use, the summary sheets provide the slope factor in units adjusted for body weight (e.g., mg/kg/day). If a different fraction of the agent is absorbed in humans from the human diet than is absorbed from the animal diet, an appropriate correction is needed when applying the animal-derived value to humans.

In summary, the quantities appropriate for calculating upper bound risks for air, drinking water, and food are, respectively, the air unit risk (risk per yg/cu. m of air), the drinking water unit risk (risk per H~1 of drinking water), and slope factor (risk per mg/kg/day of the agent). However, a smaller dose unit (e.g., yg/kg/day for dietary intake risk) is often used if the risk corresponding to the dose unit (e.g., mg/kg/day) exceeds 10-2.

5. Statement of Confidence in Dose-Response Parameters

A judgment about the degree of confidence the Agency has in the accuracy of the risks derived from the data is given in the summary sheets as a high, medium, or low rating. The factors increasing the Agency's confidence in the accuracy of these risk bounds includes the following:

(1) The existence of experimental data to replace default assumptions.

(2) Close agreement in the risk parameters derived from experiments in different animal species.

(3) Similarity in the route of exposure between the tested species and route of interest in humans.

(4) The existence of experimental data on the effective dose for the exposure route of interest.

(5) A large number of animals or people in the studies used.

(6) A large number of dose groups or a large range of doses in the studies being used.

(7) Sufficient purity of the test agent so that contamination is not a factor in interpretation of results.

(8) Similarities between the animal strain and humans as to metabolism and pharmacokinetics of the agent.

(9) For human occupational studies, determination of exposure or different worksites as opposed to an average exposure for the entire workplace.

(10) For epidemiologic studies, exposure measurements concurrent to the period being evaluated (e.g., time period of employment).

(11) Lack of concurrent exposures in epidemiologic studies which would reasonably have been expected to modify the dose-response.

(12) The ranking of epidemiologic study designs according to their usefulness in deriving accurate risk assessments: cohort case-control ecologic studies.

(13) The epidemiologic studies provided sufficient information on dose, duration of exposure, and age to permit one to separate the effects-of each on the dose-response relationship.

(14) An adequate time period was allowed in epidomiologic studies for a cancer latency period.

(15) Time regimens of animal exposure are similar to those of human exposure.

The factors decreasing the Agency's confidence, in addition to factors contrary to the points above, are as follows:

(1) The use of non-continuous dosing when we have reason to believe that there is an effective continuous dose but pharmacokinetic information is inadequate to esti'nate it.

(2) The use of vehicles, such as corn oil, which may confound or interact wish the agent under study in producing tumors at specific sites.

(3) Situations in which special test systems (such as mouse skin painting, strain A mouse pulmonary adenomas, and in vitro tests) are not similar enough to human systems to justify their use as a basis for human quantitative risk estimates.

(4) Lack of concurrent control groups.

(5) Poor animal husbandry.

APPENDIX F

PROCEDURES FOR DETERMINING THE HARMONIC MEAN FLOW

Appendix F

PROCEDURES FOR OBTAINING THE HARMONIC MEAN FLOW

Direct Calculation of the Harmonic Mean

Direct calculation of the harmonic mean flow, hm[x], can be done by retrieving several years of daily flow records at an appropriate USGS gage, taking the reciprocal of each value, calculating the average, and taking the reciprocal of the average. That is,

$$hm[x] = N/\Sigma\ (1/x) \qquad (1)$$

where x is daily flow and N is the number of daily values.

It is important to note that the harmonic mean must be determined for the flow downstream (not upstream) of the discharger. Unlike arithmetic means, harmonic means are not additive. Where the effluent flow may be a significant portion of the streamflow, the downstream harmonic mean is not the upstream harmonic mean plus the effluent flow. Rather it is the harmonic mean of the combined daily upstream plus effluent flows:

$$hm[downstream] = N\ /\Sigma\ \{1/(upstream + effluent)\} \qquad (2)$$

It might also be noted that the harmonic mean is zero if any value in the distribution is zero. However, the dilution can never actually be zero unless the pollutant load were discharged without an accompanying effluent flow, or if the downstream flow were evaporated to dryness (as opposed to disappearing beneath the stream bed, or temporarily accumulating behind a controlled outlet dam during periods of zero release from the impoundment).

As the analyst may not wish to undertake the computer programming needed to obtain the reciprocals of daily flows for the period of record, it is useful to consider simpler approximations. Such approximations can be made using data from the "duration table" generated by the USGS FLOSTAT program. This data is very easy to obtain. STORET users simply (a) log on to the NCC computer system, (b) issue the command WQAB FLOW, and in response to prompts, (c) supply the USGS gage number and years to be retrieved, (d) select option 2 (data analysis), and (e) specify the remote terminal where the data is to be printed. The WQAB FLOW procedure is one of several WQAB procedures intended to be of help to permit writers. Other procedures can assist in finding a USGS gage near a discharger having a particular NPDES number. STORET users may issue the command WQAB HELP to obtain a summary of available procedures.

An example of duration table output and accompanying statistics is attached. Summarizing the entire period retrieved, the table indicates the number of occurrences of each of 34 classes of flow magnitude. It shows, for example, four occurrences of flows of approximately 51 cfs, 31 occurrences of flows of approximately 60 cfs, and so forth up to flows of 9000 cfs. If n is the number of occurrences of flows having magnitude x , then the harmonic mean can be approximated by:

$$hm[x] = N/\Sigma\ (n_i\ /X_i) \qquad (3)$$

This procedure should generally yield a result close to Equation 1.

Indirect Methods for Approximating the Harmonic Mean

Even simpler methods for estimating the harmonic mean are derived below. These methods, however, hinge on the flows being log-normally distributed, and are thus subject to greater error.

Given a log-normally distributed variable, x, the following derivation shows the relationship between its harmonic mean, hm[x]; arithmetic mean, am[x]' geometric mean, gm[x]; and median, x(50). Since x is log-normally distributed, a variable y = ln x is normally distributed with mean and median μ and standard deviation σ, as noted on p. E-4 of the Technical Support Document for Water Quality Based Toxics Control. Furthermore, if x is log-normally distributed, 1/x must also be log-normally distributed; a variable z = ln(1/x) = -ln x is then normally distributed with mean $-\mu$ and standard deviation σ.

The geometric mean of x corresponds to the arithmetic mean of ln x, as follows:

$$gm[x] = \exp\ \mu \qquad (4)$$

And, as noted on p. E-4 of the TSD:

$$am[x] = \exp(\mu + \sigma^2/2) \qquad (5)$$

It follows that:

$$am[1/x] = \exp(-\mu + \sigma^2/2) \qquad (6)$$

Since by definition, am[1/x]={Σ (1/x)}/N, and hm[x] = N/Σ (1/x), where N is the number of observations in any distribution, it follows that:

$$am[1/x] = 1/hm[x] \qquad (7)$$

Therefore, combining Equation 6 and 7:

$$hm[x] = 1/\exp(-\mu + \sigma^2/2)$$

$$= \exp(\mu - \sigma^2/2) \qquad (8)$$

It might be noted that Equations 5 and 8 indicate that on a log scale, the geometric mean of a log-normal distribution lies midway between the harmonic mean and arithmetic mean.

If the duration table is obtained for a USGS gage, then statistics accompanying the retrieval will provide the mean and standard deviation of the base 10 logarithms. Then one may calculate equal to 2.3026 times the mean of the base 10 logs, and equal to 2.3026 times the standard deviation of the base 10 logs, and use Equation 8 to calculate the harmonic mean.

Alternatively, Equation 8 can be rewritten as follows:

$$hm[x] = \exp \mu/\exp(\sigma^2/2) = (\exp \mu)^2/\{\exp \mu \cdot \exp(\sigma^2/2)\}$$

$$= (\exp \mu)^2 \ / \ \exp(\mu + \sigma^2/2) \qquad (9)$$

Equations 4 and 5 can now be substituted into Equation 9:

$$hm[x] = gm^2[x]/am[x] \qquad (10)$$

Information on the geometric and arithmetic means is available in the statistics accompanying the duration table. Alternatively, since for a log-normal distribution, the geometric mean, gm[x], equals the median, x(50), also provided in the duration table statistics:

$$hm[x] = \{x(50)\}^2/am[x] \qquad (11)$$

Finally, since as noted on p E-4 of the TSD, the coefficient of variation is given by $CV[x] = [\exp(\sigma^2) - 1\}^{1/2}$, it also follows that:

$$hm[x] = gm[x] \ / \ \{CV^2[x] + 1\}^{1/2} \qquad (12)$$

While Equations 8, 10, 11, and 12 are equivalent for perfectly log-normal distributions with very large N, their results can ordinarily be expected to differ slightly from each other for actual flow distributions.

The usual pattern of flow distributions is that the lowest flows tend to be higher than expected for a log-normal distribution. In this case, Equations 8, 10, 11, and 12 will tend to underestimate the harmonic mean flow.

Again, whether using Equation 1, 3, 8, 10, 11, or 12, the harmonic mean must be determined for the combined upstream and effluent flow.

Example of FLOSTAT Duration Table Output

STATION NUMBER 04149000

DURATION TABLE OF DAILY VALUES FOR YEAR ENDING SEPTEMBER 30

DISCHARGE, IN CUBIC FEET PER SECOND
MEAN
FLINT RIVER NEAR FOSTERS, MICH.

CLASS	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
YEAR	NUMBER OF DAYS IN CLASS																																		
1961				6	29	38	41	27	19	14	17	37	11	10	13	9	12	15	16	13	12	5	7	4	1	3	3	2	1						
1962								1	14	61	50	51	23	28	30	16	12	11	9	12	7	4	3	4	3	5	2	3	3	4	8		1		
1963			1	5	8	33	53	78	81	28	16	17	6	8	17	13	6	3	1	3	3	1	2	4	10	1									
1964		4	29	47	59	47	49	13	23	17	14	9	4	9	7	5	14	8	4	3	1														
1965			1	35	61	40	45	13	21	23	7	9	6	5	6	12	8	8	6	5	11	7	2	5	6	6	5	3	3	2	3	1			
1966					22	44	33	20	16	31	14	15	7	13	16	18	12	10	19	15	13	11	3	5	6	2									
1967				1	22	9	15	11	32	17	12	21	30	11	17	21	26	26	15	12	8	5	12	5	3	3	5	4	11	2	8		1		
1968							4	3	2	12	9	32	25	44	36	37	33	19	13	8	14	21	9	14	2	11	3	4	3		4	2	1		1
1969							1	2	17	20	22	25	13	27	40	47	26	19	13	9	19	17	15	11	7	5	6	1	1			1		1	
1970									17	62	45	56	43	26	16	18	16	13	12	10	7	11	5	3	4	1									
1971				2	2	1	3	1	32	24	24	28	27	28	33	36	29	18	12	11	7	12	5	4	6	5	4	3	3	4	1				
1972						1	5	15	24	14	21	60	28	32	44	16	19	16	14	10	9	5	3	4	11	4	1	3	3	2	1	1			
1973								1	5	11	13	21	19	23	11	11	10	10	37	36	28	24	26	13	7	5	5	7	9	12	9	3		1	
1974								1	3	39	25	29	11	11	19	24	33	13	20	14	23	10	7	12	10	9	11	12	12	6	6	5			
1975											6	48	14	18	32	29	22	30	18	16	23	16	8	19	9	15	8	2	8	4	5	12	1	2	
1976								4	11	6	6	14	12	30	32	10	11	17	46	16	32	9	9	13	19	15	9	4	9	4	11	6	6	4	1
1977							5	18	65	30	53	54	18	30	18	2	4	6	6	11	13	10	10	9	1	2									
1978								2	9	22	31	28	18	51	54	22	20	19	20	14	6	13	3	2		2		2	1	2	12	4			
1979								17	15	32	48	69	36	26	14	13	11	8	10	7	15	16	7	7	2	6	4			1		1			
1980								1	11	9	16	36	26	43	28	20	27	29	29	19	11	8	13	11	11	12	4	1	1						

