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Toxicity Reduction Evaluation Protocol for Municipal Wastewater Treatment Plants

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Foreword

Today's rapidly developing and changing technologies and industrial products and practices frequently carry with them the increased generation of materials that, if improperly dealt with, can threaten both public health and the environment. The U.S. Environmental Protection Agency is charged by Congress with protecting the nation's land, air, and water resources. Under a mandate of national environmental laws, the agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. These laws direct the EPA to perform research to define our environmental problems, measure the impacts, and search for solutions.

The Risk Reduction Engineering Laboratory is responsible for planning, implementing, and managing research, development, and demonstration programs to provide an authoritative, defensible engineering basis in support of the policies, programs, and regulations of the EPA with respect to drinking water, wastewater, pesticides, toxic substances, solid and hazardous wastes, and Superfund-related activities. This publication is one of the products of that research and provides a vital communication link between the researcher and the user community.

This guidance document on municipal toxicity reduction evaluations (TRE) was prepared to provide technical support for water quality-based toxicity control in the National Pollution Discharge Elimination System (NPDES). It was designed to provide guidance for conducting TRE assessments at municipal wastewater treatment plants. This guidance document describes the state-of-the-art procedures and the general decision-making process for conducting municipal TREs.

E. Timothy Oppelt, Director
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Abstract

This document presents a generalized protocol for conducting Toxicity Reduction Evaluations (TREs) at municipal wastewater treatment plants (WWTPs). This protocol is designed to provide guidance to municipalities in preparing TRE plans, evaluating the information generated during TREs, and developing a technical basis for the selection and implementation of toxicity control methods. A TRE involves an evaluation of the municipal WWTP performance; an identification of the specific toxicants causing effluent toxicity; a review of the pretreatment and local limits programs; a characterization of the nature, variability and sources of toxicity; and the evaluation, selection and implementation of the toxicity control options.

Because of the broad scope of this protocol, it is to be expected that site specific considerations may to some extent warrant modifications and tailoring of the protocol approach for a given facility. The protocol has been developed based on current research and experience. TRE methods and procedures will be updated and refined based on the results of ongoing research and case studies.

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Abbreviations and Symbols

AA	– Atomic absorption
ASTM	– American Society for Testing and Materials
ATP	– Adenosine triphosphate
BOD	– Biochemical oxygen demand
BOD_5	– 5-day biochemical oxygen demand
C. dubia	– *Ceriodaphnia dubia*
CERCLA	– Comprehensive Environmental Response, Compensation and Liability Act
CFR	– Code of Federal Register
COC	– Chain-of-Custody
COD	– Chemical oxygen demand
Cr	– Chromium
CSO	– Combined sewer overflow
Cu	– Copper
DMR	– Discharge monitoring report
DO	– Dissolved oxygen
EC_{50}	– Effective concentration causing a 50% effect in the test species
EDTA	– Chelating agent
EP	– Extraction procedure
EPA	– Environmental Protection Agency
F/M	– Food to microorganism ratio
g/l	– Grams per liter
GAC	– Granular activated carbon
GC	– Gas chromatography
H&S	– Health and Safety
HNO_3	– Nitric acid
HPLC	– High pressure liquid chromatography
ICP	– Inductively coupled plasma spectrometry
IU	– Industrial user
IWS	– Industrial waste survey
LC_{50}	– Lethal concentration causing a 50% mortality in exposed test organisms
MCRT	– Mean cell residence time
mg/l	– Milligrams per liter
MLSS	– Mixed liquor suspended solids

Abbreviations and Symbols (continued)

MLVSS – Mixed liquor volatile suspended solids
MS – Mass spectrometry
MSDS – Material safety data sheet
NPDES – National Pollutant Discharge Elimination System
O_2 – Oxygen
OSHA – Occupational Safety and Health Administration
OUR – Oxygen uptake rate
PAC – Powdered activated carbon
PACT – Powdered activated carbon treatment
pH – Negative logarithm of hydrogen ion concentration or hydrogen potential
POTW – Publicly owned treatment works
PPE – Plant performance evaluation
PPR – Pretreatment program review
QA/QC – Quality assurance/quality control
RAS – Return activated sludge
RBC – Rotating biological contactor
RCRA – Resource Conservation and Recovery Act
RTA – Refractory toxicity assessment
SARA – Superfund Amendments and Reauthorization Act
$SBOD_5$ – Five-day soluble biochemical oxygen demand
SCOD – Soluble chemical oxygen demand
SIC – Standard industrial code
SOR – Surface overflow rate
SOUR – Specific oxygen uptake rate
SPE – Solid phase extraction
SRT – Sludge retention time or sludge age
SS – Suspended Solids
SSUR – Specific substrate utilization rate
TBTO – Total brominated toxic organics
TCIP – Toxics control implementation plan
TCLP – Toxicity characteristic leaching procedure
TDS – Total dissolved solids
TIE – Toxicity identification evaluation
TKN – Total kjeldahl nitrogen
TP – Total phosphorus
TRC – Total residual chlorine
TRE – Toxicity reduction evaluation
TSDF – Treatment, storage, and disposal facility
TSS – Total suspended solids
TTO – Total toxic organics
WWTP – Wastewater treatment plant
xg – Times gravity
Zn – Zinc
ZSV – Zone settling velocity

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Section 1
Introduction

Background

The Toxicity Reduction Evaluation Protocol for Municipal Wastewater Treatment Plants provides a systematic framework for conducting a toxicity reduction evaluation (TRE) and a description of the available methods and procedures, which experience to date have shown to be most useful. This protocol is designed for use by municipal facilities [Publicly Owned Treatment Works (POTW)] which are conducting a TRE to meet NPDES whole effluent toxicity permit limits. It presents methods and procedures that will be useful for: 1) the design of a TRE, 2) the development and review of a TRE plan, 3) the evaluation of the results and data generated during the TRE, and 4) the development of a sound scientific and engineering basis for the selection and implementation of a toxicity control method.

This document supports the integrated toxics control strategy using whole effluent toxicity and pollutant specific limits described in previous EPA guidance, notably, the Technical Support Document for Water Quality-based Toxics Control (1985a) and The Permit Writer's Guide to Water Quality-based Permitting for Toxic Pollutants (1987b). It has become well recognized that while POTWs may achieve effluent limits for conventional pollutants, pass-through of effluent toxicity, volatilization, and contamination of sewage sludges can still occur. The focus of this protocol is on the reduction of toxicity in municipal wastewater treatment plant effluents. It is the responsibility of the POTW to conduct a toxicity reduction evaluation in order to achieve the TRE objectives and meet the applicable NPDES permit limits. The regulatory authority will review the TRE plan and carefully monitor the progress of the TRE, providing direction as needed.

This municipal TRE protocol provides guidance for municipalities and their consultants on how to conduct a TRE at a POTW. The methods and decision points which comprise a TRE are described in the context of an overall generalized approach. Because the regulatory issues and treatment operations are unique for each POTW, not all elements of this protocol will apply to every case, and each municipality will need to develop its own site-specific TRE plan. The protocol has been developed based on the research and experience to date. The methods and procedures described will be updated and refined based on the results of on-going research and case studies.

TRE Definitions and Objectives

EPA's Permit Writer's Guide to Water Quality-Based Permitting for Toxic Pollutants (1987) defines a TRE as "a step-wise process which combines toxicity testing and analysis of the physical and chemical characteristics of causative toxicants to zero in on the toxicants causing effluent toxicity and/or on treatment methods which will reduce the effluent toxicity." Because a TRE is conducted in a tiered approach, judgement is required in selecting the appropriate steps for characterizing the causative toxicants and for evaluating options for controlling effluent toxicity.

To support TRE studies, EPA has developed a guidance manual entitled "Methods for Aquatic Toxicity Identification Evaluations (Mount and Anderson-Carnahan, (1988a) which describes procedures for identifying the toxicants causing effluent toxicity. The TIE procedures are a basic component of the municipal TRE protocol and should be carefully studied prior to their application in municipal TREs. In addition EPA has developed a general protocol for conducting industrial TREs (Fava, et al., 1988).

