



Toxicity Reduction Evaluation Guidance for Municipal Wastewater Treatment Plants

Toxicity Reduction Evaluation Guidance for Municipal Wastewater Treatment Plants

**Office of Wastewater Management
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Notice and Disclaimer

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Foreword

This document is intended to provide guidance to permittees, permit writers, and consultants on the general approach and procedures for conducting toxicity reduction evaluations (TREs) at municipal wastewater treatment plants. TREs are important tools for Publicly Owned Treatment Works (POTWs) to use to identify and reduce or eliminate toxicity in a wastewater discharge. TREs may be required by the discharger's National Pollutant Discharge Elimination System (NPDES) permit or through state or federal enforcement actions. Dischargers can use the guidance to evaluate the nature and sources of effluent toxicity before a TRE becomes a regulatory requirement. Whether the TRE is voluntary or mandated, this guidance can be helpful in preparing and executing a plan to address effluent toxicity.

This guidance describes the general approaches that have been successfully used in municipal TREs. Each TRE will be different; therefore, the strategy for conducting TREs should be tailored to address site-specific conditions. The components of a TRE may include the collection and review of pertinent data; an evaluation of the treatment facility to identify conditions that may contribute to effluent toxicity; identification of effluent toxicants using toxicity identification evaluation (TIE) procedures (USEPA 1991a, 1992a, 1993a, 1993b, 1996); location of the sources of toxicants and/or toxicity using chemical analysis or refractory toxicity assessment (RTA) procedures; and the evaluation, selection, and implementation of toxicity control measures. Dischargers are encouraged to develop a TRE plan that describes the initial components to perform in the TRE. Following initial testing, the results can be used to provide direction for further testing to identify the cause(s) and source(s) of toxicity and evaluate and select methods for toxicity control.

This document is an update of the municipal TRE protocol that was published in 1989 (USEPA, 1989a). Much experience has been gained since 1989, including the use of a number of freshwater and estuarine/marine species in acute and chronic TRE studies and the development of additional procedures for TIE and RTA studies. In most cases, the approaches and methods described in the municipal TRE protocol have been validated through TRE studies and other municipal TREs (Amato et al., 1992; Bailey et al., 1995; Botts et al., 1990, 1992, 1993, 1994; Collins et al., 1991; Fillmore et al., 1990; Lankford and Eckenfelder, 1990; Morris et al., 1990, 1992). Important lessons have been learned and this information has been incorporated in this guidance where possible. Additions to this guidance include considerations in evaluating the operation and performance of current publicly owned treatment works (POTW) technology, descriptions of current TIE procedures for acute and short-term chronic toxicity (USEPA 1991a, 1992a, 1993a, 1993b, 1996), updated methods for tracking sources of acute and chronic toxicity in POTW sewer collection systems, and additional recent case studies on acute and chronic TREs using freshwater and estuarine/marine species. Information is also provided on sampling requirements, equipment and facilities, quality assurance/quality control, and health and safety.

The updated TIE guidance procedures are important tools for conducting TREs including *Toxicity Identification Evaluation: Characterization of Chronically Toxic Effluents, Phase I* (USEPA, 1992a), *Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures, Second Edition* (USEPA, 1991a), *Marine Toxicity Identification Evaluation (TIE) Guidance Document, Phase I* (USEPA, 1996), *Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity* (USEPA, 1993a), and *Methods for Aquatic Toxicity Identification Evaluations: Phase III Toxicity Confirmation Procedures for Samples Exhibiting Acute and Chronic Toxicity* (USEPA, 1993b). The acute and chronic whole effluent toxicity testing manuals should also be reviewed during the TRE process (USEPA 1993c, 1994a, 1994b, 1995). These manuals describe procedures for the toxicity tests that are the core of the TREs.

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Acronyms, Abbreviations, and Symbols

AA	Atomic absorption
ACO	Administrative consent order
Ag	Silver
Alum	Aluminum sulfate
A/O [®]	Patented biological nutrient removal process
AQUIRE	Aquatic Information Retrieval Toxicity Data Base
APE	Alkylphenol ethoxylates
ASCE	American Society of Civil Engineers
ASM	American Society for Microbiology
ASTM	American Society for Testing and Materials
ATP	Adenosine triphosphate
BNR	Biological nutrient removal
BOD	Biochemical oxygen demand
BOD ₅	Five-day biochemical oxygen demand
BOD ₂₀	Twenty-day biochemical oxygen demand
°C	Degrees centigrade
CaCO ₃	Calcium carbonate
CADPR	California Department of Pesticide Registration
Cb	Concentration of specified analyte in filtrate of return activated sludge biomass
CCCS	Central Contra Costa Sanitary District
<i>C. dubia</i>	<i>Ceriodaphnia dubia</i> , cladoceran
Cd	Cadmium
Ce	Effluent concentration of the specified analyte
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
ChV	Chronic value, geometric mean of the no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC)
Ci	Influent concentration of the specified analyte
CO ₂	Carbon dioxide
COC	Chain-of-custody
COD	Chemical oxygen demand
Cpe	Concentration of specified analyte in primary effluent
Cr	Chromium
CSO	Combined sewer overflow
CTAS	Cobalt thiocyanate active substances
Cu	Copper
CuSO ₄	Copper sulfate
cu ft/lb	Cubic feet per pound
CV	Coefficient of variation
CVP	Chlorfenvinphos
Cw	Concentration of specified analyte in sewer wastewater
CWA	Clean Water Act
C18	Carbon-based resin used in solid phase extraction (SPE) columns to evaluate for the presence of nonpolar organic toxicants
<i>D. excentricus</i>	<i>Dendraster excentricus</i> , sand dollar
<i>D. magna</i>	<i>Daphnia magna</i> , cladoceran
<i>D. pulex</i>	<i>Daphnia pulex</i> , cladoceran
DMR	Discharge monitoring report

Acronyms, Abbreviations, and Symbols (continued)

DO	Dissolved oxygen
EC50	Effective concentration causing a 50% effect in the test species
ECOTOX	Ecotoxicology Database Retrieval System
EDTA	Ethylenediaminetetraacetate
ELISA	Enzyme-linked immunosorbent assay
EXTOXNET	Extension Toxicology Network
F/M	Food to microorganism ratio
F/Mb	Food to microorganism ratio
Fw	Flow concentration factor for RTA testing
g/L	Grams per liter
gpd/sq ft	Gallons per day per square foot
gpm/sf	Gallons per minute per square foot
GAC	Granular activated carbon
GC	Gas chromatography
GC/MS	Gas chromatograph/mass spectrophotometer
H&S	Health and safety
Hg	Mercury
HNO ₃	Nitric acid
H ₂ O ₂	Hydrogen peroxide
HPLC	High-performance liquid chromatography
HRT	Hydraulic retention time
ICP	Inductively coupled plasma spectrometry
ICp	Inhibition concentration causing a percent effect (p) in the test species (e.g., IC25, IC50)
IWC	Instream waste concentration
IWS	Industrial waste survey
L	Liter
LC50	Lethal concentration causing a 50% mortality in exposed test organisms
LRSA	Linden Roselle Sewerage Authority
LOEC	Lowest observed effect concentration
m ³ /m ² /day	Cubic meters per square meter per day
MBAS	Methylene blue active substances
MCRT	Mean cell residence time
MCWTP	Michigan City Wastewater Treatment Plant
mg/L	Milligram per liter
mg O ₂ /hr/g	Milligram dissolved oxygen per hour per gram
mg O ₂ /L/ gMLVSS/min	Milligram dissolved oxygen per liter per gram mixed liquor volatile suspended solids per minute
<i>M. bahia</i>	<i>Mysidopsis bahia</i> , mysid shrimp
mL	Milliliter
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
MS	Mass spectrometry
MSDS	Material safety data sheet
mgd	million gallons per day
NaCl	Sodium chloride
Na ₂ HPO ₄	Disodium phosphate
NaH ₂ PO ₄	Monosodium phosphate
NCDEM	North Carolina Division of Environmental Management

Acronyms, Abbreviations, and Symbols (continued)

NETAC	USEPA's National Effluent Toxicity Assessment Center
NH ₃	Un-ionized ammonia
NH ₄ ⁺	Ammonium ion
NH ₃ -N	Total ammonia as nitrogen
Ni	Nickel
NIOSH	National Institute of Occupational Safety and Health
NJDEP	New Jersey Department of Environmental Protection
NOEC	No observed effect concentration
NO ₃ -N	Nitrate as nitrogen
NJPDES	New Jersey Pollutant Discharge Elimination System
NPDES	National Pollutant Discharge Elimination System
NPO	Non-polar organic
NTIS	National Technical Information Service
O ₂	Oxygen
<i>O. mykiss</i>	<i>Oncorhynchus mykiss</i> , trout
OSHA	Occupational Safety and Health Administration
OUR	Oxygen uptake rate, a measure of the activity of activated sludge biomass
P	Phosphorus
PAC	Powdered activated carbon
Pb	Lead
PBO	Piperonyl butoxide, a metabolic blocking agent used to detect for the presence of organophosphate insecticides
pH	Logarithm of the reciprocal of the hydrogen ion concentration
PO ₄ -P	Orthophosphate as phosphorus
POTW	Publicly owned treatment works
<i>P. promelas</i>	<i>Pimephales promelas</i> , fathead minnow
PPCR	Pretreatment program chemical review
Qi	POTW influent flow rate
Qw	Sewer wastewater flow rate
QA/QC	Quality assurance/quality control
r value	Correlation coefficient
RAS	Return activated sludge
RBC	Rotating biological contactor
RCRA	Resource Conservation and Recovery Act
RREL	Risk Reduction Engineering Laboratory
RTA	Refractory toxicity assessment
RWQCP	Regional Water Quality Control Plant
SARA	Superfund Amendments and Reauthorization Act
SBOD ₅	Five-day soluble biochemical oxygen demand
SCOD	Soluble chemical oxygen demand
sf	square foot
SIC	Standard industrial classification
SNAP	Sewer network analysis program
SNH ₃ -N	Soluble ammonia – nitrogen
SO ₂	Sulfur dioxide
SOP	Standard operating procedures
SOR	Surface overflow rate
SOUR	Specific oxygen uptake rate

Acronyms, Abbreviations, and Symbols (continued)

<i>S. purpuratus</i>	<i>Strongylocentrotus purpuratus</i>
SP	Soluble phosphorus
SPI	Simulated plant influent
SPE	Solid phase extraction
SRT	Sludge retention time
SS	Suspended solids
SSO	Sanitary sewer overflow
SSUR	Specific substrate utilization rate
SVI	Sludge volume index
TBOD ₅	Total five-day biochemical oxygen demand
TCIP	Toxics control implementation plan
TCLP	Toxicity characteristic leaching procedure
TDS	Total dissolved solids
TIE	Toxicity identification evaluation
TKN	Total Kjeldahl nitrogen
TMP	Toxicity management program
TOC	Total organic carbon
TP	Total phosphorus
TRC	Total residual chlorine
TRE	Toxicity reduction evaluation
TSD	Technical Support Document
TSDF	Treatment, storage, and disposal facility
TSS	Total suspended solids
TU	Toxic unit
TUa	Acute toxic unit
TUc	Chronic toxic unit
µg/L	Microgram per liter
µm	Micron
UMVUE	Uniformly Minimum Variance Unbiased Estimator
USD	Union Sanitary District
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
UV-VIS	Ultraviolet – visible spectrophotometer
V _b	Volume of biomass for RTA testing
V _r	Reactor volume for RTA testing
V _{nb}	Volume of non-toxic biomass for RTA testing
V _{pe}	Volume of primary effluent for RTA testing
V _{sw}	Volume of synthetic wastewater for RTA testing
V _w	Volume of sewer wastewater for RTA testing
VSS	Volatile suspended solids
WEF	Water Environment Federation
WPCF	Water Pollution Control Federation (currently Water Environment Federation)
WWTP	Wastewater treatment plant
xg	Times gravity
Zn	Zinc
ZSV	Zone setting velocity

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Section 1 Introduction

Background

The Clean Water Act (CWA) (United States Federal Water Pollution Control Act Amendments, Public Law 92-500 of 1972) prohibits the discharge of “toxic pollutants in toxic amounts.” In the CWA, the mechanism for regulating discharges to the Nation’s waterways is the National Pollutant Discharge Elimination System (NPDES). Permits issued under NPDES may contain effluent limits and other requirements based on ambient water quality standards for the protection of aquatic life and human health. The water quality-based approach applies criteria for both chemical specific parameters and whole effluent toxicity to ensure that toxic pollutants are controlled and water quality standards are maintained (*Federal Register* 23868, 1989). This integrated approach to water quality protection is described in detail in USEPA’s *Technical Support Document for Water Quality-Based Toxics Control* (hereafter referred to as the TSD, 1991b).

“Whole effluent toxicity” refers to the results of acute and chronic aquatic toxicity tests used to monitor discharges to surface waters. Acute toxicity is a measure of primarily lethal effects that occur over a short period of time (i.e., 96 hours or less). Chronic toxicity refers to sublethal effects, such as inhibition of fertilization, growth, and reproduction that occur over a longer exposure period (e.g., 7 days). Acute and chronic effects to aquatic species are measured using standard procedures (40 CFR 136.3) as specified in NPDES permits. USEPA has published manuals that describe the toxicity test methods for freshwater and estuarine/marine organisms (USEPA 1993c, 1994a, 1994b, 1995). On October 26, 1995, USEPA promulgated a final rule under the CWA that adds whole effluent toxicity testing methods to the list of nationally applicable methods in 40 Code of Federal Regulations (CFR) Part 136. These methods can be

accessed electronically along with all other approved analytical methods on CD-ROM (USEPA, 1997).

Effluents from permitted facilities are monitored, and where a reasonable potential exists to exceed numeric toxicity criteria, NPDES permit limits for whole effluent toxicity are established (40 CFR 122.44(d)(1)(iv)). Whole effluent toxicity limits may also be established where there is reasonable potential to exceed a narrative toxicity criterion in the receiving water (40 CFR 122.44(a)(1)(v)). A toxicity reduction evaluation (TRE) may be used to identify and reduce or eliminate sources of effluent toxicity whether or not there are whole effluent toxicity limits in the NPDES permits. For example, where a permittee has no whole effluent toxicity limits in its current permit but discovers a toxicity problem, it may use a TRE to reduce or eliminate effluent toxicity, ensure that there is no reasonable potential that its discharge will exceed toxicity criteria and possibly obviate the need for whole effluent toxicity limits in a subsequent permit. On the other hand, if a permit contains whole effluent toxicity monitoring requirements or limits and unacceptable toxicity is observed, the permitting authority may require the permittee to perform a TRE through special conditions in the permit or an enforcement action.

The TSD defines a TRE as “a site specific study conducted in a stepwise process designed to identify the causative agents of effluent toxicity, isolate the sources of toxicity, evaluate the effectiveness of toxicity control options, and then confirm the reduction in effluent toxicity” (USEPA, 1991b). USEPA has developed procedures that can be used to conduct TREs (USEPA 1989a, 1989b, 1991a, 1992a, 1993a, 1993b, 1996).

This document represents the first update of USEPA’s *Toxicity Reduction Evaluation Protocol for Municipal*

Wastewater Treatment Plants (1989a). This guidance provides a general framework for conducting TREs at publicly owned treatment works (POTWs) and describes the available methods and procedures that experience to date has shown to be most useful. It is designed for POTW staff, consultants, and regulatory agency staff who are implementing TREs to identify and reduce or eliminate sources of effluent toxicity. Where possible, POTW staff are encouraged to use the guidance before the discharge of whole effluent toxicity is subject to regulatory review and action.

This guidance presents methods and procedures that are useful to:

- Develop and implement a TRE plan.
- Evaluate the results and data generated during the TRE.
- Develop a sound scientific and engineering basis for the selection and implementation of toxicity control methods.

This guidance supports the strategy described in the TSD (USEPA, 1991b) for integrated toxics control using whole effluent toxicity and pollutant specific limits. It is well recognized that while POTWs may achieve effluent limits for conventional pollutants, the discharge of effluent toxicity, volatilization of toxic materials, and contamination of sewage sludges can still occur. The focus of this guidance is the reduction of whole effluent toxicity at municipal wastewater treatment plants.

The methods and decision points that comprise a TRE are described in the context of an overall generalized approach. Each municipality must address regulatory issues and treatment operations that are unique to each POTW; therefore, not all components of this guidance will apply in every case. POTW staff may also select components to address specific questions about the causes and sources of effluent toxicity; however, the decision to choose a particular step should be based on technically sound information. Given the site-specific nature of TREs, POTW staff will need to develop a TRE plan that describes the overall approach and components of the guidance to be implemented.

In most cases, the approaches and methods described in the TRE protocol (USEPA, 1989a) have been validated by USEPA TRE research studies and other municipal TREs (Amato et al., 1992; Bailey et al., 1995; Botts et al., 1990, 1992, 1993, 1994; Collins et

al., 1991; Fillmore et al., 1990; Lankford and Eckenfelder, 1990; Morris et al., 1990, 1992). Appendix A provides the original case studies from the municipal TRE protocol (USEPA, 1989a). Additional examples of successful TREs are presented in Appendices B through H of this guidance. The TRE guidance includes information learned from these studies. Major changes include:

- Information on toxicants commonly found in POTW effluents and the conditions that influence their toxicity (Section 2).
- Considerations in evaluating the operation and performance of POTWs with respect to conditions that may contribute to effluent toxicity. Additional information is provided on operations review of biological nutrient removal (BNR) processes (Section 3).
- A brief description of the use of updated toxicity identification evaluation (TIE) procedures for acute and short-term chronic toxicity (Section 4). The reader is referred to USEPA's guidance on TIE procedures for further details (USEPA 1991a, 1992a, 1993a, 1993b, 1996).
- Refined step-by-step guidance for tracking sources of acute and chronic toxicity in POTW collection systems (Section 5).
- Additional recent TRE case studies that describe approaches for identifying the causes and sources of acute and chronic effluent toxicity and practical methods for toxicity reduction (Appendices B through H).

The methods and procedures described herein will continue to be updated and refined based on the results of further studies.

Professional judgment is required in selecting the appropriate steps for identifying toxicants and for evaluating options for controlling effluent toxicity. USEPA has developed TIE procedures to use as tools for TRE studies. These TIE manuals (USEPA 1991a, 1992a, 1993a, 1993b, 1996) describe procedures for characterization, identification, and confirmation of effluent toxicants. TIE procedures are a basic component of the municipal TRE and the USEPA guidance manuals should be obtained and reviewed prior to implementing a TRE. USEPA also has developed a generalized protocol for conducting industrial TREs (USEPA, 1989b).

TRE Goals and Objectives

It is the responsibility of POTW staff to conduct a TRE to identify and reduce or eliminate sources of effluent toxicity and to fully comply with applicable toxicity-based NPDES permit limits. The goal of the TRE may be to achieve compliance with a whole effluent toxicity limit; however, POTW staff are encouraged to use the guidance to evaluate effluent toxicity before it becomes a regulatory issue. The TRE goal and implementation schedule should be clearly defined with the regulatory authority as part of the preparation of the TRE plan. The regulatory authority will review the TRE plan and carefully monitor the progress of the TRE, providing direction as needed.

The following objectives may be defined to accomplish the TRE goal:

- Evaluate the operation and performance of the POTW to identify and correct treatment deficiencies contributing to effluent toxicity (e.g., operations problems, chemical additives, or incomplete treatment).
- Identify the compounds causing effluent toxicity.
- Trace the effluent toxicants and/or toxicity to their sources (e.g., industrial, commercial, or domestic).
- Evaluate, select, and implement toxicity reduction methods or technologies to control effluent toxicity (i.e., in-plant or pretreatment control options).

These objectives are applied to meet the TRE goal of compliance with regulatory requirements.

Components of the Municipal TRE

A generalized flow diagram for a TRE program is illustrated in Figure 1-1. A brief description of each major TRE component is presented below along with the section number in the guidance in which additional information is provided.

Information and Data Acquisition (Section 2)

The first step in a TRE is the collection of information and analytical data pertaining to effluent toxicity. This information includes data on the operation and performance of the POTW, such as plant design criteria and discharge monitoring reports (DMRs), and data from the POTW's pretreatment program, such as industrial waste survey (IWS) information, permit applications, and industrial user compliance reports. The POTW performance data and pretreatment program information are used in the second stage of

the TRE, as described below.

Facility Performance Evaluation (Section 3)

Operations and performance data can be reviewed in a POTW performance evaluation to indicate possible in-plant sources of toxicity or operational deficiencies that may be contributing to the effluent toxicity. If a treatment deficiency is causing noncompliance with conventional pollutant permit limits, studies should be conducted to evaluate treatment modifications before proceeding further in the TRE. These studies should evaluate the toxicity reduction that can be achieved by correcting treatment deficiencies. If plant performance is not a principal cause of toxicity or treatment modifications do not reduce effluent toxicity, a logical next step is to identify the cause(s) of toxicity using TIE procedures.

Pretreatment program data also can be gathered to prepare a data base on the wastewaters discharged to the POTW collection system. These data can be used in the latter stages of the TRE to assist in tracking the sources of toxicity and/or toxicants that are contributing to POTW effluent toxicity.

Toxicity Identification Evaluation (Section 4)

This section provides a brief overview of the TIE procedures. TIE procedures are available to evaluate the causes of acute and short-term chronic toxicity. When implementing a TIE, the reader is advised to consult USEPA's TIE procedures for freshwater species (1991a, 1992a, 1993a, 1993b) or estuarine/marine species (1996). The generic TIE protocol is performed in three phases: toxicity characterization (Phase I), toxicant identification (Phase II), and toxicant confirmation (Phase III). Phase I characterizes the types of effluent toxicants by testing the toxicity of aliquots of effluent samples that have undergone bench-top manipulation (e.g., pH adjustment, filtration). An evaluation of common POTW effluent toxicants such as ammonia, chlorine, and organophosphate insecticides may be included in Phase I. Phases II and III involve further treatments in conjunction with chemical analyses to identify and confirm the compounds causing effluent toxicity. USEPA's Phase II and III procedures (1993a, 1993b) for freshwater species are generally applicable for estuarine/marine species.

Toxicity Source Evaluation (Section 5)

A toxicity source evaluation involves the sampling and analysis of wastewaters discharged from sewer lines

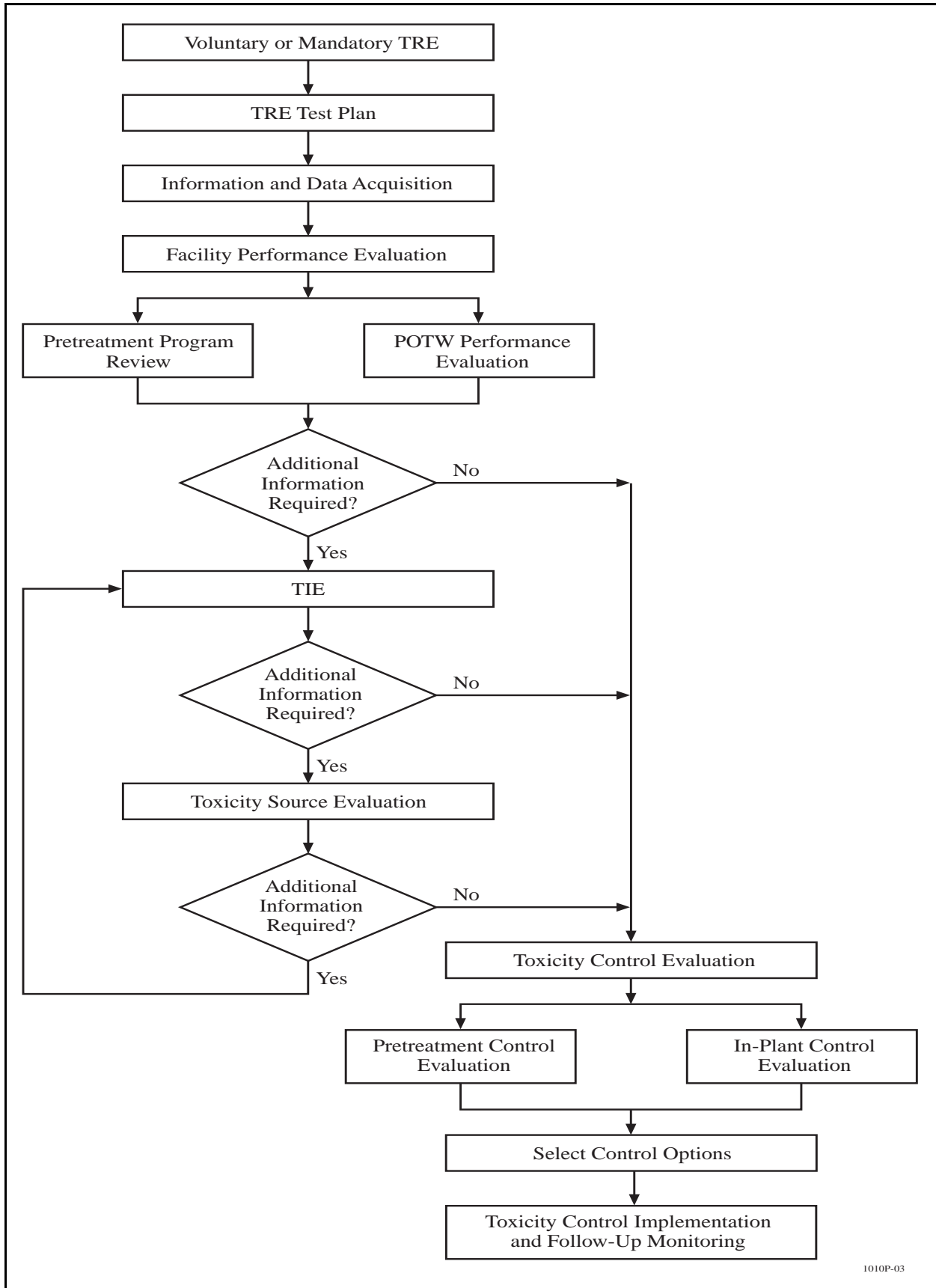


Figure 1-1. TRE flow diagram for municipal wastewater treatment plants.

and indirect dischargers such as industrial users and commercial facilities. Two types of source evaluation studies may be performed: chemical tracking or toxicity-based tracking.

Chemical-specific tracking is recommended when the POTW effluent toxicants have been identified and confirmed in the TIE, and can be readily traced to the responsible sewer dischargers. Toxicity tracking is used when TIE data indicate the type of effluent toxicant, but the specific toxicant(s) is not identified. Toxicity tracking involves treating the sewer samples in a bench-scale treatment simulation prior to toxicity measurements to account for the toxicity removal that is provided by the POTW.

The sampling strategy for toxicity source evaluations involves two tiers. Tier I focuses on sampling and analysis of the main sewer lines in the collection system. Tier II involves testing sewer lines and indirect dischargers upstream of the main lines identified as being toxic in Tier I. This tiered approach can be used to identify the contributors of toxicity and/or toxicants by eliminating segments of the collection system that do not contribute toxicity/toxicants.

Toxicity Control Evaluation (Section 6)

Using the results of each of the above TRE elements, alternatives for effluent toxicity reduction are evaluated and the most feasible option(s) is selected for implementation. Effluent toxicity may be controlled either through pretreatment regulations or in-plant treatment modifications or additions. In some cases, several control methods may be required to achieve the desired toxicity reduction. Selection of control options is usually based on technical and cost criteria.

If the toxicity source evaluation is successful in locating the sources that are contributing the POTW effluent toxicants, local limits can be developed and implemented. If in-plant control appears to be a feasible approach, treatability testing may be used to evaluate methods for optimizing existing treatment processes and to assess options for additional treatment.

Toxicity Control Implementation (Section 7)

The toxicity control method or technology is implemented and follow-up monitoring is conducted to ensure that the control method achieves the TRE objectives and meets permit limits.

Limitations of the TRE Guidance

This guidance describes procedures for evaluating and implementing controls for reduction of whole effluent toxicity. Procedures for the reduction of toxic pollutants in residuals, biosolids, and air emissions at POTWs are not discussed. The reader may consult the *Standards for the Use or Disposal of Sewage Sludge* (40 CFR Part 503) regarding the control of toxic materials in biosolids.

The municipal TRE guidance was developed based on the results and findings of TRE and TIE studies. The following limitations have been identified in these studies:

- Intermittent or ephemeral toxicity may be challenging to characterize using TIE/TRE procedures. In these cases, modifications to TRE procedures may be needed to achieve the best possible results (see Sections 4 and 5). Discussions with the regulatory authority also may help to identify the most appropriate approach for complying with effluent toxicity requirements.
- As described in this guidance, alternative procedures are available if traditional methods such as TIE testing are not successful. Additional TRE procedures, especially tools for toxicity source evaluations, have not been widely used, but may be helpful if careful consideration is given to their design and application.
- As more TRE studies are completed, more information is available on the feasibility and effectiveness of in-plant and pretreatment toxicity control options. Examples of TREs in which toxicity controls have been successfully implemented are provided in Appendices B, C, D, E, G, and H.
- The TRE guidance is designed to help public works managers select appropriate toxicity control approaches. As such, it does not discuss regulatory procedures that may be useful for assessing the need for, or compliance with, toxicity requirements, such as the determination of reasonable potential, dilution factors, and permit limits. The importance of these procedures in the evaluation of whole effluent toxicity is mentioned in Section 2 and is discussed more fully in USEPA's TSD (1991b).

Organization of the TRE Guidance

This guidance is organized according to the components of the TRE flow diagram shown in Figure 1-1.

Sections 1 through 7 describe the primary TRE elements noted above. Changes to the municipal TRE protocol (USEPA, 1989a) include more information on toxicants commonly identified in POTW effluents, suggestions for evaluating the effect of POTW operations on effluent toxicity, an overview of updated TIE procedures for acute and short-term chronic toxicity, and refined step-by-step procedures for tracking sources of acute and chronic toxicity in POTW collection systems.

Sections 8 through 11 provide information on the TRE requirements for quality assurance/quality control, health and safety, facilities and equipment, and sample collection and handling.

Sections 12 and 13 list the references and bibliography cited in this guidance.

Appendix A presents the original case histories (given in the municipal TRE protocol, USEPA, 1989a) along with commentary on how the TIE/TRE procedures have been updated to better address toxicity observed in future studies.

Appendices B through G provide new in-depth case examples of municipal TREs. These new examples include summaries of four chronic TRE studies and two acute TRE studies.

Appendix H is a new appendix that describes approaches for addressing effluent toxicity caused by organophosphate insecticides.

Appendix I describes a chemical-specific approach for TREs that may be applied in limited circumstances.

Appendix J is referenced in Section 5 (toxicity source evaluation) and presents an updated step-by-step procedure for tracking sources of toxicity in POTW collection systems.

Section 2

Information and Data Acquisition

Introduction

The first step in a TRE is to gather all information and data that may relate to effluent toxicity and that might prove useful in planning and conducting the TRE. This information can be categorized as POTW treatment plant data and pretreatment program data. The pertinent POTW information includes historical effluent toxicity data as well as information on the treatment plant's design capabilities, treatment performance, and operation and maintenance practices. Appropriate pretreatment program information consists of IWS data, industrial user permits, pretreatment inspection reports, and monitoring and compliance reports. If a pretreatment program is not in place, POTW staff may need to collect monitoring data on the POTW industrial users and, where necessary, appropriate controls should be considered to ensure good effluent quality.

Background information may provide insight into the nature of effluent toxicity and can be used to select the initial steps to take in the TRE. However, it is important *not* to draw conclusions about the causes and sources of toxicity in the beginning of the TRE unless corroborative testing is performed. A summary of information recommended for a TRE is provided in the following subsections.

Review of Effluent Toxicity Data

Information and data acquisition should include a careful review of recent effluent toxicity data. This review should be used to confirm the effluent toxicity results and the potential to cause adverse instream effects. The data also can be used to evaluate general toxicity characteristics, such as temporal variability, species sensitivity, and whether the toxicant(s) is fast or slow acting.

In some states, laboratories are required to be certified to perform toxicity tests. Toxicity test data reports also

may be reviewed by regulatory staff to confirm that the tests meet basic quality assurance/quality control (QA/QC) requirements. However, this is usually the exception; most state and regional regulatory agencies do not have certification programs for toxicity testing and reports may not be formally reviewed. As an initial step in the TRE, POTW staff should conduct an independent review of the toxicity test reports to verify the quality of the reported data, especially results that have triggered TRE requirements.

It often is beneficial to develop a profile on the characteristics of effluent toxicity using the available historical data. Information on toxicity variability, the relative sensitivity of various test species to the effluent, and effluent characteristics [e.g., pH, alkalinity, hardness, conductivity, total residual chlorine (TRC), and dissolved oxygen (DO)] can provide important clues about the nature of the toxicity. These characteristics can be compared to POTW and pretreatment information to help determine if effluent toxicity may be related to operational practices or sewer discharges. This information also can be used as part of the TIE (Section 4) to help identify the causes of effluent toxicity.

The data review may show that some test conditions such as pH may artificially change during testing. Typically, the pH of toxicity test solutions tends to drift upward over time, which can cause pH sensitive compounds such as ammonia and metals to exhibit toxicity. With the consent of the regulatory authority, it may be possible to modify the test procedures to control pH drift (USEPA 1993c and T. Davies, USEPA, Office of Water, *Memorandum on Clarifications Regarding Flexibility in 40 CFR Part 136 Whole Effluent Test Methods*, April 10, 1996). Modifications may also be allowed to better reflect the range of temperatures and hardness observed in the receiving water. Depending on the temperatures to be

considered, it is recommended to use a different test species rather than modify the recommended temperature range for a given test species.

Prior to a TRE, POTW staff or the regulatory agency may evaluate the “reasonable potential” for exceeding a toxicity-based water quality standard to determine if a permit limit is required. If there is reasonable potential to cause instream toxicity or contribute to an excursion above a narrative criterion, a statistical approach may be used to calculate a toxicity-based permit limit. This approach may also be applied during the course of a TRE to assess compliance with a permit limit or a water quality standard. For example, improvements in effluent quality resulting from the TRE could lower effluent toxicity to a point where there is no longer a reasonable potential to exceed the permit limit. Or, these improvements may reduce effluent variability. The reduced variability could result in a smaller coefficient of variation (CV), which would lessen the potential for excursions above the TRE goal. The reader is referred to USEPA’s TSD for details on these procedures (USEPA, 1991b).

The TSD also discusses the use of dilution, and particularly the use of high-rate diffusers, in achieving compliance with toxicity-based water quality standards. The dilution determination, if allowed by applicable regulations, is one of the first steps in characterizing the effluent for toxicity-based permitting (USEPA, 1991b). Public works managers, who are initiating a TRE, may choose to evaluate the application of appropriate mixing zone allowances to eliminate the potential for instream effects. A shoreline outfall, for instance, may not qualify for any dilution when determining an acute toxicity requirement. Use of a diffuser constructed in deeper water may allow the effluent to achieve sufficient dilution in the rapid-mixing, near-field area to meet permit requirements. Similar results may be obtained by moving an outfall from a small or intermittent stream, where no dilution is available under low flow conditions, to a larger permanent stream, with greater dilution. It should be noted, however, that less costly toxicity control approaches than outfall relocation may be identified during the course of the TRE. The process of selecting the most feasible and practical control option(s) is described in Section 6 of this guidance.

Toxicants Identified in POTW Effluents

As noted, the occurrence of toxicity and the treatment process operations are unique to each POTW; therefore, the causes of effluent toxicity are likely to be different for each case. Nonetheless, some toxicants have been identified at many POTWs. A list of toxicants commonly found in POTW effluents, the levels of concern, and potential sources is presented in Table 2-1. The levels of concern are to be used as a general guide, *not as absolute values*. Due to the site-specific nature of effluent toxicity, these data are intended only as background information to consider in the process of conducting a TRE. It is important to stress that a direct comparison of chemical concentrations to toxicity data reported in the literature often provides misleading information. The toxicity of effluent constituents is affected by many factors including the effluent matrix and toxicity test conditions. The most effective way to identify causes of effluent toxicity is by applying the TIE procedures, which are described in Section 4 of this guidance.