CLASS	VALUE	TOTAL	ACCUM	PERCT
0	0.0	0	7305	100.00
1	51.0	4	7305	100.00
2	60.0	31	7301	99.95
3	70.0	96	7270	99.52
4	82.0	203	7174	98.21
5	95.0	233	6971	95.43
6	110.0	254	6738	92.24
7	130.0	225	6484	88.76
8	150.0	387	6259	85.68
9	180.0	472	5872	80.38
10	210.0	449	5400	73.92
11	240.0	659	4951	67.78

CLASS	VALUE	TOTAL	ACCUM	PERCT
12	290.0	377	4292	58.75
13	330.0	473	3915	53.59
14	390.0	483	3442	47.12
15	460.0	379	2959	40.51
16	540.0	367	2580	35.32
17	630.0	298	2213	30.29
18	730.0	320	1915	26.21
19	860.0	244	1595	21.83
20	1000.0	262	1351	18.49
21	1200.0	205	1089	14.91
22	1400.0	149	884	12.10
23	1600.0	149	735	10.06

CLASS	VALUE	TOTAL	ACCUM	PERCT
24	1900.0	110	586	8.02
25	2200.0	112	468	6.41
26	2600.0	70	356	4.87
27	3000.0	51	286	3.92
28	3500.0	68	235	3.22
29	4100.0	[illegible]	167	2.29
30	4800.0	[illegible]	124	1.70
31	5600.0	38	56	0.77
32	6600.0	18	20	0.27
33	7700.0	8	10	0.14
34	9000.0	2	2	0.03

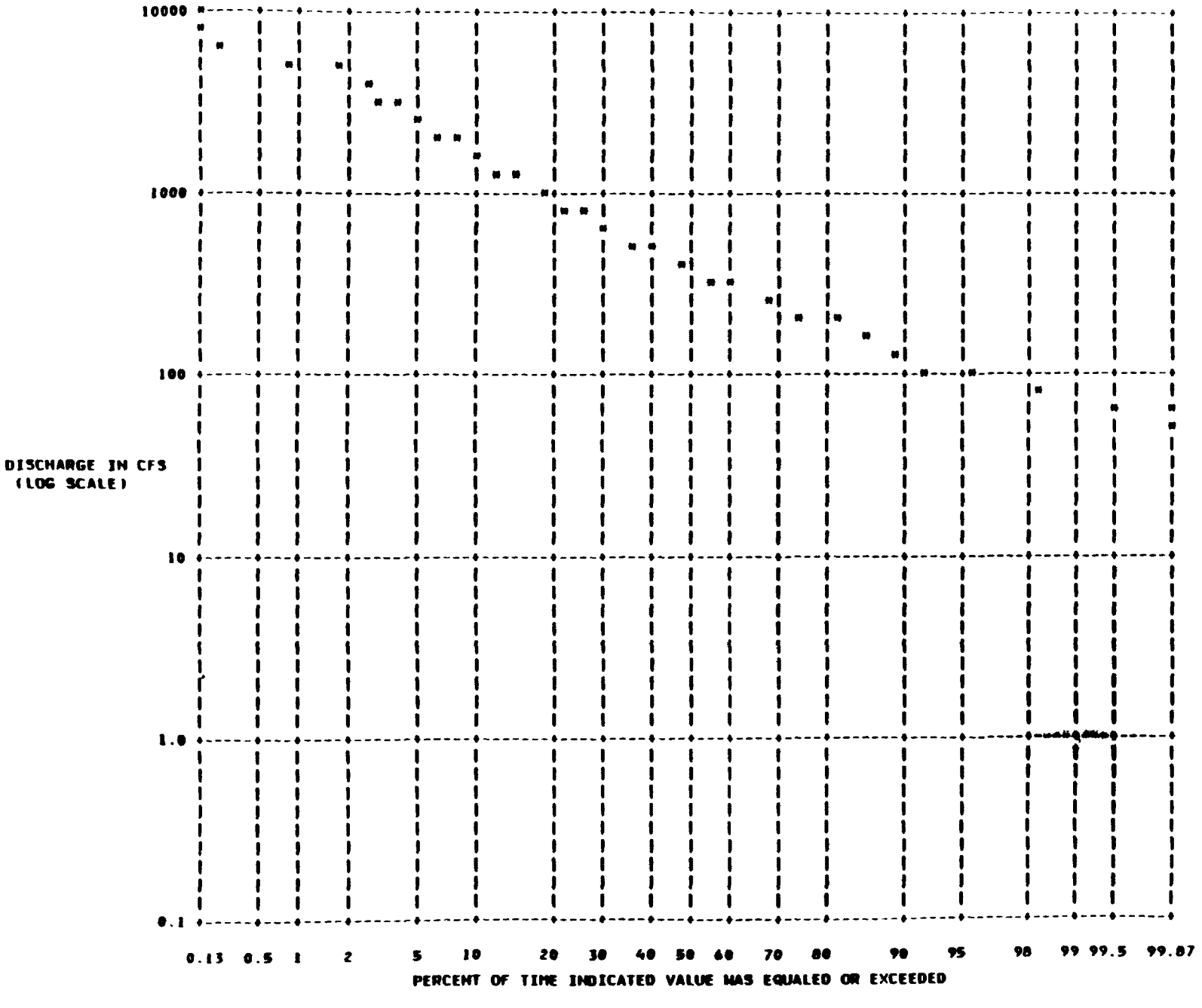
VALUE EXCEEDED 'P' PERCENT OF TIME

P95 =	97.0		
P90 =	123.0		
P75 =	205.0	P25 =	706.0
P50 =	353.0	P10 =	1610.0

DURATION PLOT OF DAILY DATA FOR ... YEAR ENDING SEPTEMBER 30 (YEARS 1961 - 1968)

STATION ID: 04149000 FLINT RIVER NEAR FOSTERS, MICH. DRAINAGE AREA = 1188.00 SQ MI

* = SINGLE POINT X = MULTIPLE POINTS



STATION NUMBER 04149000

ANNUAL VALUES

DISCHARGE, IN CUBIC FEET PER SECOND
MEAN
FLINT RIVER NEAR FOSTERS, MICH.

ANNUAL MEAN VALUE AND RANKING IN YEAR ENDING MARCH 31			ANNUAL MEAN VALUE AND RANKING IN YEAR ENDING SEPTEMBER 30		
1962	702.00	13	1961	433.00	16
1963	424.00	3	1962	669.00	10
1964	196.00	1	1963	294.00	19
1965	372.00	2	1964	180.00	20
1966	526.00	6	1965	510.00	14
1967	490.00	5	1966	425.00	18
1968	981.00	16	1967	793.00	6
1969	630.00	10	1968	848.00	5
1970	577.00	8	1969	730.00	9
1971	755.00	14	1970	438.00	15
1972	446.00	4	1971	653.00	11
1973	1170.00	18	1972	610.00	12
1974	1110.00	17	1973	1250.00	3
1975	967.00	15	1974	1140.00	4
1976	1760.00	19	1975	1260.00	2
1977	682.00	12	1976	1460.00	1
1978	630.00	11	1977	427.00	17
1979	588.00	9	1978	765.00	7
1980	573.00	7	1979	541.00	13
			1980	733.00	8

END OF FLOSTAT PROGRAM.

DURATION CURVE STATISTICAL CHARACTERISTICS FOR ...
STATION ID: 04149000 FLINT RIVER NEAR FOSTERS, MICH.
DISCHARGE, IN CUBIC FEET PER SECOND

DURATION DATA VALUES ARE INTERPOLATED FROM DURATION TABLE;
DATA ARE NOT ANALYTICALLY FITTED TO A PARTICULAR STATISTICAL DISTRIBUTION;
USER IS RESPONSIBLE FOR ASSESSMENT AND INTERPRETATION.

ADDITIONAL CONDITIONS FOR THIS RUN ARE:
BASED ON LOGARITHMS (BASE 10) OF UP TO 19 VALUES FROM P5 TO P95 BY INCREMENTS OF 5.
NUMBER OF VALUES IS REDUCED FOR EACH NEAR-ZERO OR ZERO VALUE.

NUMBER OF VALUES = 19 (NUMBER OF NEAR-ZERO VALUES = 0)
VALUES USED ARE:

P95 =	97.0	(LOG = 1.98677)
P90 =	123.0	(LOG = 2.08990)
P85 =	154.0	(LOG = 2.18752)
P80 =	182.0	(LOG = 2.26007)
P75 =	205.0	(LOG = 2.31175)
P70 =	229.0	(LOG = 2.35983)
P65 =	255.0	(LOG = 2.40654)
P60 =	283.0	(LOG = 2.45179)
P55 =	319.0	(LOG = 2.50379)
P50 =	363.0	(LOG = 2.55991)
P45 =	412.0	(LOG = 2.61490)
P40 =	468.0	(LOG = 2.67025)
P35 =	546.0	(LOG = 2.73719)
P30 =	637.0	(LOG = 2.80414)
P25 =	766.0	(LOG = 2.88423)
P20 =	937.0	(LOG = 2.97174)
P15 =	1190.0	(LOG = 3.07555)
P10 =	1610.0	(LOG = 3.20683)
P05 =	2570.0	(LOG = 3.40993)

MEAN OF LOGS = 2.60487

STANDARD DEVIATION OF LOGS = 0.38496 (VARIABILITY INDEX)

COEFFICIENT OF VARIATION = 0.14778

COEFFICIENT OF SKEW = 0.42127

APPENDIX G

SAMPLE PERMIT LANGUAGE

Appendix G

Sample Permit Language

A. Bioconcentration Evaluation Requirement for Characterizing Effluent Bioconcentration Potential

The permittee shall perform effluent bioconcentration evaluation, according to methods in "Assessment, Reference Concentration Development and Control of Bioconcentratable Contaminants in Surface Waters" (EPA 600/x-xx-xxx), on the discharge(s) from outfall(s) _____, as described below:

1. The permittee shall initiate effluent bioconcentration evaluation within 90 days of the effective date of this Part to determine effluent bioconcentration potential. Such testing will determine if an effluent sample contains compounds with a log P greater than 3.5 ("bioconcentratable compounds") in an amount sufficient to present a bioconcentration hazard in the receiving water, (i.e., in exceedance of relevant reference ambient concentration(s)).

2. The effluent bioconcentration evaluation will:

a) consist of the procedures in Appendix B of "Assessment and Control of Bioconcentratable Contaminants in Surface Waters" (EPA 600/x-xx-xxx). All tests will be conducted on 24-hour composite samples. A minimum of 2 replicates will be used in the tests.

b) be conducted every four months for a period of one year following the effective date of this permit. If no identifiable bioconcentratable compounds are found, no further analysis is necessary unless a change in process or discharge occurs. Data will be reported according to Appendix B (EPA 600/x-xx-xxx), Section 10.8 "Reporting of Data," and shall be submitted to the permitting authority in the appropriate monthly Discharge Monitoring Report. Following review of these results, the permitting authority may require the permittee to confirm the identity and quantity of any compounds for which the permitting authority determines this additional analysis is necessary.

3. Upon determination by the permitting authority that the potential exists for discharge of bioconcentratable pollutants in exceedance of reference ambient concentrations, the permit may be reopened and modified

to incorporate necessary effluent limitations for the individual bioconcentratable pollutants.