The overall objectives to be achieved in a municipal TRE are to:

- Evaluate the operation and performance of the POTW to identify and correct treatment deficiencies causing effluent toxicity;

- Identify the toxic compounds causing effluent toxicity;

- Trace the effluent toxicants and/or toxicity to their sources; and
- Evaluate, select and implement toxicity reduction methods and technologies to control effluent toxicity.

Components of the Municipal TRE Protocol

The overall flowchart for a TRE program is illustrated in Figure 1-1. A brief description of each major TRE component is presented as follows.

Information and Data Acquisition

The first step in a TRE is the collection of all 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, and data from the POTW's pretreatment program such as industrial waste survey applications and local limits compliance reports. The POTW performance data are evaluated in the second stage of the TRE as described below. The pretreatment program data are used in the latter stages of the TRE to assist in tracing the influent sources of toxicity and/or toxics which are contributing to the POTW effluent toxicity.

POTW Performance Evaluation

POTW operating and performance data can be evaluated to indicate possible in-plant sources of toxicity or operational deficiencies that may be allowing toxicity pass-through. In parallel with this evaluation an optional toxicity characterization test (TIE Phase I) can be performed to indicate the presence of in-plant toxicants caused by incomplete treatment (e.g., ammonia) or routine operating practices (e.g., chlorine). If a treatment deficiency or operating practice is causing effluent toxicity, treatability studies should be conducted to evaluate treatment modifications for reducing the toxicity. If plant performance is not a principal cause of the toxicity problem or treatment options do not reduce the toxicity, the TRE proceeds to TIE testing.

Toxicity Identification Evaluations

TIE procedures utilize aquatic toxicity tests of the effluent following bench top treatment steps to characterize the classes of toxicants causing effluent toxicity. Subsequent effluent manipulations in conjunction with chemical analyses are used to identify and confirm the specific toxicity-causing compounds.

The TIE protocol is performed in three phases: toxicity characterization (Phase I), toxicant identification (Phase II) and toxicant confirmation (Phase III). In some situations POTW pretreatment program data may help in identifying the effluent toxicants. If the specific effluent toxicants are identified, a control method such as local pretreatment limits may be implemented. If additional data are required to determine the nature and sources of the toxicants, a toxicity source evaluation is conducted.

Toxicity Source Evaluation (Tier I)

The initial stage of a toxicity source evaluation involves sampling the effluent of sewer dischargers or sewer lines and analyzing the wastewaters for toxics and/or toxicity. Because the toxicity of the POTW influent wastewater is not necessarily the same toxicity that is observed in the POTW effluent, sewer samples are treated in a simulation of the POTW's biological treatment process prior to toxicity analysis to account for the toxicity removal provided by the POTW.

The choice of chemical-specific analyses or toxicity tests for source tracking will depend on the quality of the TIE results. Chemical-specific tracking is recommended where the specific toxicants have been identified and can be readily traced to the responsible sewer dischargers. Toxicity tracking is required where TIE data on specific toxicants are not definitive.

If Tier I testing is successful in locating the sources that are contributing the POTW effluent toxicants, a toxicity control method such as local limits can be developed and implemented. If additional information on toxic indirect discharges is needed, further toxicity source testing is conducted.

Toxicity Source Evaluation (Tier II)

A Tier II evaluation is performed to confirm the suspected sources of toxicity identified in Tier I. Tier II testing involves testing the toxicity of selected sewer dischargers following simulated POTW treatment as in Tier I. Additional characterization steps are used in Tier II to determine the relative amount and the types of toxicity contributed by each discharger. Tier II information is used to rank the indirect dischargers with respect to their toxicity/toxics loading and to evaluate local limits as a toxicity control option.

POTW In-Plant Control Evaluation

A POTW control evaluation is conducted in parallel with the Tier II assessment to evaluate in-plant options for reducing effluent toxicity. If in-plant control appears to be a feasible approach, treatability testing is used to evaluate methods for optimizing existing treatment processes and to assess options for additional treatment.

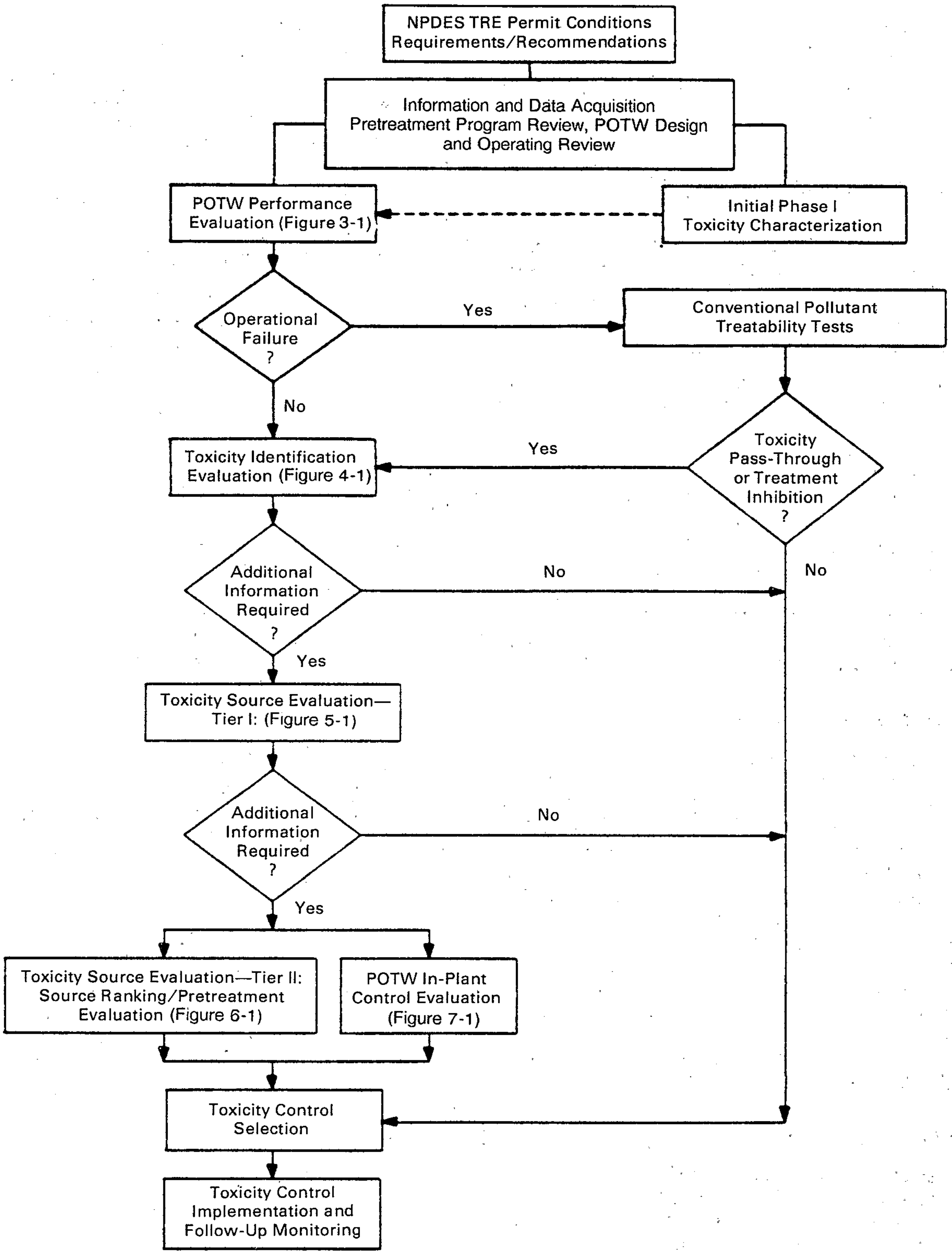

Figure 1-1. TRE flow diagram for municipal wastewater treatment plant.

Toxicity Control Selection

Using the results of the TIE testing, the Tier I and Tier II toxicity source evaluation and the POTW treatability testing, alternatives for effluent toxicity reduction are evaluated and the most feasible option(s) is selected for implementation. The choice of a control option(s) is based on several technical and cost criteria.