Some of the information that can be collected to help evaluate the contribution of the toxicants to effluent toxicity is provided in Table 2-1. Toxicity information on specific parameters can be obtained from USEPA’s *Aquatic Information Retrieval Toxicity Data Base* (AQUIRE, 1992), TIE manuals (USEPA 1991a, 1992a, 1993a, 1993b, 1996), EXtension TOXicology NETwork (EXTOXNET, 1998), peer-reviewed journal articles, and other sources. AQUIRE information can be obtained through the National Technical Information Service (NTIS) in Springfield, Virginia, or through several commercial vendors. USEPA’s Mid-Continent Ecology Division (Duluth, Minnesota) will be offering Internet access to AQUIRE data in early 1999 through its Ecotoxicology Database Retrieval System (ECOTOX). The EXtension TOXicology NETwork is currently available on the Internet at <http://ace.orst.edu/info/extoxnet/>. When reviewing toxicological data, it is important to ensure that the references for the data have been peer-reviewed and the values given are considered to be accurate.

In some cases, toxicological data may be presented in toxic units (TUs) instead of in lethal concentrations causing a 50% mortality in exposed test organisms (LC50) or no observed effect concentrations (NOEC). TUs are the inverse of the percent concentration

Table 2-1. Toxicants Identified in POTW Effluents

Toxicant Type	Level of Concern*	Potential Source	Information Needed to Assess Toxicity
Chlorine	0.05 to 1 milligram per liter (mg/L)	POTW disinfection process	TRC, temperature, and pH upon receipt of effluent sample and during toxicity test Toxicity degradation tests TIE Phase I tests†
Ammonia	5 mg/L as NH ₃ -N	Domestic and industrial sources POTW sludge processing sidestreams	Ammonia-nitrogen upon receipt of effluent sample pH, temperature, and salinity during toxicity test TIE Phase I tests†
Non-polar organics, such as organophosphate insecticides (e.g., diazinon, malathion, chlorpyrifos, and chlorfenvinphos)	Diazinon: 0.12–0.58 microgram per liter (µg/L) Chlorpyrifos: 0.03 µg/L	Homeowners, apartments, veterinarians, pest control, lawn care, and commercial businesses	High resolution analysis of organophosphate insecticides TIE Phase I tests†
Metals [e.g., cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb), nickel (Ni), zinc (Zn)]	Varies	Treatment additives in POTW Industrial users	Dissolved metals, effluent hardness (mg/L as CaCO ₃), and alkalinity upon receipt of sample TIE Phase I tests†
Other treatment chemical additives such as dechlorination chemicals and polymers	Varies	Disinfection, dechlorination, sludge processing, and solids clarification in the POTW	Vendor information on toxicity of products Dosage rates Effluent characteristics that affect toxicity (e.g., pH) TIE Phase I tests†
Surfactants	Varies	Industrial users	Methylene blue active substances (MBAS) and cobalt thiocyanate active substances (CTAS) TIE Phase I tests†
Total dissolved solids (TDS)	1,000–6,000 µhos/cm depending on endpoint, species tested, and TDS constituents	Industrial users Sludge processing sidestreams	TDS, ion analysis, and anion/cation balance TIE Phase I tests†

* As referenced by USEPA (1992a) and D. Mount (personal communication, AScI Corp, Duluth, Minnesota, 1991) for chlorine; USEPA (1992a) for ammonia; TRAC Laboratories (1992), Bailey et al. (1997) for diazinon and chlorpyrifos; and USEPA (1992a) for TDS.

† The contribution of effluent constituents such as chlorine, ammonia, organic compounds, metals, and TDS to effluent toxicity can be most effectively evaluated using the TIE Phase I procedures described in Sections 3 and 4 of this guidance and the USEPA manuals (1991a, 1992a, 1996).

values and are calculated by dividing 100% by acute or chronic percent effluent values or chemical concentration data. For example, a chronic TU (TU_c) of 2 is equivalent to an NOEC value of 50% effluent (i.e., 100%/50%). Likewise, if the LC50 of a compound is 20 µg/L, an effluent sample with 100 µg/L contains 5 acute TU (TU_a) of the compound (i.e., 100 µg/L/20 µg/L). TU values are helpful in understanding the relative contribution of toxicants to effluent toxicity and are often used in interpreting TIE data (Section 4). For example, if one of two compounds is contributing to effluent toxicity (e.g., 4 TU_c of compound A and 1.5 TU_c of compound B), it may be possible to focus on controlling the major toxicant if compliance with the permit limit (e.g., 3 TU_c) can be achieved. However, consideration should be given to possible antagonistic effects between the toxicants such that removal of one toxicant may cause the other toxicant to exhibit greater toxicity. An overview of this is provided in Section 4 and the TIE manuals (USEPA 1991a, 1992a, 1993a, 1993b, 1996) provide thorough guidance.

POTW Design and Operations Data

POTW design and operations information can indicate possible in-plant sources of toxicity or operational problems that might be contributing to treatment interferences and the pass through of toxicity. In the beginning of the TRE, it is often helpful to briefly review the operations and performance of the major unit treatment processes. Notes can be added to POTW data base about initial impressions and potential problem areas that should be investigated further in the POTW Performance Evaluation (see Section 3).

The types of POTW data to be gathered include:

- Background information on treatment plant design and operation.
- Data routinely collected for NPDES DMRs and treatment process control.
- Existing data on potential effluent toxicants, including chlorine, ammonia, organophosphate insecticides, surfactants, metals, and treatment additives (e.g., polymers, chlorine, dechlorination chemicals).

A list of useful POTW data is provided in Table 2-2.

The POTW data can be compared to the profile on effluent toxicity characteristics to determine if toxicity

may be related to operation and performance. Several questions can be posed, including:

- Is toxicity apparent during certain operational events, such as when treatment upsets are observed, when treatment units are taken offline for maintenance, or as a result of other operating practices (e.g., excess chlorine addition)?
- Does toxicity exhibit a weekly, monthly, or seasonal pattern? For example, if the POTW is operated to achieve seasonal ammonia removal, is toxicity present in the period when ammonia removal is not practiced? What process control parameters may be related to toxicity? Is toxicity apparent with changes in hydraulic and pollutant loadings to the primary sedimentation process, changes in biological treatment parameters [e.g., mixed liquor suspended solids (MLSS) concentration, DO concentration, sludge volume index (SVI), mean cell residence time (MCRT)], changes in filtration rates in filters, or changes in application rates of chlorine and dechlorinating agents?
- Is toxicity apparent when the type and dose of treatment additives change? For example, did toxicity occur when a different polymer or other coagulant/flocculent aid was used?

In the beginning of the TRE, emphasis should be placed on effluent concentrations of ammonia and chlorine, which are common toxicants in POTW effluents (USEPA 1991a, 1992a, 1993a, and 1993b). The toxicity of ammonia is dependent on effluent characteristics such as the pH, temperature, and salinity of the sample, as well as the sensitivity of the species being tested. Therefore, it will be difficult to determine the toxicity of ammonia based solely on a comparison of literature values to effluent concentrations. Likewise, the toxicity of chlorine will depend on the form of chlorine, which may be in the free form as chlorine, hypochlorous acid, or hypochlorite ion, or in the combined form as chloramines or nitrogen trichloride. The sum of the free and combined chlorine, termed total residual chlorine or TRC, is matrix dependent. Chloramines, which are formed by chlorine combining with ammonia, can be more toxic than free chlorine (AQUIRE, 1992).

Assessments of the contribution of ammonia, chlorine, and other compounds to effluent toxicity can be made using Table 2-1 as a guide. As stated in Table 2-1, if

Table 2-2. Example POTW Design and Operation Data

1. NPDES permit requirements
 - a. Effluent limitations
 - b. Special conditions
 - c. Monitoring data and compliance history
 - d. Dilution studies or modeling results
2. POTW design criteria
 - a. Hydraulic loading capacities
 - b. Pollutant loading capacities
 - c. Biodegradation kinetics calculations and assumptions
3. Influent and effluent pollutant data
 - a. Ammonia
 - b. Residual chlorine
 - b. Other pollutants of concern such as non-polar organic compounds (e.g., organophosphate insecticides), metals, and TDS (see Table 2-1)
 - c. Conventional pollutant data, including five-day biochemical oxygen demand (BOD₅), chemical oxygen demand (COD), total organic carbon (TOC), total suspended solids (TSS), volatile suspended solids (VSS), total Kjeldahl nitrogen (TKN), ammonia-nitrogen (NH₃-N), total phosphorus (TP), orthophosphate (PO₄-P), and nitrate-nitrogen (NO₃-N), to evaluate treatment performance
 - d. Parameters, including pH, hardness, and alkalinity, to evaluate the toxicity of suspect compounds (see Table 2-1)
4. Process control data
 - a. Chemical usage for each treatment process (e.g., coagulants for primary sedimentation, lime for biological treatment, polymers for tertiary clarification; see Table 2-1)
 - b. Process control data for primary sedimentation (i.e., hydraulic loading capacity and BOD₅ and TSS removal)
 - c. Process control data for activated sludge [e.g., food to microorganism (F/M) ratio, MCRT, MLSS, sludge yield, removal efficiency of BOD₅, COD, TKN, NH₃-N, TP, PO₄-P, NO₃-N, and other pollutants specified in the permit].
 - d. Process control data for secondary and tertiary clarification [e.g., hydraulic and solids loading capacity, SVI, sludge blanket depth]
 - e. Number of process units online and number offline for maintenance
5. Operations Information
 - a. Reports on previous operation and maintenance evaluations, including engineering studies and USEPA and state compliance inspections
 - b. Operating logs
 - c. Standard operating procedures
 - d. Operation and maintenance practices (e.g., filter backwash procedures)
6. Process sidestream characterization data
 - a. Chemical usage for sludge processing, including thickener, digester, and dewatering processes
 - b. Pollutant data for sludge processing sidestreams, including ammonia, metals, organophosphate insecticides, and TDS (see Table 2-1)
 - c. Incinerator scrubber waste stream, including data on possible formation of cyanide (see discussion in Section 3)
 - d. Tertiary filter backwash
 - e. Cooling water
7. Wastewater bypass, combined sewer overflow (CSO), and sanitary sewer overflow (SSO) for bypasses or overflows that are discharged to the POTW effluent
 - a. Frequency
 - b. Volume

chlorine is suspected of contributing to toxicity, TRC should be measured when the sample arrives and when toxicity tests are initiated because chlorine usually dissipates rapidly. If ammonia is a concern, conditions that affect its toxicity, including sample pH, should be carefully monitored during toxicity tests. This information is necessary to determine the concentration of the toxic un-ionized form of ammonia (NH₃) in the toxicity test. In addition to toxicity data, USEPA's AQUIRE data base (1992) includes information on the conditions of the toxicity test that may have influenced the reported toxicity of chemicals of concern. However, not all conditions may be recognized or reported, which may limit the utility for TIE tests.

Surfactants also have been identified as toxicants in POTW effluents (Diehl and Moore, 1987; Ankley and Burkhard, 1992; Botts et al., 1994). These studies focused on characterizing the type or source of surfactants rather than trying to identify the toxic surfactant compound because analytical methods are not readily available to detect and quantify surfactant compounds in complex effluents. Municipal effluents contain numerous substances that interfere with surfactant analysis. Also, surfactants are actually mixtures of many homologues and oligomers; therefore, the composition and toxicity of surfactants is complex and often variable (USEPA, 1993a). One exception is a class of surfactants, referred to as alkyl phenol ethoxylates (APEs), that can be analyzed by a gas chromatograph/mass spectrometer (GC/MS) (Giger et al., 1981). Also, it may be helpful to characterize surfactants by the nature of their polar segment; surfactants may be classified as nonionic, anionic, cationic, and amphoteric. Many surfactants tend to sorb to C18 resin and analyses of the methanol extracts from solid phase extraction (SPE) columns can help to indicate the type of surfactant causing toxicity (USEPA, 1993a). The American Public Health Association (1995) describes methods for determining anionic surfactants as MBAS and nonionic surfactants as CTAS.

The contribution of effluent constituents such as ammonia and chlorine to effluent toxicity can be most effectively evaluated using the TIE procedures described in Sections 3 and 4. Non-polar organic compounds (such as organophosphate insecticides), metals, surfactants, and TDS also can be effectively evaluated using these procedures. In Phase I of the TIE, various sample manipulations and toxicity tests

are performed to determine how toxicity is affected by removing or isolating a particular group of toxicants. These procedures establish a cause and effect relationship between toxicants and whole effluent toxicity. If a toxicant is indicated through Phase I testing, additional TIE procedures can be applied to identify (Phase II) and confirm (Phase III) the toxicant (see Section 4).

Treatment additives can be screened by obtaining toxicity data from product vendors or by performing toxicity tests on samples that have been treated using typical chemical dosages. If toxicity tests are performed, it is important to simulate the conditions occurring in the treatment process where additives are being used because some portion of the additives is usually removed in the treatment process. For example, polymers are largely bound with suspended solids in the wastewater being treated and only minor amounts may pass through in the final effluent (Hall and Mirinda, 1991).

Once the toxicants are identified, the POTW data will be useful in evaluating and selecting in-plant toxicity control options (see Section 6).

Pretreatment Program Data

Pretreatment program information may provide evidence that can be used to identify sources of toxicants or toxicity in the wastewater collection system. For this reason, pretreatment data should be briefly reviewed in the beginning of the TRE. As an initial step, pretreatment data can be compared to the profile on POTW effluent toxicity characteristics to determine if toxicity may be related to a particular type of discharge. This review may attempt to answer several questions, including:

- What changes in POTW influent characteristics may be observed during toxic periods (e.g., pH, alkalinity, suspended solids, hardness, conductivity, DO, color)? Also, does toxicity occur during changes in hydraulic and pollutant loadings to the POTW? Can these characteristics be related to certain types of discharges?
- Does toxicity occur when treatment upsets are observed at the POTW? Can the upsets be related to a particular discharge(s)?
- Does toxicity exhibit a weekly, monthly, or seasonal pattern that may be related to production schedules of certain industries? For example, is

toxicity observed when an industry is manufacturing a particular type of product? Also, does toxicity abate when the industry is shutdown for maintenance or holidays?

- If the POTW accepts hauled wastes, is toxicity apparent when a particular hauler delivers wastes?

Appropriate pretreatment program information to review includes the data on the industrial users of the POTW [e.g., industrial manufacturers, Resource Conservation and Recovery Act (RCRA) waste disposers, and Comprehensive Environmental Response, Compensation, and Liability Act

(CERCLA) dischargers] and the toxic pollutant data on the POTW waste streams. A list of suggested pretreatment data is shown in Table 2-3.

The POTW pretreatment program data can be reviewed as part of a Pretreatment Program Review (described in Section 3). The summarized data may be useful in locating the sources of toxicants identified in the TIE (see Section 4). In cases in which effluent toxicants are not identified, the pretreatment program data can be used to develop a sampling and analysis program to track sources of toxicity in the collection system (see Section 5).

Table 2-3. Example Pretreatment Program Data

1. POTW influent and effluent characterization data
 - a. Toxicity
 - b. Priority pollutants
 - c. Hazardous pollutants
 - d. Pollutants listed in Superfund Amendments and Reauthorization Act (SARA) Title 313
 - e. Other chemical-specific monitoring results (e.g., industry raw materials and products)
2. Sewage residuals characterization data (e.g., raw, digested, thickened, and dewatered sludge, composted biosolids, and incinerator ash)
 - a. Toxicity characteristic leaching procedure (TCLP)
 - b. Chemical data
3. IWS
 - a. Information on industrial users with categorical standards or local limits and other significant non-categorical industrial users
 - number of industrial users
 - discharge flow
 - chemical usage
 - b. Standard Industrial Classification (SIC) code
 - c. Wastewater flow
 - d. Types and concentrations of pollutants in the discharge
 - e. Products manufactured
 - f. Description of pretreatment facilities and operating practices
4. Industrial User Permits
 - a. Pretreatment standards
 - categorical standards
 - local limits
 - prohibited discharge standards
 - b. Monitoring requirements
5. Annual pretreatment program report
 - a. Schematic of sewer collection system
 - b. Industrial user monitoring and inspection data collected by POTW staff
 - discharge characterization data
 - spill prevention and control procedures
 - hazardous waste generation
 - c. Industrial user self-monitoring data
 - discharge characterization data
 - flow measurements
 - description of operations
 - compliance schedule (if out of compliance; e.g., notice of slug loading)
6. Headworks analysis for local limits
7. Industrial user compliance reports
8. Waste hauler monitoring data and manifests
9. RCRA reports [if the POTW is considered a hazardous waste treatment, storage, and disposal facility (TSDF)]
 - a. Hazardous waste manifests
 - b. Operating record
 - c. Biennial report
 - d. Unmanifested waste report
10. CERCLA reports (if the POTW accepts wastes from a superfund site)
 - a. Preliminary site assessment
 - b. Site investigations
 - c. Remedial investigations
 - d. Feasibility studies
 - e. CERCLA decision documents
11. Information on POTW treatment interferences (e.g., biological process inhibition); example data include:
 - a. Evidence of slug loadings
 - b. Decreased pollutant removal
 - c. Decreased oxygen uptake rates (OURs), SVI, and sludge yield in biological treatment process
 - d. Increased requirement for chemical usage (e.g., chlorine, coagulants, flocculents)
 - e. Decreased filtration rate and increased backwash frequency for filtration treatment

Section 3

Facility Performance Evaluation

Introduction

POTW treatment deficiencies that cause poor conventional pollutant removal can have an adverse effect on toxicity reduction as well. As an initial step in the TRE, effluent toxicity data (Table 2-1) and POTW operations and performance data (Table 2-2) should be evaluated to indicate potential toxicants of concern and to identify treatment deficiencies or in-plant sources of toxicity that may be responsible for all or part of the effluent toxicity. POTW pretreatment program data (Table 2-3) should also be reviewed to indicate possible sources of toxicity and summarized for use in later steps of the TRE such as the toxicity source evaluation (Section 5).

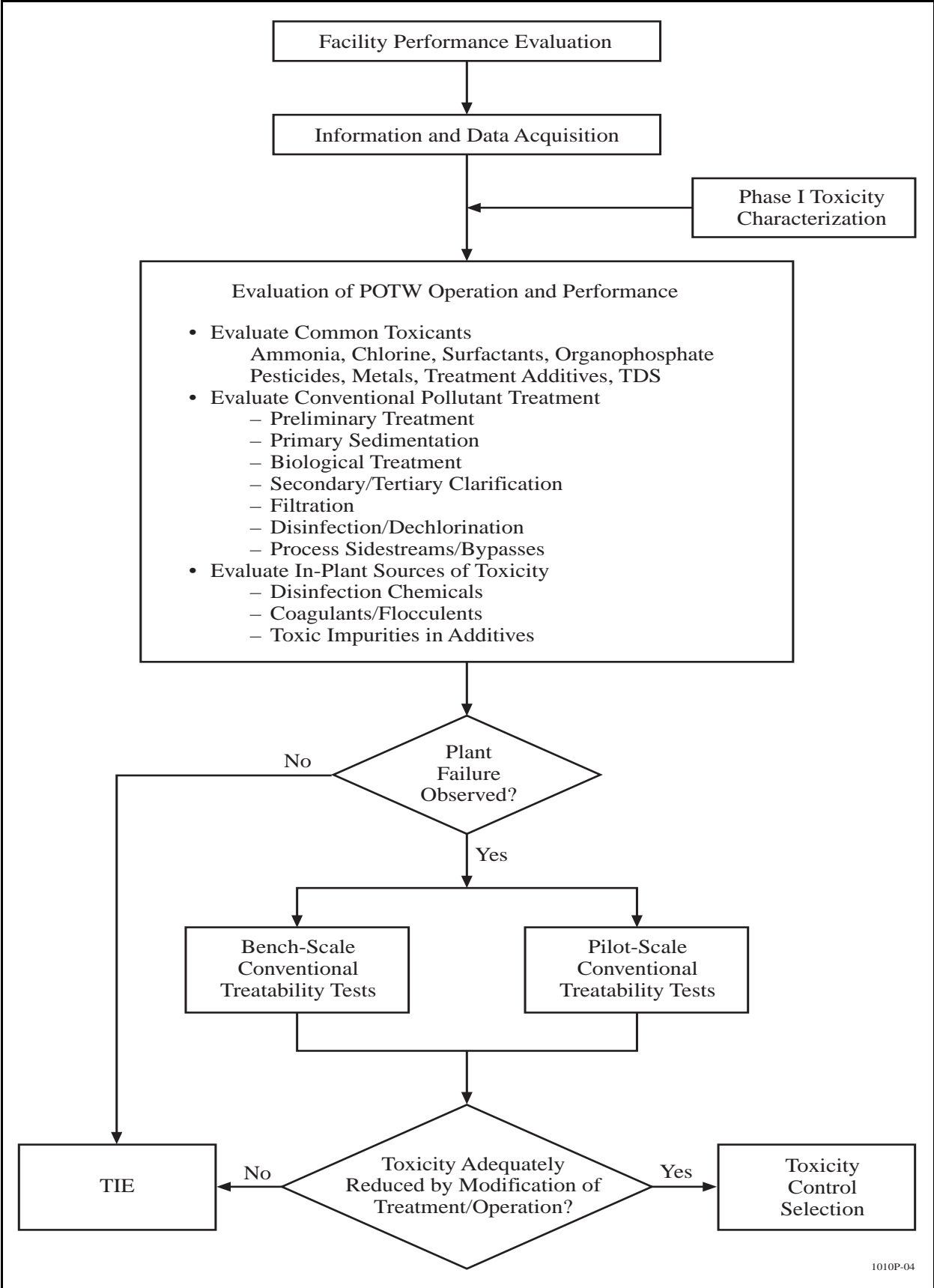
POTW Performance Evaluation

A POTW performance evaluation can be conducted to indicate conventional pollutant treatment deficiencies or in-plant sources of toxicity that may be contributing to effluent toxicity. Conventional pollutant treatment deficiencies include the inability to meet permit limits for BOD, TSS, and nutrients. These deficiencies should be corrected before initiating a full TRE because improved treatment also may reduce effluent toxicity. In-plant sources of toxicity may be present even if the POTW is meeting permit limits for pollutants other than toxicity. An example of an in-plant source of toxicity includes inadequate solids separation in the final clarifier, which may result in the discharge of toxic material bound to suspended solids. Also, incomplete biological treatment may cause the pass-through of biodegradable toxicants. Other in-plant sources of toxicity may include treatment additives used in toxic amounts or additives that contain toxic impurities. If deficiencies are found in the POTW performance evaluation, improvements can be implemented to eliminate the causes of toxicity. Several examples of operating conditions that have contributed to effluent toxicity at POTWs and the steps taken to correct the problem are included in the

following discussion of the POTW performance evaluation process.

A flowchart for conducting a POTW performance evaluation is presented in Figure 3-1. The POTW performance evaluation involves a review of the major treatment unit processes (e.g., primary sedimentation, activated sludge, and secondary clarification) using wastewater characterization data and process operations information. A TIE Phase I analysis (as described below and in Section 4) also can be performed to indicate the presence of effluent toxicants caused by incomplete treatment (e.g., ammonia), routine operating practices (e.g., chlorine), or the discharge of organophosphate pesticides in the POTW collection system. Ammonia and chlorine are commonly found to cause toxicity in POTW effluents and should be evaluated at this stage of the POTW performance evaluation. As noted in Section 2, ammonia and chlorine may be of concern at concentrations greater than 5 mg/L and 0.01 mg/L, respectively, depending on the effluent matrix and the species being tested. Levels of concern for other relatively common effluent toxicants are listed in Table 2-1. Special consideration also should be given to chemicals used in the treatment process such as used or reused waste materials and coagulants, which may contribute to toxicity due to pass-through of residual concentrations or impurities in the product.

Based on the process review results and TIE characterization data, options for improving operations and performance may be selected and evaluated in treatability studies. If treatability tests are successful in identifying options for improving conventional pollutant treatment and toxicity reduction, the TRE proceeds to the selection and implementation of those options (Section 6). If no treatment deficiencies or in-plant sources of toxicity are observed, or the treatment alternatives do not reduce effluent toxicity to



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Figure 3-1. Flow diagram for a facility performance evaluation.

acceptable levels, a complete effluent toxicity characterization should be performed using the TIE procedures described in Section 4.

POTWs are subject to both variable influent characteristics and changing operating conditions that may have a significant effect of effluent toxicity. The POTW performance evaluation should be conducted during a period when the influent loadings and facility operations are representative of average conditions. If effluent toxicity varies seasonally or as a result of a specific operational condition, the POTW performance evaluation should be scheduled to coincide with the expected toxic event. Due to the variability inherent in POTW operations, it may be necessary to conduct additional POTW performance evaluation investigations during the course of the TRE. For example, POTW performance evaluations may be useful when performed before and after implementation of facility modifications, changes in industrial user activities, or variations in effluent toxicity.

Operations and Performance Review

The operations and performance review involves the evaluation of the major POTW unit processes using the information described in Table 2-2. This review focuses on the secondary treatment system because secondary treatment is responsible for removing the majority of the conventional and toxic pollutants from municipal wastewater. Deficiencies in this system are more likely to result in incomplete treatment of wastewater toxicity. For example, problems with nitrification treatment may cause toxic concentrations of ammonia to pass through in the effluent. Other unit processes to be evaluated include primary sedimentation, disinfection, and advanced treatment processes such as filtration.

Procedures for evaluating and improving POTW operations and performance are described in USEPA's *Handbook on Retrofitting POTWs* (USEPA, 1989c). This handbook describes a two-step process to improve POTW performance: comprehensive performance evaluation and a composite correction program approach. The comprehensive performance evaluation involves a thorough review of the POTW design and operating conditions to identify problem areas. The composite correction program involves the systematic identification and implementation of improvements with an emphasis on low-cost solutions. Other useful sources of information include a joint publication by

Water Environment Federation and American Society of Civil Engineers entitled *Design of Municipal Wastewater Treatment Plants* (WEF/ASCE, 1992a, 1992b) and Metcalf and Eddy's *Wastewater Engineering Treatment, Disposal, and Reuse* (1991). Computer software programs, including USEPA's *POTW Expert* (1990), have also been developed to "troubleshoot" operations and performance problems. In addition, USEPA (1993d) has a data base on pollutant removal efficiencies (RREL Treatability Data Base, Version 5) for various treatment processes. Although this guidance does not specifically address toxicity, correcting conventional pollutant treatment problems and controlling in-plant toxicants may improve toxicity reduction. In addition to the noted guidance, public works managers are advised to use the services of a professional engineer who has experience with the POTW treatment system.

Preliminary Treatment

Preliminary treatment processes that may be used to enhance toxicity control include equalization/storage and oil and grease removal. Equalization basins can be effective in dampening the effect of slug loads of toxicity or to equalize flow and organic loadings to achieve consistent subsequent treatment of the influent wastewater. Oil and grease removal can assist in removing toxicants associated with oil and grease and to minimize the impact of oil and grease on the POTW.

TRE Example

A municipality in Texas experienced effluent toxicity that was related to a volatile organic compound entering the POTW. A pre-aeration system was to be added to the influent headworks or the grit removal system; however, before construction was started, a city employee noticed a strong odor in a sewer line that was related to the volatile toxicant. The source of the volatile compound was identified and controlled. As a result, effluent toxicity was eliminated (S. Bainter, personal communication, USEPA, Dallas, TX, 1998).

Primary Sedimentation

Primary treatment processes are designed to reduce the loading of TSS, BOD₅, and COD on the secondary treatment system. Toxic pollutant removal also can occur by sedimentation of insoluble or particulate wastewater constituents. Optimal removal of both toxic and conventional pollutants in primary

sedimentation ultimately reduces the amount of material to be treated in the biological treatment process.

Primary clarifier performance can be evaluated by comparing BOD₅ removal to the surface overflow rate (SOR), which is the average daily flow divided by the clarifier surface area. A clarifier operating at an SOR of less than 24 cubic meters per square meter per day (m³/m²/day) [600 gallons per day per square foot (gpd/sq ft)] should remove 35 to 45% of the influent BOD₅. A clarifier operating at an SOR of 24 to 40 m³/m²/day (600–1,000 gpd/sq ft) should remove 25 to 35% of the influent BOD₅ (USEPA, 1989c). In most cases, COD removal performance is comparable to the BOD₅ removal performance. If the primary clarifiers do not achieve the expected BOD₅ or COD removal, engineering studies should be initiated to determine the need for additional clarifier capacity.

Removal of toxicity associated with TSS may be enhanced by addition of coagulants to the primary clarifiers. The optimum conditions for coagulation and flocculation of toxicants can be determined by jar tests. These tests are used to establish the optimum type and dosage of coagulant, the proper mixing conditions, and the flocculent settling rates for enhanced toxicant removal (Adams et al., 1981).

A key operating parameter for controlling clarifier performance is sludge removal. Primary clarifiers generally function best with a minimum sludge blanket. Sludge withdrawal should be adjusted to maintain the primary sludge concentration in the range of 3 to 6% total solids (USEPA, 1989c).

Biological Treatment

Biological treatment is a critical process at most POTWs because it is the process that converts organic matter and nutrients to settleable microorganisms. Toxic pollutant removal during biological treatment can occur by biodegradation, oxidation, volatilization, and adsorption onto the biological floc. Key factors affecting the removal of toxic pollutants are the rates of biodegradation, tendency to volatilize, oxidize, or sorb onto solids, and the degree to which the pollutants may inhibit the treatment process.

Ammonia is a common cause of effluent toxicity at POTWs that do not include nitrification treatment. As noted by USEPA (1991a), ammonia is often present in effluents in concentrations varying from 5 to 40 mg/L.

These concentrations can cause toxicity depending on several factors that affect the toxicity of ammonia, including pH, temperature, DO, and TDS. A simple TIE procedure for checking whether effluent toxicity may be related to ammonia is described below (see “TIE Phase I Tests”). Literature data on ammonia toxicity (USEPA, 1985a) should only be used as a general guide because ammonia toxicity is significantly affected by slight pH changes.

The most commonly used biological treatment systems can be defined as either suspended growth processes, such as conventional activated sludge, contact stabilization, and extended aeration; or fixed film processes, such as trickling filters, denitrification filters, and rotating biological contactors (RBC). To simplify the discussion of biological treatment, the following subsections focus on evaluating the performance of conventional activated sludge processes and related BNR processes, which are the systems most widely used in POTWs.

Conventional activated sludge treatment is an aerobic process that can be accomplished in one stage or zone. BNR processes integrate carbon oxidation, as achieved in conventional activated sludge treatment, with treatment stages designed for nitrification, denitrification, and enhanced biological phosphorus removal. These stages require specific treatment conditions, including anaerobic, anoxic, and aerobic zones in the mixed liquors. The stages may be separated by physical divisions, non-discrete zones, or by operating cycle (WEF/ASCE, 1992b). The sequence and sizing of the BNR stages depend on the effluent nitrogen and phosphorus concentrations that must be achieved.

Conventional activated sludge processes remove phosphorus and nitrogen in the course of converting organic matter to new biomass. The typical phosphorus content of microbial cells is 1.5 to 2% on a dry-weight basis (WEF/ASCE, 1992b). BNR processes enhance phosphorus removal by utilizing the sequence of an anaerobic stage followed by an aerobic stage, which results in the selection of a biomass population capable of concentrating phosphorus from 4 to 12% of the microbial cell mass. Enhanced nitrogen removal in BNR processes is a two stage process: nitrification oxidizes ammonia to nitrite and then to nitrate, and denitrification reduces the nitrate to nitrogen gas. The nitrogen and phosphorus removal processes can be used independently (e.g., oxidation

ditches and A/O[®] process, respectively) or can be integrated into a combined nutrient removal process (e.g., A²O[®] and Bardenpho[®] processes). A wide variety of BNR systems are in operation, some of which are proprietary; therefore, specific information on the process being studied may be obtained by consultation with the system vendors.

TRE Example

A United States east coast municipality implemented nitrification to achieve a seasonal NH₃-N limit of 1 mg/L (Engineering Science, Inc., 1994). The POTW typically achieved less than 1 mg/L NH₃-N. As a result, the POTW effluent eliminated chronic toxicity to fathead minnows (*Pimephales promelas*) from May 1 through September 31 each year; however, the effluent continued to be toxic during the remainder of the year. In an effort to comply with the permit limit for chronic toxicity, nitrification was extended for the full year. This modification eliminated chronic toxicity to fathead minnows throughout the year.

The parameters that are typically used to evaluate the operational capability of an activated sludge system include organic loading, oxygen requirement, and MCRT. Additional important operating conditions include the alkalinity requirement for nitrification, and the BOD₅ requirement for phosphorus removal and denitrification. Operating values for these parameters can be compared to design specifications or recommended criteria to determine how well the processes are being operated.

Organic Loading

Organic loading affects the organic removal efficiency, oxygen requirement, and sludge production of activated sludge processes. The most common measure of organic loading in suspended growth processes is the F/M ratio, which is the organic load removed per unit of mixed liquor volatile suspended solids (MLVSS) in the aeration basin per unit time. High F/M ratios (i.e., high organic loading to MLVSS) will result in a low organic removal efficiency, low oxygen requirement, and high sludge production. Low F/M ratios (i.e., low organic loading to MLVSS) will lead to high organic removal efficiencies and low sludge production, but high oxygen requirements.

If the suspected toxicants are biodegradable or partition to activated sludge, the MLVSS of the treatment process should be increased to the maximum levels that can be maintained at the POTW. The maximum MLVSS often will be limited by the available secondary clarifier capacity. It is important to consider the effect of increased MLVSS on secondary solids separation and the TSS concentrations of the clarifier effluent. The Patapsco Wastewater Treatment Plant (WWTP) in Baltimore, Maryland, was operated at an F/M ratio of 0.40 lb BOD₅/lb MLVSS-day instead of the design F/M ratio of 0.55 lb BOD₅/lb MLVSS-day, because the POTW could not achieve consistent wastewater treatment at the higher organic loading. The increased MLVSS levels were thought to be necessary because of the toxic effect that industrial wastewaters were having on the activated sludge biomass (Slattery, 1987). For optimal treatment, it may be necessary to maintain F/M ratios that are on the low end of the range typically observed for biological treatment processes. The F/M ratio in an activated sludge system is generally maintained in the range of 0.2 to 0.4 lb BOD₅/lb MLVSS-day for conventional activated sludge, 0.05 to 0.15 lb BOD₅/lb MLVSS-day for extended aeration, and 0.2 to 0.6 lb BOD₅/lb MLVSS-day for contact stabilization (Metcalf and Eddy, 1991). The recommended F/M ratio for integrated BNR processes is generally in the range of 0.1 to 0.25 lb BOD₅/lb MLVSS-day (Metcalf and Eddy, 1991).

Influent BOD₅ concentrations should be high relative to phosphorus levels to ensure optimal phosphorus uptake and removal in BNR processes. Although optimum conditions vary according to the system design, the ratio of total influent BOD₅ (TBOD₅) to influent TP should be 20:1 to 25:1 to meet an effluent TP level of 1.0 mg/L or less. More importantly, the ratio of influent soluble BOD₅ (SBOD₅) to influent soluble phosphorus (SP) should be 15:1 (WEF/ASCE, 1992b).

The presence of biodegradable material also is necessary for denitrification in the first anoxic stage of BNR processes. For example, the Water Research Commission (1984) found that the TKN to COD ratio should be less than 0.08 to accomplish complete denitrification with the Bardenpho[®] process. In most cases, carbon must be added to the anoxic stage either by internal recycling of BOD₅ in process streams (e.g., nitrified effluent) or by chemical addition (e.g., methanol or acetate).