FACT SHEET

A. Effluent Limitations on Bioconcentratable Compounds

1. No bioconcentratable pollutant shall be discharged in an amount which would cause exceedance in the receiving water of its reference ambient concentration as determined by the procedures in Chapter 3 of "Assessment, Reference Concentration Development and Control of Bioconcentratable Contaminants in Surface Waters" (EPA 600/x-xx-xxx).

2. Where they are necessary, effluent limitations will be derived according to Chapter 6 of the "Assessment, Reference Concentration Development and Control of Bioconcentratable Contaminants in Surface Waters" (EPA 600/x-xx-xxx), for bioconcentratable compounds which have been identified in the effluent by the bioconcentration evaluation procedure of Appendix B of the above document.

3. Where final calculated limitations for individual pollutants fall below the current level of detection, the limit for the pollutant will be the one calculated; the analytical method to be used for monitoring will be specified in the permit; and the minimum level for that method (if one is available) will be specified in the permit as the threshold for compliance/non-compliance determinations. Where a minimum level is not available for the specified analytical method, an alternative threshold for compliance/non-compliance determinations will be specified in the permit (e.g., the method detection limit).

B. Effluent Limitations Compliance Monitoring Requirements

Effluent analysis for the purpose of determining compliance with limits on individual bioconcentratable compounds shall be conducted by the permittee once per week and reported on the Discharge Monitoring Report using EPA method 1625. Levels of detection and quantitation shall be reported.

Sample Section 308 Letter

CERTIFIED MAIL NO.
RETURN RECEIPT REQUESTED

Ms. Ann Powell
Plant Manager
Chemico Corporation
Anytown, USA

RE: NPDES No. xx000123
Chemico Anytown

Dear Ms. Powell,

The U.S. Environmental Protection Agency (EPA) is preparing to reissue the NPDES permit for the Chemico Anytown facility. Preliminary evaluations conducted by EPA and water quality monitoring conducted by the (State Water Control Authority) indicate that the discharges from the Chemico Anytown facility may be violating State water quality standards. In order for EPA to fulfill its responsibilities under the Clean Water act, 33 U.S.C. 1251, et seq., additional information regarding the nature of the discharges from the Chemico facility is required.

Therefore, you are hereby required (1) to perform the sampling and analysis programs described below, (2) to maintain for possible use by EPA all records regarding plant operations during the sampling and analysis programs within the time limits specified. These requirements are imposed pursuant to the authority provided in Section 308 of the Clean Water act, 33 U.S.C. Section 1318. Failure to comply with this request may result in enforcement proceedings under Section 309 of the Clean Water act, 33 U.S.C. Section 1319, which could result in the judicial imposition of civil or criminal penalties.

Effluent Bioconcentration Evaluation -

In order to identify potentially bioconcentratable pollutants discharged from Outfall 001, Chemico shall analyze effluent discharges as described below. Wastewater samples for analysis shall be composited over 24 hours once per quarter for a period of one year commencing within 30 days of receipt of this letter.

The procedures to be used shall conform to the methods in Appendix B of "Assessment, Reference Concentration Development and Control of Bioconcentratable Contaminants in Surface Waters" (EPA 600/x-xx-xxx). All tests will be conducted on 24-hour composite samples. A minimum of 2 replicates will be used in the tests. A reasonable effort shall be made to identify and confirm

those chemicals designated as chemicals of highest concern and those present in concentrations likely to cause excursions above water quality standards. Following review of the results of this evaluation, the permitting authority may direct the permittee to conduct additional confirmation on chemicals reported as present in the effluent.

Data will be reported according to Appendix B (EPA 600/x-xx-xxx), Section 10.8 "Reporting of Data," and shall be provided with a description of the analyses to the permitting authority within 45 days of sample collection.

If you have any questions please contact _____. Thank you for your cooperation.

Sincerely,

Director,
Water Division

Sample Permit Language for Fish Tissue and Sediment Evaluations

Part III Special Conditions

A. Fish Tissue and Sediment Evaluations

1. The permittee shall conduct the Tissue residue evaluation and Sediment Evaluations as described below. The purpose of these evaluations is to determine compliance with applicable State water quality standards, and to determine if pollutants accumulate in fish tissue or sediments to levels which would cause harmful effects to aquatic life and/or unacceptable risks to human health. The primary objectives of these analyses are to: 1) evaluate the discharge related bioaccumulation of organic chemical pollutants to the aquatic life in the receiving water, 2) evaluate the discharge related accumulation of organic chemical contaminants in the sediments of the receiving water, 3) provide information for evaluating the re-issuance of this permit. Following review of the results from the annual evaluations submitted by the permittee, the permitting authority may adjust the frequency and extent of the data collection and analysis if such adjustments in the sampling design are necessary to meet the objectives of the tissue residue and sediment evaluations.

2. Tissue Residue Evaluation - The objective of this evaluation is to identify and confirm the presence and concentrations of bioaccumulative pollutants in aquatic organisms in the receiving water. Monitoring will be required annually during the month of _____. Sampling stations are to be located at _____, as illustrated in Figure 1. Sampling methods, tissue residue evaluation procedures and data reporting should follow those specified in Appendix A of "Assessment, Reference Concentration Development and Control of Bioconcentratable Contaminants in Surface Waters" (EPA 600/x-xx-xxx). Species to be collected shall include ten individuals of the benthic organism species _____, and a minimum of ten individuals from at least two of the following fish species, _____. These samples shall be collected from at least two sampling stations (including a reference and effect station). One station must be in the proximity of the _____ facility mixing zone.

The results of this evaluation shall be submitted no later than 45 days after the sample collection date. Upon review of the results of this evaluation the permitting authority may require additional chemical specific effluent monitoring for any of the bioaccumulative contaminants identified in the results of the fish tissue evaluation.

3. Sediment Evaluation - The objective of this evaluation is to identify and confirm the presence and concentrations of bioaccumulative pollutants in the receiving water sediments associated with the discharge from outfall 001. Monitoring will be required annually during the month of _____. Sediment sampling stations are to be located at _____, with at least one station to be located in the _____ facility mixing zone, as illustrated in Figure 2. Sampling methods, sediment evaluation procedures and data reporting should follow those specified in Appendix C of "Assessment, Reference Concentration Development and Control of Bioconcentratable Contaminants in Surface Waters" (EPA 600/x-xx-xxx).

The results of this sediment evaluation shall be submitted no later than 45 days after the sample collection date. Upon review of the results of this evaluation the permitting authority may require additional chemical specific effluent monitoring for any of the bioaccumulative organic chemical contaminants identified in the results of the sediment evaluation.

APPENDIX H

OVERVIEW OF SELECTED AVAILABLE TOOLS

Appendix H

Overview of Selected Available Tools

A. Chemical-Specific Toxicity Data Bases

1. QSAR System [48]

Reference: "QSAR System User Manual", a joint project of the Institute for Biological and Chemical Process Analysis, Montana State University, Bozeman, Montana and the United States Environmental Protection Agency/Environmental Research Laboratory-Duluth (U.S.E.P.A./E.R.L.-D.), Duluth, Minnesota (October 1986).

To obtain:

Christine L. Russom
U.S.E.P.A./E.R.L.-D.
6201 Congdon Blvd.
Duluth, Minnesota 55804
(218)-720-5709
(FTS)-780-5709

Use: Tens of thousands of chemicals have not been studied for their environmental effects and fate. When the permit writer must decide whether to set limits for a compound for which little aquatic toxicity or rate information is available or must decide whether to require further testing by a permittee, the writer can use QSAR as screening tool to predict chemical properties including partitioning and persistence in the environment, bioaccumulation, and toxicity to certain aquatic organisms. QSAR will also screen for mutagenic functional groups. Under current discount rates, QSAR also provides EPA and State environmental regulatory agencies with inexpensive access to AQUIRE, a comprehensive data base of aquatic toxicity tests on individual compounds.

Cost: No charges are applied for Federal, State or Local governments.

Release Date: January 1991

2. Integrated Risk Information System (IRIS) [50]

Reference: "Integrated Risk Information System", Volume 1. "Supportive Documentation", Volume 11. Chemical Files (EPA-600/8-86-032a and b), Office of Health and Environmental Assessment, Office of Research and Development, U.S. EPA, Washington, D.C., March, 1987.

To obtain: IRIS is available on EPA electronic mailbox. IRIS User's Guide obtained through IRIS User Support at (FTS) 684-7254. Those outside EPA can obtain an IRIS account by contacting Mike McLaughlin, DIALCOM, Inc., Federal Systems Division, 600 Maryland Avenue S.W., Washington, D.C. IRIS is also available through the Public Health Network (Paul Johnson, (202) 898-5600) of the Public Health Foundation. IRIS will be made available on the NIH National Library of Medicine's TOXNET system ((301) 496-6531) sometime in late summer or fall of 1988.

Use: IRIS is a computer-housed, electronically communicated catalogue of Agency risk assessment and risk management information of chemical substances. The IRIS system is designed especially for federal, state, and local environmental health agencies as a source of the latest information about Agency health assessments and regulatory decisions for specific chemicals. The risk assessment information contained in IRIS, except as specifically noted, has been reviewed and agreed upon by intra-agency review groups, representing an Agency consensus. An intra-agency work group has been responsible for the development of IRIS [22]. There are currently 260 chemicals in IRIS. New chemicals are added regularly and existing chemicals are revised as warranted by new scientific findings.

Cost: No charges are applied for EPA users and anyone having EPA-paid-for electronic mail accounts. All other users are subject to charges applied by the particular system they use to access IRIS.

Caution: IRIS data is subject to update on a frequent basis.

3. Health Effects Assessment Summary Tables (HEAST)

Reference: "Health Effects Assessment Summary Tables", United States Environmental Protection Agency/Office of Solid Waste (U.S.E.P.A./O.S.W.), Washington D.C., (released quarterly)

To obtain: The U.S.E.P.A./O.S.W. requests that their users (i.e. O.S.W. staff, contractors, and State solid waste programs) call Susan Griffin of the Office of Solid Waste at (202)-382-6392 to obtain copies. Regional O.S.W. staff are reminded that copies of HEAST are sent to all Regional libraries. All others must purchase the document from:

National Technical Information Service (N.T.I.S.)
5285 Port Royal Road
Springfield, Virginia 22161
(703)-487-4650

Use: The Health Effects Assessment Summary Tables have been developed to provide information on chemicals commonly found at Superfund and RCRA sites. The reports are intended as pointer systems to identify current literature or changes in assessment criteria for many chemicals of interest to the Superfund program. The information in HEAST should be used as a secondary source to the information contained in the IRIS database.

Cost: There is a charge to receive this from N.T.I.S.

4. Risk*Assistant Prototype

Reference: "Risk*Assistant Prototype, An Overview of the Software System", The Hampshire Research Institute, Alexandria, Virginia, May 1990.

To Obtain:

RISK*ASSISTANT
Hampshire Research Institute
1600 Cameron Street, Suite 100
Alexandria, Virginia 22314
(703)-683-6695

Use: RISK*ASSISTANT is a microcomputer-based software system that provides an array of analytical tools, databases, and information-handling capabilities for individuals who must assess the health risks posed by chemicals. Requiring only estimates of the nature and

concentrations of hazardous chemicals present in a sample the system can provide information on carcinogenic and other toxic hazards of chemicals. The RISK*ASSISTANT system is designed especially for use by federal, state, and local agencies as a source of the information for evaluating the health risks posed by specific chemicals. The system can be used to retrieve information on carcinogenic and other toxic hazards of chemicals and to help direct additional data collection or set priorities for subsequent regulatory action.

Cost: The Risk Assistant Prototype is available without charge at this time.

B. Chemical-Specific Bioconcentration Tests

1. American Society of Testing and Materials [27]

Reference: "Standard Practice for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Mollusks." Designation E 1022-84, 1986 Annual Book of ASTM Standards, vol. 11.04, Publication Code Number (PCN): 01-110485-48, April 1985.