Toxicity Control Implementation

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 Protocol

This document addresses the protocol for evaluating and implementing methods for reduction of whole effluent toxicity. Because regulations concerning the transfer of toxics to sludge and air in POTWs are still under development, specific procedures for sludge and air toxics reduction are not discussed. The reader may consult EPA's Guidance Document for Writing Permit Requirements for Municipal Sewage Sludge (1988a) regarding permit conditions for sludge.

The municipal TRE protocol was developed based on the results and findings of several TRE and TIE studies. Some of the procedures used in these studies, especially tools for toxicity source evaluations, have not been widely used and will therefore require further refinement as more experience is gained. Additionally, the feasibility and effectiveness of in-plant and pretreatment toxicity control options have not been well documented and additional experience in this area is needed.

Organization of the Document

This document is organized according to the components of the TRE protocol flowchart (Figure 1-1). The section headings are as follows:

Section 2	Information and Data Acquisition
Section 3	POTW Performance Evaluation
Section 4	Toxicity Identification Evaluation
Section 5	Toxicity Source Evaluation (Tier I)
Section 6	Toxicity Source Evaluation (Tier II)
Section 7	POTW In-Plant Control Evaluation
Section 8	Toxicity Control Selection
Section 9	Toxicity Control Implementation

Sections 10 through 13 describe the TRE requirements for quality assurance/quality control, health and safety, facilities and equipment, and sample collection and handling. In Appendix A case examples of municipal TREs are discussed with respect to the study approach, important findings and problems encountered. Appendix B provides a detailed description of selected TRE procedures.

Section 2
Information and Data Acquisition

Introduction

The first step in a TRE is the collection of all information and data that may relate to effluent toxicity and that might prove useful in conducting the TRE. This information is generally divided into two main categories: POTW treatment system data and pretreatment program data. In addition to historical effluent toxicity data, the pertinent POTW information includes data on the treatment plant's design capabilities, treatment performance, and operation and maintenance practices. The appropriate pretreatment program information consists of industrial waste survey data, and pretreatment monitoring and compliance reports.

A summary of existing information required for a TRE is provided in the following subsections. It is important to emphasize that drawing preliminary conclusions based on this initial information can be misleading. Thus, the compiled data should be reserved for use as indicated in subsequent steps of the TRE protocol.

POTW Design and Operations Data

The primary objective in retrieving POTW design and operations information is to establish a data base to be used in the POTW Performance Evaluation (Section 3). This information can indicate possible in-plant sources of toxicity or operational problems that might be contributing to treatment interferences or toxicity pass-through. In addition, the POTW data will be useful in evaluating and selecting in-plant toxicity control options (Section 7).

The pertinent POTW design and operations data to be gathered include treatment system design information and the data routinely collected for NPDES discharge monitoring reports (DMRs) and for process control. A list of useful POTW data is provided in Table 2-1.

Pretreatment Program Data

The POTW pretreatment program data are used throughout the TRE. These data are reviewed in the Toxicity Identification Evaluation (Section 4) to assist in characterizing and identifying the effluent toxicants, and they are evaluated in the Toxicity Source Evaluation (Section 5) to aid in locating the influent sources of toxicity.

The appropriate pretreatment program information includes the data on the industrial users (IUs) of the POTW (i.e., industrial manufacturers, RCRA waste disposers, and CERCLA dischargers) and the toxic pollutant data on the POTW wastestreams. A list of suggested pretreatment data is shown in Table 2-2.

Table 2-1. POTW Design and Operations Data

1. NPDES permit requirements
   a. Effluent limitations
   b. Special conditions
   c. Monitoring data and compliance history

2. POTW design criteria
   a. Hydraulic loading capacities
   b. Pollutant loading capacities
   c. Biodegradation kinetics calculations/assumptions

3. Influent and effluent conventional pollutant data
   a. Biochemical oxygen demand (BOD_5)
   b. Chemical oxygen demand (COD)
   c. Suspended solids (SS)
   d. Ammonia
   e. Residual chlorine
   f. pH

4. Process control data
   a. Primary sedimentation - hydraulic loading capacity and BOD and SS removal
   b. Activated sludge - Food-to-microorganism (F/M) ratio, mean cell residence time (MCRT), mixed liquor suspended solids (MLSS), sludge yield, and BOD and COD removal
   c. Secondary clarification - hydraulic and solids loading capacity, sludge volume index and sludge blanket depth

5. Operations information
   a. Operating logs
   b. Standard operating procedures
   c. Operations and maintenance practices

6. Process sidestream characterization data
   a. Sludge processing sidestreams
   b. Tertiary filter backwash
   c. Cooling water

7. Combined sewer overflow (CSO) bypass data
   a. Frequency
   b. Volume

8. Chemical coagulant usage for wastewater treatment and sludge processing
   a. Polymer
   b. Ferric chloride
   c. Alum

9. RCRA reports [if POTW is considered a hazardous waste treatment, storage and disposal facility (TSDF)]

Table 2-2. Pretreatment Program Data

1. POTW influent and effluent characterization data
    - a. Toxicity
    - b. Priority pollutants
    - c. Hazardous pollutants
    - d. SARA 313 pollutants
    - e. Other chemical-specific monitoring results

2. Sewage residuals (i.e., raw, digested, thickened and dewatered sludge and incinerator ash) characterization data
    - a. EP toxicity
    - b. Toxicity Characteristic Leaching Procedure (TCLP)
    - c. Chemical analysis

3. Industrial waste survey (IWS)
    - a. Information on IUs with categorical standards or local limits and other significant non-categorical IUs
        - (i) number of IUs
        - (ii) discharge flow
    - 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. Annual pretreatment program report
    - a. Schematic of sewer collection system
    - b. POTW monitoring data
        - (i) discharge characterization data
        - (ii) spill prevention and control procedures
        - (iii) hazardous waste generation
    - c. IU self-monitoring data
        - (i) description of operations
        - (ii) flow measurements
        - (iii) discharge characterization data
        - (iv) notice of slug loading
        - (v) compliance schedule (if out of compliance)

5. Technically based local limits compliance reports

6. Waste hauler monitoring data and manifests

7. 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

8. CERCLA reports (if the POTW accepts wastes from a superfund site)
    - a. Preliminary assessment
    - b. Site investigations
    - c. Remedial investigations
    - d. Feasibility studies
    - e. CERCLA decision documents

9. Evidence of POTW treatment interferences (i.e., biological process inhibition)

Section 3
POTW Performance Evaluation

Introduction

POTW treatment deficiencies such as poor conventional pollutant removal can have the effect of increasing effluent toxicity. Prior to conducting effluent toxicity characterization, an evaluation of the POTW operations and performance should be made to identify and correct treatment deficiencies that may be responsible for all or part of the effluent toxicity. A POTW performance evaluation (PPE) can be conducted to indicate conventional pollutant treatment deficiencies that may be causing toxicity and to evaluate improvements in operations and treatment that may eliminate in-plant sources of toxicity. The flowchart for conducting a PPE is presented in Figure 3-1.

The PPE 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. Additionally, an optional TIE Phase I analysis (see Section 4) can be performed to indicate the presence of effluent toxicants caused by incomplete treatment (e.g., ammonia) or routine operating practices (e.g., chlorine). Based on the process review results and the Phase I data, options for improving operations and performance are selected and evaluated in treatability studies. If treatability tests are successful in identifying the necessary options for improving conventional pollutant treatment and for reducing effluent toxicity, the TRE proceeds to the selection and implementation of those options (Sections 8 and 9). If, however, the treatment alternatives do not reduce effluent toxicity to acceptable levels, further effluent toxicity characterization is required using the TIE procedures discussed in Section 4.

Operations and Performance Review

The operations and performance review involves the evaluation of the major POTW unit processes using the information described in Table 2-1. 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. Other unit processes to be evaluated include primary sedimentation and disinfection. Particular attention is paid to chlorine disinfection because residual chlorine is highly toxic to aquatic life.

Procedures for evaluating and improving POTW operations and performance are described in EPA's "Handbook on Improving POTW Performance Using the Composite Correction Approach" (1984). The composite correction approach utilizes a point system to quantify process performance and to indicate which treatment units need improvement.