Oxygen Requirement

Microorganisms in the activated sludge system require oxygen to metabolize organic material and nutrients and breakdown biodegradable toxicants. Oxygen in diffused air or pure oxygen systems also may oxidize toxicants. Oxygen deficient conditions can result in lower treatment efficiency and, as a result, a greater potential for pass-through of toxic material. To ensure an adequate supply of oxygen, the DO level for conventional activated sludge (carbon oxidation) should be at least 2 mg/L during average loading conditions and 0.5 mg/L under peak loadings (WEF/ASCE, 1992a). Typical air requirements are 1,500 cu ft/lb BOD₅ load for conventional activated sludge and contact stabilization, and 2,000 cu ft/lb BOD₅ load for extended aeration (USEPA, 1989c). Air requirements for nitrification are higher because 4.2 to 4.6 mg of oxygen are required per mg of NH₃-N oxidized as compared to 0.6 to 1.1 mg of oxygen needed per mg of BOD₅ oxidized (WEF/ASCE, 1992b). DO concentrations of 2 to 2.5 mg/L are needed for nitrification in activated sludge processes with short retention times. In integrated activated sludge/nutrient removal processes, sufficient oxygen should be provided to achieve carbon oxidation and complete nitrification at the maximum daily loading rate.

Some of the oxygen consumed in the aerobic stage is in the form of NO₃-N. This oxygen source can be recovered in the denitrification process, which is generally located in an anoxic stage at the head of the BNR system. Approximately 2.86 mg of oxygen is recovered for each mg of NO₃-N reduced by biological denitrification. Internal recycling from the aerobic stage to the anoxic stage can decrease the oxygen required for nitrification by 50 to 60%. Carryover of molecular oxygen from the aerobic stage to the anoxic stage should be minimized by regulating the internal recycle rate. Generally, the recycle rate should be no more than three to four times the influent flow rate for these systems (WEF/ASCE, 1992b).

The transfer of oxygen from the gas phase to the liquid phase is a function of the aeration equipment and the basin mixing conditions. USEPA (*Handbook for Retrofitting POTWs*, 1989c) describes a procedure for estimating the oxygen transfer capacity in aeration basins based on equipment specifications. Another estimate of oxygen transfer capacity involves comparing OUR of the biomass to the calculated theoretical oxygen demand for the aeration system

(USEPA, 1989c). If the OUR results indicate an oxygen demand that is greater than the calculated oxygen demand, the oxygen supply may be inadequate. The opposite case (i.e., higher theoretical oxygen demand than actual oxygen demand) is preferred; however, a substantial difference may indicate inhibition of biomass activity.

TRE Example

OUR measurements were used to document the start-up performance of the activated sludge treatment process at the Patapsco Wastewater Treatment Plant (Botts et al., 1987). During the start-up, the OUR of the biomass averaged 20 milligrams O₂ per liter per hour per gram MLSS (mg O₂/L/hr/g MLSS), and the POTW frequently exceeded its conventional pollutant permit limits. As the biological system became acclimated to the wastewater, the effluent quality improved and the biomass OUR increased to an average of 50 mg O₂/L/hr/g MLSS.

Oxygen is not desirable in denitrification processes and the initial (anaerobic) stage of biological phosphorus removal processes. Molecular oxygen must be absent for denitrifying organisms to reduce NO₃-N to nitrogen gas. Phosphorus removing organisms require anaerobic conditions to accomplish the initial phosphorus release step (resynthesis occurs in the subsequent aerobic zone). NO₃-N and DO concentrations in the anaerobic zone should be kept below 1 mg/L (WEF/ASCE, 1992b). Although mixing is usually required in anaerobic and anoxic basins, it is minimized to prevent oxygen transfer to the mixed liquors. Also, DO carry over from the aerobic zone to the anoxic (denitrification) zone should be regulated through independent control of aeration equipment at the end of the aerobic zone.

Mean Cell Residence Time

In the course of biological treatment, activated sludge microorganisms convert some of the organic matter and nutrients in the wastewater to new cell mass. Toxic constituents may also be degraded or adsorbed onto the biomass. To achieve optimal treatment, the biomass concentration in the aeration tank is held at a constant level by routinely wasting the excess sludge. Sludge mass control can be practiced by maintaining a consistent average age of activated sludge (i.e., MCRT) in the system. The MCRT is calculated by dividing the total sludge mass in the system by the

amount of sludge that is wasted each day (i.e., lb/lb per day).

The optimal MCRT for toxicity reduction will depend on the type of toxicant(s) in the wastewater. Some compounds may be more efficiently removed by a younger biomass (low MCRT) and other toxicants are treated better with an older biomass (high MCRT) (Metcalf and Eddy, 1991). In general, biodegradable toxicants are more efficiently removed using a relatively long MCRT (WEF/ASCE, 1992a); therefore, the MCRT may be set at the high end of the range of values typically used for biological treatment. Hagelstein and Dauge (1984) also found improved toxicity reduction of a petroleum waste at MCRTs greater than 10 days. Typical MCRTs for aeration processes are 6 to 12 days for conventional activated sludge, 10 to 30 days for contact stabilization, and 20 to 40 days for extended aeration (USEPA, 1989c). System MCRTs for nitrification and BNR processes are generally long (20 to 40 days) because the growth rates of nitrifying organisms are slower compared to those for heterotrophic organisms found in conventional activated sludge systems (WEF/ASCE, 1992b). Overly long MCRTs should be avoided because subsequent denitrification treatment may be adversely affected if essential carbon has been depleted in the nitrification/carbon oxidation stage.

TRE Example

In 1992, Novartis Crop Protection, Inc., in cooperation with Makhteshim-Agan of North America, Inc., the two principal manufacturers of organophosphate insecticides in North America, evaluated the removal of diazinon and chlorpyrifos by various treatment methods (Novartis, 1997). Anecdotal evidence from other studies (Fillmore et al., 1990) and treatability studies by Novartis suggested that adsorption onto solids was the dominant mechanism for removal of organophosphate insecticides. The treatability tests performed in the 1997 study showed that about 30% of the diazinon and 85 to 90% of the chlorpyrifos present in POTW primary influent samples is adsorbed onto primary influent solids and approximately 65 to 75% of the diazinon added to the mixed liquor is adsorbed onto the biomass. Diazinon adsorption was greater for a 30-day MCRT biomass than for a 15-day biomass. Chlorpyrifos strongly adsorbed to the biomass; none remained after biological treatment. These results suggest that longer MCRTs may improve removal of organophosphate insecticides.

Additional Considerations for BNR Process Control

Additional considerations for BNR process control include maintaining sufficient alkalinity, proper management of sludge processing sidestreams, and achieving efficient solids separation in the secondary clarifier. Nitrification reduces the wastewater alkalinity by 7.2 mg/L as CaCO₃ for each mg of NH₃-N oxidized. However, about 40 to 50% of the alkalinity destroyed by nitrification can be restored in the denitrification process. The carbonate/CO₂ equilibrium in the mixed liquor determines the pH. As a general rule, alkalinity should be maintained above 50 mg/L in the nitrification process in order to keep the pH high enough for optimal treatment.

Secondary Clarification

In order for activated sludge and BNR processes to operate efficiently, the secondary clarifier must effectively separate solids from the liquid phase and concentrate the solids for subsequent return to the aeration basin. In addition to clarifier design, solids-liquid separation is influenced to a large degree by the aeration basin operating conditions such as DO levels, F/M ratio, and MCRT. If the MLVSS and MCRT of the aeration basin is to be maximized for toxicity control, it is important to consider the impact of this change on secondary solids separation and effluent TSS concentrations. Sludge settling characteristics are affected by how the aeration basin is operated. Low or high DO levels in the aeration basin can result in the growth of filamentous bacteria (e.g., *Nocardia* spp. and *Sphaerotilus natans*, respectively) that can hinder solids settling, whereas DO levels of 2 to 4 mg/L promote the growth of “zoogloeal-type” bacteria, which aggregate into fast settling flocs. At very high organic loadings (high F/M), the activated sludge can be dispersed and will not settle well. This condition was observed at the East Side Sewage Treatment Plant in Oswego, New York, which experienced sludge bulking due to high effluent organic loadings (USEPA, 1984a). Sludge settleability was improved by increasing the MCRT and the sludge return rate.

The performance of secondary clarifiers in solids-liquid separation is dependent on a variety of factors including clarifier configuration, SOR, clarifier depth at the weirs, the type of sludge removal mechanism, and the return sludge flow rate. USEPA’s *Handbook on Retrofitting POTWs* (1989c) describes a system for scoring secondary clarifier performance based on these factors. Design clarifier SORs for conventional

activated sludge processes are typically in the range of 400 to 800 gpd/sq ft (Metcalf and Eddy, 1991). In general, integrated BNR processes require larger clarifier capacity than conventional activated sludge processes, particularly where effluent quality must be high.

Clarifier capacity is important for phosphorus removal, because phosphorus is associated with the biomass, which can be carried over into the final effluent as residual solids. At peak sustained flow, a clarifier SOR of 800 gpd/sq ft is recommended to achieve a final effluent TP concentration of 2 mg/L. Lower effluent TP limits may require chemical treatment using a metal salt of iron, aluminum, or calcium, and, perhaps, a follow-on filtration process. For example, effluent phosphorus levels at the Jerry Sellers POTW in Cocoa, Florida, were reduced from an average of 2.9 mg/L to less than 0.2 mg/L with the addition of aluminum sulfate, commonly referred to as alum (WEF/ASCE, 1992b). In general, effluent TP levels of 0.2 to 0.5 mg/L can be met through chemical addition with a clarifier capacity of 500 gpd/sq ft (WEF/ASCE, 1992b).

Process Sidestreams and Wastewater Bypasses

Some wastewater and sludge treatment processes can produce sidestream wastes that may have a deleterious effect on the wastewater treatment system or might contribute to effluent toxicity. In addition, raw or partially treated wastewater that bypasses part or all of the treatment system can add substantial toxicity to POTW discharges (Mosure et al., 1987).

Examples of POTW sidestreams include sludge processing wastewaters (from thickening, digestion, and dewatering of sludges), cooling water blowdown, incinerator scrubber blowdown, and backwash from tertiary filters. Sidestreams from anaerobic digestion and sludge dewatering can contain high concentrations of BOD₅, COD, nitrogen, and phosphorus that can represent a significant loading to the aeration basin. Nitrogen and phosphorus in sidestreams are a concern for BNR processes, which can be compromised unless the loadings are removed, equalized, or separately treated. Also, some sidestreams may contain toxic materials such as metals and cyanide that may pass through the POTW. For example, cyanide may be formed during incineration of biosolids. Once formed, the cyanide may be captured in the incinerator

scrubbers and introduced into the treatment system via the scrubber waste stream. If the treatment process does not degrade the cyanide, toxic concentrations may be discharged in the POTW effluent. Also, sufficient amounts of cyanide may be present to cause inhibition of the biological treatment process, which leads to the release of more cyanide in the POTW effluent.

In some municipalities, storm water and sewage are still collected in the same sewer system. When a large storm event occurs, the CSO is often diverted away from all or part of the treatment system to prevent hydraulic over loading. In some cases, untreated overflows or bypasses may be directed into the POTW effluent, which can cause the discharge of relatively high concentrations of toxic and conventional pollutants.

The POTW performance evaluation should include a review of data on process sidestreams, wastewater bypasses, and overflows that are discharged into all or part of the POTW or into the final effluent. Additional analytical and toxicity data may be needed to characterize the levels of toxic pollutants and toxicity in these waste streams. Information on the frequency, volume, and toxicity of sidestream discharges, bypasses, and overflows also can be compared to historical effluent toxicity data to evaluate possible trends or relationships. This information can be used to determine if the discharges are a significant source of pollutants or toxicity, and whether current treatment practices are sufficient to remove toxicity. If necessary, consideration may be given to enhanced treatment of process sidestreams to remove toxicants such as metals. Enhanced treatment may involve changing the dosage of currently used coagulants applied for solids separation or adding new coagulant and flocculent aids. Bench-scale jar tests can be performed to determine the optimum type and dosage of coagulant and the appropriate treatment conditions.

Advanced Treatment Processes

Advanced treatment processes may be included in some POTWs to achieve pollutant removal beyond what is provided by biological treatment. These processes may include filtration, adsorption, chemical treatment, air stripping, and breakpoint chlorination. Of these processes, chemical treatment and filtration are most commonly used in POTWs, particularly for enhanced phosphorus removal.

Chemical Treatment

Chemical treatment can contribute to toxicity where toxic residual concentrations or contaminants in the product are present in the final effluent. Chemicals used in the latter stages of wastewater treatment are of particular concern because final treatment processes (e.g., tertiary clarification, chlorination) are less likely to remove residual concentrations (Note: potentially toxic disinfection byproducts, including residual chlorine, are discussed in a following subsection). When used wisely, treatment additives such as coagulants, flocculent aids, and hydrogen peroxide (H₂O₂) can also improve toxicity treatment.

Chemicals of concern in POTWs include chemicals formerly classified as hazardous waste. Under RCRA, a hazardous waste sold to a POTW is no longer considered a hazardous waste. According to 40 CFR Part 261.21(c)(5)(ii):

“A material is ‘used’ or ‘reused’ if it is... employed in a particular function or application as an effective substitute for a commercial product (for example, spent pickle liquor used as phosphorus precipitant and sludge conditioner in wastewater treatment).”

TRE Example

An example of the potential problems that may occur with process chemicals was the use of a dechlorination agent at several City of Houston wastewater treatment plants (S. Bainter, personal communication, USEPA, Dallas, TX, 1998). The City had routinely passed effluent toxicity tests until a dechlorination chemical was obtained from a new vendor. When the chemical was applied, effluent toxicity was observed at each of the POTWs. At the time, the City did not know the chemical may be a problem and proceeded to retain consultants to conduct TREs at the facilities. In the meantime, the supply of the dechlorination chemical was depleted and the city turned to a new source of the chemical. When the new chemical was applied, the POTWs started to pass the effluent toxicity tests. POTW staff can avoid similar problems if vendors are queried about potential contaminants in the waste chemicals or toxicity tests are performed on product samples to verify their suitability.

Chemical treatment is often practiced for phosphorus removal at POTWs. Typical coagulant aids include

lime, alum, sodium aluminate, ferric chloride, and ferrous sulfate. These coagulants have generally not been found to be toxic at the concentrations typically used for phosphorus removal. For example, alum dosages as high as 20 mg/L did not cause chronic effluent toxicity in treatability tests conducted at the City of Durham, North Carolina (Appendix D). Nonetheless, steps should be taken to prevent excessive and inadvertent chemical use. Also, each chemical additive used for treatment should be evaluated as a potential source of toxicity, not just suspect chemicals.

If toxicity is associated with suspended solids, chemical treatment conditions may be modified to enhance toxicity removal. The optimum conditions for coagulation can be determined by conducting jar tests. These tests can be used to establish the optimum type and dosage of coagulant, the proper mixing conditions, and the flocculent settling rates for improved phosphorus and/or toxicity removal (Adams et al., 1981).

TRE Examples

Studies have been performed to evaluate the reduction of organophosphate insecticides by chemical treatment (Novartis, 1997). Chemical precipitation using ferric chloride and polymer was found to only slightly reduce diazinon levels. No major change in diazinon concentrations was observed whether the coagulants were added to primary wastewater or secondary treated wastewater prior to clarification. Chlorination treatment was effective in reducing diazinon from secondary clarifier effluent; however, chronic toxicity was unchanged. Qualitative results suggest that the chlorine oxidized diazinon to diazoxon, a byproduct that exhibits similar toxic effects as diazinon. The results of additional treatments for diazinon are given in Appendix H.

A study conducted for San Francisco Bay area POTWs also evaluated the effect of chlorine on organophosphate insecticide concentrations (AQUA-Science, 1995). This study evaluated the use of household bleach as a measure that residential customers could use to degrade diazinon in spray container rinsate and chlorpyrifos from pet flea washes prior to disposal into the sewer. Samples of tap water were spiked with diazinon (60.0 µg/L) and chlorpyrifos (10.0 µg/L) and treated with either 0.005 or 5% solutions of household bleach for 24 hours. Results showed that both bleach concentrations

reduced concentrations of the insecticides by 86 to 92%. The study suggested that household bleach may be an effective pretreatment for waste solutions of the two insecticides prior to disposal. Additional studies are planned to further define bleach exposure times and concentrations under actual use conditions and to characterize the chemical oxidation products produced by the chlorine treatment. Additional information on this study is presented in Appendix F.

H₂O₂ has been used by a North Carolina municipality to control toxicity associated with non-polar organic toxicants (Aquatic Sciences Consulting, 1997). Although the specific toxic compounds were not identified; jar tests with H₂O₂ showed a substantial reduction in chronic toxicity to *Ceriodaphnia dubia* (*C. dubia*) at dosages ranging from 1 to 10 mg/L H₂O₂. Since the City began adding H₂O₂ to the POTW effluent (final concentration of 5 to 7 mg/L), results of a single *C. dubia* test show that the effluent NOEC was reduced from <15 to 90%.

Granular Media Filtration

Granular media filtration is usually applied after biological treatment to remove residual suspended solids, particulate BOD₅, or insoluble phosphorus. Filter influent is often chemically pretreated to enhance removal of suspended solids and phosphorus. In addition to the metal salts noted above, polyelectrolytes may be added to improve coagulation and flocculation of chemically treated influents. Typical polymer dosages are 0.5 to 1.5 mg/L for settling of flocculent suspensions before filtration and 0.05 to 0.15 mg/L when added directly to the filter influent. Some polymers can be toxic to aquatic life (Hall and Miranda, 1991); therefore, polymers used in the filtration process should be evaluated for the potential to contribute to effluent toxicity.

Poor filter performance should be investigated, especially the pass-through of potentially toxic material. Loss of suspended solids and other pollutants may result from high hydraulic and solids loadings, excessively long filtration cycles, and incomplete backwashing and cleaning of the filter.

Disinfection

Disinfection is generally achieved by treating the secondary effluent with chlorine and allowing a sufficient contact period prior to discharge. Alternative disinfection practices such as ultraviolet (UV) radiation are becoming more popular because of

concerns about the effects of chlorine on aquatic life and human health.

The chlorine disinfection process should be carefully evaluated because residual chlorine and other by-products of chlorination (i.e., mono- and dichloroamines, nitrogen trichloride) are toxic to aquatic life (Brungs, 1973). Chlorine dosages are usually based on the level of residual chlorine to be maintained in the final effluent as specified in the NPDES permit. The POTW performance evaluation should focus on the minimum amount of chlorine that can be applied to achieve the required residual chlorine concentration. In some cases, the TRC level specified in the NPDES permit may be sufficient to cause effluent toxicity. In general, TRC concentrations above 0.05 mg/L are a concern (D. Mount personal communication, AScI Corp, Duluth, Minnesota, 1991), although its toxicity will depend on the effluent matrix and the species used for effluent monitoring. Residual chlorine levels can be compared to toxicity data reported in the literature (USEPA, 1984b) to determine if chlorine may be a *potential* cause of effluent toxicity. If dechlorination is practiced following chlorination, information on the type and amount of oxidant-reducing material also should be obtained.

A chlorination process that is not continuously adjusted to varying flow and chlorine demand may cause effluent toxicity. Fortunately, this problem can be corrected easily by more frequent monitoring of the chlorine residual in effluent samples and more frequent adjustments in the addition of chlorine and dechlorination chemical. Flow-proportional feed equipment for chlorine and dechlorinating agents should be used to minimize the potential for excess chemical addition.

TIE Phase I Tests

TIE Phase I tests (USEPA 1991a, 1992a) can be conducted in parallel with the above operations and performance review to obtain information on the types of compounds causing effluent toxicity. An overview of the Phase I procedure is described in Section 4 of this guidance.

TIE Phase I testing in the POTW performance evaluation focuses on characterizing toxicants that may be present in the effluent because of inadequate treatment performance or routine operating practices. Phase I results, when taken together with the POTW performance evaluation data, may provide important

clues about possible in-plant toxicants. Based on this information, treatability tests may be designed to evaluate methods for removing the suspected effluent toxicants.

TIE Phase I testing includes several characterization steps that can be used to indicate the presence of “in-plant toxicants” such as ammonia and chlorine. One step involves pH adjustment of the effluent sample to three distinct pHs, such as pH 6, 7, and 8 prior to toxicity testing to indicate the effect of pH changes on effluent toxicity. The pH adjustment will shift the equilibrium concentration of ammonia between its toxic form (NH_3) and its essentially nontoxic form (NH_4). As pH increases, the percentage of total ammonia (NH_3 and NH_4) present as NH_3 increases. If adjusting the effluent sample pH to 8 increases the toxicity and if lowering the effluent sample pH to 6 decreases the toxicity, the identity of the effluent toxicant would be consistent with ammonia (Section 4). Another Phase I step is designed to indicate whether wastewater oxidants, such as TRC (i.e., free chlorine and mono- and dichloroamines), are causing toxicity. Sodium thiosulfate, a reducing agent, is added to eliminate TRC and other oxidants. The thiosulfate is added to serial dilutions of the effluent sample with 1 or 2 levels added across the dilutions. Toxicity tests on samples with and without thiosulfate treatment are used to indicate if oxidants such as TRC may be causing effluent toxicity.

It is important to note that each of the TIE Phase I characterization steps described above addresses a broad class of toxicants rather than specific effluent constituents, such as ammonia and TRC (USEPA, 1991a). For example, the toxicant affected by pH adjustment may be a pH sensitive compound that behaves in the same manner as ammonia. Also, the oxidants that are neutralized in the thiosulfate treatment step include bromine, iodine, and manganous ions in addition to TRC. Also, some cations, including selected heavy metals, are complexed by thiosulfate and may be rendered nontoxic (Hockett and Mount, 1996). Therefore, the Phase I results should be compared with information from the POTW operations and performance review to substantiate the evidence for a particular toxicant. Using the previous example, the assumption that TRC is causing oxidant toxicity would be corroborated if operations data show that toxic concentrations of chlorine are maintained in the final effluent (see Table 2-1 for levels of concern).

Organophosphate insecticides also have been identified as causes of effluent toxicity at POTWs (Ankley et al., 1992; Amato et al., 1992; Bailey et al., 1997; Botts et al., 1990; Burkhard and Jenson, 1993). TIE Phase I procedures that affect organophosphate insecticides include C18 SPE and treatment with a metabolic blocker, piperonyl butoxide (PBO). PBO can be added to effluent samples or methanol eluates from C18 SPE columns to block the toxicity of metabolically activated toxicants like organophosphate insecticides. PBO has been shown to block the acute toxicity of diazinon, parathion, methyl parathion, and malathion to cladocerans, but does not decrease the acute effects of dichlorvos, chlorfenvinphos, and mevinphos (Ankley et al., 1996). A reduction in toxicity by PBO treatment together with toxicity removal by the C18 SPE column, recovery from the C18 SPE column, and effluent concentration data can provide strong evidence for the presence of organophosphate insecticides. An exception is chlorpyrifos, which is not recovered well from C18 SPE columns (see Appendix F).

Conventional Wastewater Treatability Testing

The operations and performance information may identify areas in the POTW where improvements in conventional pollutant treatment may reduce the pass-through of toxicity. This information and the optional TIE Phase I data also may indicate in-plant sources of toxicants such as process sidestreams or over chlorination. Using these data, a wastewater treatability program may be devised and implemented to assess in-plant options for improving conventional treatment and eliminating in-plant sources of toxicity.

Treatability studies are recommended prior to comprehensive TIE testing (Section 4) in situations where improvements in treatment operations and performance are needed to attain acceptable conventional pollutant treatment. Otherwise, TIE testing of poor quality effluents could lead to erroneous conclusions about the nature of effluent toxicity. For example, inadequate conventional pollutant treatment could cause toxic materials to pass through the POTW that would otherwise be removed. In the POTW performance evaluation, treatability studies should focus on conventional pollutant treatment deficiencies that are suspected of contributing to effluent toxicity. The scope of the treatability studies program should be based on clear evidence of a consistent treatment deficiency causing toxicity over time. If sufficient

information is not available to develop a straightforward treatability program, additional data must be gathered in the subsequent stages of the TRE before in-plant toxicity control (Section 6) can be evaluated.

Treatability studies can vary from a simple evaluation such as testing the effect of TRC reduction on effluent toxicity to an extensive effort involving long-term bench- and pilot-scale work. Prior to beginning these studies, the POTW operations and performance data and the optional TIE Phase I results should be carefully reviewed and an appropriate treatability test program should be developed using best professional judgment. The nature and variability of effluent toxicity must be completely assessed (Section 4) prior to implementing an extensive treatability effort.

A treatability program can be devised to evaluate modifications in existing treatment processes. Evaluating new or additional treatment units should be attempted only after further effluent characterization studies (i.e., TIE) have been performed. POTW performance evaluation treatability testing may involve physical/chemical treatment approaches, such as coagulation and precipitation, solids sedimentation, granular media filtration, powdered activated carbon adsorption, or biological treatment approaches, such as activated sludge or sludge digestion.

Toxicity control is the ultimate goal of the TRE; therefore, toxicity tests should be performed in addition to the conventional pollutant analyses normally conducted in treatability studies. Toxicity tests are used to assess the capability of the treatment modifications for toxicity reduction. In some cases, the waste streams to be tested may exert a high oxygen demand and aeration may be needed to maintain a minimum DO level of 4 mg/L in the toxicity test. Aeration may affect toxicant characteristics; therefore, it may be necessary to use an alternative test method, such as Microtox[®], that is not affected by low DO. Side-by-side testing with alternative methods or species and the definitive test can be used to select a procedure that correlates well with the definitive test. This initial testing will help to ensure that the alternative test method or species is sensitive to the effluent toxicants of concern.

The following subsections briefly describe some of the treatability tests that can be used to determine if

improvements in existing conventional pollutant treatment will reduce effluent toxicity. If bench-scale tests suggest that toxicity can be reduced, follow-up pilot-scale or full-scale testing is recommended to confirm the initial results. As shown in Figure 3-1, if this testing is successful in identifying improvements in conventional pollutant treatment that will achieve acceptable levels of effluent toxicity, the TRE proceeds to the selection and implementation of those options (Sections 6 and 7). If, however, the treatability data indicate that improved in-plant treatment will not reduce effluent toxicity to acceptable levels, other approaches must be investigated, including TIE testing (Section 4).

Chemical Treatment

Chemical treatment may be applied in primary sedimentation, secondary clarification, filtration, and sidestream treatment processes. As noted above, jar tests can be used to determine the optimum type and dosage of chemical, the proper mixing conditions, and the flocculent settling rates for improved conventional pollutant and toxicity removal (Adams et al., 1981). As noted, some chemical additives, including polymers (Hall and Miranda, 1991), can be toxic; therefore, the toxicity of the chemicals should be evaluated as part of treatability testing.

Sedimentation

Sedimentation processes remove suspended solids or flocculent suspensions from the wastewater. In general, sedimentation in POTWs is characterized by flocculent settling for wastewater (i.e., primary clarification) and zone settling for mixed liquors (i.e., secondary clarification) and sewage sludges (sludge thickening).

Flocculent settling rates can be converted to a clarifier SOR by measuring the flocculent percent removal with time in a settling column test (Adams et al., 1981). If coagulants are needed, the optimum conditions for flocculation can be determined from jar tests, as noted above. A series of settling column tests can then be performed to compare particle settling profiles for various coagulant doses and mixing conditions.

Zone settling also can be evaluated in settling column tests. The settling velocity of mixed liquor or sludge is determined by measuring the subsidence of the liquid-solids interface over time (Adams et al., 1981). A series of tests are performed using the anticipated

range of suspended solids loadings to the clarifier. Test results are used to calculate a solids flux curve that can be used for clarifier design.

Activated Sludge

Continuous flow and batch biological reactor tests can be used to assess pollutant and toxicity treatability, and to predict the process kinetics of an activated sludge system. A series of bioreactors are generally operated under a range of MCRT values to determine optimum operating conditions (Adams et al., 1981).

The operational performance of bioreactors can be evaluated by measuring pollutant removals, OUR, MLVSS, and the zone settling velocity (ZSV) of the sludge. These measurements are used to determine the biodegradation kinetics of the wastewater, the potential for treatment inhibition, and the preferred sludge settling conditions. Samples of the influent, intermediate treatment stages, and effluent of the bioreactors can also be tested for toxicity to evaluate the system's toxicity reduction capability. Appendix D provides an example of the use of batch treatability tests to evaluate toxicity reduction in a BNR process.

If results of bench-scale treatability tests suggest that full-scale treatment will reduce effluent toxicity, follow-up pilot-scale or full-scale tests are recommended to confirm the results.

Granular Media Filtration

Toxicity removal by filtration can be evaluated in bench-scale tests or in full-scale tests of existing processes. The main parameters to be evaluated in filtration testing include hydraulic loading rate, media type and configuration, and, if necessary, type and dose of chemical coagulant (Adams et al., 1981). Filtration testing results can be used to correlate removal of suspended solids and toxic compounds with loss of toxicity. These results are ultimately used to establish the optimum design and operational conditions for conventional pollutant and toxicity removal, including filter type and loading rates, media characteristics, backwashing, and headloss development. Examples of the use of filtration in toxicity treatability studies are presented in Appendices C and D.

Activated Carbon Adsorption

Activated carbon may be applied in powdered form to the activated sludge process or may be used in granular form in a post treatment process (e.g., columns). The capability of carbon adsorption for treatment of organic

wastewater constituents or toxicity is determined by conducting batch isotherm tests and continuous-flow tests (Adams et al., 1981).

The effectiveness of carbon in removing BOD₅, selected organic contaminants (e.g., phenols), or toxicity is predicted by adding varying amounts of powdered activated carbon (PAC) to wastewater samples and measuring removal of the organic constituents or toxicity. The equilibrium relationship between a wastewater and carbon usually can be described either by a Langmuir or Freundlich isotherm. A plot of equilibrium concentration versus carbon capacity is used to select the required PAC concentration to add to activated sludge processes.

Continuous-flow tests are required to confirm the batch isotherm results. PAC tests involve adding PAC to bench- or pilot-scale biological reactors and monitoring the removal of the organic wastewater constituents or toxicity.

TRE Example

Activated carbon was investigated as a toxicity control option in the TRE at the Linden Roselle Sewerage Authority's (LRSA) POTW in New Jersey (Appendix G). Both PAC and granular activated carbon (GAC) were expected to remove non-polar organic toxicity in the effluent; however, the costs were determined to be prohibitive. It was also anticipated that carbon would concentrate the toxicants in the mixed liquor and cause unacceptable sludge quality.

Pretreatment Program Review

POTW pretreatment program data (Table 2-3) may provide information that can be used in subsequent steps of the TRE such as the toxicity source evaluation (Section 5). Information on the main trunk lines and the types of indirect dischargers in the sewer collection system can be used to devise a sampling strategy for tracking the sources of toxicants or toxicity. In some cases, the pretreatment program data may be sufficient to identify the sources of effluent toxicants identified in the TIE. In most cases, however, additional data, such as wastewater flow and toxicant concentrations in indirect discharges, will be needed to track the sources of toxicants or toxicity.

The information needed to conduct a toxicity source evaluation is presented in Section 5. In a USEPA TRE

research study conducted in Linden, New Jersey, pretreatment program data on wastewater characteristics of the main sewer lines and industrial dischargers were used to develop a comprehensive toxicity tracking program (see Appendix G). Sources of toxicity were successfully identified by devising a sampling schedule that accounted for periods of normal industry activity and periods of temporary shut-down for industry maintenance. The level of toxicity from the industries was found to vary with the industry production schedules.

It may be possible, in a few cases, to identify the toxic sources by comparing chemical-specific data on the POTW effluent to information on suspected sources of the toxic pollutants. This pretreatment program chemical review (PPCR) approach is recommended *only* in situations where the POTW has only a few indirect dischargers that have relatively non-complex wastewaters.

PPCR methods are described in Appendix I. These methods involve a direct comparison of industry chemical data to POTW effluent toxicity. It is important to emphasize that drawing preliminary conclusions based on PPCR results can be misleading because pretreatment monitoring information could be incomplete, analytical techniques may not be sensitive to low levels of effluent toxicants, and the estimated toxicity of individual compounds may not reflect the whole effluent toxicity. Domestic sources of toxicants

TRE Example

The PPCR approach was applied at the Mt. Airy POTW in North Carolina that receives industrial wastewater from only a few sources, all of which are textile industries (Diehl and Moore, 1987). Detailed information on the manufacturing processes and wastewater discharges of the industries was gathered, including data on the toxicity and biodegradability of raw and manufactured chemicals as provided in material safety data sheets (MSDS) and the scientific literature. This information was used to identify industrial chemicals with a relatively high potential to cause toxicity. Subsequent chemical analysis of the POTW effluent was performed to evaluate the presence of the suspected industrial toxicants. Effluent results were then compared to literature toxicity values for individual compounds. Using this approach, APE surfactants, largely attributed to textile industries, were identified as the primary causes of POTW effluent toxicity.

such as organophosphate insecticides also may be responsible for effluent toxicity (see Appendices A and F). In summary, comparisons of toxic pollutant concentrations to effluent toxicity may yield false correlations. Whenever possible, results of TIE testing should be used in lieu of PPCR results because the TIE establishes a cause and effect relationship between toxicants and effluent toxicity.

Section 4

Toxicity Identification Evaluation

Introduction

The TIE is an integral tool in the TRE process and is applied to evaluate the acute and short-term chronic toxicity of effluents and other samples. Toxicity is the trigger for TREs; therefore, the toxicity test is used in the TIE as the detector for chemicals causing effluent toxicity. Many types of test species and test conditions, including lethal (acute) and sublethal (chronic) measures for both freshwater and saltwater, can be adapted for use in the TIE. The use of modified effluent monitoring procedures, which incorporate the permit test species or a suitable surrogate, will help to ensure that the toxicants identified are the ones that specifically affect the species of concern. In the TIE, the toxicity test is used to track changes in the presence and magnitude of toxicity as the effluent is manipulated to isolate, remove, or render biologically unavailable specific types of constituents (e.g., volatile, filterable, oxidizable). These procedures relate toxicity to the wastewater's physical/chemical characteristics to determine the compound(s) causing effluent toxicity.