To obtain: ASTM, Customer Service, 1916 Race St., Philadelphia, PA 19103, (215) 299-5400, (FTS) 299-5400. It is not necessary to order the entire Annual Book of Standards. A copy of Standard Practice E 1022-84 costs $8.00.

Use: Where protection against bioaccumulating compounds is warranted and individual, potentially bioaccumulative compounds in the effluent can be identified, the permit writer should consider requiring the permittee to perform bioconcentration tests on these compounds using the methods described in Standard Practice E 1022-84.

Cost: The ASTM bioconcentration test is very time consuming and costly.

Cautions: Some techniques described in the method were developed for tests on non-ionizable organic chemicals and may not apply to ionizable or inorganic compounds. The bioaccumulation potential of many non-ionizable organic compounds can be predicted much more cheaply using physical-chemical properties such as the log P. For example, QSAR (see A.1.) uses log P to calculate BCFs.

2. log P values [49]

Reference: Hansch, C. and A.J. Leo. "Substituent Constants for Correlation Analysis in Chemistry and Biology." John Wiley and Sons: New York, 1979.

C. Models

1. Food and Gill Exchange of Toxic Substances (FGETS, GETS)

Reference: "FGETS (Food and Gill Exchange of Toxic Substances: A Simulation Model for Predicting Bioaccumulation of Nonpolar Organic Pollutants by Fish" by M.C. Barber, L.A. Suarez, and R.R. Lassiter. (EPA-600/3-87-038) [25]. Also, "GETS, A Simulation Model for Dynamic Bioaccumulation of Nonpolar Organics by Gill Exchange: A User's Guide," by L.A. Suarez, M.C. Barber, and R.R. Lassiter. 1987 (EPA-600/S3-86-057) [26].

To obtain: Environmental Research Laboratory, Office of Research and Development, U.S. EPA, Athens, GA 30613.

Use: This model is for the bioaccumulation of nonpolar, non-metabolized organic chemicals by fish. The FGETS model simulates thermodynamically-driven chemical exchange by fish assuming either aqueous exposure only or joint aqueous and food chain exposure.

Cost: Single copies free of charge.

Caution: The FGETS model is still in development. Initial analysis indicates that FGETS can simulate quite well observed patterns of bioaccumulation and depuration of a single, nonpolar, slowly or non-metabolized organic pollutant by individual fish. Extensions of the model to incorporate simulation of mixtures and to account for other variables are planned.

D. Supporting Information and Guidance

1. Health Risk Assessment Procedure for Consumption of Contaminated Fish and Shellfish [44]

Reference: "Assessing Human Health Risks from Chemically Contaminated Fish and Shellfish: A Guidance Manual", United States Environmental Protection Agency/Office of Marine and Estuarine Protection (U.S.E.P.A./O.M.E.P.), Washington D.C., September 1989.

To obtain:

Paula Monroe
U.S.E.P.A./O.M.E.P.
WH-556-F
401 M Street S.W.
Washington D.C. 20460
(FTS/202)-475-6182

Use: This guidance manual provides step-by-step assistance for assessing health risks from exposure through consumption of chemically contaminated aquatic organisms. The guidance is applicable to freshwater, brackish water, and saltwater fish and shellfish. Guidance is provided on mathematical models used to estimate chemical exposure and risk. Information on sampling design is provided. Sources of information on toxic chemicals and model variables are noted. Additionally, suggestions for presentation of risk assessment results are provided and uncertainties are summarized. The guidance provided in this manual is directed primarily at risk assessment related to recreational fisheries.

Cost: No charges are applied for Federal, State or Local governments.

2. State Procedures for Calculating RACs in the Absence of IRIS Values

Reference:

1) Department of Natural Resources, "Guidelines for Rule 57(a) (5 and 6), Michigan Department of Natural Resources, Environmental Protection Bureau, 1987 [51].

2) Department of Natural Resources, "Proposed Chapter NR10S.08", Wisconsin Department of Natural Resources, Bureau of Water Resources Management, 1987 [52].

Use: Michigan's procedures for deriving a dose factor depend upon the type and quality of the toxicity data base used [51]. Wisconsin uses toxicity data in conjunction with feeding habits for mammalian and avian test species to estimate RTCs for fish consumption by wildlife. Where data for multiple species are lacking, Wisconsin applies an uncertainty factor of 0.1 to account for differences in species sensitivity [52]. These procedures have not been formally reviewed or approved by the EPA and the inclusion of these procedures, pending such review, is intended to be advisory only.

APPENDIX I

FIELD VALIDATION STUDIES

The Field Validation Report Will be Added When Completed

APPENDIX J

EXAMPLE DATA AND REPORTS

Appendix J

Illustrative Data and Reports for Effluent, Tissue, and Sediment Analytical Procedures.

1.0 General Considerations

In this appendix, example data and discussion on data evaluation are provided for the tissue, effluent, and sediment analytical procedures. The reports generated by the different analytical procedures are similar. The formats for reporting the data and some of the reported data in this document are illustrative. Reporting formats for laboratories and consultants performing these procedures may differ.

2.0 Tissue Alternative

In evaluating any report, the QA/QC report should be examined first since this information will provide a good indication of the quality of the analytical data. The QC requirement for recovery of the surrogates is 25% < % recovery < 120% and all three surrogates, d_{10}-biphenyl, $^{13}C_6$-1,2,4,5-tetrachlorobenzene, and $^{13}C_6$-hexachlorobenzene, should have recoveries within this range. If recovery data is out of this range, the analytical data should not be used. Provided acceptable recoveries are obtained, the GC/MS chromatograms and the QA/QC data for GPC resolution, silica gel performance, etc. should be examined. This examination should take 5 to 15 minutes and will provide a good indication of the overall quality of the analysis.

In Table J-1, an illustrative report for the tissue analytical procedure is provided. After examining the recovery data for the surrogate chemicals, the GC/MS chromatograms for the procedural blank and the sample extract should be examined. The procedural blank should contain few peaks as illustrated in the report. Procedural blanks should always contain fewer peaks than the sample extract. Peaks in the GC/MS chromatograms should be narrow, e.g., 10 to 20 seconds base widths, and when large fat peaks exist, e.g., base widths exceeding 1 minute, overloading of the capillary column on the GC/MS has occurred. Efforts should be directed at determining why these large peaks exist in the sample extract. Sometimes these large peaks exist because the cleanup procedure for the tissue extract was performed poorly, the capacity of the cleanup procedure was exceeded, and/or severe blank problems exist. In these situations, additional cleanup or extraction of another portion of the tissue composite should be requested.

The evaluation of the QC data for GPC resolution, silica gel performance, GC resolution, MS sensitivity, MS calibration, DFTPP performance, and precision are all straight forward. The evaluation criteria for these QC procedures are:

a) GPC Resolution: Baseline resolution between the three

performance chemicals, Dacthal, pyrene, and di-2-ethylhexylphthalate, must exist. The data in Table J-1 presents excellent resolution.

b) Silica Gel Performance: The eluate from the column using the silica performance solution must not contain more than 10% of the cholesterol while at least 90% of the dieldrin must be recovered. This is performed for each lot of silica gel and/or every two months, which ever comes first. This data can be presented using control charts or in tabular form.

c) GC Resolution: One of two solutions can be used to evaluate GC resolution. For the solution containing β-BHC, -BHC, endrin ketone and d_{12}-chrysene, baseline resolution between the β-BHC/ -BHC and endrin ketone/d_{12}-chrysene pairs should exist. For the solution containing anthracene, phenanthrene, benz[a]anthracene, and chrysene, anthracene and phenanthrene should be baseline resolved and benz[a]anthracene and chrysene should be separated by a valley whose height is less than 25% of the average peak height of these two compounds. The data in Table J-1 is for the first solution above. Note, when using the first solution, fresh solution is required since endrin ketone degrades.

d) MS Sensitivity: For the 0.5 ppm calibration solution, the GC/MS peaks for the three surrogate chemicals should have a signal to noise ratio of 3 or more.

e) MS Calibration: For d_{12}-chrysene, the ratio of the abundance of the 241 mass/charge relative to that of 240 should be >15% and <25%. In the example data, this ratio is 20%.

f) DFTPP Performance: Comparison of the mass spectrum for DFTPP to the performance criteria is required. A DFTPP mass spectrum is reported in Table J-1.

g) Precision: A duplicate analysis should be performed with every 10 sample analyses. This data can be presented by using control charts or tabular reports. Precision data should not exceed 150%.

If any of the QC/QA procedures are not met, the analytical data is of questionable quality and use of the data is not recommended. Depending upon which factors are out of range, reanalysis of the sample extract (c,d,e,f) or extraction of another portion of the tissue composite (a,b,g) will be required. Some judgement will be required in this evaluation.

If the QA/QC Report is acceptable, the data in Reports 1, 2, and 3 can be used.

For **Tissue Report 1**, GC/MS components tentatively identified using the CHC mass spectral library search are reported. Those GC/MS components with fits of 70% and greater are considered

tentatively identified. For each tentatively identified GC/MS component, the following information is require:

1) GC retention time of the component, minutes
2) Amount of the component, ug/kg
3) A list of the best mass spectral library identifications (up to a total of ten identifications)
4) For each tentative identification:
   Library searching fit, %
   CAS number

This report often contains none or few tentatively identified components.

For **Tissue Report 2**, GC/MS components tentatively identified using the EPA/NIH/NBS mass spectral library search are reported. Those GC/MS components with fits of 70% and greater are considered tentatively identified. This report contains only those components with concentrations of 5 ug/kg and greater. For each tentatively identified GC/MS component, the following information is require:

1) GC retention time of the component, minutes
2) Amount of the component, ug/kg
3) A list of the best mass spectral library identifications (up to a total of ten identifications)
4) For each tentative identification:
   Library searching fit, %
   CAS number

For **Tissue Report 3**, GC/MS components not tentatively identified, i.e., fits <70%, using the EPA/NIH/NBS mass spectral library search are reported. This report contains only those components with concentrations of 5 ug/kg and greater. For each GC/MS component not tentatively identified, the following information is require:

1) GC retention time of the component, minutes
2) Amount of the component, ug/kg
3) If the fit parameter is >25%, the two best mass spectral library identifications and for each tentative identification, their library searching fit and CAS number.
4) If the fit parameter is <25%, compound is labeled as unknown.

3.0 Effluent Alternative

In evaluating any report, the QA/QC report should be examined first since this information will provide a good indication of the quality of the analytical data. The QC requirement for recovery of the surrogates is 25% < % recovery < 120% and all three surrogates, d_{10}-biphenyl, $^{13}C_6$-1,2,4,5-tetrachlorobenzene, and $^{13}C_6$-hexachlorobenzene, should have recoveries within this range. In addition, HPLC fraction 1 should contain d_{10}-biphenyl and should not

contain $^{13}C_6$-1,2,4,5-tetrachlorobenzene or $^{13}C_6$-hexachlorobenzene. HPLC fraction 2 should contain $^{13}C_6$-1,2,4,5-tetrachlorobenzene and should not contain d_{10}-biphenyl or $^{13}C_6$-hexachlorobenzene. HPLC fraction 3 should contain $^{13}C_6$-hexachlorobenzene and should not contain $^{13}C_6$-1,2,4,5-tetrachlorobenzene or d_{10}-biphenyl.

If recovery data is out of range and/or the surrogates are in the wrong fraction, the analytical data should not be used. Provided acceptable recoveries and surrogate location in the HPLC fractions are obtained, the GC/MS chromatograms and the QA/QC data for HPLC resolution, GC resolution, etc. should be examined. This examination should take 5 to 15 minutes and will provide a good indication of the overall quality of the analysis.