Primary Sedimentation

Primary treatment processes are designed to reduce the loading of suspended solids (SS), 5-day biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD) on the secondary treatment system. Toxics removal can also occur during primary sedimentation from the settling of insoluble or particulate toxic wastewater constituents. Optimal removal of both toxic and conventional pollutants in primary sedimentation will ultimately reduce the amount of material passing through the biological treatment process.

Primary clarifier performance can be evaluated by comparing surface overflow rate (SOR), which is the average daily flow divided by the clarifier surface area, to the expected BOD_5 removal. A clarifier operating at an SOR of less than 25 m^3/m^2/day (600 gpd/sq ft) should remove 35 to 45 percent of the influent BOD_5. A clarifier operating at an SOR of 25 to 40 m^3/m^2/day (600-1,000 gpd/sq ft) should remove 25 to 35 percent of the influent BOD_5 (USEPA, 1984). In most cases COD removal performance will be comparable to the BOD removal performance.

Aerobic Biological Treatment

Aerobic biological treatment is a critical process in a POTW, because it is the process that converts organic matter to settleable microorganisms. Toxics

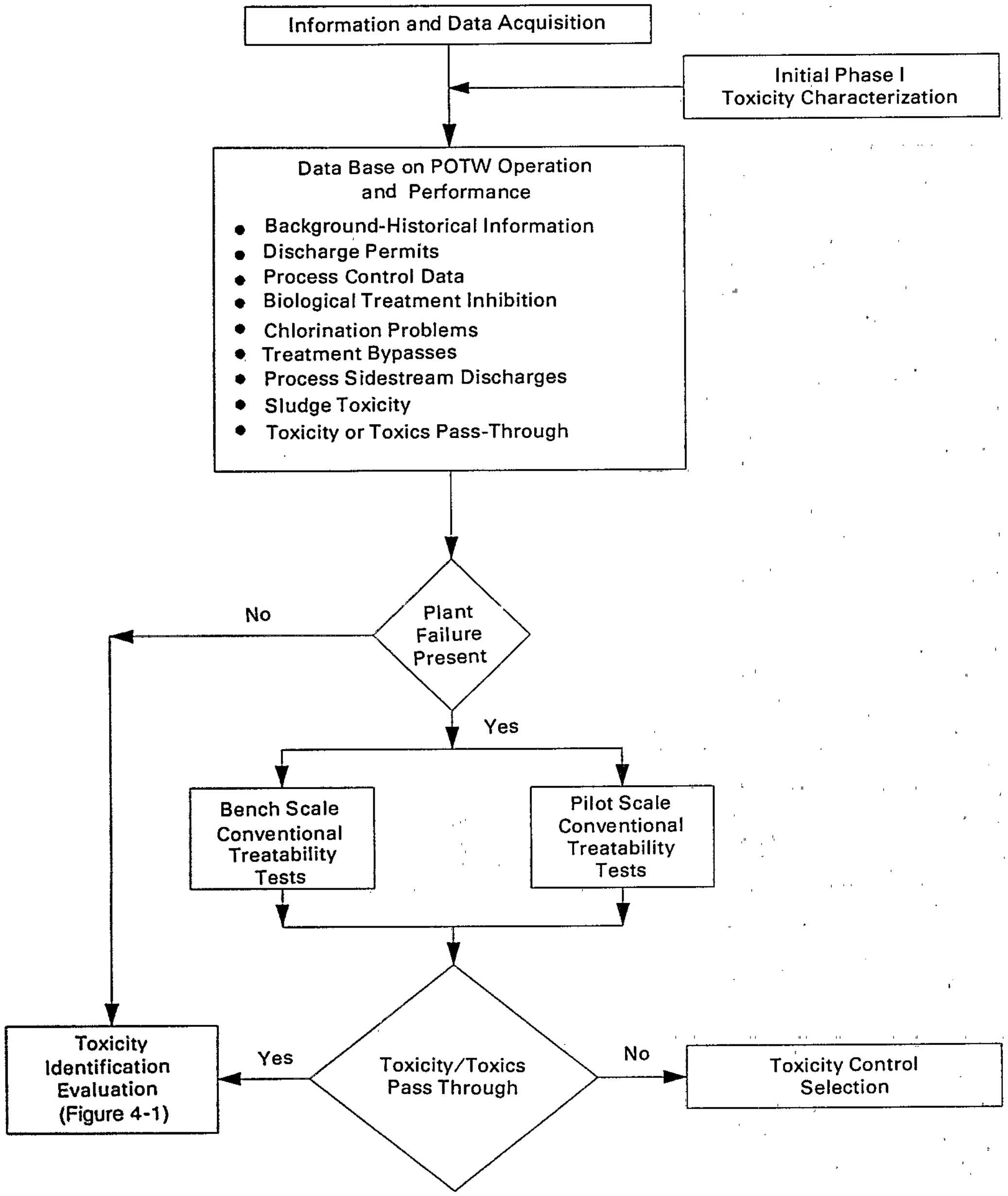

Figure 3-1. Plant performance evaluation.

removal during aerobic biological treatment can occur by biodegradation, adsorption onto the biological floc, and by volatilization of volatile constituents. Key factors affecting toxics removal are the tendency for toxics to biodegrade, volatilize, or sorb onto solids and the degree to which toxics will inhibit the biological process.

Aerobic biological treatment systems that are typically used in POTWs are activated sludge, trickling filter and rotating biological contactor (RBC) processes. To simplify the discussion of aerobic biological treatment systems, the following subsections will focus on the performance evaluation of the activated sludge process, which is the process most widely used in POTWs.

The parameters that are used to evaluate the operational capability of an activated sludge system include organic loading, oxygen requirement and mean cell retention time (MCRT). Operating values for these parameters can be compared to design specifications or recommended criteria to determine how well the activated sludge process is being operated.

Organic Loading --

Organic loading on an activated sludge system affects the organic removal efficiency, oxygen requirement and sludge production of the process. The most common measure of organic loading in suspended growth processes is the food-to-microorganism (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) will result in low organic removal efficiency, low oxygen requirement and high sludge production. Low F/M ratios (i.e., low organic loading) will cause high organic removal efficiencies and low sludge production, but high oxygen requirements. For optimal biological treatment, the F/M ratio in an activated sludge system is typically maintained in the range of 0.2 to 0.4 lb BOD_5/lb MLVSS-day for conventional activated sludge, 0.05 to 0.15 lb BOD_5/lb MLVSS-day for extended aeration, and 0.2 to 0.6 lb BOD_5/lb MLVSS-day for contact stabilization (Metcalf and Eddy, 1979).

An activated sludge process operating at an F/M ratio that is substantially lower than the design F/M ratio may indicate biological treatment performance problems. For example, the Patapsco Wastewater Treatment Plant in Baltimore, Maryland was operated at an F/M ratio of 0.40 lb BOD_5/lb MLVSS-day instead of the design F/M ratio of 0.55 lb BOD_5/lb MLVSS-day, because the POTW could not achieve consistent wastewater treatment at the higher organic loading. The decreased treatment capacity at the POTW was thought to be due to the toxic effect that industrial wastewaters had on the activated sludge biomass (Slattery, 1987).

Oxygen Requirement --

Microorganisms in the activated sludge system require oxygen to metabolize the organic material in the wastewater. Oxygen deficient conditions can result in lower treatment capacity, and hence a greater potential for toxics pass-through. To ensure an adequate supply of oxygen, the dissolved oxygen (DO) level should be maintained above 2 mg/l. Typical air requirements are 1,500 cu ft/lb BOD_5 load for conventional activated sludge and contact stabilization, and 2,000 cu ft/lb BOD_5 load for extended aeration (USEPA, 1984).

The transfer of oxygen from the gas phase to the liquid phase is a function of the aeration equipment and the basin mixing conditions. EPA's Composite Correction Program Manual (1984) describes a procedure for estimating oxygen transfer capacity in aerators based on equipment specifications. Another estimate of oxygen transfer capacity involves comparing the measured oxygen uptake rate (OUR) of the biomass to the calculated theoretical oxygen demand (USEPA, 1984) for the aeration system. 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.