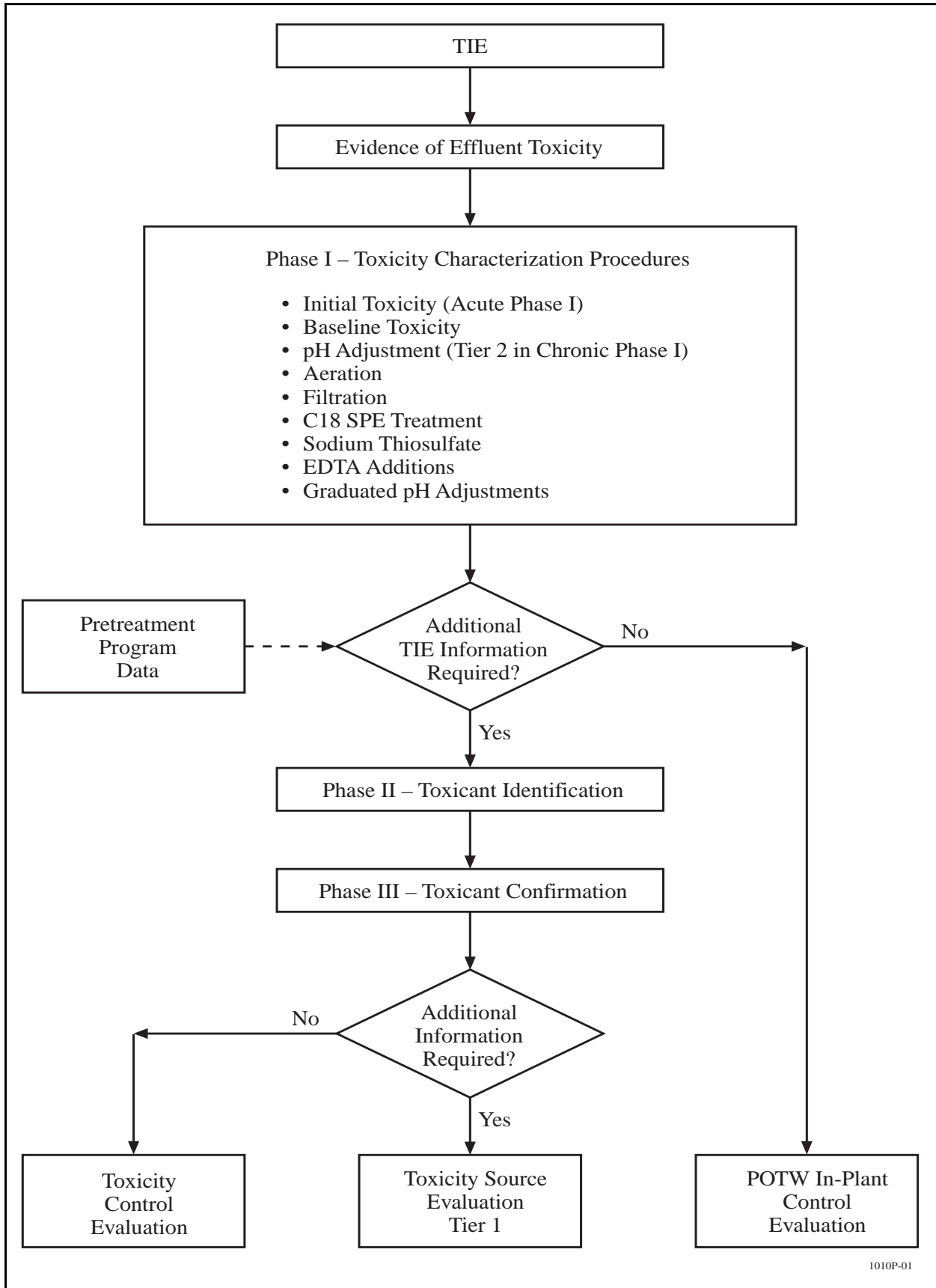
This section of the guidance is intended to be a general guide for TIEs. For specific guidance on how to conduct TIEs, the reader should consult USEPA's TIE manuals (USEPA 1991a, 1992a, 1993a, 1993b, 1996). The TIE procedures consist of three phases: Phase I involves characterization of the toxic wastewater components, Phase II is designed to specifically identify the toxicants of concern, and Phase III is conducted to confirm the causes of toxicity. Figure 4-1 presents the logical progression of these three phases within the framework of a municipal TRE. USEPA has published guidance documents for performing each phase of the TIE procedures. Phase I procedures are available to characterize acute (USEPA 1991a, 1996) and short-term chronic toxicity (USEPA 1992a, 1996). Phase II procedures (USEPA, 1993a) and Phase III procedures (USEPA, 1993b) are used to identify and confirm the causes of acute or chronic toxicity,

respectively. Each TIE is unique and a strategy should be developed for each study that accounts for site-specific conditions and allows flexibility in the study design, including the use of alternative tools and techniques noted in the TIE documents.

Several effluent samples should be tested to characterize the magnitude and variability of effluent toxicity over time. Failure to understand the variability in whole effluent toxicity and individual toxicants could lead to the selection of controls that do not consistently reduce toxicity to compliance levels. Sampling requirements for TIEs are described in Section 11. In addition to effluent testing, the TIE procedures can be applied in toxicity source evaluations (Section 5) to obtain information about the causes of toxicity in sewer wastewater or industrial discharges.

Toxicity Tests

The choice of acute or short-term chronic tests in the TIE should be determined based on discharge permit requirements and the toxicity exhibited by the effluent. Modifications to the whole effluent toxicity test procedures specified in the permit (USEPA 1993c, 1994a, 1994b, 1995) have been made to streamline the TIE process. These modifications are described in the respective TIE characterization, identification, and confirmation manuals and include smaller test volumes, shorter test duration, smaller number of replicates, reduced number of test concentrations, and reduced frequency of sample renewal (USEPA 1991a, 1992a, 1996). In addition, it is often more useful to evaluate only one effluent sample in chronic TIEs instead of multiple samples (e.g., two, three, or seven) as is typically used for chronic toxicity monitoring. Reducing the scale of the toxicity tests improves the efficiency of processing the large number of subsamples usually generated in the TIE. During the



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Figure 4-1. Flow diagram of a toxicity identification evaluation.

confirmation stage of the TIE (Phase III), whole effluent toxicity test methods are applied to confirm that the toxicant(s) identified in Phases I and II is the cause of the observed effluent toxicity.

TIE procedures have been designed to utilize both freshwater and estuarine/marine species in acute and short-term chronic tests (USEPA 1991a, 1993a, 1993b, 1996). Most POTW discharges to freshwater are monitored with the cladoceran, *C. dubia*, and/or *P. promelas* or, less commonly, the cladocerans, *Daphnia magna* or *Daphnia pulex*, and the trout, *Oncorhynchus mykiss*. *C. dubia* and *P. promelas* were used in the development of the TIE procedures and many subsequent TIEs have been performed successfully with these species (USEPA 1991a, 1992a, 1993a, 1993b), including the case studies presented in Appendices A, E, and F. TIEs also have been performed with trout (Goodfellow et al., 1994) and the green alga, *Selenastrum capricornutum* (Walsh and Garnas, 1983).

In addition, USEPA has provided guidance for the use of Atlantic, Pacific, and Gulf coast estuarine/marine species in TIEs (USEPA, 1996). A compilation of marine TIE studies has been prepared (Burgess, personal communication, USEPA, Narragansett, Rhode Island, August, 1998). TIEs have been performed using mysid shrimp, *Mysidopsis bahia* (Morris et al., 1990; Collins, et al., 1994; Burgess et al., 1995; Douglas et al., 1996), the grass shrimp, *Palaemonetes pugio* (Goodfellow and McCulloch, 1993), the mussels, *Mytilus edulis* (Edile et al., 1995) and *Mytilus californianus* (Higashi et al., 1992), the sheephead minnow, *Cyprinodon variegatus* (Goodfellow and McCulloch, 1993; Burgess et al., 1995; Douglas et al., 1996), the inland silverside, *Menidia beryllina* (Burgess et al., 1995), the purple urchin, *Strongylocentrotus purpuratus* (Bailey et al. 1995; Jirik et al., 1998), the urchin, *Arbacia punctulata* (Burgess et al., 1995), the sand dollar, *Dendraster excentricus* (Bailey et al. 1995), the red abalone, *Haliotis rufescens* (Griffin et al., 1993), the alga, *Champia parvula* (Burgess et al., 1995), the giant kelp, *Macrocystis pyrifera* (Higashi et al., 1992; Griffin et al., 1993), and other estuarine/marine species (Higashi et al., 1992; Weis et al., 1992). Case studies that utilized the echinoderms, *S. purpuratus* and *D. excentricus*, and the mysid shrimp, *M. bahia*, are presented in Appendices B and G, respectively. Although many species can be used in TIEs, the use of the species that is specified in the NPDES permit or

that triggered the TRE is encouraged to ensure that the toxicants identified are the ones that affect the species of concern. Also, NPDES permit species are more widely used in TIEs; therefore, extensive published data are generally available to help characterize and identify the toxicants affecting these species.

The TIE should incorporate modifications in toxicity test procedures that are specified in the permit, to the extent practicable. If pH control in the toxicity tests is allowed (T. Davies, USEPA, Office of Water, *Memorandum on Clarifications Regarding Flexibility in 40 CFR Part 136 Whole Effluent Test Methods*, April 10, 1996), the effects of pH should be addressed when evaluating effluent toxicants. However, procedural modifications should be limited to steps that are easy and practical to implement.

Effluents with intermittent and ephemeral toxicity may be challenging to characterize using TIE procedures. Intermittent toxicity may require adjustments in the TIE such as performing frequent toxicity screening tests over time to ensure that toxic samples are collected. Some effluents also may exhibit toxicity that dissipates after the samples are received and the initial and baseline toxicity tests are performed. If possible, this ephemeral toxicity may be characterized by conducting both the baseline test and TIE treatments immediately upon sample receipt. Also, it may be possible to shorten the time between sample collection and testing (i.e., <36 hours) or use grab samples in addition to composite samples. Depending on the level of effluent toxicity, it also may be challenging to discern differences in toxicity following the various TIE treatments. Steps that may improve characterization of these samples include adding more replicates and/or effluent concentrations in toxicity tests used in the TIE and testing more samples to evaluate trends in the toxicity characteristics. Additional information on this topic is given in USEPA's TIE manuals (1991a, 1992a, 1996).

Effluent monitoring data often includes information on the relative sensitivity of test organisms. It is generally recommended that initial TIE testing be performed using the test species that has been shown to be most sensitive to the effluent. In cases where equal sensitivity is observed, the organism that is easiest to use in the TIE should be selected. Phase III confirmation tests should utilize each of the species required by the discharge permit to ensure that all toxicants of concern have been determined.

A brief description of the TIE procedures is provided below. Examples of TIE applications in municipal TREs are presented in Appendices A through G.

TIE Procedures

Acute Toxicity Characterization (Phase I)

The first step in the TIE is to characterize effluent toxicity using the Phase I approach (USEPA, 1991a). This procedure involves several bench-top treatment steps to indicate the general types of compounds that are causing effluent toxicity. An initial toxicity test is performed to determine if the sample is acutely toxic. Simple manipulations for removal or alteration of effluent toxicity are then performed and the resulting treated samples and the original sample are tested for toxicity. The physical/chemical characteristics of the toxicants are indicated by the treatment steps that reduced toxicity relative to the baseline test.

The Phase I characterization includes the following tests:

- Initial toxicity (unaltered effluent)
- Baseline toxicity (unaltered effluent)
- pH adjustment (pH 3 and 11)
- Filtration/pH adjustment (pH 3 and 11)
- Aeration/pH adjustment (pH 3 and 11)
- C18 SPE/pH adjustment (pH 3 and 11)
- Sodium thiosulfate additions
- Ethylenediaminetetraacetate (EDTA) additions
- Graduated pH adjustments.

USEPA recommends performing the full suite of Phase I procedures on initial effluent samples (USEPA 1991a, 1996). As information is obtained on the nature and variability of toxicity, additional Phase I tests may focus on the steps that are successful in affecting toxicity. The aeration procedure is used to determine if toxicity is associated with volatile or oxidizable compounds. The filtration procedure is designed to evaluate whether toxicity is in the suspended particulate phase or in the soluble fraction. Aeration and filtration, in conjunction with pH adjustments, are used to evaluate the volatility and solubility of toxicants such as ammonia, hydrogen sulfide, and metals. The toxicity of oxidants and certain metals is evaluated by adding sodium thiosulfate. Cationic metal toxicity is determined by ethylenediaminetetraacetate EDTA additions and, possibly, by the graduated pH procedure. The graduated pH step is used to evaluate for the presence of pH sensitive compounds such as ammonia. An

aliquot of the effluent sample also is used to evaluate the presence of pH sensitive compounds such as ammonia. In addition, an aliquot of the effluent sample is passed through a C18 SPE column that selectively removes non-polar organic compounds (USEPA, 1991a).

In general, the TIE procedures used for marine species are similar to those used for freshwater species, except that samples used in marine TIEs must be adjusted to the salinity appropriate to the species being tested (USEPA, 1996). As part of the development of the marine TIE procedures, USEPA found that marine species can tolerate EDTA and sodium thiosulfate additions at concentrations that can affect toxicants of concern. Marine species can also tolerate methanol at concentrations that are necessary to evaluate non-polar organic compounds with the C18 SPE column. However, there are exceptions to the methods used for freshwater species for TIE steps. Due to the strong carbonate buffering capacity of seawater, it is difficult to characterize pH dependent toxicants using acids, bases, and organic buffers. The only efficient method for maintaining pH in the pH manipulation procedures is to use controlled atmospheric chambers. Also, a higher range of pH values is used in the graduated pH procedure because of the sensitivity of some marine species to lower pH.

When characterizing toxicity to marine species, USEPA recommends adjusting the salinity of samples before performing Phase I manipulations (USEPA, 1996; Ho et al., 1995). However, if a Phase I TIE is being conducted to help identify potential treatment options for the POTW, the salinity of the samples may be adjusted after the TIE manipulations are performed. This approach is necessary to ensure that toxicity removal in the TIE reflects the conditions that would occur in the POTW (i.e., mimics treatment before discharge to saline waters).

Subsequent tests are recommended to further characterize effluent toxicity. These tests are described in the acute Phase I document (USEPA, 1991a) in the "Interpretation of Results/Subsequent Tests" sections for each procedure. Some of these procedures include elution of the C18 SPE column with methanol to retain possible toxicants for further testing. If Phase I does not adequately characterize the toxicants, other techniques can be used, such as ion exchange resins for anions and cations; XAD (a commercially available ion exchange resin) and activated carbon for various

inorganic and organic compounds; and molecular sieves, such as Sephadex resins that separate compounds by molecular weight (Walsh and Garnas, 1983; Lankford and Eckenfelder, 1990; Burgess et al., 1997). TDS is an example of a toxicant that may not be well characterized in the TIE. Methods for characterizing and identifying TDS toxicity are presented below.

The characterization procedures are relatively broad and can indicate more than one class of toxicant. Additional tests are needed to delineate the nature of the toxicity if significant toxicity changes occur following the Phase I tests. For example, the C18 SPE column procedure, which is designed to determine if non-polar organic compounds contribute to toxicity, also can remove other compounds such as metals. USEPA (1991a, 1992a) reports that aluminum, nickel, and zinc concentrations may be adsorbed onto the C18 SPE resin. Confirmation that the C18 SPE column removed non-polar organic compounds is obtained by eluting the column with methanol to try to recover the toxicity. If toxicity can be recovered in the methanol eluate, then a non-polar organic toxicant is likely causing toxicity because metals do not elute with methanol. If toxicity adsorbed by the C18 SPE column is *not* recovered by the methanol elution, the column may have removed toxicants other than non-polar organic compounds, such as metals, or the non-polar organic compounds may have a higher affinity for the SPE column resin than methanol. Appendix E provides a case example in which toxicity due to metals was removed by the C18 SPE procedure.

When the primary toxicant is present in high concentrations, it may mask other potential toxicants, making it difficult to detect changes in toxicity following the TIE treatments. Modified procedures can be designed to control or account for the toxicity of the primary toxicant. Ammonia is a common example of a toxicant that may need to be controlled in the TIE (e.g., pH control) in order to evaluate secondary toxicants (see Appendix G).

Pretreatment program data and chemical-specific effluent data may provide useful information to assist in the Phase I characterization. By reviewing available information, compounds that are known to be problematic can be compared to the Phase I results to assist in indicating the effluent toxicants. This data comparison should not, however, replace the Phase II and III analyses.

After successful completion of Phase I, it may not be necessary to proceed to Phases II and III. If the effluent toxicity can be isolated to a class of compounds, POTW staff may opt to evaluate the treatment of effluent toxicity. These studies may involve bench-scale or pilot-scale testing procedures described in Section 6. However, if toxicity remains following implementation of toxicity control methods, the TIE should begin again with Phase I. In most cases, a complete TIE using all three phases will provide results that will lead to a more cost-effective evaluation of toxicity control approaches.

Chronic Toxicity Characterization (Phase I)

The chronic TIE Phase I procedures (USEPA 1992a, 1996) are similar to the acute Phase I procedures and include aeration, filtration, C18 SPE treatment, chelation with EDTA, oxidant reduction and/or precipitation with sodium thiosulfate, and graduated pH testing. The chronic test measures sublethal effects, such as reproduction, fertilization, cyst development, and/or growth. These measurements may be affected by the TIE manipulations. Adjustments have been made in the TIE procedures to limit toxicity artifacts. As in acute TIEs, additional steps are recommended to evaluate potential toxicity artifacts, including use of system blanks and replicate tests.

The same freshwater species typically used in acute TIEs (i.e., *C. dubia*, *P. promelas*, and, less commonly, *D. magna* or *D. pulex*) can be applied in chronic TIEs. Species that have been used in chronic marine TIEs include those noted above in the section titled "Toxicity Tests."

Two tiers of the Phase I characterization are recommended for the chronic TIE. Tier 1 is performed without major pH adjustments. Consistent, representative blank tests with reconstituted water are not readily obtained at higher pHs; therefore, the pH adjustment procedures used in the acute TIE are separated into Tier 2. Tier 2 is performed only when Tier 1 does not provide sufficient information about the types of compounds causing toxicity, and includes adjusting the effluent sample to pH 3 and 10 as part of the filtration and aeration steps and pH 9 for C18 SPE treatment.

Tier I of the chronic Phase I characterization consists of the following:

- Baseline toxicity
- Aeration
- Filtration
- C18 SPE treatment (including tests on post C18 SPE treatment and methanol eluate)
- Sodium thiosulfate additions
- EDTA additions
- Graduated pH adjustments.

The Tier 2 tests are to be conducted when Tier 1 does not provide sufficient information and consists of filtration, aeration, and the C18 treatment technique of Tier 1 with an effluent sample adjusted to both pH 3 and 10. Tier 2 of the chronic Phase I characterization consists of the following:

- pH adjustment
- Aeration and pH adjustment
- Filtration and pH adjustment
- C18 SPE treatment and pH adjustment (including tests on post C18 SPE treatment and methanol eluate).

Additional Characterization Procedures for Evaluating the Effect of Ion Composition

Although toxicity caused by ion composition is more commonly found in industrial effluents, ion-based toxicity has been reported at POTWs (Rodgers 1989a, 1989b; Douglas and Horne, 1997; Dawson et al., 1997). Ion composition can cause toxicity in two ways: relatively high levels of TDS can inhibit osmotic regulation in freshwater species, and an imbalance in ion composition, particularly calcium carbonate levels, can adversely affect marine crustaceans (Ward, 1989; MacGregor et al., 1996; Mickley et al., 1996). The later mechanism primarily affects crustaceans such as the mysid shrimp, *M. bahia*, which require minimum concentrations of calcium carbonate for survival, growth, and reproduction.

Procedures for evaluating toxicity caused by ion composition are available (USEPA 1991a, 1992a; Goodfellow et al., 1998). The following summary is intended to provide an overview of procedures that can be used to evaluate ion-based toxicity.

TDS Toxicity

As a general guide, TDS may contribute to acute toxicity when conductivity exceeds 3,000 and 6,000 $\mu\text{mhos/cm}$ at the LC50 for *C. dubia* and fathead minnows, respectively (USEPA, 1991a). For chronic toxicity, TDS may be a concern when conductivity

exceeds 1,000 and 3,000 $\mu\text{mhos/cm}$ at the lowest observed effect concentration (LOEC) for *C. dubia* and *P. promelas*, respectively (USEPA, 1992a). The conductivity of 100% effluent is not the relevant reading, but rather the conductivity at the concentrations bracketing the effluent LC50 and NOEC.

C. dubia's higher sensitivity to TDS as compared to *P. promelas* can provide additional evidence for TDS toxicity. Also, the cladoceran, *D. magna*, exhibits less sensitivity to TDS than the cladocerans, *C. dubia* and *D. pulex* (API, 1998). These species generally show similar sensitivities to most toxicants (Mount and Gulley, 1992); therefore, the difference in sensitivity to TDS can be useful in characterizing TDS toxicity. It is the toxicity of the individual ions that actually constitutes TDS toxicity; therefore, it is important to review the literature for toxicity data on specific ions. A thorough review of the toxicity of common ions to freshwater and marine organisms was recently published by the American Petroleum Institute (API, 1998).

An approach for evaluating TDS toxicity may consist of the following steps:

1. Monitor the effluent for TDS and if the conductivity exceeds the levels given above, measure the major cations (calcium, magnesium, sodium, potassium) and anions (carbonate, bicarbonate, sulfate, chloride). A cation/anion balance should be performed to ensure that all major ions have been accounted for.
2. For freshwater effluents, conduct toxicity tests using *D. magna*, *C. dubia*, and *D. pulex*. A greater sensitivity by *C. dubia* and *D. pulex* compared to *D. magna*, together with high conductivity readings, provides a weight of evidence for TDS toxicity.
3. If TDS toxicity is suspected, review the ion analysis data gathered above and prepare a stock solution of the ions in proportion to the amounts typically observed in toxic effluent samples. Collect an effluent sample and immediately measure the constituent cations and anions. Prepare a mock effluent by adding the solution to deionized water to yield the same cation/anion concentrations observed in the effluent sample. Measure the toxicity of the effluent sample and mock effluent. If the toxicity is similar, additional evidence is provided for TDS toxicity.

4. If TDS toxicity is indicated, additional procedures can be used to determine the extent to which TDS contributes to effluent toxicity. A sample of the effluent can be prepared for toxicity testing by setting up an appropriate dilution series and then adjusting the TDS levels in each dilution to the same TDS level as the 100% effluent using the stock solution (prepared above). Each effluent dilution is then tested individually for toxicity. Comparable results for each effluent dilution provides additional evidence for TDS toxicity.
5. Additional testing can be performed to identify the TDS constituent(s) that are causing toxicity. The toxicity of various cations and anions is well known and a review of the literature (e.g., ENSR, 1998) can be helpful in indicating potential ions of concern. The ions of concern can be evaluated by spiking the ions into dilution water and measuring the resulting toxicity. It should be noted that toxicity may be caused by a combination of many ions that exert their influence together. Therefore, a single salt may not be solely responsible for the observed toxicity.

TRE Example

Some of these procedures were used in a TIE at a POTW in Georgia (Dawson et al., 1997) where chronic effluent toxicity to *C. dubia* was observed. TIE characterization tests conducted on the effluent did not show a reduction in toxicity as a result of the Phase I manipulations. Independent analyses of the effluent indicated elevated chloride concentrations. A mock effluent was prepared as described in step 3, above, and the ion mixture was found to be as toxic to *C. dubia* as the POTW effluent. Laboratory toxicity data for sodium chloride (NaCl) were used to confirm that the effluent chloride levels would impair reproduction in *C. dubia* at the effect concentration.

Additional TIE studies were performed on the Georgia POTW effluent using calcium addition and species sensitivity tests. Calcium has been found to reduce chloride toxicity in waters with similar ion composition as the effluent and addition of calcium to effluent samples reduced toxicity. Toxicity tests using *D. magna*, which has been shown to be less sensitive to chloride than *C. dubia*, also provided evidence for chloride toxicity. Overall, the TIE results identified chloride as a major contributor to effluent toxicity.

Ion Imbalance

Calcium and carbonate, in proper balance, with other natural ions, are essential for the formation of new exoskeleton for mysid shrimp and other crustaceans. At low calcium carbonate levels (i.e., 15 mg/L CaCO₃), Ward (1989) observed 60% mortality in mysids between the 48-hour and 72-hour exposure periods, which corresponds well with the mysid molting cycle. Low CaCO₃ concentrations also appear to enhance mysid sensitivity to other toxicants. Ward (1989) observed a significant increase in the toxicity of Cd to mysids when calcium carbonate levels were reduced.

The investigator should consider the potential effect of ion balance as part of the TIE. Ion imbalance can contribute to apparent toxicity in some marine crustaceans when CaCO₃ concentrations are 15 mg/L or less.

Interpretation of Phase I Characterization Results

The following information on the interpretation of Phase I characterization results is paraphrased from the TIE manuals. The Phase I characterization provides information on the types of toxicants in the POTW effluent. In reviewing the Phase I data, whether for acute or chronic toxicity characterization, caution is needed to avoid making inaccurate conclusions about the results. For example, as noted above, toxicity removal by C18 SPE treatment does not necessarily mean that non-polar organic toxicants are present. Toxicity must be recovered in the methanol eluate test to provide evidence for non-polar organic toxicants.

The following guidance is given by USEPA (1992a) for interpreting Phase I data on various types of toxicants. Note that the reduction or elimination of toxicity is determined by comparing toxicity before treatment, as measured by the baseline test, with toxicity after treatment.

Non-Polar Organic Toxicants

Non-polar organic toxicants may be indicated if:

- Toxicity in the *post C18 SPE column test* was absent or reduced.
- Toxicity is recovered in the *methanol eluate test*. However, in those instances where methanol does not recover toxicity from the C18 SPE column, other solvents may be needed to elute the toxicants (USEPA, 1993a).

- Toxicity is reduced by adding PBO to effluent samples or methanol from the *methanol eluate test*. PBO blocks the toxicity of metabolically activated toxicants like organophosphate insecticides (USEPA, 1992a).

Cationic Metals

Cationic metals may be indicated if:

- Toxicity is removed or reduced in the *EDTA addition test*.
- Toxicity is removed or reduced in the *post C18 SPE column test*.
- Toxicity is removed or reduced in the *filtration test*, especially when *pH adjustments* are coupled with filtration.
- Toxicity is removed or reduced in the *sodium thiosulfate addition test*.
- Erratic dose response curves are observed.

None of these characteristics is definitive, with the possible exception of EDTA. In addition, toxicity may be pH sensitive in the range at which the graduated pH test is performed, but may become more or less toxic at lower or higher pH depending on the particular metal involved. This characteristic has not been demonstrated for chronic toxicity to the extent it has for the acute toxicity of several metals (USEPA, 1991a).

Surfactants

Surfactants may be indicated if:

- Toxicity is reduced or removed in the *filtration test*.
- Toxicity is reduced or removed by the *aeration test*. In some cases, toxicity may be recovered from the walls of the aeration vessel using a dilution water or methanol rinse.
- Toxicity is reduced or removed in the *post C18 SPE column test*. The toxicity may or may not be recovered in the *methanol eluate test*. If a series of methanol concentrations (e.g., 25, 50, 75, 80, 85, 90, 95, and 100% in water) is used to elute the column, toxicity may be observed in multiple fractions.
- Toxicity is reduced or removed in the *post C18 SPE column test* using unfiltered effluent. Toxicity reduction/removal is similar to that

observed in the *filtration test* and toxicity may or may not be recovered in the *methanol eluate test* or by extraction from the glass fiber filter used in the *filtration test*.

- Toxicity degrades over time as the effluent sample is held in cold storage (4°C). Degradation is slower when the effluent sample is stored in glass containers instead of plastic containers.

Ammonia

Ammonia may be indicated if:

- Toxicity increases in the *graduated pH test* at higher pH.
- The effluent is more toxic to *P. promelas* than to *C. dubia*.
- Note: If the concentration of total ammonia (as nitrogen) is 5 mg/L or more and chronic toxicity is a concern, the potential for ammonia toxicity should be evaluated.

Drawing conclusions about ammonia toxicity based solely on observed concentrations can be misleading, especially where chronic toxicity is a concern because of the uncertainty about the chronic effects of ammonia. Ammonia is an example of a toxicant that acts independently of other toxicants in effluents. Even though ammonia concentrations may appear to be sufficient to cause all of the effluent toxicity, other toxicants may be present and may contribute to toxicity when ammonia is removed.

Oxidants

Oxidants may be indicated if:

- Toxicity is removed or reduced in the *sodium thiosulfate addition test*.
- Toxicity is removed or reduced in the *aeration test*.
- The sample is less toxic over time when held at 4°C (and the type of container does not affect toxicity).
- *C. dubia* are more sensitive to the effluent than *P. promelas*.

The presence of TRC in the effluent is not enough to conclude that toxicity is due to an oxidant. However, TRC concentrations of 0.05–0.1 mg/L or more in 100% effluent provides strong evidence for oxidant

toxicity. Further evidence would be provided, if dechlorination with sulfur dioxide (SO₂) or another dechlorinating agent removes or reduces toxicity (USEPA, 1992a).

TDS

TDS may be indicated if:

- pH adjustments do not remove or reduce toxicity and a precipitate is not visible in the *pH adjustment test*, *pH adjustment and filtration test*, or *pH adjustment and aeration test*.
- There is no loss of toxicity in the *post C18 SPE column tests*, or a partial loss of toxicity, but no change in conductivity measurements.
- There is no change in toxicity with the *EDTA addition test*, *sodium thiosulfate addition test*, or the *graduated pH test*.
- There is a greater sensitivity by *C. dubia* and *D. pulex* compared to *D. magna*, together with high conductivity readings.
- A mock effluent prepared with the same ions as the effluent exhibits similar toxicity as the effluent.
- Toxicity is removed or reduced by ion exchange resin.
- Toxicity is not removed or reduced by passing the effluent over activated carbon.

Appendices A, B, E, F, and G provide example Phase I data and describe how results are used to select additional TIE procedures for testing. The Phase II and Phase III procedures (USEPA 1993a and 1993b) are applicable to both acutely and chronically toxic samples.

Acute and Chronic Toxicity Identification (Phase II)

The Phase II guidance manual (USEPA, 1993a) describes procedures that can be used to identify specific toxicants such as non-polar organic compounds, ammonia, cationic metals, chlorine, or toxicants removed by filtration. The Phase II procedures are applicable to both acute and chronic toxicant identification. Phase II uses treatment and toxicity testing techniques similar to Phase I and incorporates chemical-specific analyses to identify the toxicants. Examples of TIE Phase II studies are provided in Appendices A, B, E, F, and G. Appendices A, F, and G describe the use of Phase II techniques for non-polar organic compounds, including high-performance liquid chromatography (HPLC) for the isolating toxicants. Appendices B and E describe the application of Phase II procedures for identifying toxic metals. Appendix G describes how Phase II procedures were used to identify ammonia toxicity.

Acute and Chronic Toxicity Confirmation (Phase III)

The toxicants identified in Phase II may be confirmed by a series of Phase III steps, including correlating toxicity and toxicant concentration from multiple samples, observing test organism symptoms, evaluating species sensitivity, spiking effluent samples with suspected toxicants, and performing a mass balance to account for all of the effluent toxicity. In many cases, it will be appropriate for the Phase I, II, and III evaluations to overlap because confirmation information can be obtained during Phases I and II. Examples of TIE confirmation testing are provided in Appendices A, B, E, F, and G.

Section 5

Toxicity Source Evaluation

Introduction

Once the effluent toxicants have been identified, a follow-up evaluation can be conducted to locate the sources of the toxicants. This evaluation may involve a review of existing pretreatment program data or data from the collection and analysis of additional samples from industrial users. In some cases, the TIE may not identify the specific compounds causing effluent toxicity and, in the absence of data on toxicants, the sources of toxicity must be tracked. Examples of compounds that are not easily identified in the TIE include surfactants and some non-polar organic compounds (other than organophosphate insecticides). Although the class of compounds may be indicated in the TIE, it may not be possible to locate the sources without information on the specific toxic compounds. In these cases, a guidance is available to track the sources of toxicity.

A toxicity source evaluation is conducted to locate the sources of influent toxicity or toxicants that are contributing to the POTW effluent toxicity. This evaluation is performed in two tiers whether chemical-specific or toxicity tracking is to be performed:

- Tier I—generally involves sampling and analysis of wastewater samples collected from the main POTW sewer lines.
- Tier II—is performed using samples collected from tributary sewer lines or point sources on the main sewer lines found to be toxic in Tier I.

This tiered tracking approach can be used to identify the sources of toxicity and/or toxicants through a process of eliminating segments of the collection system that prove to be non-toxic.

The flow diagram for the toxicity source evaluation is presented in Figure 5-1. The choice of chemical-

specific analyses or toxicity tests for source tracking will depend on the TIE data on the POTW effluent toxicants. A chemical-specific investigation is recommended in cases where the effluent toxicants have been confirmed and can be traced to the responsible sewer dischargers. If the sources of toxicants are located, the TRE can then proceed to the evaluation of local pretreatment limits as described in Section 6. Toxicity tracking, using the refractory toxicity assessment (RTA) approach described herein, is required in situations where the TIE does not provide conclusive data on the effluent toxicants. Prior to toxicity analysis, sewer samples are subjected to the same type of treatment as is provided by the POTW for its influent wastewaters. This treatment step allows a measurement of “refractory” wastewater toxicity, which is the toxicity that passes through the POTW and causes effluent toxicity. If toxicity tracking is successful in locating the sources of toxicity, pretreatment requirements can be set to reduce the refractory toxicity contributed to the POTW.

In some cases, industrial users may modify or cease the discharge of toxicity before specific sources are identified. The abatement of effluent toxicity during the course of TREs is not uncommon; however, efforts to ensure ongoing compliance can be difficult when the original sources of toxicity are not located. These situations dramatize the importance of collecting information on industrial pretreatment activities and POTW operations in the early stages of the TRE. As part of the toxicity source evaluation, POTW staff can request industrial users to submit weekly or daily reports of production and waste discharge activities that can be used to indicate potential sources of toxicity. This information also is helpful in subsequent pretreatment control studies, if an industrial user is identified as a source of toxicity (Botts et al., 1994).

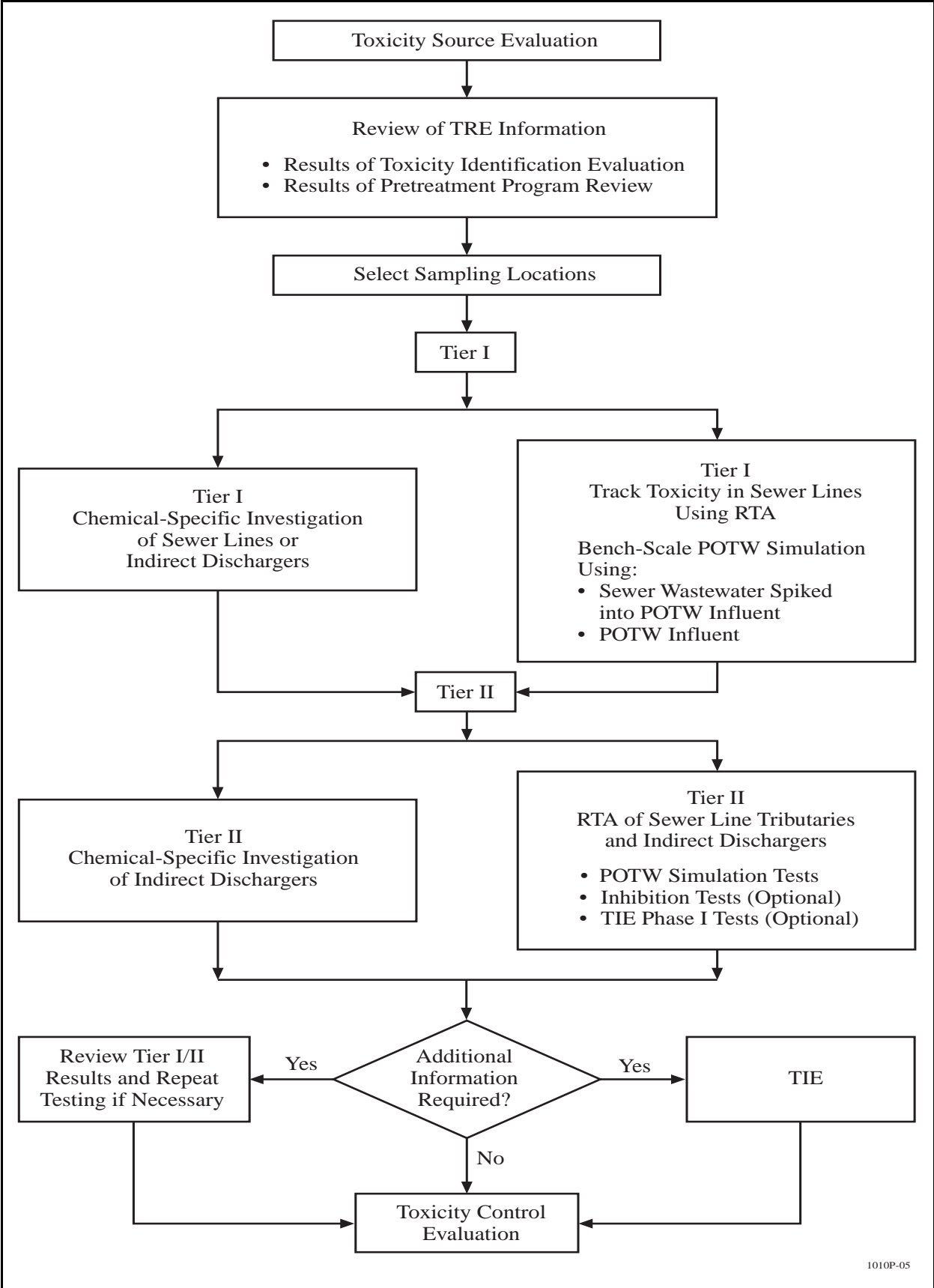


Figure 5-1. Flow diagram for a toxicity source evaluation.