In Table J-2, an illustrative report for the effluent analytical procedure is provided. After examining the recovery data for the surrogate chemicals, the GC/MS chromatograms for the procedural blank and the sample extract should be examined. There will be six GC/MS chromatograms, three for the procedural blank and three for the effluent sample as a result of the HPLC fractionation procedure. The GC/MS chromatograms for the HPLC fractions for the procedural blank should contain few peaks as illustrated in the report. Procedural blanks should always contain fewer peaks than the sample extract. Peaks in the GC/MS chromatograms should be narrow, e.g., 10 to 20 seconds base widths, and when large fat peaks exist, e.g., base widths exceeding 1 minute, overloading of the capillary column on the GC/MS has occurred. Efforts should be directed at determining why these large peaks exist in the sample extract. Sometimes these large peaks exist because the cleanup procedure for the effluent extract was performed poorly, the capacity of the cleanup procedure was exceeded, and/or severe blank problems exist. In these situations, additional cleanup or extraction of another portion of the effluent sample should be requested.

The evaluation of the QC data for HPLC resolution, GC resolution, MS sensitivity, MS calibration, DFTPP performance, and precision are all straight forward. The evaluation criteria for these QC procedures are:

a) HPLC Resolution: Baseline resolution between the three performance chemicals, biphenyl, 1,3-diethylbenzene, and bibenzyl, must exist. In Table J-2, chromatographic data with excellent resolution is shown.

b) GC Resolution: One of two solutions can be used to evaluate GC resolution. For the solution containing β-BHC, -BHC, endrin ketone and d_{12}-chrysene, baseline resolution between the β-BHC/ -BHC and endrin ketone/d_{12}-chrysene pairs should exist. For the solution containing anthracene, phenanthrene, benz[a]anthracene, and chrysene, anthracene and phenanthrene should be baseline resolved and benz[a]anthracene and chrysene should be separated by a valley whose height is less than 25% of the average peak height

of these two compounds. Note, when using the first solution, fresh solution is required since endrin ketone degrades.

c) MS Sensitivity: For the 0.5 ppm calibration solution, the GC/MS peaks for the three surrogate chemicals should have a signal to noise ratio of 3 or more.

d) MS Calibration: For d_{12}-chrysene, the ratio of the abundance of the 241 mass/charge relative to that of 240 should be >15% and <25%.

e) DFTPP Performance: Comparison of the mass spectrum for DFTPP to the performance criteria is required. A DFTPP mass spectrum is presented in Table J-1.

f) Precision: A duplicate analysis should be performed with every 10 sample analyses. This data can be presented by using control charts or tabular reports. Precision data should not exceed 150%.

If any of the QC/QA procedures are not met, the analytical data is of questionable quality and its use is not recommended. Depending upon which factors are out of range, reanalysis of the HPLC fractions (b,c,d,e) or extraction of another portion of the effluent sample (a,f) will be required. Some judgement will be required in this evaluation.

If the QA/QC Report is acceptable, the data in Reports 1, 2, 3, and 4 can be used.

For **Effluent Report 1**, GC/MS components tentatively identified using the CHC mass spectral library search are reported. Those GC/MS components with fits of 70% and greater are considered tentatively identified. For each tentatively identified GC/MS component, the following information is require:

1) GC retention time of the component, minutes
2) Amount of the component, ng/l
3) HPLC fraction number of the component
4) A list of the best mass spectral library identifications (up to a total of ten identifications)
5) For each tentative identification:
   Library searching fit, %
   CAS number

This report often contains none or few tentatively identified components.

For **Effluent Report 2**, GC/MS components tentatively identified using the EPA/NIH/NBS mass spectral library search are reported. Those GC/MS components with fits of 70% and greater are considered tentatively identified. This report contains only those components with effluent concentrations of 100 ng/l and greater and with predicted tissue concentrations of **1 ug/kg and greater.** For each

tentatively identified GC/MS component, the following information is require:

1) GC retention time of the component, minutes
2) Amount of the component, ng/l
3) HPLC fraction number of the component
4) A list of the best mass spectral library identifications (up to a total of ten identifications)
5) For each tentative identification:
   Library searching fit, %
   CAS number

For **Effluent Report 3**, GC/MS components not tentatively identified, i.e., fits <70%, using the EPA/NIH/NBS mass spectral library search are reported. This report contains only those components with concentrations of 100 ng/l and greater. For each GC/MS component not tentatively identified, the following information is require:

1) GC retention time of the component, minutes
2) Amount of the component, ng/l
3) HPLC fraction number of the component
4) If the fit parameter is >25%, the two best mass spectral library identifications and for each tentative identification, their library searching fit, %, and CAS number.
5) If the fit parameter is <25%, compound is labeled as unknown.

For **Effluent Report 4**, GC/MS components tentatively identified using the EPA/NIH/NBS mass spectral library search are reported. Those GC/MS components with fits of 70% and greater are considered tentatively identified. This report contains only those components with effluent concentrations of 100 ng/l and greater and with predicted tissue concentrations of **less than 1 ug/kg**. For each tentatively identified GC/MS component, the following information is require:

1) GC retention time of the component, minutes
2) Amount of the component, ng/l
3) HPLC fraction number of the component
4) A list of the best mass spectral library identifications (up to a total of ten identifications)
5) For each tentative identification:
   Library searching fit, %
   CAS number

4.0 Sediment Analytical Procedure

In evaluating any report, the QA/QC report should be examined first since this information will provide a good indication of the quality of the analytical data. The QC requirement for recovery of the surrogates is 25% < % recovery < 120% and all three surrogates, d_{10}-biphenyl, $^{13}C_6$-1,2,4,5-tetrachlorobenzene, and $^{13}C_6$-

hexachlorobenzene, should have recoveries within this range. In addition, HPLC fraction 1 should contain d_{10}-biphenyl and should not contain $^{13}C_6$-1,2,4,5-tetrachlorobenzene or $^{13}C_6$-hexachlorobenzene. HPLC fraction 2 should contain $^{13}C_6$-1,2,4,5-tetrachlorobenzene and should not contain d_{10}-biphenyl or $^{13}C_6$-hexachlorobenzene. HPLC fraction 3 should contain $^{13}C_6$-hexachlorobenzene and should not contain $^{13}C_6$-1,2,4,5-tetrachlorobenzene or d_{10}-biphenyl.

If recovery data is out of range and/or the surrogates are in the wrong fraction, the analytical data should not be used. Provided acceptable recoveries and surrogate location in the HPLC fractions are obtained, the GC/MS chromatograms and the QA/QC data for HPLC resolution, GC resolution, etc. should be examined. This examination should take 5 to 15 minutes and will provide a good indication of the overall quality of the analysis.

In Table J-3, an illustrative report for the effluent analytical procedure is provided. After examining the recovery data for the surrogate chemicals, the GC/MS chromatograms for the procedural blank and the sample extract should be examined. There will be six GC/MS chromatograms, three for the procedural blank and three for the effluent sample as a result of the HPLC fractionation procedure. The GC/MS chromatograms for the HPLC fractions for the procedural blank should contain few peaks as illustrated in the report. Procedural blanks should always contain fewer peaks than the sample extract. Peaks in the GC/MS chromatograms should be narrow, e.g., 10 to 20 seconds base widths, and when large fat peaks exist, e.g., base widths exceeding 1 minute, overloading of the capillary column on the GC/MS has occurred. Efforts should be directed at determining why these large peaks exist in the sample extract. Sometimes these large peaks exist because the cleanup procedure for the extract was performed poorly, the capacity of the cleanup procedure was exceeded, and/or severe blank problems exist. In these situations, additional cleanup or extraction of another portion of the sediment sample should be requested.

The evaluation of the QC data for HPLC resolution, GC resolution, MS sensitivity, MS calibration, DFTPP performance, and precision are all straight forward. The evaluation criteria for these QC procedures are:

a) HPLC Resolution: Baseline resolution between the three performance chemicals, biphenyl, 1,3-diethylbenzene, and bibenzyl, must exist. The data in Table J-2 presents excellent resolution.

b) GC Resolution: One of two solutions can be used to evaluate GC resolution. For the solution containing β-BHC, -BHC, endrin ketone and d_{12}-chrysene, baseline resolution between the β-BHC/ -BHC and endrin ketone/d_{12}-chrysene pairs should exist. For the solution containing anthracene, phenanthrene, benz[a]anthracene, and chrysene, anthracene and phenanthrene should be baseline resolved and benz[a]anthracene and chrysene should be separated by a valley whose height is less than 25% of the average peak height

of these two compounds. Note, when using the first solution, fresh solution is required since endrin ketone degrades.

c) MS Sensitivity: For the 0.5 ppm calibration solution, the GC/MS peaks for the three surrogate chemicals should have a signal to noise ratio of 3 or more.

d) MS Calibration: For d_{12}-chrysene, the ratio of the abundance of the 241 mass/charge relative to that of 240 should be >15% and <25%.

e) DFTPP Performance: Comparison of the mass spectrum for DFTPP to the performance criteria is required. A DFTPP mass spectrum is presented in Table J-1.

f) Precision: A duplicate analysis should be performed with every 10 sample analyses. This data can be presented by using control charts or tabular reports. Precision data should not exceed 150%.

If any of the QC/QA procedures are not met, the analytical data is of questionable quality and its use is not recommended. Depending upon which factors are out of range, reanalysis of the sample extract (b,c,d,e) or extraction of another portion of the sediment sample (a,f) will be required. Some judgement will be required in this evaluation.

If the QA/QC Report is acceptable, the data in Reports 1, 2, and 3 can be used.

For **Sediment Report 1**, GC/MS components tentatively identified using the CHC mass spectral library search are reported. Those GC/MS components with fits of 70% and greater are considered tentatively identified. For each tentatively identified GC/MS component, the following information is require:

1) GC retention time of the component, minutes
2) Amount of the component, ug/kg
3) HPLC fraction number for the component
4) A list of the best mass spectral library identifications (up to a total of ten identifications)
5) For each tentative identification:
   Library searching fit, %
   CAS number

This report often contains none or few tentatively identified components.

For **Sediment Report 2**, GC/MS components tentatively identified using the EPA/NIH/NBS mass spectral library search are reported. Those GC/MS components with fits of 70% and greater are considered tentatively identified. This report contains only those components with concentrations of 5 ug/kg and greater. For each tentatively identified GC/MS component, the following information is require:

1) GC retention time of the component, minutes
2) Amount of the component, ug/kg
3) HPLC fraction number for the component
4) A list of the best mass spectral library identifications (up to a total of ten identifications)
5) For each tentative identification:
   Library searching fit, %
   CAS number

For **Sediment Report 3**, GC/MS components not tentatively identified, i.e., fits <70%, using the EPA/NIH/NBS mass spectral library search are reported. This report contains only those components with concentrations of 5 ug/kg and greater. For each GC/MS component not tentatively identified, the following information is require:

1) GC retention time of the component, minutes
2) Amount of the component, ug/kg
3) HPLC fraction number for the component
4) If the fit parameter is >25%, the two best mass spectral library identifications and for each tentative identification, their library searching fit, %, and CAS number.
5) If the fit parameter is <25%, compound is labeled as unknown.

