Water

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Assessment and Control of Bioconcentratable Contaminants in Surface Waters

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GUIDANCE ON

ASSESSMENT AND CONTROL OF BIOCONCENTRATABLE CONTAMINANTS IN SURFACE WATERS

March 1991

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).

18661

Chapter 1

Approach to Assessment and Control of
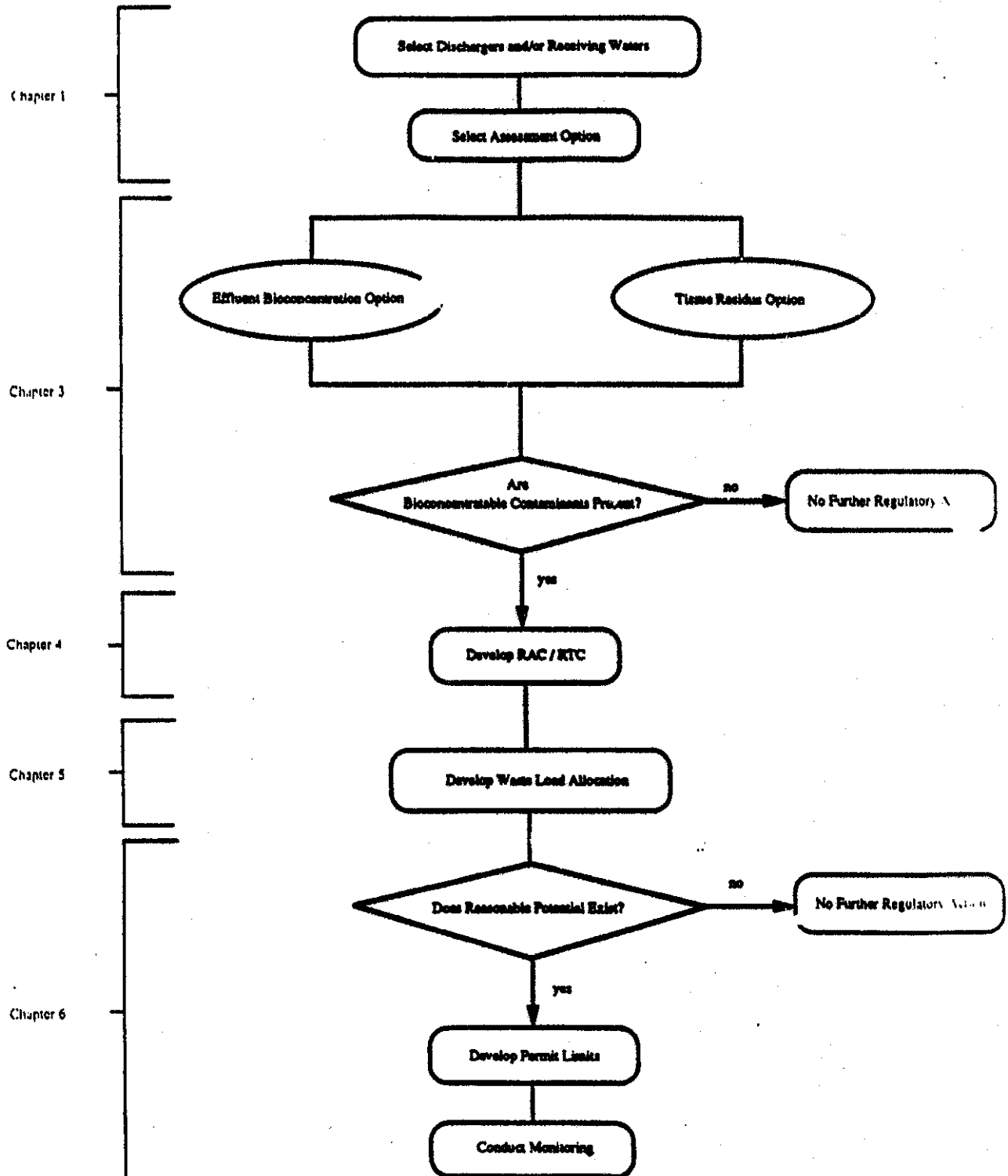## Bioconcentratable Contaminants

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.

I-1

18662

Figure 1.1
Procedure for Assessment and Control of Bioconcentratable Contaminants in Surface Waters

Chapter 1

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Select Dischargers and/or Receiving Waters

Select Assessment Option

Effluent Bioconcentration Option

Tissue Residue Option

Are Bioconcentratable Contaminants Present? — no → No Further Regulatory

yes

Develop RAC / RTC

Develop Waste Load Allocation

Does Reasonable Potential Exist? — no → No Further Regulatory

yes

Develop Permit Limits

Conduct Monitoring

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 does not address other types of chemicals known to bioconcentrate, such as metals (e.g. mercury, selenium) and organometals (e.g. tributlytin). Also,

I-3

18664

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

I-6

18667

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

I-7

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
nonresidue 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

I-8

18669

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 timeframes 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.

I-9

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:

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

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

II-2

are more water soluble and have shorter retention times on a
reverse phase high performace 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 chemcials, 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   (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 flowthrough 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, lifestage, 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 lifestage 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

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