Sampling Approach

The “Tier I” and “Tier II” designations refer to the sampling approach to be taken in tracking the sources of toxicity and/or toxicants.

Tier I – Toxicity Source Evaluation

Sampling locations for Tier I testing are established by reviewing the pretreatment program data (Section 3) and selecting appropriate sampling points on the main sewer lines. In some cases, industrial users or tributary sewer lines may be selected when substantial evidence is available on potential sources of toxicity or toxicants.

If the toxicants have been identified and chemical-specific tracking is to be performed in Tier I, sampling locations can include industrial users who have manufacturing processes or use raw materials that are known or suspected of containing the toxicants (e.g., metals from metal finishers). If the toxicant is contributed by a large number of dischargers, sewer line sampling is recommended in Tier I. For example, sewer line sampling was conducted to determine the sources of organophosphate insecticides in the City of Fayetteville, North Carolina, sewer system and several sewer systems in the San Francisco Bay Area in California (see Appendix F). These studies indicated that two insecticides, diazinon and chlorpyrifos, are widely distributed in POTW collection systems.

If toxicity tracking is to be performed in Tier I, each major sewer line should be sampled to ensure that all possible sources in the collection system are considered. Indirect discharger sampling is not recommended in Tier I because of the large number of sources that may need to be evaluated. Sewer line testing may ultimately reduce the number of sampling points by eliminating segments of the collection system where toxicity is not observed (USEPA, 1983a).

In the RTA study conducted at Fayetteville, North Carolina (Fillmore et al., 1990), sewer wastewater samples were initially collected from manholes throughout the collection system because of the large number of potential sources of toxicity. Sources of toxicity were subsequently identified by testing the indirect dischargers located on the toxic sewer lines.

Tier II – Toxicity Source Evaluation

Results of Tier I are used to establish the sampling locations for Tier II. The toxic sewer lines identified by toxicity or toxicant tracking in Tier I can be further

evaluated in Tier II by sampling indirect dischargers or tributary sewers on the toxic sewer lines.

Information on classes of toxicants such as surfactants can be obtained by coupling the RTA protocol with selected TIE procedures. For example, in the TRE at the LRSA, New Jersey, sources of non-polar organic toxicants were identified by passing RTA test samples through C18 SPE columns (see Appendix G). Sources of toxicity were indicated if toxicity was observed in methanol eluates from the columns.

Sampling Conditions

Whether sampling of sewer lines or indirect dischargers is conducted, 24-hour flow proportional composite samples are recommended to characterize daily variations in toxicant concentrations or toxicity. In some cases, samples may be collected over less than 24 hours to observe the contribution of potential intermittent sources of toxicants or toxicity.

Flow data must be gathered in order to determine the relative contributions of toxicants or toxicity from the sewer lines or indirect dischargers. Flow data can be used to calculate the toxicant loadings, which will be needed to develop local pretreatment limitations (Section 6). Flow data also will be needed to conduct RTA testing, as described later in this section.

The sampling period for both sewer lines and indirect dischargers should account for:

- Discharge schedules for indirect dischargers (i.e., intermittent versus continuous).
- Temporary shut-down schedules for industry maintenance.
- Coordination with routine pretreatment program monitoring, if possible.

For example, in the LRSA TRE (Appendix G), sources of refractory toxicity were identified by sampling during periods of normal industry activity and during a period of temporary industry shut-down.

Other considerations for sampling are described in Section 11. QA/QC sampling requirements are discussed in Section 8.

Chemical-Specific Investigation

A chemical-specific approach can be used to trace the influent sources of toxicants if definitive TIE data on the causes of POTW effluent toxicity are available.

This approach is not recommended in cases in which the TIE data only indicate a broad class of compounds (e.g., polar organic compounds), because the toxicants may be contributed by a variety of sources that will be difficult to pinpoint by chemical tracking. Chemical-specific tracking should be conducted after the effluent toxicants have been identified and confirmed in TIE Phase II and III tests (USEPA 1993a and 1993b).

The chemical-specific approach involves testing indirect dischargers or sewer line samples for toxicants using chemical analysis techniques. In some cases, existing pretreatment program data may be adequate to identify the indirect dischargers that are contributing the toxicants. It is likely, however, that further sampling and analysis will be necessary, because pretreatment program data generally do not include information on toxicants typically identified in TIE tests (e.g., compounds other than regulated pollutants). Existing pretreatment program data may be used to reduce the amount of sampling and analysis by indicating which sources contribute toxic pollutants that are similar to the effluent toxicants.

Chemical analysis methods for potential toxicants such as ammonia, metals, and organic compounds are described in several USEPA documents (USEPA 1979b, 1983b, 1985b) and *Standard Methods for the Examination of Water and Wastewater* (APHA, 1995). USEPA (1997) provides all of USEPA's methods for analysis of water on a CD-ROM. USEPA's Phase II TIE manual (1993a) also provides guidance for the analysis of organophosphate insecticides, surfactants, and metals. Analytical methods for organophosphate insecticides have been improved to achieve the lower detection limits necessary to assess insecticide toxicity (USEPA, 1993a; Durhan et al., 1990). Enzyme-linked immunosorbent assay (ELISA) procedures also are available for selected organophosphate insecticides, metals, and other compounds. ELISA methods offer the advantage of low cost, rapid sample processing, and field portability; however, these methods may not be specifically approved by USEPA. Additional analytical techniques can be found in American Society for Testing and Materials (ASTM) manuals and peer-reviewed journals such as the *Analytical Chemistry Journal*. A qualified chemist should verify the selected analytical method in the laboratory prior to sampling and analysis.

A literature search also can be made to determine if the toxicant could be a biodegradation product resulting from POTW treatment. Where clear evidence is available to show that the toxicant is a treatment by-product, the sewer sample should be analyzed for the precursor form(s) of the toxicant as well as the toxicant itself.

In cases where chemical tracking is successful in locating the sources of the POTW effluent toxicants, the TRE can proceed to the selection and development of toxicity control options such as local pretreatment regulations (Section 6). Information on toxicant distribution can be used in developing pretreatment control options. For example, although a primary contributor of ammonia was identified in the LRSA TRE (Appendix G), system-wide pretreatment limitations were adopted to address all non-domestic sources of ammonia. In other situations, control methods other than pretreatment limitations, such as public education, may be needed to control the discharge of a widely used toxicant. Public education has been successfully used at a number of POTWs (Appendix H) to control the use of organophosphate insecticides, which can be contributed from many domestic and commercial areas of the collection system.

If the responsible indirect dischargers are not located, the TIE results should be reviewed to confirm previous conclusions. The chemical analysis results also should be carefully reviewed to determine if errors or wastewater matrix effects may have caused inaccurate results. In cases where the chemical-specific approach is ultimately not successful, the source evaluation testing should be repeated using toxicity tests in lieu of chemical analyses, as described below.

Refractory Toxicity Assessment

Toxicity tracking may be required when the TIE characterizes the toxicity as broad classes of toxicants or identified toxicants cannot be confirmed. Toxicity tracking also may be useful in situations in which there are multiple effluent toxicants and the occurrence of these toxicants in the POTW effluent is highly variable. In such cases, toxicity testing may be more cost-effective than chemical tracking.

The toxicity found in influent wastewaters is not necessarily the same toxicity that is observed in the

POTW effluent because the POTW is capable of removing some toxic wastewater constituents. The amount of sewer wastewater toxicity that could potentially pass through the POTW must be estimated by treating sewer samples in a simulation of the POTW prior to toxicity analysis. This treatment step accounts for the toxicity removal provided by the POTW.

A protocol has been developed for predicting the potential for a sewer discharge to contribute to acute or chronic toxicity in POTW effluents. This protocol, referred to as the RTA procedure, has been successfully used to track sources of acute and chronic toxicity using both freshwater and estuarine/marine species (Morris et al., 1990; Botts et al., 1992, 1993, 1994). Examples of RTA studies are presented in Appendices C, D, and G.

The RTA protocol has been designed to simulate conventional activated sludge processes, although it has also been adapted to other POTW treatment processes including single and two-stage nitrification systems (Collins, et al. 1991), BNR processes (Appendix D), and filtration treatment systems (Appendices C and D). The RTA procedure described herein involves treating sewer samples in a bench-scale, batch simulation of a conventional activated sludge process and measuring the resulting toxicity. Batch simulations are appropriate for plug-flow biological systems because batch processes behave over time as plug-flow processes do with flow

time. Batch biological reactors have been used by several researchers to screen wastewaters for activated sludge inhibition (Grady, 1985; Adams et al., 1981; Philbrook and Grady, 1985; and Kang et al., 1983) and non-biodegradable aquatic toxicity (Hagelstein and Dauge, 1984; Lankford et al., 1987; and Sullivan et al., 1987). Hagelstein and Dauge (1984) and Lankford et al. (1987) have found that toxicity measurements coupled with bioreactor tests can be a pragmatic way to evaluate refractory wastewater toxicity.

The RTA protocol was developed in the USEPA TRE research study at the City of Baltimore's Patapsco POTW (Botts et al., 1987) to evaluate the potential for indirect dischargers to contribute refractory toxicity. Additional USEPA TRE research studies in Linden, New Jersey; High Point, North Carolina; Fayetteville, North Carolina; and Bergen County, New Jersey were conducted to improve the RTA approach (Morris et al., 1990; DiGiano, 1988; Fillmore et al., 1990; Collins et al., 1991). The RTA procedure described herein is a refined version of the method given in the municipal TRE protocol (USEPA, 1989a).

The batch reactor used in RTA testing is designed to simulate, as close as possible, the operating characteristics of the POTW's activated sludge process (e.g., MLSS concentration, DO level, and F/M ratio). Two types of batch reactors are used, as shown in Figure 5-2. One reactor serves as the control and consists of the POTW influent and return activated

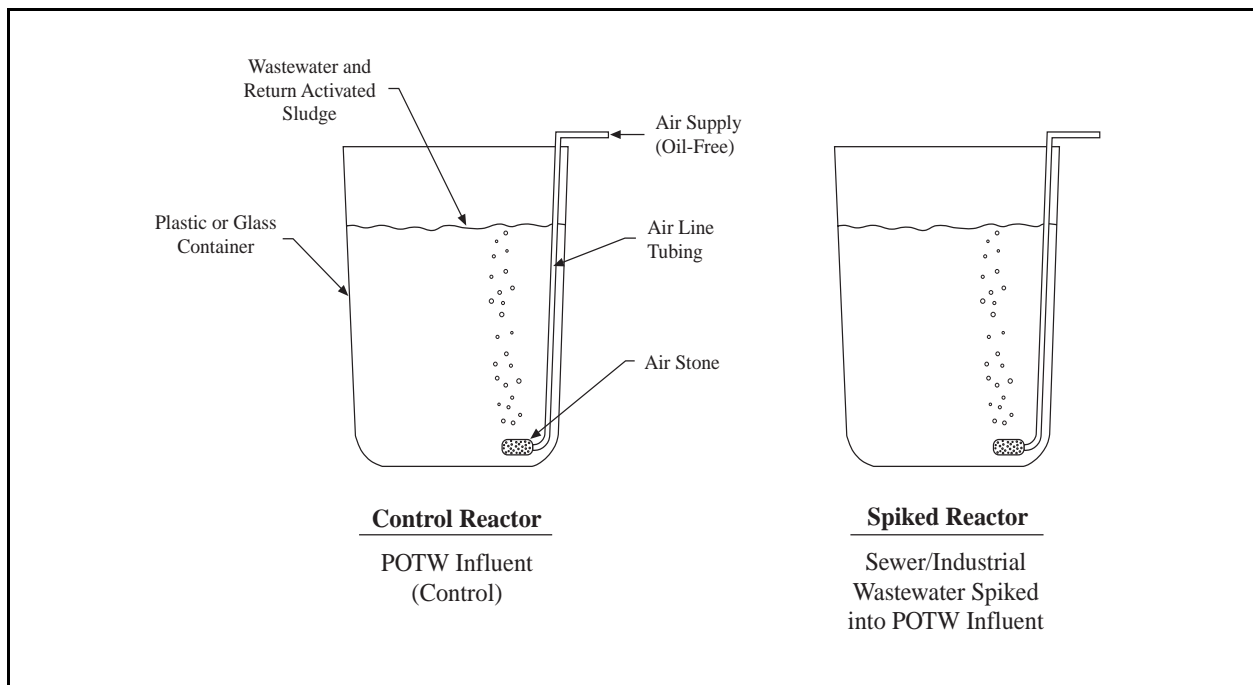


Figure 5-2. Schematic of a refractory toxicity assessment test.

sludge (RAS). The other reactor consists of sewer wastewater spiked into the mixture of POTW influent and RAS. If the effluent toxicity of the reactor spiked with sewer wastewater is increased relative to the unspiked reactor, the sewer wastewater would be considered a source of refractory toxicity. In the spiked reactor, sewer wastewater is tested together with the POTW influent in order to observe possible interactive effects (e.g., additivity, antagonism) that can occur when the wastewater and the total POTW influent are combined and treated in the POTW.

A general description of the RTA procedure is presented below. A step-by-step protocol for RTA testing is provided in Appendix J. The basic steps in the RTA approach are:

- Conduct conventional pollutant analyses to develop a profile for each wastewater to be tested in the RTA.
- Perform toxicity tests on the POTW's RAS (filtrate) to determine its potential to cause an interference in RTA testing.
- Collect, characterize, and prepare wastewater samples for RTA tests.
- Calibrate the RTA batch reactors to achieve a treatment level comparable to that of the POTW's activated sludge process.
- Calculate wastewater volumes to be used in RTA tests.
- Set-up and operate the RTA batch reactors.
- Analyze batch effluent toxicity.
- Evaluate the potential for the sewer wastewaters to inhibit activated sludge treatment (optional).
- Conduct TIE Phase I tests to indicate the types of refractory toxicants in the sewer wastewater (optional).
- Interpret the results.

It is important to emphasize that the RTA protocol should be modified to address site-specific conditions. For example, Appendix C describes an RTA study that simulated a filtration treatment process in addition to a nitrification treatment process. The following summary of the RTA protocol is intended to be a general guide to evaluating sources of toxicity using simulations of suspended biological growth processes. Best professional judgment is important in adapting the procedures to treatment processes and conditions that are unique to each facility.

POTW Wastewater Profile

The first step in the RTA is to characterize the POTW influent (primary effluent), sewer wastewaters, and RAS to be used in RTA testing. The POTW influent wastewater should be collected from the effluent of the POTW primary treatment process because primary effluent is treated by the activated sludge process, which is the main process to be simulated in the RTA. RAS is recommended for use in batch testing because it is in a concentrated form that can be easily diluted to the target MLSS concentration. Mixed liquor from the POTW's aeration basins can be used in lieu of RAS; however, the activated sludge will need to be thickened to the same suspended solids concentration as the RAS before use.

Table 5-1 presents the analyses and information that are needed to characterize the wastewaters to be used in RTA testing. This information will be useful for determining the following operating conditions for the RTA batch reactors, including:

- Determining the volume of sewer wastewater to use in testing based on sewer line and indirect discharger flow-rate data.
- Determining whether nutrient addition is necessary using information on the ratio of organic content (BOD₅ or COD) to nutrient concentrations (TKN and TP).
- Selecting a test period for the RTA reactors that is based on the organic content of the sewer wastewater. Some sewer wastewaters may have substantially higher COD concentrations, which will increase the initial COD level in the RTA reactor. A longer treatment time may be needed to ensure that the wastewater is treated to the same level as the POTW influent.

Biomass Toxicity Measurement

Sometimes the RAS used in testing can cause an interference in the measurement of refractory toxicity. In the Patapsco TRE, filtered samples of RAS were found to be acutely toxic to *C. dubia* (Botts et al., 1987). This toxicity was related to residual biosolids that passed through the filter [10-micron (µm) pore size]. The toxic biosolids caused the batch reactor effluents in RTA tests to be acutely toxic and masked the refractory toxicity of the wastewaters being tested. This biomass interference reduced the effectiveness of the RTA test for determining the sources of refractory

Table 5-1. POTW Wastewater Profile Analyses for a Refractory Toxicity Assessment

Waste Stream	Information Required
RAS	TSS VSS NH ₃ -N pH
Primary effluent	BOD ₅ COD TSS TP TKN NH ₃ -N pH
Sewer line or indirect discharger wastewater	Location in collection system Number/type of indirect dischargers Flow, million gallons per day (mgd) BOD ₅ COD TSS TKN NH ₃ -N TP pH
Other indirect discharger data	Type of discharger Wastewater pretreatment system Operations/production schedule

toxicity at the Patapsco WWTP. Additional tests demonstrated that RAS toxicity could be removed by filtration of the coarse filtrate through a 0.2 µm pore-size filter or by centrifugation of the coarse filtrate at 10,000 times gravity (xg) for 20 minutes (Botts et al., 1987).

Additional information obtained in the Linden Roselle, New Jersey; Fayetteville, North Carolina; and Bergen County, New Jersey USEPA TRE research studies indicated that the POTW RAS filtrate was not acutely toxic, and therefore did not cause an interference in RTA testing (Morris et al., 1990; Fillmore et al., 1990; Collins et al., 1991). The existing data on the toxicity of sewage sludges are not sufficient, however, to evaluate the occurrence of biomass toxicity at POTWs. The following discussion provides information on how to proceed, if POTW biomass toxicity is observed. Prior to conducting the RTA, toxicity tests of the POTW activated sludge should be performed to determine if the biomass is toxic. This testing involves

toxicity measurement of two aliquots of RAS: coarse RAS filtrate and coarse RAS filtrate subjected to centrifugation to remove colloidal particles. The RAS should first be filtered through a coarse glass fiber filter (e.g., 10 µm pore size), which is the same type of filter used for suspended solids analysis (APHA, 1995). Following coarse filtration, an aliquot of the RAS filtrate should be further treated by centrifugation at 10,000 xg for 10 to 15 minutes. Alternatively, the coarse filtrate could be filtered through a 0.2 µm membrane filter. However, tests should be conducted to confirm that soluble toxicity is not removed by sorption onto the filter.

Both the RAS filtrate and centrate should be tested for acute or chronic toxicity using limited-scale tests (USEPA 1993c, 1994a, 1994b). If results show that centrifugation does not reduce biomass toxicity relative to the coarse filtrate, then the RAS is not likely to cause an interference in RTA testing. In this case, the POTW biomass can be used directly in RTA testing

and centrifugation of the RTA batch effluents will not be required. Samples of RAS should be periodically analyzed for toxicity during RTA testing to monitor for possible biomass toxicity.

If the biomass coarse filtrate is observed to be more toxic than the RAS centrate, the biomass toxicity may interfere with RTA testing. Two options are available in this case: removal of the toxic biosolids by fine particle filtration (or centrifugation) of batch test effluents, and use of an alternate biomass.

Biomass toxicity may be removed by applying the fine particle filtration or centrifugation treatment steps to the batch test effluents. In this case, the resulting RTA effluent toxicity will only indicate *soluble* refractory toxicity, not the *total* refractory toxicity (i.e., soluble and particulate).

Another approach to remove biomass toxicity is to use a non-toxic biomass such as another POTW biomass or a commercially available (freeze-dried) preparation. A surrogate biomass will not be acclimated to the influent wastewaters of the POTW being evaluated; therefore, it may not treat the wastewaters as well as the POTW's biomass. Nonetheless, an alternate biomass can provide a level of treatment that will approximate the refractory toxicity of the sewer wastewater. It may be helpful to conduct a parallel series of RTA tests using the toxic POTW biomass. The use of toxic POTW biomass is suggested because it is acclimated to the POTW influent wastewaters and will therefore provide a level of batch treatment that is more similar to the POTW treatment than that provided by the unacclimated alternate biomass. In this case, fine particle filtration or centrifugation is required to remove the interfering biomass particles prior to toxicity analysis. By performing RTA tests with POTW biomass in parallel with RTA tests with alternate biomass, both the soluble and total refractory toxicity of the wastewater may be estimated.

An alternate biomass may be useful in cases where it is necessary to simulate future modifications or additions to the POTW activated sludge treatment process (e.g., conversion from conventional activated sludge to nitrification). In these cases, a biomass that is indicative of the future activated sludge, may not be directly available at the POTW. An alternate biomass can be obtained from another POTW that has a biological treatment process similar to the treatment

process planned for the POTW. A TRE conducted by the City of Durham, North Carolina, used this approach to evaluate the toxicity reduction capability of planned nutrient removal treatment systems (Appendix D).

RTA Reactor Calibration Testing

Generally, ideal plug-flow conditions do not occur in activated sludge processes; therefore, it will be necessary to adjust the RTA batch treatment conditions to account for the actual level of treatment achieved in the POTW. One method of controlling the treatment efficiency of activated sludge processes is to adjust the biomass concentration, measured as MLVSS concentration. Batch calibration tests can be performed using a series of MLVSS concentrations and the MLVSS concentration that most closely simulates the POTW treatment efficiency can be selected for RTA testing.

Prior to calibration testing, a target MLVSS concentration can be estimated using mathematic models. In the Fayetteville TRE, a steady state, completely mixed, multi-stage model (Grady and Lim, 1980) was used to determine biokinetic coefficients that best modeled the POTW treatment performance (Fillmore et al., 1990). The biokinetic coefficients were then used in a steady state plug-flow model (Kornegay, 1970) to calculate a batch MLVSS concentration that would theoretically simulate the POTW treatment efficiency. The model results were confirmed in bench-scale, batch reactor tests using a range of MLVSS concentrations, including the theoretical MLVSS concentration and several MLVSS concentrations that bracketed the theoretical value. In this case, the MLVSS concentration determined from the calibration tests matched the theoretical MLVSS value (Fillmore et al., 1990).

POTW primary effluent is typically used in RTA calibration testing. The treatment efficiency of the batch reactors can be evaluated by periodically collecting and analyzing samples for COD and toxicity. TKN, NH₃-N, and TP may also be monitored if the batch reactors are simulating BNR treatment systems. Results of the batch reactor tests are then compared to COD, nutrient, and toxicity data for the POTW final effluent to indicate which batch reactor achieved treatment comparable to the POTW. If there are large differences between batch effluent results and POTW effluent results, it may be necessary to evaluate

different MLVSS concentrations in additional calibration tests. In the Durham TRE, calibration tests were used to define batch operating conditions for a five-stage BNR process (Appendix D).

The RTA calibration study can also be used to establish an appropriate test dilution series for the toxicity tests of batch effluents. Where possible, the dilution series for toxicity tests should bracket the acute or chronic toxicity value (i.e., LC50 or ICp) as closely as possible in order to reduce the span of the 95% confidence limits. Increased confidence in the data is important because sources of refractory toxicity are indicated based on a comparison between effluent toxicity results for the sewer wastewater-spiked reactor and the POTW influent (control) reactor (Figure 5-2). The following example illustrates this point.

A wastewater from an industrial user is spiked into POTW influent sample and tested using the RTA procedure. The acute toxicity of the RTA effluent is measured using two different dilution series: one test series encompasses a wide range of sample concentrations and the other series more closely brackets the expected LC50. The dilution series and resulting survival and LC50 values are shown in Table 5-2.

Using dilution series #1, the LC50 for the industrial wastewater test would be 35% sample with confidence limits (95%) of 25% to 50% (based on binomial model). Using series #2, the LC50 would also be 35% sample, but the confidence limits (95%) would be much tighter at 31 to 39% (based on probit method).

The results for the RTA control test together with the industrial wastewater spiked test are shown in Table 5-3.

In this example, if the control reactor LC50 had been 50% with confidence limits of 42 to 62%, the industrial wastewater would have been indicated as a possible source of toxicity based on the results of series #2, because the 95% confidence limits do not overlap (i.e., 31 to 39% versus 42 to 62%). However, if dilution series #1 had been used, the industrial wastewater may not have been judged to be a toxic source because the confidence limits overlap (25 to 50% versus 42 to 62%). The partial mortality in the 35% concentration in series #2 (Table 5-3) helps to more precisely define the LC50. The narrow confidence limits in series #2 support the conclusion that the refractory toxicity of the wastewater is significantly greater than the POTW influent control (i.e., confidence limits do not overlap).

In the Reidsville and LRSA TREs (Appendices C and G), the results of preliminary toxicity analyses were used to adjust the dilution series to closely bracket the expected IC25 and LC50 value of the batch effluent samples. This approach allowed the identification of sources of refractory toxicity that would not have been indicated using a standard toxicity test dilution series.

Sample Collection

Wastewater and activated sludge samples should be collected according to the procedures described in Section 11. Sample volumes will be based on the subsample volumes needed for periodic reactor measurements and batch effluent toxicity testing.

Table 5-2. Example of Bracketing the LC50 Concentration in the RTA Sewer Wastewater Test

Test	Percent Survival in Sample Concentration					
	100	50	25	12.5	6.25	0
Series #1	100	50	25	12.5	6.25	0
	0	0	100	100	100	100
Series #2	100	50	35	25	12.5	0
	0	0	50	100	100	100

Source: Fillmore et al., 1990.

Table 5-3. Comparison of Control Test and Industrial Wastewater Spiked Test Results

Test	Control Test LC50 (CI)*	Industry Spiked Test LC50 (CI)	Potential Source of Toxicity?
Series #1	50% (42–62)	35% (25–50)	No
Series #2	50% (42–62)	35% (31–39)	Yes

* Confidence intervals (95%) shown in parentheses.

The volumes of wastewater and RAS to be used in RTA testing will depend, in part, on whether acute or chronic toxicity will be measured. Generally, a batch reactor volume of 3 liters (L) is sufficient when standard freshwater and marine/estuarine species (i.e., *C. dubia*, *Daphnia* sp., *P. promelas*, *M. bahia*) are to be used for testing the acute toxicity of batch effluents. A batch reactor volume of 10 L is adequate to measure chronic batch effluent toxicity using the 7-day *C. dubia* test.

Sample Characterization

Average characteristics of the sewer wastewater and POTW primary effluent can be determined using the wastewater profile data (Table 5-1). These data should include historical results of BOD₅, COD, TKN, TP, TSS, NH₃-N, and pH analyses. Analyses should also be performed on the samples collected for RTA tests to ensure that the wastewater characteristics are consistent with historical data.

Preparation of RTA Test Mixtures

Two batch influent solutions are prepared for each test of a sewer wastewater sample: sewer sample spiked into POTW primary effluent, and primary effluent alone. The sewer sample may be collected from a sewer line or an industrial discharge. The amount of sewer sample to be used in testing should reflect the percent volume of sewer wastewater in the POTW influent. In some cases, the wastewater toxicity from small contributors may not be readily observed when the wastewater is mixed by percent volume with POTW influent. In these cases, it may be necessary to use a greater volume of sewer wastewater than is typically contributed to the POTW.

The volume of sewer wastewater (V_w) sample to be added to the batch reactor is calculated as follows:

$$V_w(L) = \frac{Q_w}{Q_i} \times (V_r - V_b) \times F_w,$$

where: Q_w is the sewer wastewater flow rate (mgd).
 Q_i is the average POTW influent flow rate (mgd).
 V_r is the total reactor volume (gal or L).
 V_b is the volume of RAS biomass (gal or L).
 F_w is the sewer wastewater flow concentration factor (e.g., 1, 2, 10 times the sewer wastewater flow).

The selection of a flow concentration factor (F_w) will depend on the percent flow of the sewer wastewater in the POTW influent. A conservative, yet realistic, approach would be to use a F_w that is based on the maximum daily wastewater flow from the sewer discharge in the past year. The F_w should not cause the sewer wastewater to be 100% of the reactor wastewater volume. For example, if the sewer wastewater flow is greater than 20% of the POTW influent flow, a F_w of less than 5 should be used. It is necessary to test the mixture of sewer wastewater and POTW primary effluent in order to evaluate the interactive effects (e.g., additive or antagonistic) that can realistically occur when these wastewaters are combined at the POTW. All sewer samples should be tested using the same F_w to allow a comparison of batch effluent toxicity between the various sewer wastewaters.

After determining the V_w, the volume of primary effluent (V_{pe}) to be added to the batch reactors can be calculated as:

$$V_{pe} = (V_r - V_b - V_w).$$

The batch reactor influents are prepared by mixing the V_w and V_{pe} for the sewer wastewater spiked reactor and measuring V_{pe} for the control reactor. In some cases, it may be necessary to adjust the nutrient levels or pH of the batch influents prior to testing as described below.

The BOD₅/TKN/TP ratio of the batch reactor influents should be compared to the average BOD₅/TKN/TP ratio of the POTW influent, as determined from historical or profile data. The sewer wastewater added in the batch reactor influent may be deficient in nutrients, especially if industrial wastewaters are used. If necessary, nitrogen and/or phosphorus should be added so that the BOD₅/TKN/TP ratios of the batch reactor influent and POTW influent are similar. Unless the BOD₅ to nutrient ratios for the batch reactor influent and POTW influent are clearly dissimilar, nutrient addition is not recommended because of the potential for added nutrient salts to change the sample's toxicity.

Using the profile data, BOD₅ and nutrient (TKN, TP) concentrations (C) in the batch reactor influent (spiked) are calculated as follows:

$$C(\text{mg/L}) = \frac{(V_{pe} \times C_{pe}) + (V_w \times C_w)}{(V_{pe} + V_w)},$$

where: V_{pe} is the volume of primary effluent in reactor (L).

C_{pe} is the BOD_5 or nutrient concentration in primary effluent (mg/L).

V_w is the volume of sewer wastewater in reactor (L).

C_w is the BOD_5 or nutrient concentration in sewer wastewater (mg/L).

The typical BOD_5 /TKN/TP ratio for municipal sewage is 100:5:1 (WEF/ASCE, 1992a). This ratio will ensure that sufficient nutrients are available for consistent batch treatment of the sewer wastewaters. If necessary, phosphorus should be added in the form of three parts monosodium phosphate (NaH_2PO_4) to four parts disodium phosphate (Na_2HPO_4). Nitrogen should be added as urea nitrogen, except in cases where ammonia is suspected as a cause of effluent toxicity, because urea nitrogen can be converted to ammonia during biological treatment.

Following nutrient addition, the pH of the batch influents may need to be adjusted to within the average range of pH for the POTW influent. Typically, the range of POTW influent pH values will be pH 6 to 9. Hydrochloric acid and sodium hydroxide can be used for pH adjustment.

Following nutrient addition and pH adjustment, the batch influent toxicity should be measured to determine if the added nutrients or pH adjustment cause a change in sample toxicity. Substantial differences between the initial toxicity and the adjusted sample toxicity may indicate the presence of specific types of toxicants. Use of pH adjustment for toxicity characterization is discussed in the USEPA TIE Phase I manuals (USEPA 1991a and 1992a).

The volume of RAS biomass (V_b) to be used in batch testing should yield a batch MLVSS concentration that is equal to the target MLVSS concentration determined in calibration testing (see above). The amount of RAS to be added to the total reactor volume (V_r) is calculated as follows:

$$V_b(L) = \frac{\text{Target MLVSS (mg/L)}}{\text{RAS VSS (mg/L)}} \times V_r(L).$$

This equation also is used to determine the alternate (non-toxic) biomass volume (V_{nb}), if required.

Synthetic Wastewater Testing (Optional)

In some cases it may be important to determine the amount of refractory toxicity of the sewer wastewater excluding the effects of other influent wastewaters. A batch influent solution containing sewer sample spiked into a synthetic wastewater can be used to determine the individual refractory toxicity of the sewer sample. The synthetic wastewater will provide a standard substrate that will allow consistent treatment of the sewer wastewaters.

A synthetic wastewater should be prepared that has a COD concentration that is equal to the average COD concentration of the POTW primary effluent. The volume of synthetic wastewater (V_{sw}) to be added to the batch reactor is calculated using the same equation that is used to calculate the volume of POTW primary effluent. A synthetic wastewater has not been developed that is consistently non-toxic (DiGiano, 1988). Prior to use in RTA testing, the synthetic wastewater should be tested for toxicity to ensure that it will not interfere with the measurement of refractory toxicity.

Performance of RTA Tests

RTA testing is initiated when the batch influent solutions are mixed with RAS and diffused air is applied to the mixture. The aeration rate should be adjusted to maintain a DO concentration equal to the DO level observed in the POTW activated sludge treatment process. Mechanical mixing using a magnetic stirrer and teflon-coated stir bars may be required to ensure complete mixing in the reactor. The RTA tests must be performed in appropriate laboratory fume hoods to prevent exposure of laboratory staff to any toxic vapors stripped from the wastewater samples (Section 9).

The organic loading to the batch reactors can vary substantially depending on the type of sewer wastewater being tested. For example, a wastewater with a high COD concentration (e.g., >5,000 mg/L) is likely to increase the COD loading to the RTA reactor. The effect of this variation on batch treatment can be minimized by adjusting the reactor treatment time to achieve a constant "food-to-microorganism ratio" in the batch reactor (F/M_b). F/M_b should be similar to

the F/M of the POTW biological treatment process. This adjustment will allow the biodegradable material in the batch influent to be reduced to approximately equal levels in all RTA tests. The required batch test period (d) can be calculated as follows:

$$d = \frac{\text{Batch Influent COD (mg/L)}}{\text{MLVSS (mg/L)} \times F/\text{Mb}},$$

where: F/Mb is equal to the calculated F/M of the primary effluent reactor (i.e., COD/MLVSS \times treatment period in days).

Both acute and chronic refractory toxicity can be measured in RTA testing. In order to obtain comparable toxicity results, RTA testing should utilize the same species that was used for TIE tests or routine compliance monitoring. Use of toxicity screening tests such as bacterial bioluminescence tests (e.g., Microtox[®]) in conjunction with the preferred test species may provide additional information. These screening tests are recommended when the waste streams to be tested exert a high oxygen demand (i.e., high BOD concentration) which would otherwise require aeration during testing and a possible loss of toxicants. Standard procedures for toxicity measurement are not practical due to the large number of samples that will need to be processed in the RTA. Instead, simplified acute toxicity test procedures, like those presented in the USEPA TIE Phase I manuals (USEPA 1991a, 1992a, 1996) are recommended. Likewise, simplified procedures for short-term measurement of chronic toxicity (USEPA 1992a, 1996) are recommended for chronic refractory toxicity assessments. Oris et al. (1991) and Masters et al. (1991) describe the use of an abbreviated version of the 7-day chronic *C. dubia* test, referred to as the 4-day test. However, the 7-day test has been the method of choice for most RTA studies because the use of younger test animals provides more consistent results. Therefore, 7-day test data are better for discerning differences between toxic and nontoxic sources.

The batch test mixtures are prepared for toxicity analysis by allowing the mixed liquors to settle, decanting the clarified supernatant, and filtering the supernatant through a coarse glass fiber filter. The coarse filtration step is used to more closely simulate the POTW clarification process because solids settling in bench-scale containers is not as efficient as the POTW settling process. Note that this step may not be required if the RTA includes a simulation of effluent

filtration processes at the POTW (see Appendix C). If toxic biomass is used in the RTA tests, further particulate removal is required to measure the soluble refractory toxicity in the sewer wastewater. In this case, the coarse filtrate can be filtered through a 0.2 μm pore-size glass filter or centrifuged at 10,000 xg for 10 to 15 minutes (American Society for Microbiology, 1981) to remove colloidal size particles from the wastewater. Membrane filters such as cellulose nitrate filters may not be appropriate because some soluble organic constituents may adsorb onto the filter. Prior to sample filtration, all filters should be washed and filter blanks should be prepared using the steps described in Section 8 and Appendix J.