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

Mean Cell Residence Time --

In the course of biological treatment, the activated sludge microorganisms convert some of the organic matter in the wastewater to new cell mass. 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., mean cell residence time) in the system. Mean cell residence time (MCRT) is calculated by dividing the total sludge mass in the system by the amount of sludge that is wasted each day.

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, 1984). During the Patapsco TRE, the MCRT of the pure oxygen activated sludge process increased from an average 13.8 days to 16.9 days over a 9 month period (Botts et.al., 1987). This increase in MCRT appeared to cause a corresponding decrease in OUR in the biomass from 46.7 mg O_2/hr/g MLSS to 25.2 mg O_2/hr/g MLSS over the same period. The increase in MCRT appeared to be limiting biomass activity, however, effluent quality as measured by toxicity and conventional pollutant removal was not affected.

Secondary Clarification

In order for the activated sludge process 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. Solids-liquid separation is influenced to a large degree by the aeration basin operating conditions such as DO levels, F/M ratio and MCRT. Secondary clarifier factors also have an important effect on solids clarification.

Sludge settling characteristics are affected by how the aeration basin is operated. Low DO levels in the aeration basin promote the growth of filamentous bacteria which can hinder solids settling, whereas high DO levels lead to the growth of fast settling zoogleal-type bacteria. At very high organic loadings (high F/M) the activated sludge can be dispersed and will not settle well. An example of this condition was observed at the East Side Sewage Treatment Plant in Oswego, New York, which experienced sludge bulking due to high influent organic loadings (USEPA, 1984). 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 wiers, the type of sludge removal mechanism, and the return sludge flow rate. The Composite Correction Program Manual (1984) describes a system for scoring secondary clarifier performance based on these factors.

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 the effluent toxicity. In addition raw or partially treated wastewater that bypasses part or all of the wastewater treatment system can add substantial toxicity to POTW discharge(s).

Examples of POTW sidestreams include sludge processing wastewaters from thickening, digestion, and dewatering of sludges, cooling water blowdown, and backwash from tertiary filters. Of these wastewaters, anaerobic digestion and sludge dewatering sidestreams can contain high concentrations of organic material (BOD_5) and nutrients (nitrogen and phosphorus) which can represent a significant loading to the aeration basin. In addition, these and other sidestreams may contain toxic material such as metals from sludge dewatering treatment that may pass through the POTW.

In some municipalities, stormwaters and sewage are still collected in the same sewer system. When a large storm event occurs, the combined sewer wastewater is often diverted away from all or part of the POTW to prevent hydraulic over loading, and the bypass is discharged directly to the receiving water. Combined sewer overflows (CSOs) can cause short-term exceedances in discharge permit limits for both toxic and conventional pollutants.

The PPE should include a listing of all process sidestreams and wastewater bypasses in the POTW, and a review of the data on each of these wastestreams. Additional analytical and toxicity data may be needed to characterize the possible toxic constituents and toxicity of the wastestreams. This information can be used to determine if process sidestreams are a significant source of pollutants or toxicity, and whether or not current treatment practices are sufficient to remove the toxicity. Information on the frequency and volume of CSOs, relative to the receiving wasteflow, can indicate if CSOs are a problem.

Disinfection

Disinfection is generally achieved by treating the secondary effluent with chlorine and allowing a sufficient contact period prior to discharge. The chlorine dosage is usually based on the required level of residual chlorine to be maintained in the final effluent which is specified in the NPDES permit.

The chlorine disinfection process should be carefully evaluated because residual chlorine and other by-products of chlorination (i.e., mono- and dichloro-amines) are toxic to aquatic life (Brungs, 1973). The PPE focuses on the minimum amount of chlorine that must be applied to achieve the desired residual chlorine concentration. Observations should be made to determine how close the actual residual chlorine level is to the required minimum level. For example, an average residual chlorine level of 3.0 mg/l for a POTW with a minimum permit level of 1.0 mg/l chlorine would indicate excessive chlorination.

If dechlorination is practiced following chlorination, information on the type and amount of oxidant-reducing material used should be obtained. Some dechlorinating agents such as sulfur dioxide may be toxic to aquatic life at high concentrations.

Optional TIE Phase I Tests

Optional TIE Phase I analyses (Mount and Anderson-Carnahan, 1988a) can be conducted in parallel with the above operations and performance review to obtain information on the types of compounds causing the effluent toxicity. An overview of the Phase I procedure is described in Section 4 of this document.

The TIE Phase I testing in the PPE focuses on characterizing toxicants that may be present in the effluent because of inadequate treatment performance or routine operating practices. Although the Phase I results provide only an initial indication of the effluent toxicants, when taken together with the PPE data and a knowledge of the POTW treatment operations, possible in-plant toxicants can be suggested based on the "weight of evidence". Using this information, treatability tests can be devised to evaluate procedures for removing the suspected toxicants from the effluent.

The TIE Phase I testing includes several characterization steps that can be used to indicate the presence of "in-plant toxicants" such as suspended solids, ammonia and chlorine. One step involves filtration to remove effluent particles, which include residual suspended solids resulting from biological treatment. By testing the toxicity of unfiltered and filtered samples, it is possible to determine whether or not the effluent toxicity is associated with these particles. Another Phase I step involves pH adjustment of the effluent sample to 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 + NH_4^+) present as NH_3 increases. If adjusting the effluent sample pH to 8 increases the toxicity or if lowering the effluent sample pH to 6 decreases the toxicity, the identity of the effluent toxicant would be consistent with ammonia. Caution should be used in drawing preliminary conclusions about these data, because the toxicant could actually be something that behaves in the same manner as ammonia. A third Phase I step is designed to indicate whether or not wastewater oxidants such as total residual chlorine (i.e., free chlorine and mono- and dichloroamines) are causing the toxicity. Thiosulfate, a reducing agent, is added to aliquots of the effluent sample to eliminate total residual chlorine (TRC). Toxicity tests prior to and following thiosulfate treatment are used to indicate if TRC is present in toxic amounts in the effluent.

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 (Mount and Anderson-Carnahan, 1988a). For example, the oxidants that are neutralized in the thiosulfate treatment step include bromine, iodine and manganous ions in addition to TRC. Thus, to substantiate the initial indication that specific toxicants are causing effluent toxicity, the Phase I results should be compared with information from the POTW operations and performance review. Using the previous example, the assumption that TRC is causing oxidant toxicity would be corroborated if operations data show that the POTW maintains toxic concentrations of chlorine in the final effluent.

Conventional Wastewater Treatability Testing

The operations and performance review information may identify areas in the POTW where improvements in conventional pollutant treatment may reduce toxicity pass-through. This information and the optional TIE Phase I data may also indicate in-plant sources of toxicants such as process sidestream by-passes or over chlorination that are causing effluent toxicity. Using these data, a wastewater treatability program can 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) only in situations where improvements in treatment operations and performance are needed to attain acceptable conventional pollutant treatment. These studies should focus on conventional pollutant treatment deficiencies which 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 7) can be evaluated.

Treatability studies can range from a simple evaluation such as testing TRC removal by dechlorination 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 judgement. It may be necessary to more completely assess the nature and variability of the effluent toxicity (Section 4) prior to implementing an extensive treatability effort.

A treatability program can be devised to evaluate modifications in existing treatment processes. The evaluation of additional treatment units should be attempted only after further effluent characterization studies (i.e., TIE) have been performed. PPE treatability testing may involve physical/chemical treatment approaches such as coagulation and precipitation, solids sedimentation, granular media filtration, and powdered activated carbon adsorption, or biological treatment approaches such as activated sludge or sludge digestion. Because toxicity control is the ultimate goal of the TRE, 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.

The following subsections briefly describe some of the treatability tests that can be used to determine if improvements in conventional pollutant treatment will reduce effluent toxicity. 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 8 and 9). If, however, the treatability data indicate that improved in-plant treatment will not reduce effluent toxicity to acceptable levels, further effluent toxicity characterization is required using the TIE procedures (Section 4).