Table J-1. Example Data for the Tissue Analysis Procedures

Tissue QA/QC Report

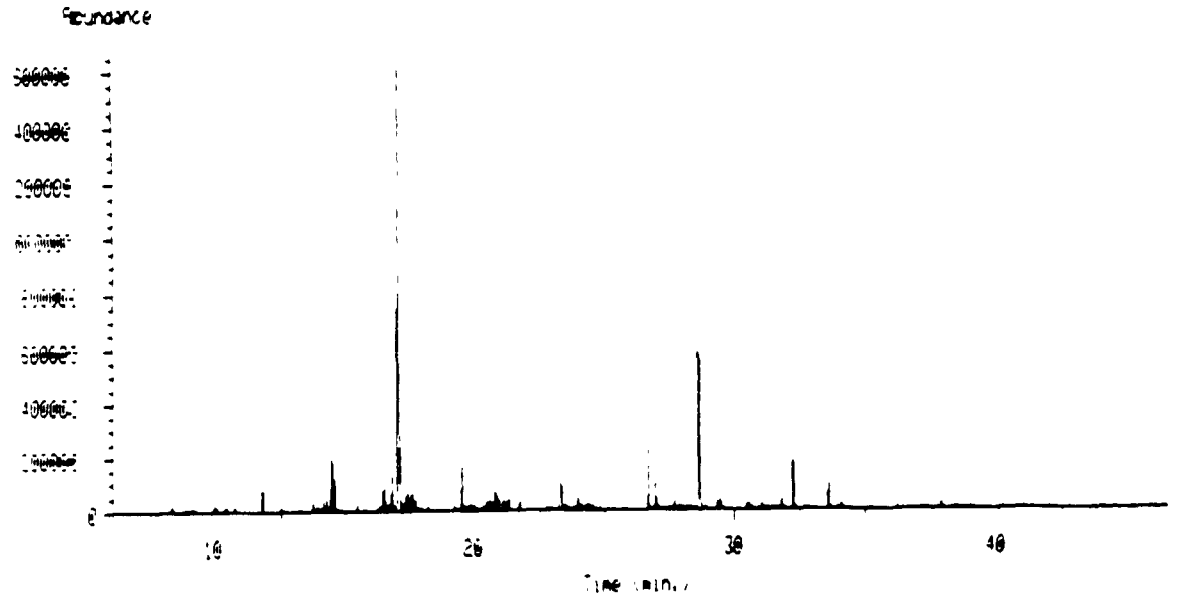
Percent lipid content of tissue sample

Tissue mass	20.0 g	
Residue	0.200 g	(10% of total extract)
Percent lipid =	10.0%	

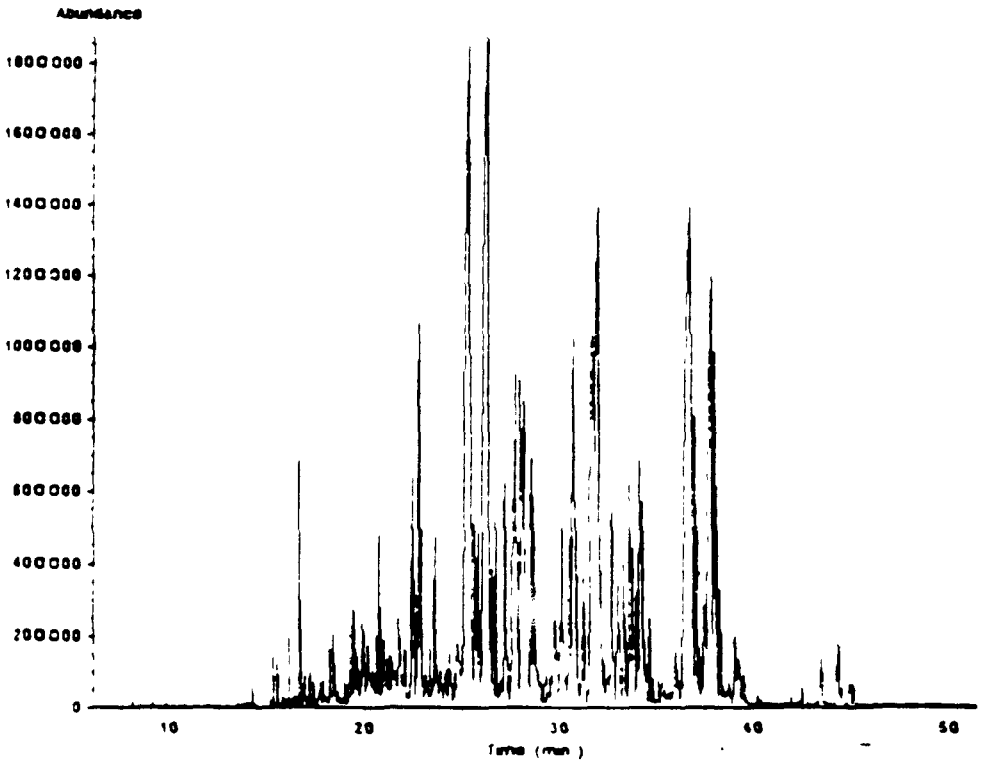
Recoveries

d_{10}-biphenyl	67%
$^{13}C_6$-1,2,4,5-tetrachlorobenzene	50%
$^{13}C_6$-hexachlorobenzene	46%