Data Evaluation

Results of RTA testing are used to locate the sources that are contributing refractory toxicity to the POTW. A discussion of the evaluation of RTA results is provided as follows.

Results of RTA Tests if POTW Biomass is Nontoxic

Results for each sample analysis will consist of data on two types of batch tests: tests of sewer sample spiked into primary effluent, and a control test using primary effluent alone. The batch test of the sewer sample/primary effluent will indicate the toxicity that would realistically occur upon mixture of the sewer wastewater with POTW influent. Results of this test are compared to results of the primary effluent control test to determine if the addition of sewer wastewater decreases the refractory toxicity (e.g., dilution or antagonistic effect) or increases the refractory toxicity (e.g., additive effect) of the primary effluent.

If the effluent toxicity of the sewer sample/primary effluent test is greater than the effluent toxicity of the primary effluent control test, the sewer wastewater source may be a contributor of refractory toxicity. POTW influent and sewer wastewater toxicity is known to vary significantly over time; therefore, each wastewater source should be tested several times over an extended period (e.g., three times during both cold and warm weather months) to determine the overall potential for the discharge to cause POTW effluent toxicity. Results of Tier I sewer line tracking can be used to prepare a list of the toxic sewer lines. This list can be compared to a sewer collection system map to indicate tributary sewer lines or indirect dischargers to be tested in Tier II.

The TRE case study summaries in Appendices C and G describe how RTA results were used to indicate sources of refractory toxicity in the Reidsville and LRSA TREs, respectively. These studies illustrate the need to test several samples from each wastewater source in order to account for the variability in refractory toxicity over time.

Results of RTA Tests if POTW Biomass is Toxic

In situations where the RAS coarse filtrate is found to be more toxic than the RAS centrate, RTA tests may use alternate (non-toxic) biomass in addition to tests with the POTW biomass. The data for each sewer sample analysis will consist of results of two batch tests using alternate biomass (i.e., one test of sample/primary effluent, and one test of primary effluent) and results of two batch tests using toxic POTW biomass. The results of tests that use alternate biomass will provide an estimate of the total refractory wastewater toxicity. The disadvantage of these tests is that the alternate biomass is not acclimated to the POTW influent wastewaters; therefore, it may not provide the same level of treatment as the POTW acclimated biomass.

Batch tests using toxic POTW biomass better reflect the treatment efficiency of the activated sludge process; however, manipulation of the batch effluent (i.e., centrifugation or small particle filtering) removes particles that normally are present in the POTW effluent. Batch effluent treatment is necessary to remove the interfering toxic biomass, but this treatment may artificially change batch effluent toxicity. The advantage of toxic biomass tests is that the soluble refractory toxicity of source wastewaters can be determined. The non-toxic biomass tests cannot provide as good an estimate of soluble toxicity, because alternate biomass is not acclimated to the POTW influent wastewaters. If both toxic biomass and alternate nontoxic biomass are used in testing, results are obtained on both the soluble and total refractory toxicity of the sewer wastewater.

Inhibition Testing (Optional)

Inhibitory wastewater may upset the normal operation of the POTW biological treatment process to the extent that it causes toxicity pass-through. Biological treatment inhibition may occur by three primary mechanisms: competitive inhibition, non-competitive inhibition, and substrate inhibition. The effect of competitive inhibitors is most pronounced at low

substrate concentrations. Inhibition by non-competitive inhibitors such as chromate or other heavy metals is observed over a range of substrate concentrations. The third mechanism of biological inhibition, substrate inhibition, occurs at high substrate concentrations.

Only substrate inhibition can be practically evaluated in batch treatment tests. An example of the effects of substrate inhibition on biological activity is shown in Figure 5-3. This figure shows that substrate utilization normally achieves a constant maximum rate as the wastewater concentration is increased. If inhibitory substances are present in the wastewater, the substrate uptake rate would decrease as the wastewater concentration is increased further.

Substrate inhibition can be assessed by monitoring removal of substrate (e.g., BOD₅, COD, TKN, and TP) and oxygen uptake rates in the RTA batch reactors. A series of dilutions of the sewer line or indirect discharger wastewater is tested with POTW biomass: one with 100% indirect discharger wastewater and at least three consisting of serial dilutions (e.g., 50%, 25%, and 12.5%) of sewer wastewater. A range of wastewater dilutions is necessary to compare organic, nutrient, and oxygen removal rates over a range of substrate concentrations. At high wastewater strengths [e.g., 1 mg/L soluble COD (SCOD) to 4 mg/L MLVSS], biomass activity will generally reach a maximum rate (Figure 5-3). When wastewater concentrations are increased, a decrease in COD, nutrient, and oxygen removal rates would indicate the presence of inhibitory materials.

SCOD, ammonia (SNH₃-N), and phosphorus (SP) removal can be used to calculate the specific substrate utilization rate (SSUR). The SSUR is reported in units of mg/L of soluble substrate per gram MLVSS per minute (g MLVSS/min), and is calculated using the equation:

$$SSUR = \frac{C_i (\text{mg/L}) - C_e (\text{mg/L})}{MLVSS (\text{g/L}) \times \text{Test Period (min)}}$$

where: C_i is the influent substrate concentration as SCOD, SNH₃-N, or SP.

C_e is the substrate concentration in periodic samples collected from the batch reactor.

The POTW biomass used in batch testing contains residual SCOD, SNH₃-N and SP remaining from

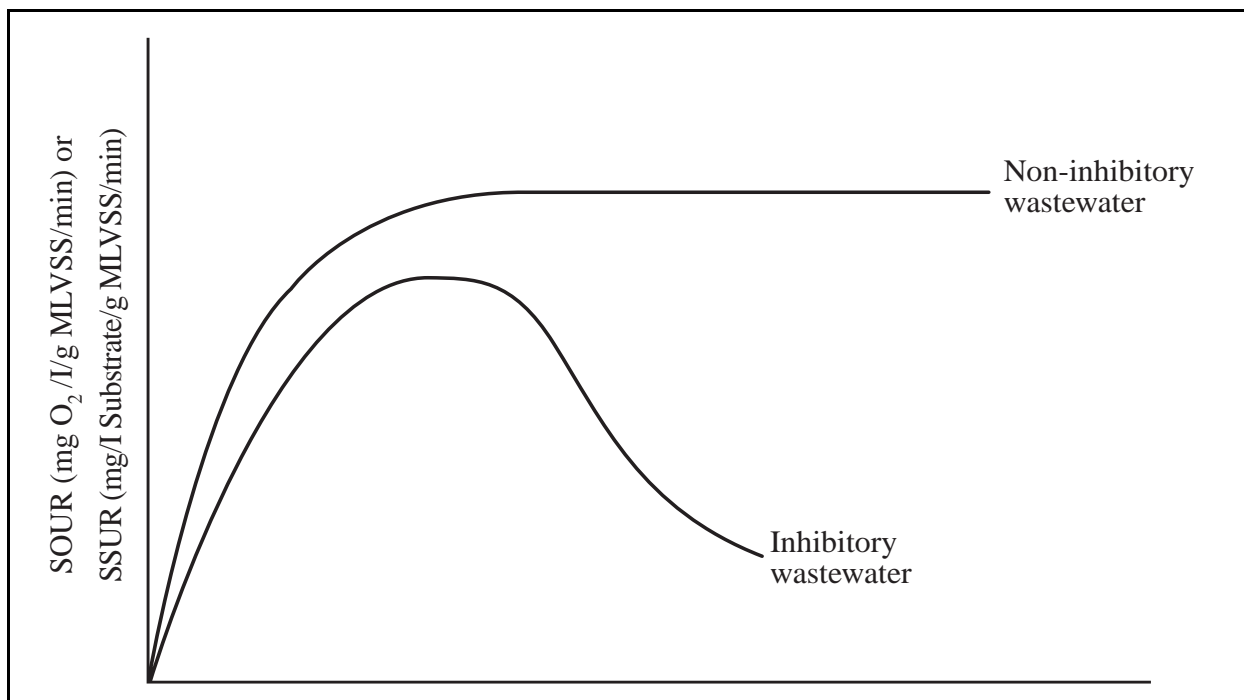


Figure 5-3. Theoretical results of inhibition testing.

biological treatment that must be accounted for when calculating batch effluent concentrations. The correction for biomass SCOD, $\text{NH}_3\text{-N}$, and SP is calculated by the following equation:

$$\text{SCOD} = \frac{[(V_r) \times (C_e, \text{mg/L})] - [(V_b) \times (C_b, \text{mg/L})]}{V_r}$$

where: C_b is the concentration of SCOD, $\text{NH}_3\text{-N}$, and SP in the RAS filtrate.

V_r is the total volume in the batch reactor (L).

V_b is the volume of RAS added to the reactor (L).

Oxygen utilization can be measured as a specific oxygen uptake rate (SOUR). SOUR is reported in units of $\text{mg O}_2/\text{L/g MLVSS}/\text{min}$ and is calculated as follows:

$$\text{SOUR} = \frac{\text{Oxygen Consumed (mg/L)}}{\text{MLVSS (g/L)} \times \text{DOMeasurement Period (min)}}$$

The SSUR and SOUR data for the four wastewater concentrations can be plotted as shown in Figure 5-3. A reduction in the SSUR and SOUR rates for the full strength sample test relative to the SSUR and SOUR rates for the sample dilution tests would indicate the presence of inhibitory material in the sewer wastewater sample. The degree of inhibition can be inferred by the

amount of deviation in biomass activity rates between the full strength sample test and the sample dilution tests.

Phase I Toxicity Characterization (Optional)

TIE Phase I tests can be applied to the batch effluent of the indirect discharger/primary effluent test to determine the types of toxicants causing refractory toxicity in the sewer wastewater. Results of the TIE Phase I testing can be compared to TIE results for the POTW effluent to determine if the sewer wastewater contains the same types of refractory toxicants that were observed in the POTW effluent. Sources that discharge the same types of toxicants as those found in the POTW effluent would be candidates for a pretreatment control evaluation (Section 6). The TIE Phase I procedure is described in Section 4.

Findings of the Toxicity Source Evaluation

The results of Tier I and Tier II testing should be sufficient to confirm the sources of POTW effluent toxicants or refractory toxicity. This information can be used to evaluate and select pretreatment control options (Section 6).

It is possible that the toxicity source evaluation results will suggest that no single sewer line or indirect discharger is a source of refractory toxicity. This case may occur if the sources of toxicants or toxicity are

widely dispersed throughout the collection system. Examples of dispersed toxicants include organophosphate insecticides (e.g., diazinon) and ammonia. The inability to locate the toxicant or toxicity sources may also indicate that the sewer sampling points did not include all possible sources of the toxicants or toxicity. In this case, it may be necessary to evaluate additional sewer lines in the collection system.

In situations where the toxicity source evaluation proves to be a prodigious task, the permittee may elect to evaluate alternatives for in-plant toxicity control (Section 6). The choice of pretreatment or in-plant controls may be determined by assessing the best use of the resources that are available for the TRE. In this regard, POTW staff have the option to recover costs associated with toxicity source evaluation through the process of local limits development.

Section 6

Toxicity Control Evaluation

Introduction

The goal of the TRE is to select and implement toxicity control methods and technologies that will achieve compliance with the permit limits for effluent toxicity. Toxicity control evaluation involves assessing the potential control options and selecting the best option(s) for toxicity reduction based on technical and cost considerations. Figure 6-1 illustrates the process of evaluation and selection of toxicity control options. Toxicity control may be accomplished either through the implementation of pretreatment requirements or POTW modifications. Examples of pretreatment controls include local limits development and waste minimization/pollution prevention requirements. POTW modifications may include changes in treatment chemical usage, enhanced operational strategies, or addition of treatment processes.

Criteria for the selection of the preferred toxicity control option(s) should be defined at the beginning of the toxicity control evaluation. Recommended criteria include:

- Compliance with effluent toxicity limits
- Compliance with other permits
- Capital, operational, and maintenance costs
- Ease of implementation
- Reliability
- Environmental impact.

Cost will be a primary selection criterion; however, the selected control option must offer the best potential for consistent, reliable toxicity reduction with the least impact on other permit requirements. The selection criteria should be used initially to screen all candidate control options to determine which alternatives merit further study. The preferred options can then undergo an in-depth review in a pretreatment control evaluation (e.g., local limits development) or in-plant control evaluation (e.g., treatability studies). Information from

these evaluations will be used to select the most feasible option(s) based on a more thorough comparison of the criteria listed above. The final selection process may require a quantitative examination of the options using a scoring and ranking system. Table 6-1 presents a matrix of in-plant toxicity control options for the TRE case example provided in Appendix G. Further discussion of the final selection process is provided at the end of this section.

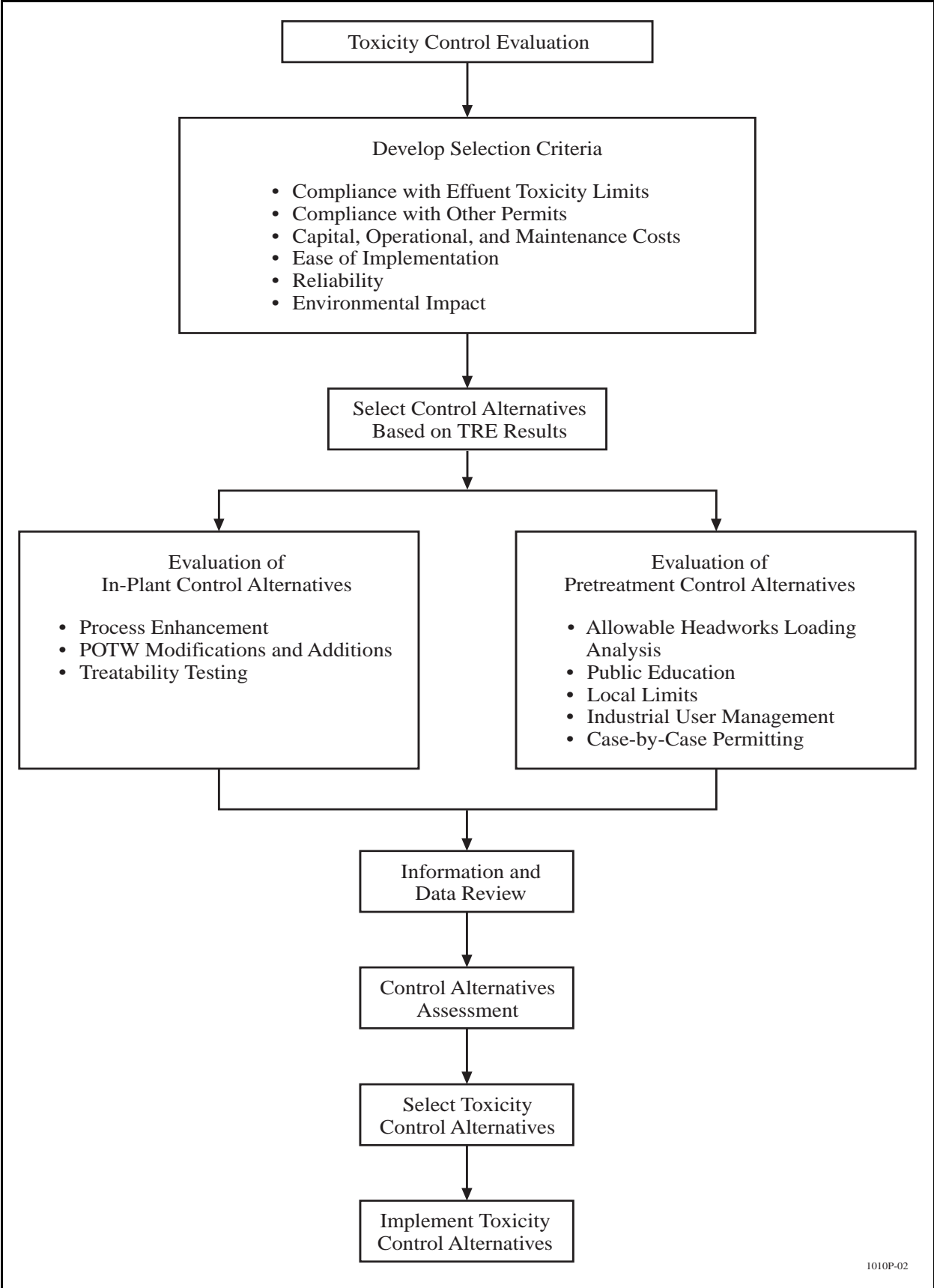
Identifying Toxicity Control Options

The TRE guidance is designed to identify possible methods for toxicity reduction at the earliest possible stage in the TRE process. As shown in the overall schematic of the TRE process (Figure 1-1), sufficient information may be available for toxicity control evaluation at the completion of the POTW performance evaluation conventional pollutant treatability tests, TIE tests, and toxicity/toxicant tracking. Control options must be identified based on ample data that clearly demonstrates the option's technical feasibility.

POTW Performance Evaluation Treatability Tests

Treatability testing in the POTW performance evaluation may identify options for improved conventional pollutant treatment that also reduce effluent toxicity to acceptable levels (Section 3). In addition, the optional TIE Phase I tests may provide information on the presence of in-plant toxicants such as suspended solids or chlorine that is corroborated in the operations and performance review. The treatment steps in TIE Phase I also may provide information on treatment options for control of the in-plant toxicants.

Potential control options may involve treatment modifications or additions that are necessary to improve conventional pollutant treatment and to reduce or eliminate in-plant sources of identified toxicants. Examples of these control options include dechlorin-



1010P-02

Figure 6-1. Flow diagram for a toxicity control evaluation.

Table 6-1. An Example of the Comparison of In-Plant Ammonia Treatment Alternatives (Ammonia Concentrations of 90 mg/L NH₃-N or Higher)

Treatment Technology	Capital Costs Millions*	O&M Costs Millions†	Equivalent Annual Cost Millions‡	Relative Practicality§	Relative Reliability#
1. Single-stage biological nitrification	9.1	1.5	3.4	Low	Low
2. Two-stage biological nitrification	11.5	2.4	5.0	Impractical	Low
3. Biological nutrient removal with nitrification	18.7	2.8	6.4	Low	Low
4. Ammonia air stripping	11.2	1.3	3.3	Very Low	Low
5. Selective ion exchange (including resin regeneration)	28.0	6.2	12.7	Very Low	Low
6. Breakpoint chlorination	7.5	6.8	11.5	Very Low	Low

* Approximate capital costs based in part on WPCF Nutrient Control Manual cost curves (WPCF, 1983). Values reflect conditions of 17 mgd and 90 mg/L NH₃-N. The values presented here have been modified from the cost curves to reflect engineering and contingency costs at 25% and contractor's overhead and profit at 15%.

† Approximate overhead costs based on WPCF Nutrient Control Manual cost curves. Values reflect conditions of 17 mgd and 90 mg/L NH₃-N.

‡ Approximate equivalent costs amortized over 20 years, assuming an annual 5.00% increase in operation and maintenance costs and an estimated annual interest rate of 8.86%.

§ Relative practicality based on typical technology applications, available land space, overall costs, and/or chemical usage requirements.

Relative reliability based on potential inhibition, temperature and pH sensitivity, and evidence that the technology is proven reliable at 17 mgd and 90 mg/L NH₃-N. Scores of "low" to "high" were used.

Source: LRSA (1991). Additional information on this TRE is presented in Appendix G. All costs shown are in 1991 dollars.

ation treatment to eliminate toxic levels of chlorine and biological treatment optimization (e.g., increased MCRT) to remove toxic ammonia concentrations.

TIE Tests

Results of TIE Phase I testing (Section 4) may indicate the types of treatment that can be used to remove broad classes of effluent toxicants (e.g., filterable material, metals, organic compounds). For example, filterable toxicants may be removed by granular media filtration. The feasibility of options for removing classes of toxicants can be evaluated in the POTW in-plant control evaluation.

Alternatively, results of TIE Phases II and III may help to identify and confirm the specific effluent toxicants (Section 4). If the pretreatment program data are adequate to determine the sources of the toxicants, local limits can be developed and evaluated in the pretreatment control evaluation. In this case, pretreatment control would be preferred over in-plant

control because the costs of implementation are usually lower. If pretreatment program data on the toxicants are not available, chemical-specific testing will be necessary to track the sources of the toxicants before toxicity control selection can proceed.

Chemical-Specific Investigation

Chemical-specific tracking in the Tier I – toxicity source evaluation may locate the sources of the POTW effluent toxicants (Section 5). Once the sources have been identified, pretreatment control options such as local limits or waste minimization requirements can be developed and evaluated.

Refractory Toxicity Assessment

Results of the Tier II RTA testing may identify the indirect dischargers contributing refractory toxicity to the POTW. Based on these results, POTW staff can require the indirect discharger to limit the discharge of wastewater toxicity even though the toxic wastewater constituents have not been identified. In some cases,

POTW staff may elect to perform optional TIE Phase I analyses to provide information on the toxic constituents in the indirect discharger wastewater. This additional testing may be conducted so that numerical pretreatment limits can be set.

Toxicity Control Screening Process

Using appropriate selection criteria, the preferred toxicity control options are identified. Available options can be compared using a ranking system (e.g., on a scale of 1 to 10). This screening process may be relatively simple, although some estimate of costs (i.e., order of magnitude) will be useful in selecting the most practical options. The selected options are then studied in the pretreatment control evaluation and in-plant control evaluation, as described below.

The example matrix in Table 6-1 compares in-plant control options for ammonia toxicity. In this case, costs and qualitative measures were used to rank the various options. All of the in-plant control options were found to be impractical or costly; therefore, the sewerage authority investigated pretreatment controls. The source of a majority of the ammonia loading was an industry, which was considered to be controllable. As a result, the sewerage authority required the industry to implement ammonia control methods. The cost to the authority was relatively low and involved a headworks analysis for ammonia and reissuance of discharge permits. Additional information on this TRE is provided in Appendix G.

Pretreatment Control Evaluation

Pretreatment control options can be developed by public works managers to prevent the pass-through of toxicants, toxicity, and inhibitory material that have been traced to indirect dischargers. The primary advantages of pretreatment control of toxicity are that a smaller volume of waste can be managed by addressing individual sources and the costs are usually the responsibility of the industrial users. Pretreatment requirements may involve a public education effort or the implementation of narrative or numerical limitations for POTW users.

The toxicants to be controlled may not be the same parameters that are currently regulated under the pretreatment program. Examples of these types of toxicants include organophosphate insecticides, TDS, biocides, and specialty chemicals used by industries. In cases where current pretreatment regulations are

inadequate to address sources of toxicants or toxicity, POTW staff should revise or adopt new permit regulations or ordinances, as appropriate. In these cases, it may be necessary to initiate the following steps to control toxicants or toxicity:

- Investigate public education approaches, if the toxicant is widely used in the service area (e.g., organophosphate insecticides).
- Perform an allowable headworks loading analysis.
- Decide whether to establish local limits or implement a more directed approach, such as industrial user management or case-by-case requirements.
- Develop a monitoring program to evaluate compliance with the requirements.

These steps are described below.

Public education has been successfully used to control toxicity at POTWs. Organophosphate insecticides such as diazinon and malathion have been identified as effluent toxicants at many POTWs, especially in the southeast and southwest United States (Norberg-King et al., 1989). Insecticides can be discharged by many users in the POTW service area, including pest control businesses, veterinarians, lawn care businesses, apartment complexes, restaurants, hotels/motels, office buildings, and homeowners. These users are usually not included under pretreatment programs and it may be impractical to control these sources by regulating each discharge. Studies at POTWs in California (Singhasemanon et al., 1997), Texas (City of Greenville, 1991), Oklahoma (Engineering-Science, Inc., 1992), and North Carolina (Fillmore et al., 1990) have determined that public education is a viable option for control of organophosphate insecticide toxicity attributed to multiple sources. Recommended steps in a successful public awareness program include identifying the significant users of insecticides, developing education materials targeted to users, and distributing the materials on an ongoing basis during periods of expected insecticide use. The City of Greenville also enacted an ordinance to encourage the environmentally sound use of insecticides and require merchants to display public education materials where insecticides are sold (City of Greenville, 1991). Additional information on the identification and control of organophosphate insecticides is presented in Appendices F and H. Public education efforts may be applied to control other effluent toxicants that are widely used in POTW service areas and are not

practical to regulate through local pretreatment limitations.

POTW staff have successfully used revised or new pretreatment regulations to reduce POTW effluent toxicity (Appendices C, E, and G). Local pretreatment limits can be developed to control sources of toxicants or toxicity identified in the toxicity source evaluation. USEPA's *Guidance Manual on the Development and Implementation of Local Discharge Limitations Under the Pretreatment Program* (USEPA, 1987b) describes several approaches for developing local limits. These approaches include:

- **Allowable Headworks Loading Method:** Numerical limits are defined based on the maximum pollutant loadings that will allow compliance with receiving water quality criteria, sludge quality criteria, or protection against treatment interferences.
- **Industrial User Management Method:** Based on an in-depth review of indirect discharger practices, POTW staff can set narrative limits for chemical management practices (e.g., chemical substitution, spill prevention, and slug loading control).
- **Case-by-Case Permitting:** Technology-based limits are established based on levels that can be feasibly and economically achieved by industries.

Some of the local limits approaches can be adapted to address effluent toxicants or toxicity. For example, the allowable headworks loading method is well-suited for developing limits to prevent the pass-through of toxicants identified in POTW effluent TIE tests and located by chemical-specific analyses in the toxicity source evaluation. This method can be used to establish the maximum level of the toxicant that can be safely received by the POTW without exceeding the effluent toxicity limit. The LRSA, New Jersey, conducted an allowable headworks loading analysis to address industrial sources of ammonia (see Appendix G). The results of the analysis were used to develop local limits for controllable sources in order to reduce effluent toxicity caused by ammonia.

The industrial user management method provides a framework for implementing chemical management practices including slug discharge control. In cases in which slug loadings contribute to POTW effluent toxicity, spill prevention or load equalization can be implemented at the industrial facility to moderate the

slug loadings. USEPA's *Guidance Manual for Control of Slug Loadings to POTWs* (1988b and 1991c) describes methods for the development of slug loading control programs.

The case-by-case permitting method can be used when the POTW effluent toxicants cannot be identified, but TIE information on the general classes of toxicants is available or sources of toxicity have been located in the toxicity source evaluation. Using TIE data, an engineer may be able to select a pretreatment technology that can remove general types of toxicants (i.e., non-polar organic compounds). In cases where the sources of toxicity have been identified, POTW staff have the authority to require the indirect discharger to take steps to limit the discharge of refractory or inhibitory toxicity (USEPA, 1987b).

Although USEPA and the States with approved pretreatment programs have overview authority, the choice of which approach to use for local limits development is the municipal government's decision. The goal in developing local limits is to implement pretreatment regulations that are technically and legally defensible. Local limits can include provisions for equitable recovery of costs associated with the toxicity source evaluation and limits development.

In-Plant Control Evaluation

The objective of the in-plant control evaluation is to select and evaluate feasible treatment options for the reduction of effluent toxicity at the POTW. Treatability testing may be conducted to determine the toxicity removal effectiveness and operating characteristics of the candidate treatment options. These tests should use acute or chronic toxicity tests and chemical analyses to evaluate the removal of specific toxicants and/or toxicity. The resulting data provide a basis for the final selection and conceptual design of feasible POTW process modifications or additions.

It is important to consider that major changes in treatment plant facilities or operations may not be practical due to the cost of new facilities or the complexity of additional process operations. In these situations, pretreatment control of toxicity may be preferred over in-plant control. Wherever possible, the in-plant control evaluation should be performed in conjunction with the pretreatment control evaluation to identify the most technically feasible and cost-effective control option.

Review Existing Information

The first step in the in-plant control evaluation is to review the POTW performance evaluation data on the POTW design (Section 3) to establish the physical space available for new process additions and to determine the idle facilities and equipment that could be used for toxicity control. Operations and maintenance information also should be reviewed to determine if the POTW is capable of handling the increased operational control that may be required with process modifications or additions. In addition, POTW performance evaluation information should be reviewed to determine how the control options might be integrated into the overall treatment system design.

TIE results on identified effluent toxicants can be used to determine in-plant control options. Although information on specific toxicants is well suited for the application of pretreatment control limitations, POTW staff may choose to evaluate in-plant control of these toxicants. An example is the treatment of ammonia by optimizing the POTW activated sludge process (e.g., increase MCRT) to achieve nitrification. In some cases, TIE Phase I data on the classes of effluent toxicant can be used to select options to be examined. For example, if filterable material is the principal effluent toxicant, possible options would include improved solids clarification or granular media filtration.

In-plant toxicity control may be achieved by enhancement of the existing treatment system or by the implementation of additional treatment processes. In-plant control alternatives for different categories of toxicants are summarized in Table 6-2. A description of these control alternatives is provided as follows.

Process Enhancement

Biological Process Control

Biological process control is most easily applied to suspended growth systems (e.g., conventional activated

sludge and BNR processes), although some modifications to fixed film processes (e.g., trickling filters and RBCs) may be feasible. The performance of activated sludge and BNR systems is generally controlled by adjusting several process parameters, including MCRT, MLSS, DO levels, recycle ratio, and F/M ratio. The treatment efficiency of the activated sludge system is optimized by varying these interrelated process parameters. A description of the use of operational parameters for toxicant control is provided as follows: "Removal of biodegradable toxic compounds in suspended growth systems may be improved by increasing the MCRT" (Adams et al., 1981). MCRT can be increased by lowering the excess sludge wasting rate. Longer MCRTs are necessary for nitrification and can be beneficial for the biodegradation of some types of organic compounds. An example of this approach was practiced at a POTW on the United States' east coast (Judkins and Anderson, 1992). The facility was retrofitted to achieve nitrification to reduce ammonia. Existing treatment capacity, including aeration basins and secondary clarification, was available to accommodate the longer MCRTs and detention times needed to accomplish nitrification and denitrification. The retrofits involved increasing the air supply, changing the air diffuser pattern, adding an anoxic zone in the aeration basins, increasing the MCRT, and modifying the return sludge flow. Usually, mixed liquor from the aerobic zone of the biological treatment process is recycled to the anoxic zone to accomplish denitrification. However, it was possible in this case to use the existing return sludge pumps to recycle the secondary clarifier underflow to the anoxic zone. The cost of the retrofit consisted of approximately \$100,000 in capital costs and an increase in annual operating costs of about 25%.

High MLSS concentrations have been shown to minimize the effects of inhibitory pollutants on activated sludge treatment systems (WEF/ASCE,

Table 6-2. POTW In-Plant Control Technologies for Categories of Toxic Compounds

Biodegradable Organic Compounds and Ammonia*	Non-Biodegradable Organic Compounds	Volatile Organic Compounds	Heavy Metals and Cationic Compounds
Biological process control	Filtration	Biological process control	Filtration
Nutrient addition	Activated carbon	Aeration	Coagulation/precipitation
Coagulation/precipitation	Coagulation/precipitation		pH adjustment

* Air stripping, breakpoint chlorination, and ion exchange also may be considered for ammonia removal; however, the cost of these technologies and the use of toxic additives such as chlorine often preclude their use.

1992a). High MLSS concentrations increase the potential for biodegradation and sorption of toxic wastewater constituents and can help to protect the treatment process from shock loadings. The maximum MLVSS will often be limited by the available secondary clarifier capacity. It is important to consider the effect of increased MLVSS on secondary solids separation and the TSS concentrations of the clarifier effluent.

A decrease in F/M (based on BOD_5) effectively decreases the organic waste loading per unit of biomass, which may improve the biodegradation of some toxic compounds (Adams et al., 1981). The F/M ratio is inversely related to MCRT.

Biological process control is not as easily accomplished for fixed film processes, such as trickling filters or RBCs. Some adjustments can be made, however, such as varying the amount and point of wastewater recirculation in a trickling filter to potentially increase the removal of toxicants or toxicity. In addition, secondary clarifier effluent can be recirculated to dilute high-strength wastes prior to treatment in a trickling filter or RBC. In some cases, inhibitory pollutants may cause excessive sloughing of the fixed film biomass. This problem may be rectified by returning thickened secondary clarifier solids to the fixed film process to help maintain a proper biomass population.

Chemical Addition

The addition of chemicals or additives to waste streams in existing POTW treatment processes may improve toxicant or toxicity removal. Nutrients can be added to influent wastewaters that have low nutrient levels (relative to their organic strength) to improve biological treatment. Lime or caustic chemicals can be used to adjust wastewater pH for optimal biological treatment or for coagulation and precipitation treatment. Other chemical coagulants are used to aid in removal of insoluble toxicants and to improve sludge settling. Powdered activated carbon may be applied in activated sludge systems to remove toxic organic compounds. A description of each of these treatment additives is provided as follows.

Addition of phosphorus, nitrogen, or sulfur may in some cases improve biological treatment of industrial wastewaters with low nutrient concentrations. The optimal BOD_5 /TKN/TP ratio for municipal activated sludge treatment is 100:5:1. Lime and caustic

chemical addition may be used to increase influent wastewater pH prior to primary sedimentation in order to enhance the precipitation of heavy metals. Chemical addition may also be appropriate for removal of metals in sidestreams from sludge processing. Some metals, however, such as iron and chromium will go into solution rather than precipitate at alkaline pH. The optimum pH range for metals precipitation varies for each type of metal and the solubility/precipitation equilibrium can be affected by other factors such as dissolved solids concentrations in the wastewater. Lime and caustic chemicals also provide additional alkalinity, which is essential for biological treatment, especially nitrification treatment, processes.

Polymers and inorganic coagulants such as alum and ferric chloride can be introduced to POTW waste streams to help remove insoluble pollutants. Coagulants may be added to influent wastewater to increase the sedimentation of toxic constituents in primary treatment and thereby minimize the loading of toxicants on the biological treatment process. Coagulants also can be added after the activated sludge aeration basins to control sludge bulking or reduce effluent suspended solids, which may be associated with effluent toxicity. The optimum conditions for coagulation can be determined by conducting bench-scale jar tests. These tests are used to establish the optimum coagulant type and dose, the proper mixing requirements, and the flocculent settling rates for treatment (Adams et al., 1981).

Coagulants can adversely affect the characteristics of sewage sludges, which could affect the sludge disposal method. Coagulants may increase the toxicity of the sludge (as measured by a TCLP) as a result of the removal of toxic wastewater constituents or as a result of the toxicity of the coagulant itself (e.g., metal salts). Therefore, coagulants should be evaluated carefully prior to use.

The addition of PAC to an activated sludge unit may increase the removal of toxic organic chemicals. Organic pollutants that are not biodegraded can be removed by adsorption onto the surfaces of activated carbon particles. Activated carbon also improves sludge settleability by providing a substrate onto which sludge flocs can agglomerate. Although PAC processes have been used in municipal wastewater treatment, studies (Deeny et al., 1988) have shown that PAC regeneration by wet-air oxidation breaks down the activated carbon particles to carbon fines, which

carry over the secondary clarifier weirs. In some cases, periodic additions of PAC to an aeration basin can be used to minimize the effects of toxic slug loadings, thereby improving the stability of the activated sludge system.

POTW Modifications and Additions

Where process enhancement is not feasible or will not provide adequate toxicant removal, physical addition to or modification of the POTW can be undertaken. Additional treatment processes could include equalization prior to treatment, instrumentation control, BNR, and advanced wastewater treatment processes such as coagulation/flocculation, granular media filtration, and GAC treatment. Public works managers also may consider enhancing effluent dilution through the addition of an outfall diffuser or relocation of the outfall to a larger water body.

Equalization

Equalization can be used prior to the biological treatment process to dampen the effect of slug or diurnal loadings of high-strength industrial wastes. Equalization facilities can be provided to either equalize wastewater flows or wastewater concentrations. Flow equalization is partially provided by existing primary sedimentation tanks and can be enhanced by increasing the size of the primary tankage. Concentration equalization requires mixing of the wastewater to moderate intermittent pollutant loadings; therefore, separate facilities must be provided.