Coagulation and Precipitation

Coagulation is the addition of a chemical such as polymer or ferric chloride to wastewater that causes the formation of flocculant suspensions or insoluble precipitates. Coagulation can be applied in POTWs to improve suspended solids settling or remove phosphorus, heavy metals, and particulate toxicity.

The optimum conditions for coagulation can be determined by conducting a series of jar tests. These tests are used to establish the optimum type and dose of coagulant, the proper mixing conditions, and the flocculant settling rates (Adams et al., 1981).

Sedimentation

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

Flocculant settling rates can be converted to a clarifier SOR by measuring the flocculant percent removal with time in a settling column test (Adams et.al., 1981). Using the optimum range of flocculant conditions determined in jar tests, a series of settling column tests can be performed to compare particle settling profiles for various coagulant doses and mixing conditions.

Zone settling can also 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). The test is repeated using the anticipated range of suspended solids loading to the clarifier. Test results are used to calculate a solids flux curve that can be used for clarifier design.

Activated Sludge

Activated sludge is an aerobic treatment process that converts organic matter to settleable microorganisms. Continuous flow and batch biological reactor tests are used to assess pollutant or toxicity treatability, and predict the process kinetics of an activated sludge system. A series of bioreactors are generally operated under a range of F/M values to determine optimum operating conditions (Adams et al., 1981).

Bioreactor performance is 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 and the preferred sludge settling conditions.

Granular Media Filtration

Some POTWs utilize filtration processes following secondary clarification to enhance suspended solids removal. Filtration performance is influenced by the physical/chemical properties of the granular media and the wastewater.

The main parameters to be varied in filtration testing include hydraulic loading rate, type and configuration of the media, and, if necessary, type and dose of chemical coagulant (Adams et al., 1981). Results of filtration testing are used to correlate suspended solids removal with flow rate, headloss through the filter with time, and differential headloss with solids accumulation on the filter media.

Activated Carbon Adsorption

Activated carbon may be applied in powdered form to the activated sludge process. 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 BOD_5, selected organic contaminants (e.g., phenols) or toxicity is predicted by adding known amounts of powdered activated carbon to a series of batch wastewater samples and measuring removal of the organic constituents or toxicity. The equilibrium relationship between a wastewater and carbon can usually be described either by a Langmier or Freundlich isotherm. A plot of equilibrium concentration versus carbon capacity is used to select the required carbon concentration for powdered activated carbon (PAC)-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.

Section 4
Toxicity Identification Evaluation

Introduction

Once the presence of toxicity has been established, the primary components of toxicity can be characterized and possibly identified through the use of toxicity identification evaluation procedures. These procedures relate the wastewater components', physical/chemical characteristics to their toxicological characteristics in a attempt to identify the compound(s) causing effluent toxicity.

The TIE procedures as described by Mount and Anderson-Carnahan (1988a, 1988b) and Mount (1988) 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 suspected toxicants. Figure 4-1 presents the logical progression of these three phases within the framework of a municipal TRE.

The TIE procedures are applied to POTW effluent wastewater samples. A sufficient number of effluent samples should be collected to characterize the magnitude and variability of the effluent toxicants over time. To ensure that the samples are representative of the effluent wastewater, a large number of samples may need to be collected. Sampling requirements for TIE testing are described in Section 13. In addition to POTW effluent TIE testing, the Phase I protocol may be applied to batch treated sewer wastewaters, as described in Section 6, to characterize the components of the POTW influent toxicity.

Toxicity Tests

Toxicity identification evaluations rely on the use of aquatic toxicity tests to detect the compounds causing wastewater toxicity. Acute toxicity tests are generally used to indicate the presence of toxicants as the effluent is manipulated in TIE testing. Presently, the TIE procedures do not address chronic toxicity directly; however, TIE testing can be performed on effluents violating chronic toxicity limits, provided that the effluent causes significant lethality (e.g., >20% mortality in 100% effluent) in an acute exposure period (i.e., 48 to 96 hr.). In this case the compound(s) causing acute toxicity are assumed to be the same compound(s) causing chronic toxicity. This hypothesis is tested in the Phase III tests (Mount, 1988).

The sensitivities of the test organisms used in toxicity tests can vary from species to species, therefore it is recommended that multiple species testing be performed in the initial stages of the TIE to select an appropriately sensitive species for the TIE program. Another criterion in test species selection is that the species be cost-effective to use, because of the large number of samples that may need to be tested. Test species commonly used for TIE toxicity tests are *Ceriodaphnia* or *Daphnia magna*; however, other species such as fathead minnows (*Pimephales promelas*) can also be used.

Saltwater species are not recommended, because the tolerances of marine organisms to the TIE effluent manipulations have not been measured (Mount and Anderson-Carnahan, 1988a). In situations where the POTW effluent is discharged to saline receiving waters, a freshwater species that is responsive to the same toxicants as the marine species should be chosen. Phase III involves minimal effluent manipulation and a marine species should be used to confirm the Phase I and II freshwater species results.

Toxicity test procedures for TIEs are described in the Phase I manual (Mount and Anderson-Carnahan, 1988a). The test organisms are placed into six test vessels: one containing the highest test sample concentration; four containing the appropriate range of sample dilutions; and one containing only dilution water which serves as the control. Following an appropriate exposure period, the test organism mortality is determined and an LC_{50} value (i.e. percent sample concentration causing 50% mortality) is calculated. These tests are usually performed with minimal quality assurance (e.g. duplicates may not be required) in an effort to collect as much data on the sample toxicity, as quickly and inexpensively as possible.

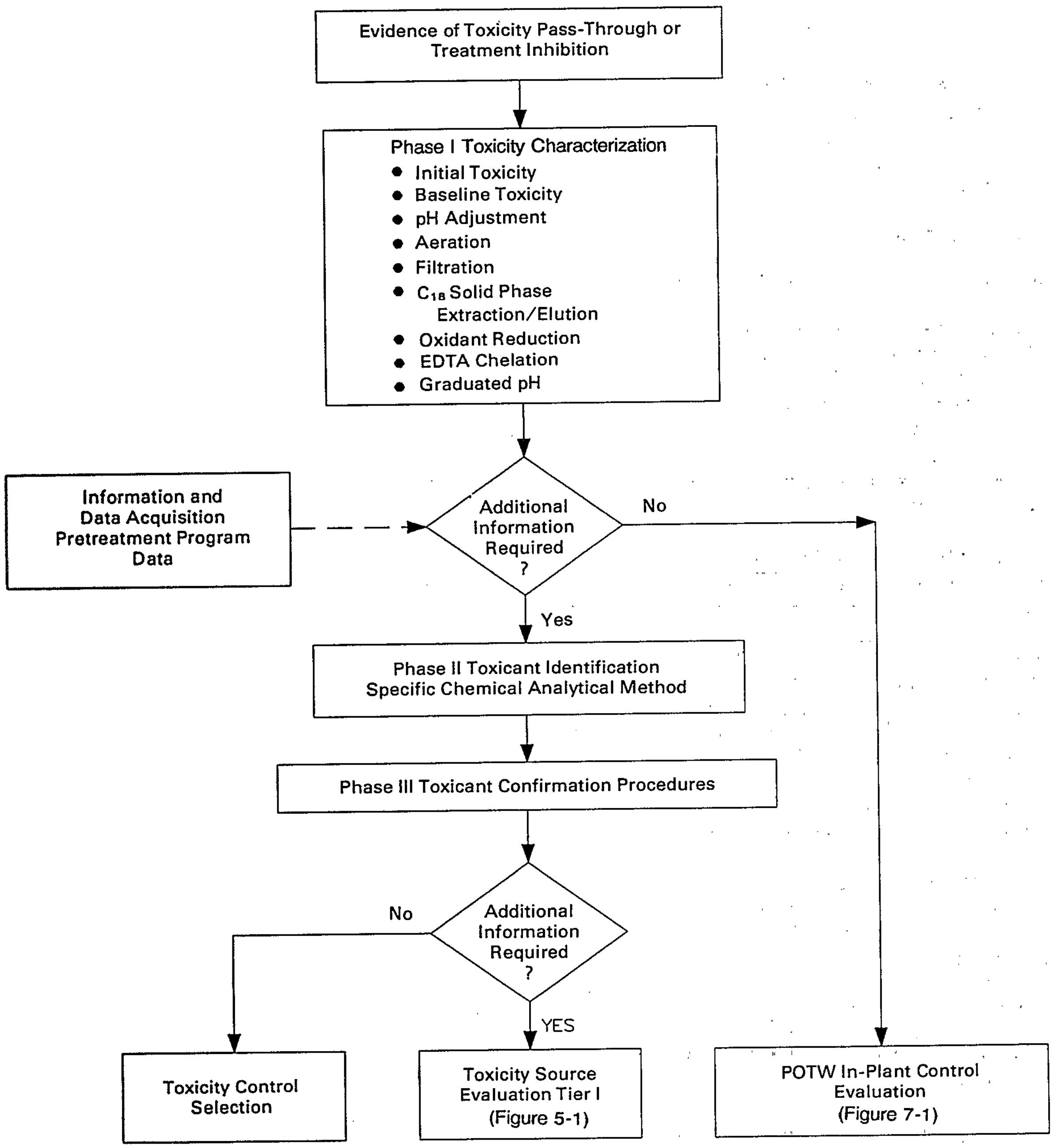

Figure 4-1. Toxicity identification evaluation.