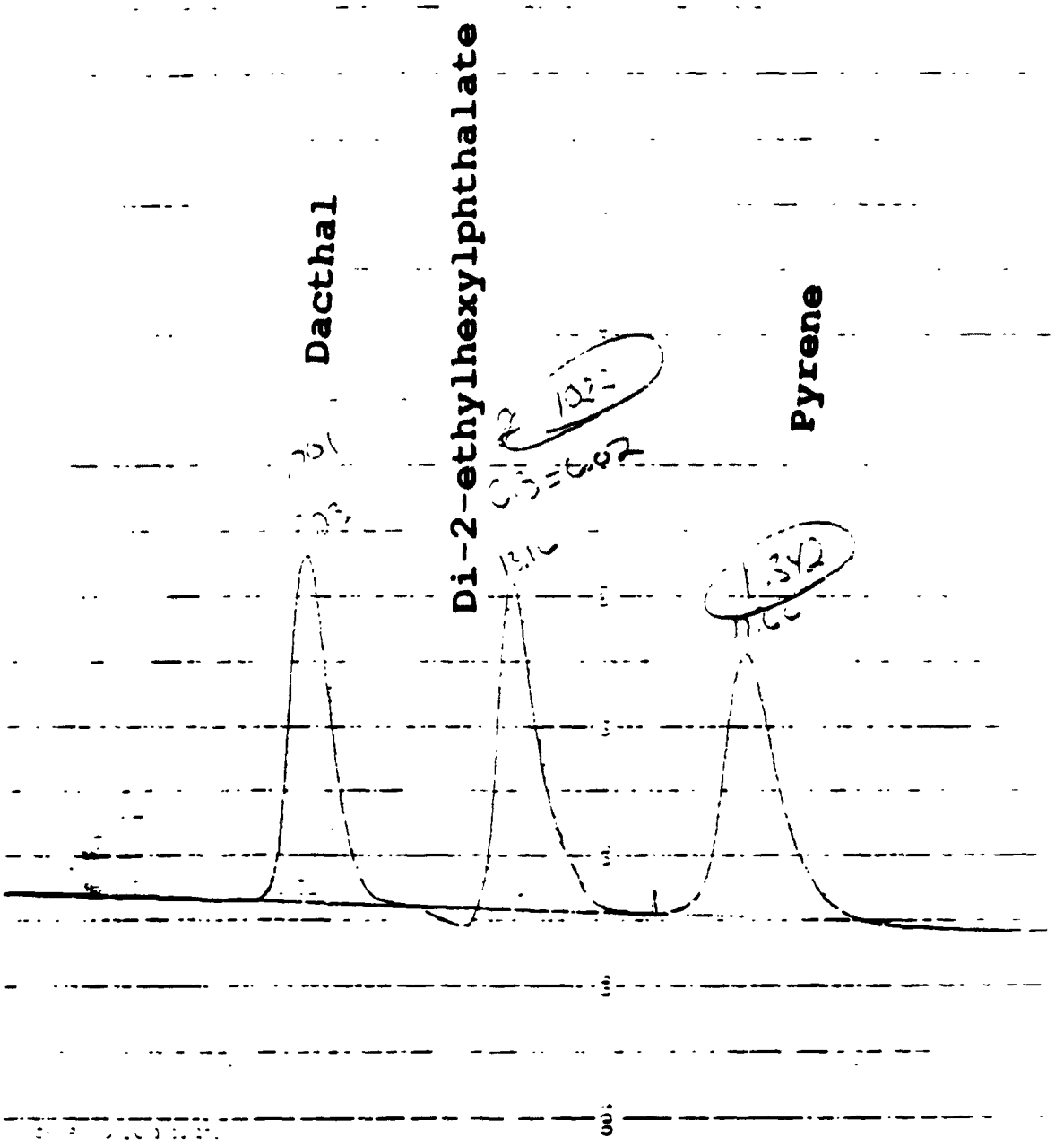
GC/MS Chromatogram
Procedural Blank



GC/MS Chromatogram
Sample Extract



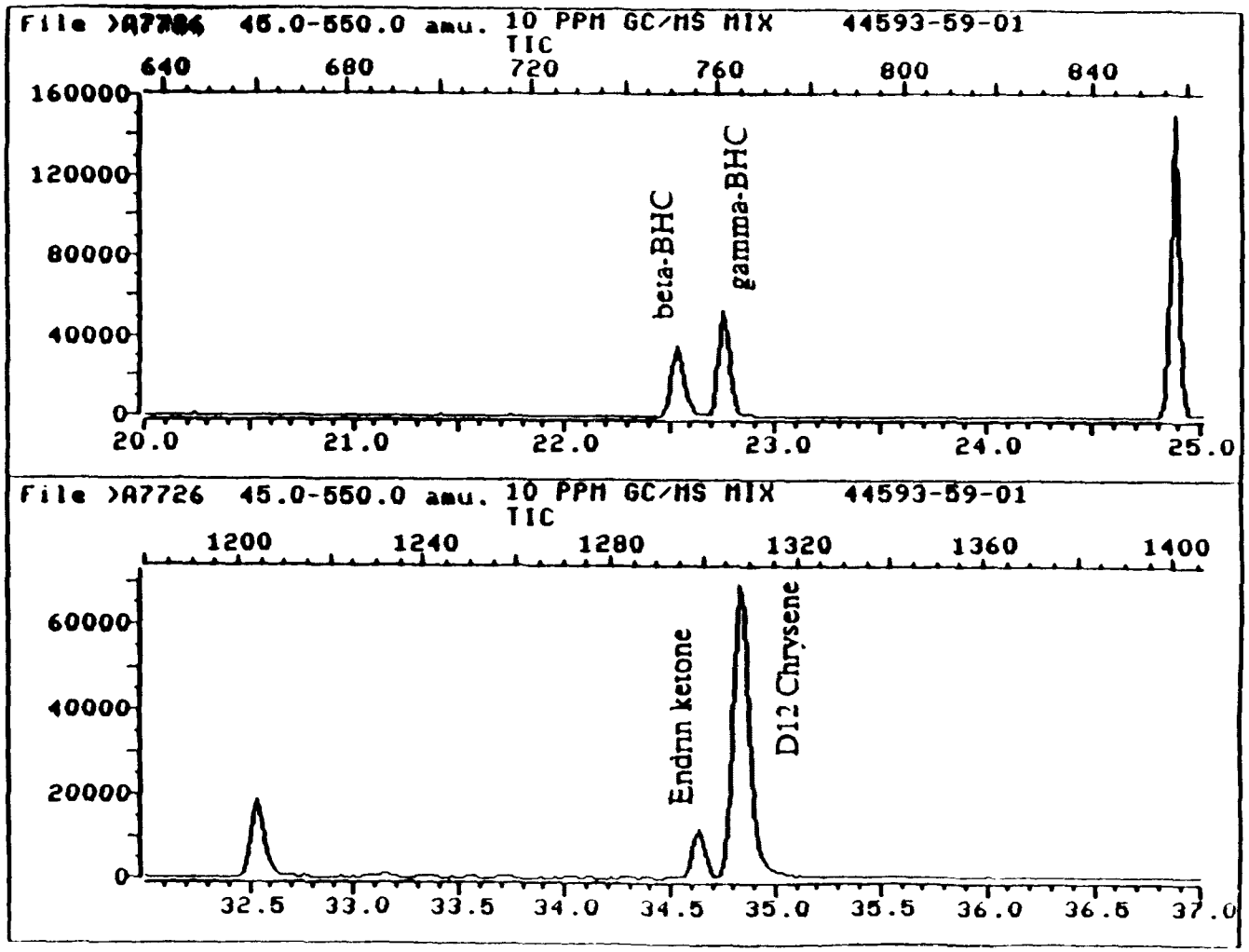
GPC Resolution Check



Silica Gel Performance Check

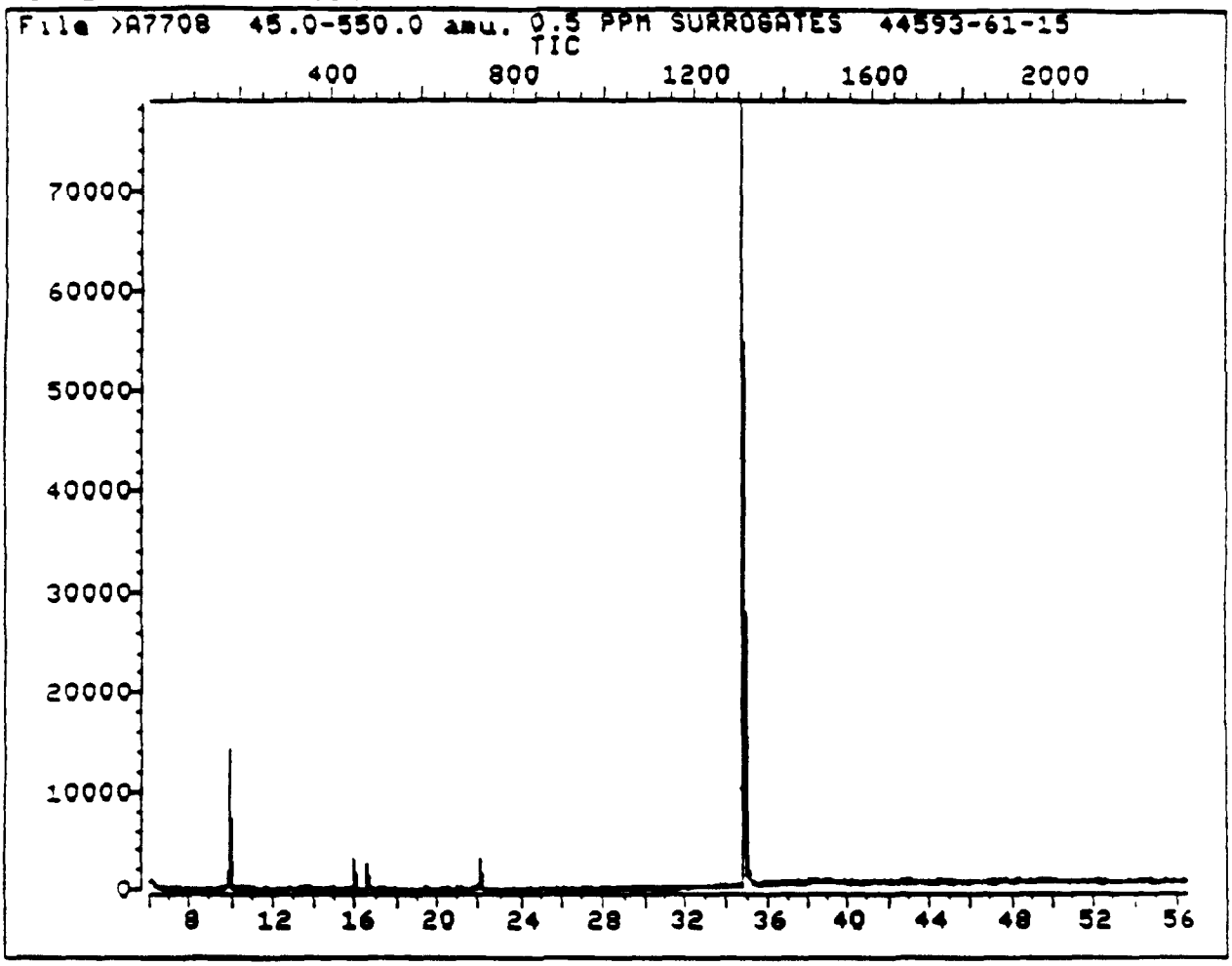
Date of Analysis	Silica Gel Lot #	Analyst	Chemical	Recovery %	Elution Solvent (MECL:HEX)
2/8/89	Baker A27345	JJ	Cholesterol Dieldrin	8 95	15:85
4/4/89	Baker A27345	LB	Cholesterol Dieldrin	5 90	15:85
6/5/89	Baker A27345	JJ	Cholesterol Dieldrin	9 97	15:85
7/8/89	Baker A27345	CJ	Cholesterol Dieldrin	4 92	15:85

GC/MS Resolution Check



GC/MS Sensitivity Check

TOTAL ION CHROMATOGRAM

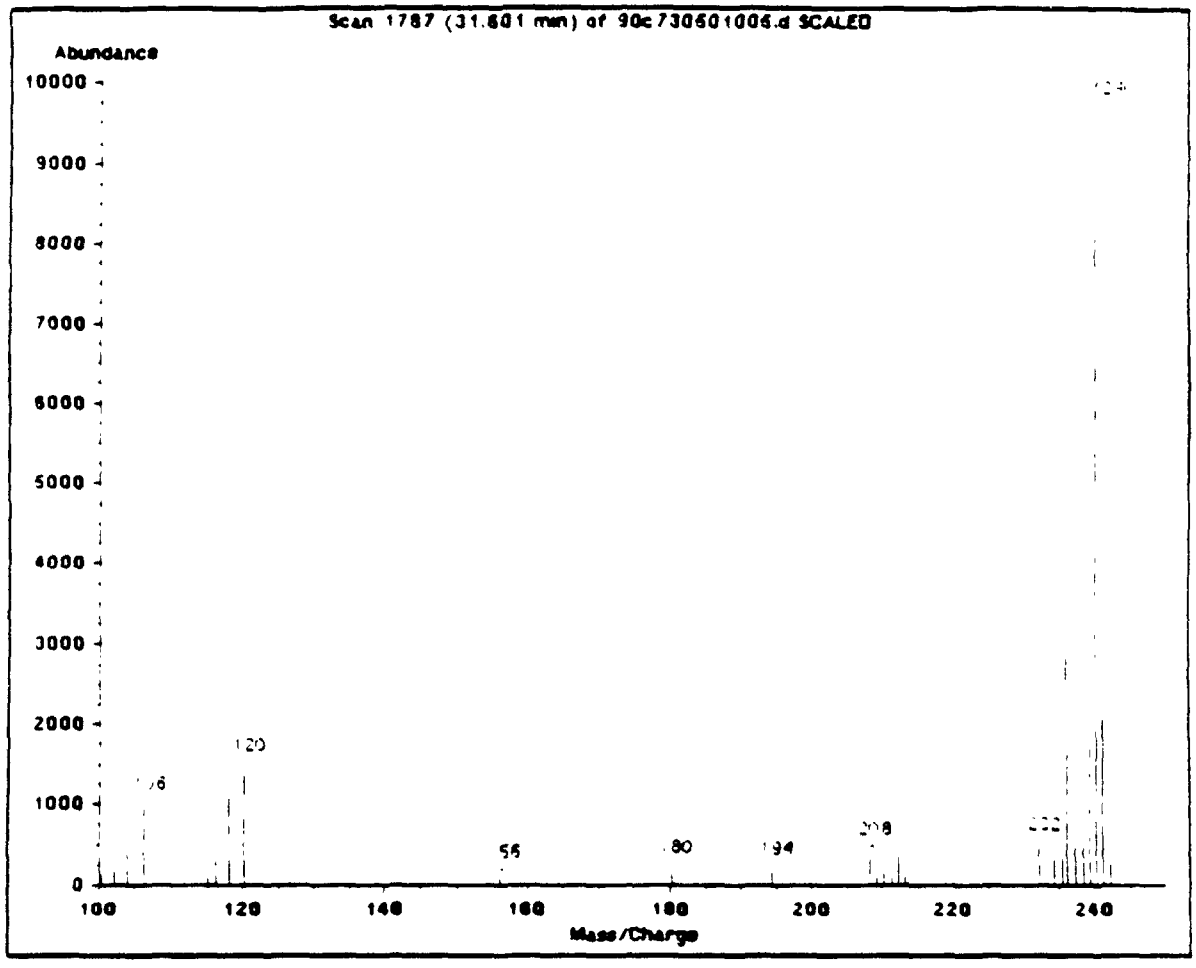


Data [redacted] >A7708::DO

Name: [redacted] PPM SURROGATES

Quant Output File: ^A7708::DO

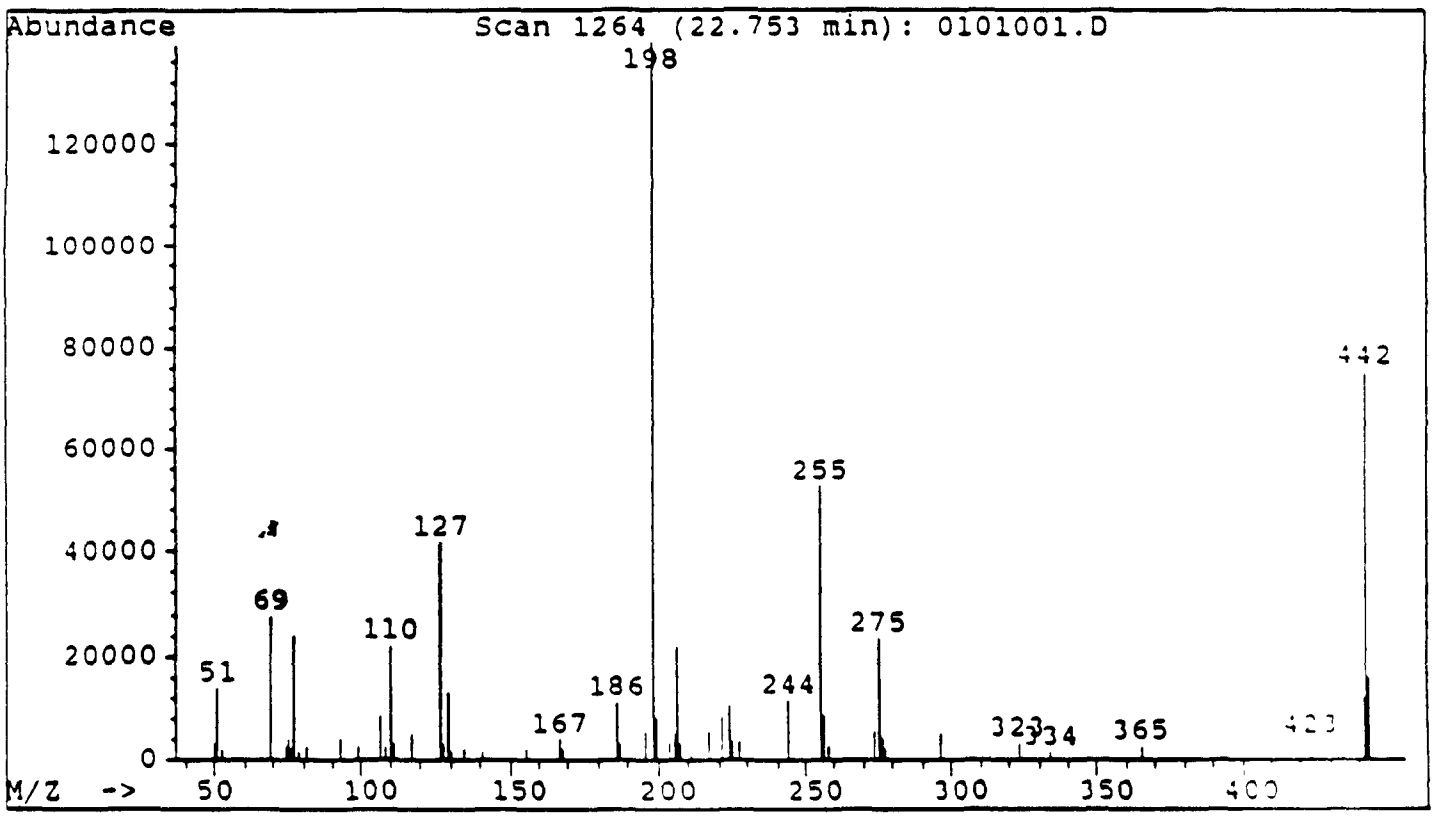
GC/MS Calibration Check



DFTPP Performance Check

DFTPP Ion Abundance Criteria.