Instrumentation Control

Instrumentation/monitoring can be used to help control slug loadings of toxic constituents in the POTW influent wastewater. For example, transient metals loadings may be monitored by continuously measuring the pH and conductivity of the influent wastewater. A significant change in pH or an increase in conductivity may indicate a slug loading of toxic material, which can be manually or automatically diverted to a holding basin. After equalization, the diverted wastewater can be slowly added back to the influent waste stream to dilute the material prior to treatment.

Outfall Diffuser/Relocation

Public works managers may choose to evaluate the alternative of installing a diffuser or relocating the outfall to achieve better dilution. For example, the extension of a shoreline outfall to a submerged high-rate diffuser in deeper water may promote rapid mixing and achieve an acute dilution factor of 10 or more. If

allowed by applicable state water quality standards, the effectiveness of outfall relocation or diffuser installation can be evaluated along with other control options. The reader is referred to USEPA's TSD (1991b) for a discussion of the role of dilution in permitting for whole effluent toxicity control and details on mixing zone analyses and high-rate diffusers.

Advanced Wastewater Treatment

POTWs that only utilize primary and secondary wastewater treatment may achieve toxicity reduction by the addition of advanced wastewater treatment processes such as coagulation/flocculation, sedimentation, granular media filtration, and granular activated carbon. Each of these processes can provide enhanced removal of some toxicants and toxicity. Treatability tests used to evaluate treatment process additions are described below.

Treatability Testing

Bench-scale and pilot-scale treatability tests are commonly used to evaluate treatment options that have been selected for testing. Bench-scale or pilot-scale tests offer several advantages compared to full-scale testing, including a more manageable test unit size and the ability to vary the operating conditions to evaluate toxicity reduction. Treatability methods can range from simple jar tests for testing coagulation/flocculation options to flow-through bioreactors for investigating the biodegradation kinetics of wastewater treatment.

During treatability testing, influent, effluent, and sidestream wastewaters of the treatment simulation are tested for acute or chronic toxicity. Toxicity testing is used to assess the effectiveness of the treatment option in reducing wastewater toxicity and to determine the fate of toxicity in the treatment process. Initial testing should use the simplified toxicity test methods described in the TIE manuals (USEPA 1991a, 1992a, 1996) because of the large number of samples that may need to be tested. Toxicity screening tests such as Microtox[®] also may be used in conjunction with the required test species to provide additional information. These tests are recommended for waste streams with a high oxygen demand (i.e., high BOD₅ concentration), which would otherwise require aeration when testing with permit species. Aeration should be avoided because it may remove volatile or oxidizable toxicants.

Definitive acute or chronic toxicity tests (USEPA 1993c, 1994a, 1994b, 1995) should be used at the

completion of treatability testing to verify the option's capability in meeting the NPDES permit limit. Optional TIE Phase I analyses also may be performed on treatability test samples to confirm toxicant removal by the treatment option.

Activated Sludge/BNR Treatment

The basic parameters of interest in the design of activated sludge/BNR systems include organic loading, oxygen requirements, nutrient requirements, sludge production, sludge settleability, and internal recycle rates. Continuous flow systems are most useful for evaluating activated sludge/BNR systems; however, batch systems may provide sufficient treatability information in some cases. An example of the use of batch treatment tests in a TRE is provided in Appendix D. This study determined that an upgrade of a conventional activated sludge process to a five-stage BNR process would achieve compliance with chronic toxicity limits. Follow-up monitoring upon completion of the upgrades confirmed the toxicity reduction.

Consideration should be given to evaluating design specifications and operating conditions that are expected to optimize the treatment of toxicants and toxicity. These parameters may include relatively long MCRTs and high MLVSS levels, which have been shown to improve toxic pollutant removal and protect the process from inhibitory wastes (Hagelstein and Dauge, 1984; WEF/ASCE, 1992a).

Coagulation/Flocculation

The evaluation of coagulation and flocculation treatment involves the use of bench-scale jar tests or zeta potential tests to provide information on the optimum coagulant type and dosage, mixing rates, and flocculent settling rates for removal of solids and flocculent suspensions (Adams et al., 1981). Results of these tests are used to devise treatability tests to evaluate the sedimentation of flocculent suspensions.

Sedimentation

Sedimentation involves the removal of suspended solids or flocculent suspensions by gravity settling. Sedimentation is evaluated by conducting a series of settling column tests that measure the settling rates of solids or flocculent suspensions (Adams et al., 1981). Test results are used to calculate a settling profile that can be used for clarifier design.

Granular Media Filtration

Filtration testing involves scaled-down models (usually pilot-scale) of full-sized filters. The choice of filter media and test-flow rates should correspond to the intended design and operating criteria. Although the process scale is reduced, the bed gradation and thickness should be equivalent to anticipated full-scale processes in order to predict actual treatment performance (Adams et al., 1981).

Granular Activated Carbon

The carbon adsorption isotherm test is used to determine the optimum type and dosage of activated carbon for wastewater treatment (Adams et al., 1981). Results of this test are used to prepare bench-scale or pilot-scale carbon columns that can be used to evaluate carbon exhaustion rates and the effect of carbon regeneration on toxicity removal performance.

Toxicity Control Selection

The final process of toxicity control selection involves an assessment of potential control options and selection of the best option(s) for toxicity reduction based on several criteria. In most cases, both a pretreatment control evaluation and an in-plant control evaluation will have been performed; therefore, the review information should include the data developed in both evaluations.

The choice of in-plant toxicity control or pretreatment toxicity control will depend largely on the technical and economic feasibility of POTW treatment modifications and pretreatment controls. Pretreatment control will be feasible in situations where the TIE data and the toxicity source evaluation data are sufficient to definitively identify the sources of toxicity. These data should provide an indication of the variability of toxicity and toxicants in the indirect discharge. If these conditions are satisfied, POTW staff can set local limits using the methods outlined above. In-plant control will be preferred in cases where the implementation of feasible treatment modifications or additions is more practical than pretreatment control. Data obtained in treatability studies should include information on the variability of toxicity treatment performance and the design criteria for implementing the treatment option. In-plant options provide POTW staff a direct method of controlling effluent toxicity; however, in-plant modifications or additions may substantially increase process operation requirements and maintenance costs.

Selection of Toxicity Control Options

Final selection of the preferred toxicity control option(s) involves a comparison of the options using appropriate criteria (see example in Table 6-1). It may be necessary to select and implement more than one control option to ensure compliance with effluent toxicity requirements. The preceding evaluations should provide sufficient information to document the technical and cost considerations for each option.

Compliance with Effluent Toxicity Limits or Requirements

Data gathered through the TRE should indicate that the selected option will consistently achieve compliance with the toxicity permit requirement. Sufficient information should be provided to show that the option will reduce effluent toxicity even during periods of maximum occurrence of toxicity. If this information includes bench and/or pilot-scale treatability data, scale-up factors must be incorporated into estimates of toxicity reduction to adjust for differences in treatment efficiency between laboratory and full-scale treatment systems. Likewise, safety factors should be included in the calculation of local limits to allow for variation in toxicant loadings to the POTW. It also may be necessary to conduct a sensitivity analysis to evaluate the effectiveness of the options under variable conditions (e.g., variable toxicant loadings or treatment performance).

A relative scoring system can be used to rate the overall potential for the options to achieve permit compliance. The scores can be entered into a matrix table like that shown in Table 6-1.

Compliance with Other Permits

Steps taken to reduce effluent toxicity may have a detrimental effect on other permitted activities such as sludge disposal or air emissions. If toxicants are expected to be transferred to sludge or air, the potential effects on limitations specified in residuals and air permits should be estimated. Each option should be rated for its potential to comply with related permits.

Capital, Operation, and Maintenance Costs

Sufficient detail on costs should be presented to allow a straight-forward comparison of the control options. Cost estimates should include the effort and materials required for planning, implementation, operation, and maintenance of the options. Cost information may be obtained from equipment vendors, engineering consultants, and existing data for comparable systems.

Costs for requisite environmental and construction permits should be included.

In some cases, it may be possible to recover some of the costs of implementation from responsible dischargers. For example, municipalities may apply surcharges to local limits or request in-kind funding for POTW modifications or additions to recover the costs of toxicity control. Anticipated cash returns should be included in the final cost estimate.

Costs for all options can be ranked and a score can be assigned and entered into a matrix table. Weighting factors may be incorporated into the scoring if funding of some options is uncertain.

Ease of Implementation

Factors such as land availability, permits, operability, and maintenance will have a major influence on the selection of options involving POTW modifications or additions. Likewise, the economic impact and level of community cooperation anticipated from new pretreatment regulations will affect the selection of pretreatment control options. Public works managers should develop a list of all potential constraints as well as benefits of the candidate control methods. Benefits should address items other than effluent toxicity reduction such as improved treatment conditions or better cooperation among POTW users. Each constraint and benefit can be assigned a weighted score, the individual values can be summed for each option, and the total value entered into the matrix table.

Reliability

The selected option(s) must be dependable. Pretreatment approaches or treatment processes that tend to malfunction or fail because of difficulties in executing complicated operational plans should be avoided. Experience in implementing similar projects will be useful in defining the reliability of the options. Public works managers should consider each option's operational history, maintenance requirements, and longevity.

Environmental Impact

Some options may require the evaluation of environmental issues related to the protection of wetlands, rare and endangered species, and cultural resources. Although the costs of these evaluations are included under the above cost criteria, other factors will affect the decision-making process, including public perception, time period for permit approval (if

needed), and potential remediation issues. A score can be developed based on these factors and entered into the matrix table.

Comparison of the Toxicity Control Options

Scores developed in the criteria evaluation are summed for each option. These scores will incorporate all necessary weighting factors; therefore, the total scores

for each option can be compared directly. The options can be ranked according to their scores and the highest ranked option(s) can be selected for implementation. In some cases, it may be necessary to select more than one toxicity control option to ensure that permit compliance will be achieved. This approach is highly recommended when the control options are relatively inexpensive to implement, operate, and maintain.

Section 7

Toxicity Control Implementation

Introduction

Once the evaluation and selection of toxicity control options has been completed, the final steps in the TRE are the implementation of the selected pretreatment and in-plant control options and follow-up monitoring to ensure permit compliance. The degree of effort in the implementation step will depend on the severity of the effluent toxicity and the complexity of the selected control approaches. Depending on the findings of the TRE, implementation may involve relatively minor changes such as modifying POTW operating procedures or more complex modifications such as expanding the POTW's pretreatment program or designing and constructing new treatment facilities.

Implementation

Using the results of the previous steps in the TRE, a Toxics Control Implementation Plan (TCIP) should be developed. The TCIP should summarize the results of the TRE, results of the screening and selection of toxicity control options, and justification for selecting the preferred toxicity control option(s). For in-plant control options, the TCIP should provide the basis of design for the selected control options, including capital and operating costs, and a schedule for design and construction. For pretreatment control options, the TCIP should specify the basis of selection and technical justification for local limits and discharger monitoring methods. In addition, the procedures for implementing revised pretreatment regulations also should be defined.

Follow-Up Monitoring

Once a control technology has been implemented, a follow-up monitoring program should be developed and implemented to ensure the effectiveness of the selected control option(s). In most cases, the

conditions and frequency of monitoring will be set by the regulatory agency. If source controls are implemented, POTW staff should specify additional monitoring requirements for indirect dischargers under the pretreatment program. These requirements may include verification of statements from industries that the required reduction of toxicity has been made.

The POTW effluent monitoring program should be designed to provide data to ensure that toxicity has been reduced to acceptable levels and that the TRE objectives have been met. This program may involve more frequent monitoring than is required by the NPDES permit, including monitoring to evaluate daily, weekly, monthly, or seasonal variations in effluent toxicity that were observed during the TRE. Follow-up monitoring should utilize the test species and methods specified in the discharge permit. Additional tests, including surrogate methods applied in the TRE, may be included to re-evaluate correlations between test species that may have changed as a result of the effluent toxicity reduction.

Any effluent toxicants that were determined to be present prior to implementation of the control technology should be monitored to ensure that concentrations are below toxic levels. Approved analytical methods will generally be applied; however, screening methods such as ELISA tests or other field kits, which may not be specifically approved by USEPA, can be used to evaluate trends and identify potential problems for follow-up testing. As with toxicity monitoring, the analytical program should re-evaluate trends in toxicant concentrations observed during the TRE. A discussion of an ongoing POTW monitoring program for organophosphate pesticides is described in Appendix F.

Section 8

Quality Assurance/Quality Control

Introduction

A QA/QC program for the TRE should be developed and implemented to ensure the reliability of the collected data. The QA/QC program should include addressing the monitoring of field sampling and measurement activities, the review of laboratory analysis procedures, and the documentation and reporting of the analytical data. A QA/QC program should be designed so that corrective action can be quickly implemented to detect and eliminate erroneous or questionable data without undue expense to the project or major delays in the schedule.

The POTW laboratory manager should ensure that the specific QA/QC requirements for TRE activities are addressed by the facility's QA/QC plan. If a private consultant is to be used for all or part of the TRE testing, the POTW laboratory manager should request a QA/QC plan from the consultant and review the consultant's proposed QA/QC activities. Whether the TRE is to be performed by the POTW laboratory or by a consultant, it is essential that the project organization include competent chemists, toxicologists, and engineers who have adequate knowledge of TRE methods.

The QA/QC plan should be prepared prior to the initiation of the TRE and should contain the following elements:

- QA/QC objectives
- Sample collection and preservation techniques
- Chain of custody procedures
- Analytical QA/QC
- Laboratory equipment maintenance
- QA/QC training requirements
- Documentation and reporting procedures
- Corrective action protocols.

Sample Collection and Preservation

To ensure quality control in sample collection activities, the TRE sampling plan (Section 11) should be strictly followed. In addition, the QA/QC plan should state the minimum sample volumes, maximum sampling holding times, and sample preservation techniques for each analytical method. The sampling requirements for conventional and priority pollutant analyses are described in USEPA's *Methods for Chemical Analysis of Water and Wastes* (USEPA, 1983b) and *Standard Methods for the Examination of Water and Wastewater* (APHA, 1995). Sampling requirements for acute toxicity tests are provided in USEPA's *Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms* (USEPA, 1993c) and *Methods for Aquatic Toxicity Identification Evaluations: Phase I, Toxicity Characterization Procedures* (USEPA, 1991a). Sampling requirements for chronic toxicity tests are provided in USEPA's *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms* (USEPA, 1994a), *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms* (USEPA, 1994b), *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms* (USEPA, 1995), *Toxicity Identification Evaluation: Characterization of Chronically Toxic Effluents, Phase I* (USEPA, 1992a), and *Marine Toxicity Identification Evaluation (TIE)* (USEPA, 1996).

It is important to routinely assess the effects of sample holding times on wastewater toxicity to predict how long samples can be kept before changes in toxicity occur. For example, the acute TIE Phase I manual (USEPA, 1991a) describes how testing the sample toxicity on the day of collection and comparing this initial toxicity to its baseline toxicity (tested 1 day

later) can provide information on appropriate sampling holding times for toxicity analysis. In chronic TIEs, effluent manipulations are performed on the day the sample is received so that the possible effects of any toxicity degradation are minimized (USEPA, 1992a).

Other QA/QC considerations for TRE sample collection include routine cleaning and inspection of automatic sampling equipment, cleaning sample containers according to the requirements for each analytical method, and collecting duplicate samples and field blanks. When preserving samples for chemical analysis, only analytical grade preservatives should be used to avoid contamination and overestimation of analyte concentrations. Unpreserved samples that are to be used for toxicity and chemical analyses require sample containers that are both toxicologically and analytically clean. Equipment and containers used for toxicity test samples require special cleaning procedures outlined in USEPA manuals (1993c, 1994a, 1994b).

Chain-of-Custody

A chain-of-custody (COC) form should accompany all samples to document the collection, preservation, and handling of samples. The COC form should indicate the sample identification number, sample type (i.e., composite or grab), date and time of collection, a brief description of the sample, number of samples taken, name of the person taking the sample and performing field measurements, and sample characteristics such as temperature, pH, total and free residual chlorine, and conductivity. A field book also should be used to record any field observations or conditions noted during sampling along with other pertinent information. Each laboratory should identify a sample custodian to log in and store samples collected during the TRE. The sample custodian should acknowledge receipt of samples by signing the COC form and noting the date and time of sample receipt, the sample identification number, the laboratory accession code, and sampling information such as temperature, pH, and TRC. Upon receipt of the sample, a sample tracking form should be used to record the date, time, and volume of aliquots of the sample removed for analysis, the analyst, and any changes in the nature of the sample, including its toxicity, over time. All COC and sample tracking forms should be maintained in a permanent file so that information on specific samples can be traced easily.

TRE Procedures

Analytical tests should provide data of an acceptable quality for characterizing wastewater toxicity and for evaluating methods and technologies for toxicity reduction. Several test methods described in this document are not standard procedures and require careful attention to unique QA/QC procedures. Special QA/QC procedures for each major TRE test are discussed below. Whenever possible, these procedures should be followed to ensure precise and accurate results.

Toxicity Identification Evaluation

Special precautions for TIE tests are discussed in the Phase I, II, and III manuals (USEPA 1991a, 1992a, 1993a, 1993b, 1996). In general, strict adherence to standard quality control practices is *not* required for conducting Phase I analyses due to the large number of toxicity tests to be performed and the tentative nature of the toxicant characterization. Nonetheless, system blanks and controls should be used whenever possible to indicate toxicity artifacts caused by the characterization procedures. In Phase II more attention should be paid to quality control in order to identify interferences in toxicant characterization and identification. Even greater attention to quality control is needed in Phase III. Sample manipulation should be minimized in Phase III to prevent analytical interferences and toxicity artifacts. Field replicates, system blanks, controls, and calibration standards should be used extensively to allow a precise and accurate determination of the sample toxicants and toxicity.

Specific precautions for characterization (Phase I) and toxicity testing in TIE analyses are provided below.

Aeration

For air stripping or aeration tests, only a high quality compressed air source should be used. Oil, water, and dirt are undesirable contaminants in compressed air; therefore, it is important to use equipment and filters that generate dry, oil-free air. Oil-sealed air compressors should not be used. Simple aeration devices, such as those sold for use with aquariums, are acceptable provided that the ambient laboratory air is uncontaminated (USEPA, 1991a). Recommendations for in-line filters for air exchange systems in laboratories are provided by USEPA (1993c).

Filtration

High purity water, which has been adjusted to a specified pH, should be used to rinse filters between filtration steps (USEPA 1991a, 1992a). Filtration equipment should be rinsed with 10% nitric acid (HNO₃), acetone, and high purity water between sample aliquots. Filtration equipment should be made of plastic to avoid leaching of metals or other toxicants during acid washes. Toxicity can be checked by testing filtered dilution water.

pH Adjustments

Concerns in the pH adjustment steps involve artificial toxicity caused by excessive ion concentrations from the addition of acid and base solutions, contamination from impure acid and base solutions, and silver contamination from some pH probes (USEPA 1991a, 1992a). The baseline toxicity test acts as a control for indicating whether addition of acid and base solutions increases effluent toxicity. Ultra-pure acids and bases should be used to minimize artificial toxicity. During pH measurement, toxic concentrations of silver can leach from refillable calomel electrodes; therefore, only solid state pH probes should be used.

Methanol/C18 SPE Column

HPLC grade methanol is required for C18 SPE column preparation and extraction steps. A blank toxicity test should be conducted for each methanol reagent lot. In addition, a toxicity blank should be performed on each C18 SPE column to check for resin-related toxicity (USEPA 1991a, 1992a).

Sodium Thiosulfate Addition

The TIE manuals (USEPA 1991a, 1992a, 1996) provide information on the toxicity of sodium thiosulfate to several freshwater and marine species. These manuals prescribe the amount of sodium thiosulfate to use in testing. If alternative species are to be used, the species tolerance should be evaluated by adding increasing quantities of sodium thiosulfate to aliquots of the sample, testing the resulting toxicity, and comparing the toxicity to the sample's baseline toxicity.

EDTA Addition

The TIE manuals (USEPA 1991a, 1992a, 1996) also prescribe the concentration of EDTA ligand to be added to samples. If alternative species are to be used in the TIE, the same test approach noted above for sodium thiosulfate can be applied.

Toxicity Tests

The organisms used to test the sample toxicity prior to and following each characterization step should not be subject to undue stresses such as contamination (USEPA, 1991a). The test organisms should have had no prior exposure to pollutants and their sensitivity should be constant over time. To assess changes in the sensitivity of the test organisms, a standard reference toxicant test should be performed on a regular basis and accompanying quality control charts should be developed (USEPA 1993c, 1994a, 1994b). Reference toxicant tests should be performed monthly. If test organism cultures are not maintained in the laboratory, reference toxicant tests should be performed with each group of test organisms received, unless such information is available from the vendor. Information on obtaining and culturing species for toxicity testing is provided in the acute and chronic toxicity test manuals (USEPA 1993c, 1994a, 1994b).

The quality of the dilution water used in toxicity tests will depend on the purpose of the TIE test and whether the test is being performed for toxicant characterization (Phase I), identification (Phase II), or confirmation (Phase III). Much of Phase I and parts of Phase II rely on relative toxicity measurement; therefore, water that is of consistent quality and will support the growth and reproduction of the test species is suitable for these phases of the TIE (USEPA 1991a, 1992a, 1996). The objective of Phase III, however, is to confirm the true cause of toxicity; therefore, artifacts are to be excluded and the choice of dilution water should follow Phase III guidance (USEPA, 1993b). Guidance for preparing the dilution waters is described by USEPA (1991a, 1992a, 1996).

USEPA (1991a) recommends feeding cladocerans (i.e., *C. dubia* and *Daphnia* sp.) in the TIE test solutions at the beginning of acute TIE toxicity tests. Daily feeding is required in the chronic TIE tests (USEPA, 1992a). Feeding requirements for selected species are described in the acute and chronic toxicity test manuals (USEPA 1993c, 1994a, 1994b, 1995).

Sample pH should be recorded at each sample renewal. Additional pH measurements may be needed during the test, especially if ammonia toxicity is a concern.

DO measurements may be made at sample renewal or at the end of the exposure period in the TIE. In cases where low DO is a problem, DO adjustment should be

performed at a rate that will not intentionally change the sample toxicity.

Refractory Toxicity Assessment and Treatability Tests

RTA and treatability tests are subject to a variety of potential interferences due to the large number of variables that must be accounted for and controlled during testing. When performing RTA and treatability analyses, it is important to hold all parameters potentially affecting toxicity constant so that sample toxicity is the sole variable. Important parameters to be controlled in RTA testing include the test solution temperature, DO level, and pH.

The QA/QC concerns for toxicity analysis in RTA and treatability tests are the same as those noted above for TIE tests. Selection and use of test species and dilution water should follow procedures given in the USEPA Phase I manual (USEPA 1991a, 1992a).

Potential sources of toxicity contamination should be identified through the use of system blanks. As in TIE testing, the filters used in RTA testing should be tested to determine if toxicity is added during filtration. Each of the solutions used in RTA testing, including activated sludge, should be checked for toxicity. In the Patapsco TRE, the RAS used in the RTA batch tests was found to be acutely toxic to *C. dubia* (Botts et al., 1987). Steps for addressing RAS toxicity are described in Section 5. Similarly, the reagents used in treatability testing such as chemical coagulants should be screened for toxicity.

Field replicates, calibration standards, and analytical replicates should be routinely performed during RTA and treatability testing. Results of these quality control analyses can be used to calculate the precision, accuracy, and the sensitivity of each physical/chemical analysis method used in these studies.

Chemical Analyses

Quality control for chemical analyses includes the use of calibration standards, replicate analyses, spiked sample analyses, and performance standards. The detection limits and the recommended reagents for method calibration and spiking are discussed in USEPA's *Methods for Chemical Analysis of Water and Wastes* (USEPA, 1983b) and *Standard Methods for Examination of Water and Wastewater* (APHA, 1995). General information on laboratory quality control for chemical analyses is provided in USEPA's *Handbook for Analytical Quality Control in Water and Wastewater Laboratories* (USEPA, 1979a).

Equipment Maintenance

All facilities and equipment such as pH, DO, and conductivity meters, spectrophotometers, GC/MS, and HPLC instruments should be inspected and maintained according to manufacturers' specifications. Standard operating procedures (SOP) should be followed for routine maintenance and calibration of each analytical instrument. A maintenance log book also should be kept for each major laboratory instrument.

The measurement of toxicity or trace compounds in wastewater samples requires the use of carefully cleaned instruments and glassware. Instruments that involve flow-through analysis such as automated spectrophotometers should be inspected to ensure that flow-through parts (i.e., tubing) are periodically replaced. New glassware may be contaminated with trace amounts of metals; therefore, any glassware being used in toxicity tests for the first time should be soaked for three days in 10% HNO₃ (USEPA, 1991a). For subsequent use in TIE and toxicity tests, the glassware should be washed with phosphate-free detergent, and sequentially rinsed with 10% HNO₃, acetone, and finally high-purity water (USEPA 1993c, 1994a, 1994b).

Documentation and Reporting of Data

Basic steps in a successful QA/QC program are the documentation of the analytical data in meaningful, exact terms, and reporting the analytical data in a proper form for future interpretation and use. To ensure the reliability of the data, its handling must be periodically monitored and reviewed. This review generally consists of three elements: an assessment of laboratory record-keeping procedures, a review of the data calculations, and a review of the final reported data. On the basis of these review steps and the QA/QC analyses for precision and accuracy, the data are accepted or rejected. This review process is essential because some or all records may have to be submitted for review by State or Federal regulatory agencies.

Corrective Action

Procedures should be established to ensure that QA/QC problems such as improper sampling techniques, inadequate COC records, and poor precision and accuracy results are promptly investigated and corrected. When a QA/QC deficiency is noted, the cause of the condition should be determined and corrective action should be taken to preclude repetition.

Section 9

Health and Safety

Introduction

A health and safety (H&S) plan may be necessary to establish policies and procedures to protect workers from hazards posed by TRE sampling and analytical activities. The general guidelines outlined in this section should be integrated into existing H&S programs even if a specific H&S plan is not required. Whether a specific H&S plan is necessary or not will depend on the conditions under which the TRE is being conducted. For example, if the POTW operates under an RCRA permit by rule, then H&S must be addressed when collecting and analyzing hazardous wastes.

Important considerations for H&S for TRE studies include:

- Identification of personnel responsible for H&S matters
- H&S information and training activities
- Protective equipment required for TRE activities
- Materials cleanup and disposal procedures
- Emergency response contingencies.

Detailed information on the preparation and scope of H&S plans is provided in the Occupational Safety and Health Administration's (OSHA's) *Safety and Health Standards, General Industry* (OSHA, 1976). The following subsections discuss specific H&S considerations for selected TRE activities.

Sample Collection and Handling

Working with waste streams of unknown composition is inherent to TREs. Samples of industrial sewer discharges, municipal wastewater, and sewage sludge can contain a variety of toxic and hazardous materials (e.g., pathogens, carcinogens) at concentrations that can be harmful to human health.

It is the responsibility of the laboratory sample custodian to ensure that TRE samples are properly stored, handled, and discarded after use (see Section 8). Upon sample storage, the sample custodian should indicate the H&S considerations for sample handling and disposal.

Exposure to toxic and hazardous sample constituents should be minimized during sampling handling. The principal routes of human exposure to toxics is via inhalation, dermal absorption, and/or accidental ingestion. Exposure can be minimized through the use of proper laboratory safety equipment such as gloves, laboratory aprons or coats, safety glasses, respirators, and laboratory hoods. Laboratory hoods are especially important when testing wastewaters containing toxic volatile substances such as volatile priority pollutant compounds, hydrogen sulfide, or hydrogen cyanide. Proper dermal protection such as using neoprene gloves for solvent-containing wastes also is important. Laboratory managers should consult the manufacturers' specifications in selecting appropriate clothing materials for protection against specific chemicals.

Residual wastewater samples and wastes generated during TRE studies should be disposed of properly. Residual municipal wastewater and other non-hazardous wastes can be disposed directly into the sink drain if the TRE is being conducted at the POTW. Residual industrial samples and other wastes that may contain hazardous materials should be decontaminated and/or disposed of in accordance with hazardous waste regulations (NIOSH, 1977).

TRE Methods

Specific precautions to be followed for selected TRE techniques are described below.

Toxicity Identification Procedures (TIE)

USEPA's TIE Phase I manuals (USEPA 1991a, 1992a, 1996) address the general H&S concerns involved in performing TIE testing. Ventilation is a specific concern when performing the Phase I aeration procedure. The aeration test should be performed in laboratory hoods to prevent exposure to toxic volatile compounds or pathogens resulting from aeration.

H&S considerations for aquatic toxicity testing are addressed in USEPA's toxicity test manuals (USEPA 1993c, 1994a, 1994b, 1995). Special precautions need to be taken for on-site mobile laboratories in the handling and transportation of chemicals, supply of adequate ventilation and safe electrical power, and disposal of waste materials.

Refractory Toxicity Assessment and Treatability Tests

Proper ventilation also is important when conducting RTAs and treatability tests in the laboratory. Hoods should be used to capture and vent potential volatile compounds that are stripped from the wastewater during biological treatment tests.

Physical/chemical treatability testing may involve the use of hazardous reagents such as acids or caustics.

Caution should be taken in the handling and disposal of these chemicals.

Chemical Analyses

Several reagents used for chemical-specific analyses (e.g., priority pollutants, COD, etc.) are toxic or hazardous substances. Analysts should be familiar with safe handling procedures for all reagents used in testing, including the practice of proper chemical storage to avoid storing incompatible chemicals together (NIOSH, 1977; OSHA, 1976). After use, the waste chemicals should be converted into a less hazardous form in the laboratory before disposal or disposed of by a commercial disposal specialist.

General Precautions

USEPA (1977) and the American Chemistry Society (1979) describe additional laboratory safety procedures that can be used in TRE studies, including:

- Use of safety and protective equipment such as eye protection (safety goggles, eye wash), fire hazard protection (smoke and fire detectors, fire extinguishers), and electrical shock protection (ground-fault interrupters for wet laboratories).
- Protocols for emergency response and materials cleanup.
- Personnel training in H&S procedures.

Section 10

Facilities and Equipment

Introduction

Laboratories should be equipped with all the basic and specialized laboratory equipment required to conduct the TRE, and laboratory personnel should be skilled and experienced in operating this equipment. The facilities and equipment needed to perform a TRE will be different for each POTW and will depend on the type of testing to be performed in the TRE. In general, the minimum facilities and equipment for initiating a TRE will include the equipment needed for toxicity and TIE testing (USEPA 1991a, 1992a, 1993c, 1994a, 1994b, 1996). As additional information becomes necessary, facility and equipment needs will depend on the physical/chemical characteristics of the causative toxicants and the toxicity control approaches to be evaluated. For example, the selection of bench-scale equipment and/or pilot plant facilities for treatability studies will be dictated by the control options to be tested (e.g., physical/chemical processes such as filtration or biological processes).

The choice of whether to work on-site or off-site will depend on the stage of the TRE, the approach for tracking sources of toxicants or toxicity, and the requirements for treatability testing. In general, the equipment and time required for conducting TIE tests makes on-site testing less feasible. If the loss of sample toxicity over time is minimal, TIE samples can be shipped and tested off-site, usually at much less cost than on-site testing. If toxicity tracking using RTA tests is required, on-site testing is recommended for the treatment phase of the RTA, because fresh samples of the POTW RAS biomass must be used. Treatability tests that require continuous supplies of POTW influent or process wastewaters and/or activated sludge (i.e., flow-through bioreactor tests) also may be more efficiently conducted in on-site facilities. Some treatability evaluations require unique or sophisticated equipment (e.g., ultra-filtration apparatus) that is not readily available for on-site

work. In these situations, the equipment vendor may be able to conduct the required tests at their facility.

The general equipment requirements for each of the main TRE methods are summarized below. H&S equipment is discussed in Section 9.

Toxicity Identification Evaluations

Laboratories should be equipped with the equipment and materials needed to conduct the TIE, including filtration and air-stripping equipment, pH and DO meters, C18 SPE columns, fluid metering pumps, required reagents for the TIE manipulations, and facilities for organism handling, water preparation, sample holding, and glassware cleaning. Equipment requirements for culturing standard test species are described in USEPA's acute and chronic toxicity test manuals (USEPA 1993c, 1994a, 1994b, 1995).

More sophisticated analytical equipment is required for the TIE Phase II toxicant identification and TIE Phase III toxicant confirmation procedures. The choice of analytical instruments for these procedures will depend on the compound to be measured. Equipment may include an analytical balance, a GC/MS, an HPLC, an atomic absorption (AA) spectrometer, an inductively coupled plasma (ICP) spectrometer, an ultraviolet-visible spectrophotometer (UV-VIS), an ion chromatograph, ion selective electrodes, a pH meter, a conductivity meter, and a refractometer. Use of inert materials such as perfluorocarbon plastics for TIE Phases II and III are recommended to protect against toxicity artifacts (USEPA, 1991a).

Refractory Toxicity Assessment and Treatability Tests

Laboratories should be equipped with the basic equipment for setting up and operating the RTA batch

reactors, including an air supply, electrical supply, and a laboratory hood. Instruments for monitoring the batch reactors include respirometer and/or oxygen meter, pH meter, ion selective electrode meter and probes, total TOC analyzer, spectrophotometer for COD and nutrient (e.g., ammonia and nitrate) analyses. A drying oven, muffle furnace, and analytical balance will be needed for TSS and VSS measurements.

The equipment for toxicity testing will depend on the choice of toxicity screening tests. Depending on the species to be used, it may be more economical to

culture the test organisms than to purchase them. In some cases, rapid screening tests such as a bacterial bioluminescence test (e.g., Microtox[®]) may be used as a surrogate method for toxicity testing (see the Billerica, Massachusetts, case history in Appendix A).

General Analytical Laboratory Equipment

General laboratory equipment such as refrigerators, a water purification system, and commonly used reagents are needed to support the TIE and RTA analyses. The type of water purification system needed for testing is described by USEPA (1993c, 1994a, 1994b, 1995).

Section 11

Sample Collection and Handling

Introduction

The most important criterion in sampling is to obtain a sample that is representative of the discharge. Several samples will need to be collected to ensure that the samples represent the typical toxicological and chemical quality of the wastewater. Guidelines for sample collection and handling are presented in the acute and chronic toxicity test manuals (USEPA 1993c, 1994a, 1994b, 1995) and the Phase I TIE documents (USEPA 1991a, 1992a, 1996). The WEF also has published a useful guide to sampling at POTWs (WEF, 1996).

A sampling plan should be prepared to document the procedures to be followed in TRE sampling. This plan should include:

- A description of sampling locations
- Sampling equipment and methodology
- Sample delivery requirements.

These elements are discussed in the following subsections. QA/QC procedures for sampling are addressed in Section 8. Procedures include preparing COC forms, maintaining sampling equipment, and identifying the minimum volume requirements, holding times, and preservation techniques for samples.

Sampling Location

Sampling locations should be established where representative samples can be readily obtained. When sampling waste streams within the POTW, care should be taken to exclude unwanted waste streams and select a sampling point that is most representative of the discharge (e.g., the common discharge channel for secondary clarifiers). The sampling location for the POTW effluent should correspond with the biomonitoring sampling point stated in the NPDES permit. If the permit does not specify whether the

effluent sample is to be collected prior to or following the chlorination/dechlorination treatment process, the choice of a sampling location will depend on the toxicants of concern. Generally, sampling at the point of final discharge is the best option; however, sampling both before and after chlorination/dechlorination may help to determine if toxicity is caused by chlorination (i.e., TRC) or dechlorination. If samples are collected following the chlorination process, free chlorine and TRC should be measured when sampling is completed and upon initiation of toxicity tests. These results will provide information on the potential for chlorine toxicity.