When Phase III is reached, definitive toxicity tests using standard EPA procedures (Horning and Weber, 1985, Peltier and Weber, 1985, Weber et al., 1988) are recommended to confirm the suspected toxicants. These tests should utilize the same test species that is required for NPDES biomonitoring.

Toxicity Characterization and Toxicant Identification Procedures

The effluents of some municipal treatment facilities can be extremely complex, particularly facilities which receive a significant amount of industrial wastewater.

These effluents have posed a problem in attempting to determine the compound(s) causing toxicity. Furthermore, effluents from POTWs with little or no industrial inputs have proven to be difficult to characterize. Nonetheless, the TIE procedures are useful in the identification of the toxic compound(s) in POTW effluents. These procedures allow the toxicity to be traced to a particular class of compounds and sometimes to a particular compound(s).

Phase I - Toxicity Characterization

The first step in the TIE is to characterize the effluent toxicity using the Phase I approach (Mount and Anderson-Carnahan, 1988a). This procedure involves the use of several bench-top treatment steps to determine the types of toxic components in the effluent. Initially, the whole wastewater sample is evaluated for "baseline" toxicity. If it is toxic, aliquots of the sample are treated to remove certain types of compounds and the resulting acute toxicity of these treated aliquots is measured.

The Phase I characterization steps consist of toxicity degradation, aeration, filtration, C_{18} solid phase extraction (all of these procedures with and without pH adjustment), pH adjustment, oxidant-reduction, EDTA chelation, and graduated pH treatments. The toxicity tests on aerated wastewater samples are used to indicate if toxicity is associated with volatile or oxidizable compounds. The filtration step is designed to determine whether toxicity is in the suspended particulate phase or in the soluble fraction. Aeration, in conjunction with pH adjustment is used to evaluate toxicants with volatility, such as ammonia or hydrogen sulfide. The toxicity associated with the presence of oxidants is evaluated through sodium thiosulfate addition. Cationic metal toxicity is evaluated through graduated EDTA addition. An aliquot of the effluent sample is also passed through a C_{18} solid phase extraction (SPE) column that selectively removes non-polar organic compounds. If the effluent toxicity is removed or reduced by this treatment, the column is then eluted with methanol and the eluant is tested for toxicity. If these Phase I tests are inadequate to characterize the toxicants, other techniques can be used such as ion exchange resins for anions and cations; activated carbon for various inorganic and organic compounds; zeolite resins for ammonia; and molecular sieves such as Sephadex resins which separate compounds by molecular weight.

Pretreatment program data (Table 2-2) may provide useful information to assist in the Phase I characterization. By reviewing available information on the pretreatment program, compounds that are known to be toxic or problematic in the POTW 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 (i.e., non-polar organics, suspended particulates, volatile organics), treatability studies can be designed to evaluate removal of the compounds causing toxicity. These studies may involved bench-scale or pilot-scale testing procedures described in Section 7.

Phase II - Causative Toxicant Identification

The Phase II procedure manual (Mount and Anderson-Carnahan, 1988b) describes specific test methods that can be used to further identify specific causative agents such as non-polar organic compounds, ammonia, cationic metals, or chlorine. These methods are not intended as final proof that the compounds are causing toxicity. Phase III tests are required to confirm that the toxicants are the compounds consistently causing toxicity. Depending on the characteristics of the causative chemicals, Phase II methods may entail the use of reverse phase HPLC columns to further separate non-polar organic toxicants into more narrow fractions. This separation technique allows the identification of specific toxicants using gas chromatography/mass spectrometry (GC/MS) procedures.

Additional methods for identification of toxic effluent components include: the equitoxic solution test which evaluates the effect of pH adjustment and dilution on ammonia toxicity; the zeolite resin test which also can be used to identify ammonia toxicity; and atomic absorption or Inductively Coupled Plasma Spectrometry (ICP) to determine which cationic metals are the toxicants. Toxicant identification procedures for other groups of causative toxicants will be published by EPA as they become available.

Phase III - Causative Toxicant Confirmation

The next step in the TIE is Phase III which is the toxicant confirmation procedure (Mount, 1988). The toxicants identified in Phase II are confirmed by a series of test steps including observation of test organisms symptoms; additional species toxicity testing; and correlation of toxicity and toxicant concentration from multiple samples.

Section 5
Toxicity Source Evaluation - Tier I

Introduction

A toxicity source evaluation is conducted to locate the sources of influent toxicity or toxics that are contributing to the POTW effluent toxicity. This evaluation is performed in two tiers. Tier I, which is discussed in this section, involves sampling wastewater of indirect dischargers or sewer lines and analyzing the wastewaters for toxics and/or toxicity. Tier II, which is described in Section 6, is performed to confirm the suspected sources of toxicity identified in Tier I testing.

The flow diagram for the Tier I source evaluation is presented in Figure 5-1. Selection of sampling locations is based on evidence that a source contributes toxicants causing POTW effluent toxicity or discharges substantial levels of potentially toxic pollutants. Sampling of wastewaters from indirect dischargers is recommended where existing pretreatment program data or TIE results are adequate to indicate that the dischargers may contribute toxic pollutants that cause POTW effluent toxicity. Sewer line sampling can be used to locate toxic sources by process of elimination, if pretreatment program information and TIE data are lacking, or sampling of indirect dischargers is not feasible (e.g., large number of IUs).

The choice of chemical-specific analyses or toxicity tests for source tracking will depend on the quality of the TIE data on the POTW effluent. Chemical-specific investigation is recommended in cases where the effluent toxicants have been identified and presumably can be traced to the responsible sewer dischargers. In situations where TIE data indicate that specific industrial chemicals such as copper or phenol are the effluent toxicants, the TRE should proceed to the evaluation of local pretreatment limits as described in Section 8.

Toxicity tracking is required in situations where TIE data on specific toxicants are not definitive. Prior to toxicity analysis, sewer samples are afforded the same level of biological treatment as provided by the POTW for its influent wastewaters. If toxicity tracking is successful in locating sources that are contributing toxicity, the TRE proceeds to the Tier II source evaluation to confirm the toxic indirect dischargers. At this point, the POTW may require the IUs to conduct a TRE to reduce IU wastewater toxicity. It may also be possible in a few instances to correlate the pretreatment program data gathered at the beginning of the TRE (Section 2) with the effluent toxicity results to identify the influent sources of toxicity.

Sampling Location

Sampling locations for Tier I testing are established by reviewing the pretreatment program data (Table 2-2) and the TIE results, and selecting the sources that contribute relatively high loadings of potentially toxic pollutants or toxicants identified in TIE tests. Where possible, the selection of sampling locations should be based on TIE results, because the toxicants causing the effluent toxicity are the pollutants that must be controlled. Sampling may be conducted at the point where the IU discharges its wastewater to the sewer or in the sewer lines of the sewer collection system, if no IU appears to be the source of the toxicants. A plan for sewer line sampling can be devised to track toxic sources through a process of elimination of segments of the collection system (USEPA, 1983a).