m/z	Criteria	Measured
51	10-80% of the base peak	10%
68	≤2% of m/z 69	0%
70	≤2% of m/z 69	0%
127	10-80% of the base peak	30%
197	≤2% of m/z 198	0%
198	base peak or >50% of 442	100%
199	5-9% of m/z 198	6%
275	10-60% of the base peak	17%
365	>1% of base peak	2%
441	present and <mass 443	16%
442	base peak or >50% of m/z 198	54%
443	15-24% of m/z 442	21%



Precision Data

Sample ID	Date of Analysis	Surrogate Chemical	Surrogate Concentration		Precision
			ng/l		%
89C3015	2/8/89	Biphenyl	85	56	41
		TCB	67	28	82
		HCB	47	34	32
89C4115	3/1/89	Biphenyl	68	63	11
		TCB	50	46	8
		HCB	50	35	35
89C4185	3/25/89	Biphenyl	53	41	25
		TCB	46	52	12
		HCB	40	34	25

Table J-1 continued: Example Reports 1, 2, and 3 for the Tissue Analytical Procedures

Tissue Report 1: CHC Mass Spectral Library Tentative Identifications

Peak RT	Amount (ug/kg)	Fit %	Tentative Identification (CAS #)
12.602	31	99	Dieldrin (60-57-1)

Tissue Report 2: EPA/NIH/NBS Mass Spectral Library Tentative Identifications

Peak RT (minutes)	Amount (ug/kg)	Fit %	Tentative Identification (CAS #)
11.748	39	99	1,3-Butadiene, 1,1,2,3,4,4-hexachloro- (87-68-3)
14.889	32	90	1,1'-biphenyl (92-52-4)
		87	Naphthalene, 2-ethenyl- (827-54-3)
19.756	25	95	Phenanthrene (85-01-8)
		95	9H-Fluorene, 9-methylene (2523-37-7)
		94	Benzene, 1,1'-(1,2-ethynediyl)bis- (501-65-5)
		76	Anthracene (120-12-7)

Tissue Report 3: GC/MS Components not Identified by EPA/NIH/NBS Mass Spectral Library Search

Peak RT (minutes)	Amount (ug/kg)	Fit %	Tentative Identification (CAS #)
8.621	60	64	2-Pentene, 4,4-dimethyl-, (E)- (690-08-4)
		64	Pyridine, 2,3,4,5-tetrahydro- (505-18-0)
8.680	30	64	Benzene, 1,2,3-trichloro- (87-61-6)
		42	Benzene, 1,2,4-trichloro- (120-82-1)
19.756	25	-	Unknown

Table J-2. Example Data for the Effluent Analytical Procedures

Effluent QA/QC Report

Recoveries	HPLC Fraction Number 1	2	3
d_{10}-biphenyl	67%	nd[a]	nd
$^{13}C_6$-1,2,4,5-tetrachlorobenzene	nd	50%	nd
$^{13}C_6$-hexachlorobenzene	nd	nd	46%

[a]nd = not detected

For the effluent example, illustrative copies of the HPLC performance check and Reports 1, 2, 3, and 4 are presented on the following pages. The remaining portions of the effluent QA/QC report have previously been presented in Table J-1. Consultant Table J-1 for copies of the GC Resolution, MS Sensitivity, MS Calibration, DFTPP Performance, and Precision checks as well as the GC/MS chromatograms.

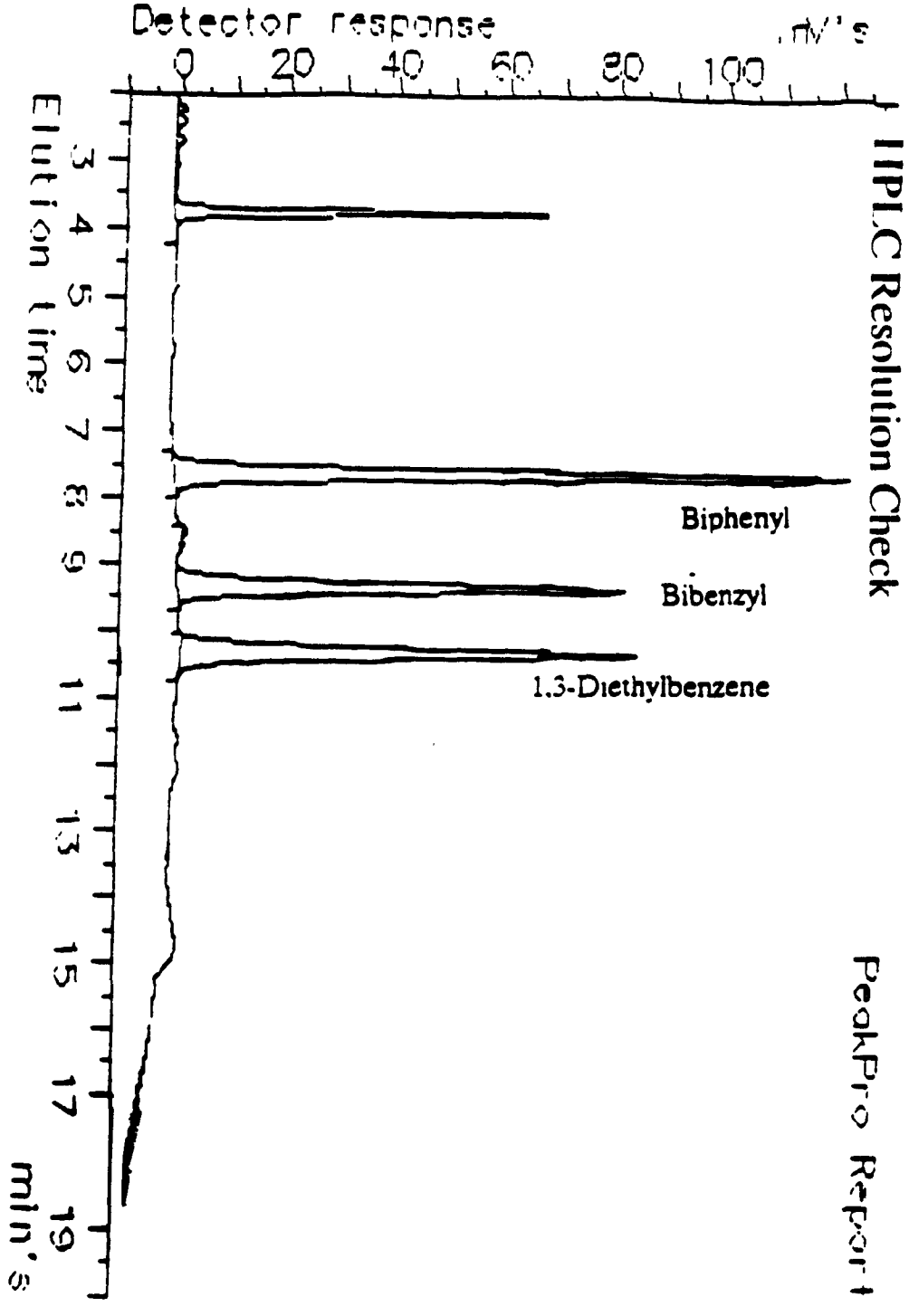

HPLC Resolution Check
PeakPro Report
Detector response
mV's
0
20
40
60
80
100
Elution time
3
4
5
6
7
8
9
11
13
15
17
19
min's
Biphenyl
Bibenzyl
1,3-Diethylbenzene

Table J-2 continued: Example Reports 1, 2, 3, and 4 for the Effluent Analytical Procedure

Effluent Report 1: CHC Mass Spectral Library Tentative Identifications

Peak RT	Amount (ng/l)	HPLC Fr #	Fit %	Tentative Identification (CAS #)
12.602	31	1	99	Dieldrin (60-57-1)

Effluent Report 2: EPA/NIH/NBS Mass Spectral Library Tentative Identifications

Peak RT (minutes)	Amount (ng/l)	HPLC Fr #	> 1 ppb	Fit %	Tentative Id. (CAS #)
11.748	391	2	Yes	99	1,3-Butadiene, 1,1,2,3,4,4-hexachloro- (87-68-3)
14.889	132	1	Yes	90	1,1'-biphenyl (92-52-4)
				87	Naphthalene, 2-ethenyl- (827-54-3)
19.756	250	2	Yes	95	Phenanthrene (85-01-8)
				95	9H-Fluorene, 9-methylene (2523-37-7)
				94	Benzene, 1,1'-(1,2-ethynediyl)bis- (501-65-5)
				76	Anthracene (120-12-7)

Dilution = 25%

Effluent Report 3: GC/MS Components not Identified by EPA/NIH/NBS Mass Spectral Library Search

Peak RT (minutes)	Amount (ng/l)	HPLC Fr #	Fit %	Tentative Identification (CAS #)
8.621	602	1	64	2-Pentene, 4,4-dimethyl-, (E)- (690-08-4)
			64	Pyridine, 2,3,4,5-tetrahydro- (505-18-0)
8.680	303	1	64	Benzene, 1,2,3-trichloro- (87-61-6)
		1	42	Benzene, 1,2,4-trichloro- (120-82-1)
19.756	250	3	-	Unknown

Table J-2 continued: Example Reports for Effluent Analytical Procedure

Effluent Report 4: EPA/NIH/NBS Mass Spectral Library Tentative Identifications

Peak RT (minutes)	Amount (ng/l)	HPLC Fr #	> 1 ppb	Fit %	Tentative Id. (CAS #)
No data with predicted tissue residues of < 1 ug/kg					

Dilution = 25%

Table J-3. Example Data for the Sediment Analytical Procedure

QA/QC Report

Percent Organic Carbon	15%	(dry mass basis)			
Percent Moisture	33%				
Recoveries		HPLC Fraction Number			
			1	2	3
d_{10}-biphenyl			67%	nd[a]	nd
$^{13}C_6$-1,2,4,5-tetrachlorobenzene		nd	50%	nd	
$^{13}C_6$-hexachlorobenzene			nd	nd	46%

[a]nd = not detected

For the sediment example, illustrative copies of the remaining portions of the sediment QA/QC report and the Reports 1, 2, and 3 have previously been presented in Tables J-1 and J-2. Consult Table J-1 for copies of the GC Resolution, MS Sensitivity, MS Calibration, DFTPP Performance, and Precision checks as well as the GC/MS chromatograms and Reports 1, 2, and 3. Consultant Table J-2 for a copy of the HPLC resolution check.

APPENDIX K

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APPENDIX K

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