Wastewater sampling for toxicity source evaluations requires knowledge of sewer discharge locations. Sampling may be conducted at the point of sewer discharge or within the sewers in the municipal sewer collection system. The choice of sampling points for sewer line tracking may be based on existing pretreatment program data. If these data are not available, a sampling scheme can be devised to locate sources of toxicity by testing and eliminating segments of the collection system that prove to be non-toxic. In some cases, indirect dischargers may have multiple sewer discharges that need to be included when sampling.

RTA testing requires samples of the POTW influent (primary effluent) and activated sludge. Primary effluent samples should be collected at the overflow weirs of the primary sedimentation tanks. Activated sludge samples can be collected from the aeration basin effluent weirs or the RAS pipelines.

POTW Sampling

The choice of grab or composite samples of POTW waste streams (i.e., effluent and influent wastewater and process waste streams) will depend on the physical/chemical characteristics and variability of the

toxicants. Initial effluent toxicity characterization (TIE Phase I) should utilize 24-hour composite samples in order to ascertain the daily, weekly, or seasonal variability of the causative agents. If effluent toxicity is not easily observed in 24-hour time composites, flow proportional composite or grab samples may be used to observe possible flow-related peaks of toxicity. In the latter phases of the TIE, grab samples may be used to determine the variability in the type and concentration of effluent toxicants (USEPA 1991a, 1992a). A discussion of the use of grab versus composite sampling for toxicity tests is provided by USEPA (USEPA 1993c, 1994a, 1994b, 1995). The choice of sampling techniques for chemical-specific analyses is dependent on the type of compounds to be measured (e.g., grab sampling for volatile organic compounds).

When evaluating the treatment efficiency of the POTW or its unit processes, collection of the influent and effluent wastewaters should be lagged by the hydraulic retention time (HRT) of the treatment process in order to obtain comparable samples. For example, if the HRT of the treatment plant is 20 hours, the effluent sampler should be timed to start 20 hours after influent sampling is initiated. Likewise, sampling of wastewater from industries or sewers should account for the travel time in the collection system (i.e., POTW influent sample collection should lag industry sample collection).

Samples also should be collected during representative discharge periods. An evaluation of the POTW operations and performance at the time of sampling can be made by comparing the effluent sample concentrations of BOD₅, TSS, and other pollutants to long-term historical averages and/or permitted values for those parameters.

Effluent samples are often collected, shipped, and stored in plastic containers. However, some toxicants such as surfactants may adsorb to plastic. A simple way to check for this characteristic is to collect and ship samples in both glass and plastic containers, then test the samples for toxicity (USEPA, 1991a). A greater loss of toxicity in plastic containers as compared to glass containers may indicate the presence of toxic surfactants.

The sample volume requirements for TIE Phase I tests are provided by USEPA (1991a, 1992a, 1996).

Volume requirements for POTW samples that are used in RTA tests are given in Section 5.

If TIE or physical/chemical treatability testing is being conducted off-site, samples should be shipped on ice to maintain the sample temperature at 4°C. RTA and some biological treatability tests require fresh or continuous samples of POTW waste streams, which requires testing to be conducted on-site. Samples of RAS and activated sludge should be delivered to the on-site laboratory and used immediately in testing to prevent changes in the biomass that can occur during long-term storage. Biomass samples should be vigorously aerated for a minimum of 15 minutes before use in the RTA or treatability tests. POTW influent and process wastewater samples required for on-site RTA or treatability studies should be used on the day of sample collection.

Sewer Discharge Sampling

The choice of grab or composite samples of indirect discharges will depend on the physical/chemical characteristics and variability of the toxicants. The sample type also will be dictated by the stage of the toxicity source evaluation. In Tier I testing, 24-hour flow proportional composite samples are recommended to characterize daily variability while accounting for variations in flow. Flow proportional sampling should be scheduled to coincide with production schedules for industrial discharges, the frequency of intermittent inputs for RCRA discharges, and the schedule of remedial activities for CERCLA discharges. This information is usually available in the POTW's pretreatment program reports.

Sampling techniques for flow proportional composites should account for the potential loss of volatile compounds. For samples collected for chemical analysis or refractory toxicity testing, zero headspace sampling methods can be used to minimize volatile losses. In some cases, grab sampling may be used in lieu of zero headspace methods to reduce sampling costs; however, care should be exercised in collecting samples that are representative of the discharge.

In Tier II, grab sampling can be used in addition to composite sampling to assess the variability of the toxicants. This type of sampling requires in-depth knowledge of the production schedules and the pretreatment operations of the discharger.

Section 12

References

- Adams, C.E., D.L. Ford, and W.W. Eckenfelder. 1981. *Development of Design and Operational Criteria for Wastewater Treatment*. Enviro Press, Nashville, Tennessee.
- Amato, J.R., D.I. Mount, E.J. Durhan, M.L. Lukasewycz, G.T. Ankley, and E.D. Robert. 1992. An Example of the Identification of Diazinon as a Primary Toxicant in an Effluent. *Environ. Toxicol. Chem.* 11: 209–16.
- American Chemistry Society. 1979. *Safety in Academic Chemistry Laboratories*. ACS Publication Committee on Chemical Safety, Third Edition. American Chemistry Society, Washington, D.C.
- American Petroleum Institute (API). API. 1998. *The Toxicity of Common Ions to Freshwater and Marine Organisms*. Washington, DC, November 1998, Doc. No. 0300–029.
- American Public Health Association (APHA). 1995. *Standard Methods for the Examination of Water and Wastewater*. 19th ed. American Public Health Association, American Waterworks Association, Water Environment Federation. Washington, D.C.
- American Society for Microbiology (ASM). 1981. *Manual for Methods for General Bacteriology*. Washington, D.C.
- Ankley, G.T., J.R. Dierkes, D.A. Jensen, and G.S. Peterson. 1991. Piperonyl Butoxide as a Tool in Aquatic Toxicological Research with Organophosphate Insecticides. *Ecotoxicol. Environ. Saf.* 21: 266–74.
- Ankley, G.T., and L.B. Burkhard. 1992. Identification of Surfactants as Toxicants in a Primary Effluent. *Environ. Toxicol. Chem.* 11: 1235–48.
- AQUA-Science. 1995. *Diazinon and Chlorpyrifos in Wastewater from Residential and Commercial Sources*. Report for Central Contra Costa Sanitary District, Martinez, California. AQUA-Science, Davis, California.
- Aquatic Sciences Consulting. 1997. *Evaluation of Sources of Toxicity and Toxicity Treatment Options at the City of Henderson's Nutbush Creek Wastewater Treatment Plant*. Prepared for the City of Henderson, North Carolina.
- AQUIRE. 1992. *Aquatic Information Retrieval Toxicity Data Base*. Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, Duluth, Minnesota.
- Bailey, H.C., J.L. Miller, M.J. Miller, and B.S. Dhaliwal. 1995. Application of Toxicity Identification Procedures to the Echinoderm Fertilization Assay to Identify Toxicity in a Municipal Effluent. *Environ. Toxicol. Chem.* 14(12): 2181–86.
- Bailey, H.C., J.L. Miller, M.J. Miller, L.C. Wiborg, L. Deanovic, and T. Shed. 1997. Joint Toxicity of Diazinon and Chlorpyrifos Under Conditions of Acute Exposure to *Ceriodaphnia dubia*. *Environ. Toxicol. Chem.* 16: 2304–08.
- Botts, J.A., J.W. Braswell, E.C. Sullivan, W.C. Goodfellow, B.D. Sklar, and A.G. McDearmon. 1987. *Toxicity Reduction Evaluation at the Patapsco Wastewater Treatment Plant*. Water Engineering Research Laboratory, Cincinnati, Ohio. Cooperative Agreement No. CR812790-01-1. NTIS # PB 88-220 488/AS.

- Botts, J.A., L.B. Fillmore, E.J. Durhan, W.A. Goodfellow, T. Pereira, and D.F. Bishop. 1990. Evaluation of the Role of Diazinon in the Toxicity of a Municipal Wastewater Treatment Plant Effluent. *Proceedings of the Third National Pesticide Conference*, November, 1990, Richmond, Virginia.
- Botts, J.A., T.L. Morris, J.E. Rumbo, and C.H. Victoria-Rueda. 1992. Case Histories-Municipalities. In *Toxicity Reduction: Evaluation and Control*. D.L. Ford, ed. Technomic Publishing Co., Lancaster, Pennsylvania.
- Botts, J.A., T.L. Morris, M.A. Collins, T.M. Schmitt, and E.K. Wilson. 1993. Evaluating the Impact of Industrial Discharges to Publicly Owned Treatment Works: The Refractory Toxicity Assessment Protocol. *Annual Conference and Exposition of the Water Environment Federation*, Anaheim, California.
- Botts, J.A., T. Schmitt, E. Wilson, M. Collins, D. Waddell, R. Diehl, and L. Ehrlich. 1994. Refractory Toxicity Assessment: An Alternative Approach for Chronic Toxicity Reduction Evaluations. *Annual Conference and Exposition of the Water Environment Federation*, Chicago, Illinois. Paper # AC944404.
- Brungs, W.A. 1973. Effects of Residual Chlorine on Aquatic Life. *Journal Water Pollut. Control Fed.* 45(10): 2180-93.
- Burgess, R.M., K.T. Ho, M.D. Tagliabue, A. Kuhn, R. Comeleo, P. Comeleo, G. Modica, and G.E. Morrison. 1995. Toxicity Characterization of an Industrial and a Municipal Effluent Discharging to the Marine Environment. *Marine Pollut. Bull.* 30: 524-35.
- Burgess, R.M., J.B. Charles, A. Kuhn, K.T. Ho, L.E. Patton, and D.G. McGovern. 1997. Development of a Cation-Exchange Methodology for Marine Toxicity Identification Evaluation Applications. *Environ. Toxicol. Chem.* 16: 1203-11.
- Burkhard, L.P., and J.J. Jenson. 1993. Identification of Ammonia, Chlorine, and Diazinon as Toxicants in a Municipal Effluent. *Arch. Environ. Contam. Toxicol.* 25: 506-15.
- City of Greenville. 1991. *TRE Phase B Final Report*. City of Greenville, Texas.
- Code of Federal Regulations, Title 40, Part 503. 1993. *Standards for the Use of Disposal of Sewage Sludge*, 58 FR 9387. Office of Federal Register, National Archives and Records Administration, Washington, D.C.
- Collins, M.A., T.L. Morris, J.A. Botts, T. Norberg-King, J. Thompson, and D.I. Mount. 1991. *Chronic Toxicity Reduction Evaluation at the Bergen County Utilities Authority Wastewater Treatment Plant*, Draft Report. USEPA, Risk Reduction Engineering Laboratory, Cincinnati, Ohio. USEPA Contract No. 68-03-3431.
- Collins, M.A., E.K. Wilson, C.A. LaRocca, and W.L. Goodfellow. 1994. Chronic Toxicity Identification Evaluation Analyses Using *Mysidopsis bahia*. *Annual Conference and Exposition of the Water Environment Federation*, Chicago, Illinois.
- Dawson, T., K. Lott, and M. Deadrick. 1997. Toxicity Identification Evaluation of Washington, Georgia, Wastewater Treatment Plant Effluent. USEPA Contract No. 68-W3-0034. Mid-continent Ecology Division, Duluth, Minnesota.
- Deeny, K., J. Heidman, and J. Condren. 1988. Evaluation of a Full-Scale Activated Sludge System Utilizing Powdered Activated Carbon Addition with Wet Air Oxidation. *Annual Conference of the Water Pollution Control Federation*, Dallas, Texas.
- Diehl, R., and S. Moore. 1987. Case History: A North Carolina Municipal TRE. *Toxicity Identification/Reduction Evaluation Workshop, Annual Conference of the Water Pollution Control Federation*, Philadelphia, Pennsylvania.
- DiGiano, F. 1988. Case History: Toxicity Reduction Evaluation at High Point, North Carolina. *Virginia Water Pollution Control Association Wastewater Toxics Management Seminar*, November 3, 1988, Richmond, Virginia.
- Douglas, W.S., S.S. Grasso, D.G. Hutton, and K.R. Schroeder. 1996. Ion Imbalance as a Source of

- Toxicity in Estuarine Effluent. *Arch. Environ. Contam. Toxicol.* 31: 426–32.
- Douglas, W.S., and M.T. Horne. 1997. The Interactive Effects of Essential Ions and Salinity on the Survival of Mysid shrimp, *Mysidopsis bahia* in 96-hour Acute Toxicity Tests of Marine and Estuarine Effluents. *Environ. Toxicol. and Chem.* 16(10): 1996–2001
- Durhan, E.J., M.T. Lukasewycz, and J.R. Amato. 1990. Extraction and Concentration of Nonpolar Organic Toxicants from Effluents Using Solid Phase Extraction. *Environ. Toxicol. Chem.* 9: 463–66.
- Edile, D., S. Newton, N. Lewnes, G.N. Cherr, L. Schipper, and J. Anderson. 1995. A Marine TIE: When Phase I Becomes a Research Project. *16th Annual Meeting of the Society of Environmental Toxicology and Chemistry*, Vancouver, B.C., Canada, November 1995.
- Engineering-Science, Inc. 1992. *Toxicity Reduction Evaluation: Toxicant Identification and Confirmation*. Submitted to the City of Lawton, Oklahoma.
- Engineering-Science, Inc. 1994. *Quarterly Progress Report (July 1 to September 30, 1994) for the Rivanna Water & Sewer Authority Toxicity Reduction Evaluation*. Prepared for the Rivanna Water & Sewer Authority, Charlottesville, Virginia.
- EXTOXNET (Extension Toxicology Network). 1998. Developed by University of California, Davis, Oregon State University, Michigan State University, Cornell University, and the University of Idaho. On the Internet at <http://ace.orst.edu/info/extoxnet/>.
- Federal Register. 1989. *USEPA: National Pollutant Discharge Elimination System; Surface Water Toxics Control Program*. USEPA, Volume 54, No. 105, Friday, June 2, 1989.
- Fillmore, L.B., T.L. Morris, T.L. Champlin, M.C. Welch, and J.A. Botts. 1990. *Toxicity Reduction Evaluation at the City of Fayetteville Cross Creek Wastewater Treatment Plant*, Draft Report, 1990. USEPA, Risk Reduction Engineering Laboratory, Cincinnati, Ohio. USEPA Contract No. 68-03-3431.
- Giger, W., E. Stephanou, and C. Schaffner. 1981. Persistent Organic Chemicals in Sewage Effluents: Identification of Nonylphenols and Nonylphenoethoxylates by Gas Capillary Gas Chromatography/Mass Spectrometry. *Chemosphere* 10(11/12): 1253–63.
- Goodfellow, W.L., and W.L. McCulloch. 1993. Toxicity or Physiological Imbalance? *Inorganic Salts TIE Meeting (work group)*, Houston, Texas, November 14, 1993.
- Goodfellow, W.L., V.A. Sohn, and Kotulak, M.A. 1994. Performance of a Second Generation Toxicity Reduction Evaluation. *15th Annual Meeting of the Society of Environmental Toxicology and Chemistry*, Denver, Colorado, October 29 - November 3, 1994.
- Goodfellow, W.L., P.B. Dorn, L.W. Ausley, D.T. Burton, D.L. Denton, D.R. Grothe, M.A. Heber, T.J. Norberg-King, and J.H. Rodgers, Jr. 1998. Submitted: Environmental Toxicology and Chemistry. *The Role of Inorganic Ion Imbalance in Aquatic Toxicity Testing*. Society of Environmental Toxicology and Chemistry, Foundation of Environmental Education, Whole Effluent Steering Committee, Pensacola, Florida.
- Grady, C.P.L. 1985. Biodegradation: Its Measurement and Microbiological Basis. *Biotechnology and Bioengineering* 27: 660–74.
- Grady, C.P.L., and H.P. Lim. 1980. *Biological Wastewater Treatment: Theory and Applications*. Marcel Dekker, Inc., New York, New York.
- Griffin, D.M., K.F. Kline, and L.S. Targgart. 1993. Development of Phase I TIE Methods for the Giant Kelp, *Macrocystis pyrifera*, and Red Abalone, *Haliotis rufescens*. *13th Annual Meeting of the Society of Environmental Toxicology and Chemistry*, Cincinnati, Ohio.
- Hagelstein K., and R.R. Dauge. 1984. Use of Long Solids Retention Times to Improve the Activated Sludge Biodegradation of a Complex Waste. *Proceedings of the Purdue Industrial Waste Conference*, Ann Arbor, Michigan.

- Hall, W.S., and R.J. Mirenda. 1991. Acute Toxicity of Wastewater Treatment Polymers to *Daphnia pulex* and the Fathead Minnow (*Pimephales promelas*) and the Effects of Humic Acid on Polymer Toxicity. *Res. Journal of Water Pollut. Control Fed.* 63(6): 895–99.
- Higashi, R.M., G.N. Cherr, J.M. Shenker, J.M. MacDonald, and D.G. Crosby. 1992. A Polar High Molecular Mass Constituent of Bleached Kraft Mill Effluent is Toxic to Marine Organisms. *Environ. Sci. and Tech.* 26: 2413–20.
- Ho, K.T., K. Mitchell, M. Zappala, and R.M. Burgess. 1995. Effects of Brine Addition on Effluent Toxicity and Marine Toxicity Identification Evaluation (TIE) Manipulations. *Environ. Toxicol. Chem.* 14: 245–49.
- Hockett, J.R., and D.R. Mount. 1996. Use of Metal Chelating Agent to Differentiate Among Sources of Aquatic Toxicity. *Environ. Toxicol. Chem.* 15: 1687–94.
- Jirik, A.W., S.M. Bay, D.J. Greenstein, A. Zellers, and S-L. Lau. 1998. Applications of TIEs in Studies of Urban Stormwater Impacts on Marine Organisms. In *Environmental Toxicology and Risk Assessment: 7th Volume, ASTM STP 1333*, E.E. Little, A.J. SeLonay, and B.M. Greenberg, eds. American Society for Testing and Materials, Philadelphia, Pennsylvania.
- Judkins, J.F., and G.A. Anderson. 1992. Using Denitrification for pH Control. *Water Environ. & Tech.* February: 62–65.
- Kang, S.J., L.B. Pugh, and J.A. Borchardt. 1983. ATP as a Measure of Active Biomass Concentration and Inhibition in Biological Wastewater Treatment Processes. *Proceedings of the 38th Purdue Industrial Waste Conference*, Ann Arbor, Michigan.
- Kornegay, B.H. 1970. The Use of Continuous Culture Theory in the Selection of Biological Reactor Systems. *43rd Annual Conference of the Water Pollution Control Federation*, Boston, Massachusetts, October.
- Lankford, P.W., and W.W. Eckenfelder. 1990. *Toxicity Reduction in Industrial Effluents*. New York, New York: McGraw-Hill.
- Lankford, P.W., W.W. Eckenfelder Jr., and K.D. Torrens. 1987. Technological Approaches to Toxicity Reduction in Municipal and Industrial Wastewaters. *Annual Meeting of the Virginia Water Pollution Control Association*, Norfolk, Virginia.
- Linden Roselle Sewerage Authority. 1991. *Linden Roselle Sewerage Authority - 1991 Toxicity Reduction Evaluation Project Report*. Prepared by Engineering-Science, Inc., Fairfax, Virginia, in association with EA Engineering, Science, and Technology, Inc., Sparks, Maryland.
- MacGregor, R., M. Tucker, and J.S. Qi. 1996. Application of Ion-balancing Methods for Marine Whole Effluent Toxicity Tests. *17th Annual Meeting of the Society of Environmental Toxicology and Chemistry*, Washington, D.C.
- Masters, J.A., M.A. Lewis, D.H. Davidson, and R.D. Bruce. 1991. Validation of a Four-Day *Ceriodaphnia* Toxicity Test and Statistical Considerations in Data Analysis. *Environ. Toxicol. Chem.* 10: 47–55.
- Metcalf and Eddy, Inc. 1991. *Wastewater Engineering Treatment, Disposal, and Reuse*. Third Edition. New York, New York: McGraw-Hill.
- Mickley, M., J. Briceno, B. Borkman, K. Fucik, J. Case, and T. Nickel. 1996. Common Ion Toxicity in R.O. Membrane Concentrate. *17th Annual Meeting of the Society of Environmental Toxicology and Chemistry*, Washington, D.C.
- Morris, T.L., J.A. Botts, J.W. Braswell, M.C. Welch, and W.L. Goodfellow. 1990. *Toxicity Reduction Evaluation at the Linden Roselle Sewerage Authority Wastewater Treatment Plant*, Draft Report, 1990. USEPA, Office of Research and Development, Risk Reduction Engineering Laboratory, Cincinnati, Ohio. USEPA Contract No. 68-03-3431.

- Morris, T.L., G. Fare, and J. Spadone. 1992. Toxicity Reduction Evaluation at the Linden Roselle Sewerage Authority Wastewater Treatment Plant. *Water Environ. Tech.* June: 8–16.
- Mosure, T.E., J.P. Pierko, J.M. Schmidt, R.A. Monteith, and J.S. Mosser. 1987. *Toxicity Reduction Evaluation at the Akron Water Pollution Control Station*, Draft Report. USEPA, Office of Research and Development, Risk Reduction Engineering Laboratory, Cincinnati, Ohio. Cooperative Agreement No. #CR813681010.
- Mount, D.R., and D.D. Gulley. 1992. *Development of a Salinity/Toxicity Relationship to Predict Acute Toxicity of Saline Waters to Freshwater Organisms*. Gas Research Institute, Environment and Safety Research Department, Chicago, IL, October, 1992.
- National Institute of Occupational Safety and Health (NIOSH). 1977. *Working With Carcinogens*. Public Health Service, Centers for Disease Control. Publication No. 77-206.
- Norberg-King, T.J., M. Lukasewycz, and J. Jenson. 1989. *Results of Diazinon Levels in POTW Effluents in the United States*. National Effluent Toxicity Assessment Center, Duluth, Minnesota. Technical Report 14-89.
- Novartis Crop Protection, Inc., and Makhteshim-Agan of North America, Inc. 1997. *Investigation of Diazinon Occurrence, Toxicity, and Treatability in Southern United States Publicly Owned Treatment Works*. Technical Report 3-7, Environmental Affairs Department, Greensboro, North Carolina.
- Occupational Safety and Health Administration (OSHA). 1976. *OSHA Safety and Health Standards, General Industry*. 29 CFR 1910. OSHA 2206 (Revised).
- Oris, J.T., R.W. Winner, and M.V. Moore. 1991. A Four-day Survival and Reproduction Toxicity Test for *Ceriodaphnia dubia*. *Environ. Toxicol. Chem.* 10: 217–24.
- Philbrook, D.M., and C.P.L. Grady. 1985. Evaluation of Biodegradation Kinetics for Priority Pollutants. *Proceedings of the Purdue Industrial Waste Conference*, Ann Arbor, Michigan.
- Rodgers, J.H., Jr. 1989a. *Species tolerances for NPDES bioassays: Volume I, freshwater organisms*. American Petroleum Institute. No. 4483. Washington, D.C.
- Rodgers, J.H., Jr. 1989b. *Species tolerances for NPDES bioassays: Volume II, marine organisms*. American Petroleum Institute. No. 4482. Washington, D.C.
- Singhasemanon, N., C. Nordmark, and T. Barry. 1997. *Diazinon and Chlorpyrifos in Central Contra Costa Sanitary District Sewer System, Summer 1996*. State of California Environmental Protection Agency, Department of Pesticide Regulation. Sacramento, California. Draft Report.
- Slattery, G.H. 1987. Effects of Toxic Influent on Patapsco Wastewater Treatment Plant Operations. *Annual Conference of the Water Pollution Control Federation*, New Orleans, Louisiana.
- Sullivan, E.C., D.F. Bishop, J.A. Botts, J.W. Braswell, G.H. Slattery, and W.L. Goodfellow. 1987. Effluent Toxicity Monitoring Methodology Evaluated for Five Industrial Dischargers. *Proceedings of the Purdue Industrial Waste Conference*, Ann Arbor, Michigan.
- TRAC Laboratories, Inc. 1992. Unpublished data. Prepared by TRAC Laboratories, Inc., Denton, Texas.
- USEPA. 1977. *Occupational Health and Safety Manual*. Office of Planning and Management, Washington, D.C.
- USEPA. 1979a. *Handbook for Analytical Quality Control in Water and Wastewater Laboratories*. EPA/600/4-79/019. Analytical Quality Control Laboratory, Cincinnati, Ohio.
- USEPA. 1979b. *Methods 624 and 625: GC/MS Methods for Priority Pollutants*. *Federal Register* 44(223):69532-58.

-
- USEPA. 1982. *Handbook for Sampling and Sample Preservation of Water and Wastewater*. EPA/600/4-82-019. Cincinnati, Ohio.
- USEPA. 1983a. *Guidance Manual for Pretreatment Program Development*. Office of Water Enforcement and Permits, Washington, D.C.
- USEPA. 1983b. *Methods for Chemical Analysis of Water and Wastes*. EPA/600/4-79-020, Cincinnati, Ohio.
- USEPA. 1984a. *Handbook on Improving POTW Performance Using the Composite Correction Program Approach*. EPA/625/6-84/008. Center for Environmental Research Information, Cincinnati, Ohio.
- USEPA. 1984b. *Ambient Water Quality Criteria for Chlorine - 1984*. Office of Water Regulations and Standards, Criteria and Standards Division, Washington, D.C.
- USEPA. 1985a. *Ambient Water Quality Criteria for Ammonia - 1985*. Office of Water Regulations and Standards, Criteria and Standards Division, Washington, D.C.
- USEPA. 1985b. *Master Analytical Scheme for Organic Compounds in Water*. EPA/600/4-85-008. Office of Research and Development, Cincinnati, Ohio.
- USEPA. 1987a. *Permit Writer's Guide to Water Quality-Based Permitting for Toxic Pollutants*. Office of Water Enforcement and Permits, Washington, D.C.
- USEPA. 1987b. *Guidance Manual on the Development and Implementation of Local Discharge Limitations Under the Pretreatment Program*. Office of Water Enforcement and Permits, Washington, D.C.
- USEPA. 1988a. *Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures*. EPA/600/3-88/034. National Effluent Toxicity Assessment Center, Duluth, Minnesota.
- USEPA. 1988b. *Development of Slug Loading Control Programs for Publicly Owned Treatment Works*. Office of Water Enforcement and Permits, Washington, D.C.
- USEPA. 1989a. *Toxicity Reduction Evaluation Protocol for Municipal Wastewater Treatment Plants*. EPA/600/2-88/062. Office of Research and Development, Risk Reduction Engineering Laboratory, Cincinnati, Ohio.
- USEPA. 1989b. *Generalized Methodology for Conducting Industrial Toxicity Reduction Evaluations*. EPA/600/2-88/070. Water Engineering Research Laboratory, Cincinnati, Ohio.
- USEPA. 1989c. *Handbook: Retrofitting POTWs*. EPA/625/6-89/020. Center for Environmental Research Information, Cincinnati, Ohio.
- USEPA. 1990. *User Documentation: POTW Expert, Version 1.0*. EPA/625/11-90/001. Office of Research and Development, Cincinnati, Ohio.
- USEPA. 1991a. *Methods for Aquatic Toxicity Identification Evaluations: Phase I, Toxicity Characterization Procedures*. Second Edition. EPA/600/6-91-003. National Effluent Toxicity Assessment Center, Duluth, Minnesota.
- USEPA. 1991b. *Technical Support Document for Water Quality-Based Toxics Control*. EPA/505-2-90-001. Office of Water Enforcement and Permits, Washington, D.C.
- USEPA. 1991c. *Guidance Manual for Control of Slug Loadings to POTWs*. 21W-4001. Office of Water Enforcement and Permits, Washington, D.C.
- USEPA. 1991d. *Methods for Determination of Metals in Environmental Samples*. EPA 600/4-91/010. Office of Research and Development, Washington, D.C.
- USEPA. 1992a. *Toxicity Identification Evaluations: Characterization of Chronically Toxic Effluents, Phase I*. EPA/600/6-91-005F. National Effluent Toxicity Assessment Center, Duluth, Minnesota.
- USEPA. 1993a. *Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures for Samples Exhibiting*

- Acute and Chronic Toxicity*. EPA/600/R-92-080. National Effluent Toxicity Assessment Center, Duluth, Minnesota.
- USEPA. 1993b. *Methods for Aquatic Toxicity Identification Evaluations. Phase III Toxicity Confirmation Procedures for Samples Exhibiting Acute and Chronic Toxicity*. EPA/600/R-92-081. National Effluent Toxicity Assessment Center, Duluth, Minnesota.
- USEPA. 1993c. *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*. Fourth Edition. EPA/600/4-90-027F. Environmental Monitoring Systems Laboratory, Cincinnati, Ohio.
- USEPA. 1993d. *Treatability Data Base, Version 5*. EPA/600/C-93/003a. Risk Reduction Engineering Laboratory, Cincinnati, Ohio.
- USEPA. 1994a. *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*. Third Edition. EPA/600/4-91/002. Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.
- USEPA. 1994b. *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms*. Second Edition. EPA/600/4-91/003. Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.
- USEPA. 1994c. *Methods for Determination of Metals in Environment Samples – Supplement I*. EPA 600/R-94/111. Office of Research and Development, Washington, D.C.
- USEPA. 1995. *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms*. EPA/600/R-95-136. National Exposure Research Laboratory, Cincinnati, Ohio.
- USEPA. 1996. *Marine Toxicity Identification Evaluation (TIE) Guidance Document, Phase I*. EPA/600/R-96/054. Office of Research and Development, Washington, D.C.
- USEPA. 1997. *Methods and Guidance for Analysis of Water CD ROM* Version 1.0. Office of Water, EPA-821-97-001. Washington, D.C.
- Walsh, G.E., and R.L. Garnas. 1983. Determination of Bioactivity of Chemical Fractions of Liquid Wastes Using Freshwater and Saltwater Algae and Crustaceans. *Environ. Sci. Tech.* 17: 180–82.
- Ward, S.H. 1989. The Requirements for a Balanced Medium in Toxicological Experiments using *Mysidopsis bahia* with Special Reference to Calcium Carbonate. *Environmental Toxicology and Hazard Assessment: 12th Volume, ASTM STP No. 1023: 402-12*. U.M. Cowgill and L.R. Williams, eds. American Society for Testing and Materials, Philadelphia, Pennsylvania. 402–12.
- Water Environment Federation. 1996. *Wastewater Sampling for Process and Quality Control - MOP OM-1*. Water Environment Federation, Alexandria, Virginia.
- Water Environment Federation and American Society of Civil Engineers (WEF/ASCE). 1992a. *Design of Municipal Wastewater Treatment Plants, Volume I, Chapters 1-13. WEF Manual of Practice No. 8 and ASCE Manual and Report on Engineering Practice No. 76*. Water Environment Federation, Alexandria, Virginia, and American Society of Civil Engineers, New York, New York.
- Water Environment Federation and American Society of Civil Engineers (WEF/ASCE). 1992b. *Design of Municipal Wastewater Treatment Plants, Volume II, Chapters 13-20. WEF Manual of Practice No. 8 and ASCE Manual and Report on Engineering Practice No. 76*. Water Environment Federation, Alexandria, Virginia and American Society of Civil Engineers, New York, New York.
- Water Pollution Control Federation (WPCF). 1983. *Nutrient Control. Manual of Practice FD-7, Facilities Design*, Alexandria, Virginia.
- Water Research Commission (WRC). 1984. *Theory, Design and Operation of Nutrient Removal Activated Sludge Processes*. Wiechers HNS, ed. Water Research Commission, Pretoria, South Africa.
- Weis, P., J.S. Weis, C-M Chen, and A. Greenberg. 1992. Treated Municipal Wastewaters: Effects of Organic Fractions on Development and Growth of Fishes. *Environ. Toxicol. Chem.* 11: 1451–59.

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Bibliography

- Ahel, M., W. Giger, and M. Koch. 1985. Behavior of Nonionic Surfactants in Biological Wastewater Treatment. In *Organic Micropollutants in the Aquatic Environment*. Proceedings of the 4th European Symposium, Vienna, Austria.
- Ahel, M., and W. Giger. 1985. Determination of Alkylphenols and Alkylphenol Mono- and Diethoxylate in Environmental Samples by High-Performance Liquid Chromatography. *Analytical Chem.* 57(8): 1577–83.
- Ahel, M., and W. Giger 1985. Determination of Nonionic Surfactants of the Alkylphenol Polyethoxylate Type by High-Performance Liquid Chromatography. *Analytical Chem.* 57(13): 2584–90.
- Ayres, G.H., 1970. *Quantitative Chemical Analysis*. New York: Harper & Bow.
- Code of Federal Regulations, Title 40, Part 503. 1993. *Standards for the Use of Disposal of Sewage Sludge*, 58 FR 9387. Office of Federal Register, National Archives and Records Administration, Washington, D.C.
- De Renzo, D.J. 1981. *Pollution Control Technology for Industrial Wastewater*. Noyes Data Corporation, New Jersey.
- Eckenfelder, W.W. 1980. *Principles of Water Quality Management*. Boston, MA: CBI Publishing Company.
- Federal Register. 1984. USEPA. Development of Water Quality-Based Permit Limitations for Toxic Pollutants; National Policy, Vol. 49, No. 48, March 9, 1984.
- Giger, W., H. Brunner, and C. Schaffner. 1984. 4-Nonylphenol in Sewage Sludge: Accumulation of Toxic Metabolites from Nonionic Surfactants. *Science* 225: 623–25.
- Goodfellow, W.L., and W.L. McCulloch, 1987. A Technique for the Rapid Evaluation of Effluent Acute Toxicity. In *Proceedings of the 8th Annual SETAC Meeting*, Pensacola, Florida.
- Jirka, A.M., and M.J. Carter. 1975. HACH COD Procedure for the Bausch & Lomb Spec 20. *Analytical Chem.* 47(8).
- Marcomini, A., and W. Giger, 1987. Simultaneous Determination of Linear Alkylbenzenesulfonates, Alkylphenol Polyethoxylates and Nonylphenol by High-Performance Liquid Chromatography. *Analytical Chem.* 59(13): 1709–15.
- McEvoy, J., and W. Giger, 1986. Determination of Linear Alkyl-benzenesulfonates in Sewage Sludge by High-Resolution Gas Chromatography/Mass Spectrometry. *Environ. Sci. Tech.* 20(4): 376–83.
- Patterson, J.W., 1985. *Industrial Wastewater Treatment Technology*. 2nd Edition. Boston, MA: Butterworth.
- Peters, R.W., and Y. Ku, 1984. Removal of Heavy Metals from Industrial Plating Wastewaters by Sulfide Precipitation. *Proceedings of the Industrial Wastes Symposium (57th Annual Conference)*.
- Techline Instruments, Inc. 1984. *Techline Laboratory Respirometer Operating Manual*, Fond du Lac, Wisconsin.

-
- USEPA. 1983. *Treatability Manual*. EPA/600/2-82-001a. Office of Research and Development, Washington, D.C.
- USEPA. 1985b. *Protocol Development for the Prediction of the Fate of Organic Priority Pollutants in Biological Wastewater Treatment Systems*. Risk Reduction Engineering Laboratory, Cincinnati, Ohio.
- USEPA. 1988. *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms*. EPA/600/4-87-028. Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.
- USEPA. 1989. *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*. Second Edition. EPA/600/4-89/001. Environmental Monitoring Systems Laboratory, Cincinnati, Ohio.
- Water Pollution Control Federation, 1982. *Wastewater Treatment Plant Design, Manual of Practice No. 8*. Alexandria, Virginia.
- Water Pollution Control Federation (WPCF). 1983. *Nutrient Control. Manual of Practice FD-7, Facilities Design*. Alexandria, Virginia.
- Water Environment Federation, 1990. *Operation of Wastewater Treatment Plants. A Manual of Practice*. Alexandria, Virginia.