The choice of IU discharge sampling or sewer line sampling will depend on the number of IUs contributing to the POTW, the quality of the pretreatment program data, and the TIE results. IU discharge sampling is recommended where the:

- Number of IUs is small enough so that sufficient test data can be collected with the allocated resources;
- Pretreatment program data are sufficient to characterize each of the IUs; and
- TIE data indicate POTW effluent toxicants that can be attributed to selected IUs.

In the Patapsco TRE indirect dischargers were selected for sampling based on evidence that the IUs contributed substantial loadings of toxicity (as

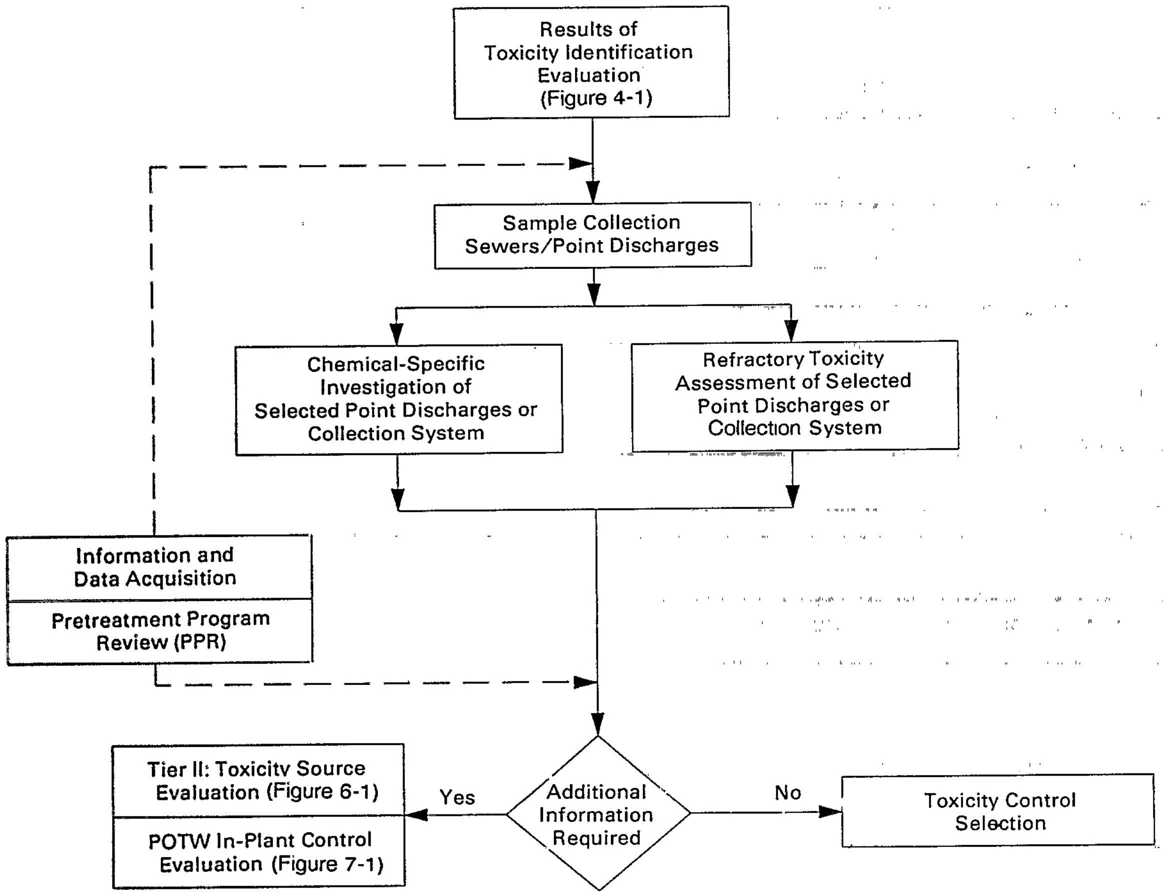

Figure 5-1. **Tier I: toxicity source evaluation.**

measured by Microtox[TM]) and toxicants (non-polar organic material) identified by TIE tests of POTW effluent (Botts et.al., 1987). A description of the Patapsco study is given in Appendix A.

Sewer line sampling is recommended where:

- A sewer line sampling plan can be developed that will allow a more efficient method of toxicity tracking than sampling IU discharges;
- Pretreatment program data are limited or unavailable; and
- Sources of toxicants identified during the TIE are not obvious.

The Town of Billerica, Massachusetts, utilized a sewer line sampling approach to identify areas within the sewer collection system that contribute toxicity to the Town's wastewater treatment plant (Durkin et.al., 1987). A description of the Billerica study is provided in Appendix A.

Whether sampling of IU discharges or sewer lines is conducted, 24-hour flow proportional samples are recommended to characterize daily variations in toxics or toxicity while accounting for variations in flow. Flow data must be gathered in order to determine the relative contributions of toxicants or toxicity from the IUs. Other considerations for Tier I sampling are described in Section 13. QA/QC sampling requirements are discussed in Section 10.

Chemical-Specific Investigation

A chemical-specific approach can be used to trace the influent sources of toxics, if definitive TIE data on the specific toxicants causing POTW effluent toxicity are available. This approach is not recommended in cases where the TIE data only indicate a broad class of compounds (e.g., polar organic compounds), because the toxicants may be contributed by a large number and variety of sources which will be difficult to pinpoint by chemical tracking.

The chemical-specific approach involves testing IU discharges or sewer line samples for specific toxicants using chemical analysis techniques. In some cases existing pretreatment program data may be adequate to determine the IUs that are contributing the toxicants. It is likely, however, that further sampling and analysis will be necessary, because many toxicants other than those typically monitored (i.e., priority pollutants) are present in IU discharges. Existing pretreatment program data can be used to reduce the amount of sampling and analysis by indicating which sources contribute toxics that are similar to the effluent toxicants.

Chemical analysis methods for priority pollutants are described in several EPA documents (USEPA 1979a, 1979b, 1980, 1985b) and Standard Methods for the Examination of Water and Wastewater (APHA, 1985). Analytical techniques for non-priority pollutants can be found in American Society for Testing and Materials (ASTM) manuals and analytical chemistry journals such as the Analytical Chemistry Journal. The selected analytical method should be verified in the laboratory prior to sampling and analysis. Prior to analysis, a literature search should 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.

In cases where Tier I chemical tracking is successful in locating the IUs that are responsible for the POTW effluent toxicants, the TRE process can move to the selection and development of local pretreatment regulations (Section 6). If the responsible IUs can not be located, the TIE results should be reviewed to confirm previous conclusions. The chemical analysis results should also 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 Tier I testing should be repeated using toxicity tests in lieu of chemical analysis as described later in this section.

Pretreatment Program Review

It may be possible in a few cases to identify the toxic influent sources by comparing pretreatment program information on suspected influent toxics to chemical-specific data on POTW effluent toxics. This pretreatment program review (PPR) approach is recommended only in situations where the POTW has only a few IUs which have relatively non-complex discharges.

The PPR approach was applied at the Mt. Airey POTW in North Carolina (Diehl and Moore, 1987) which receives industrial wastewater from only a few sources, predominately textile industries. In the Mt. Airey TRE, detailed information on the manufacturing processes and wastewater discharges of the industries was gathered (see Table 2-2), including data on the toxicity and biodegradability of raw and manufactured chemicals as provided in material safety data sheets (MSDS). This information was used to identify industrial chemicals with relatively high potential toxicity that may be present in the POTW effluent. Subsequent chemical analysis of the POTW effluent was performed using methods specifically designed to measure for the suspected industrial toxics. The chemical analysis results were compared with water quality criteria and toxicity values for individual compounds. Using this approach, alkyl phenol ethoxylate surfactants, phthalate esters and chlorinated solvents, largely attributed to textile industries, were identified as the primary toxics causing the POTW effluent toxicity.

A description of PPR methods is provided in Appendix A. The methods described involve a direct comparison of IU chemical data to POTW effluent toxicity. It is important to emphasize that drawing preliminary conclusions based on PPR results can be misleading, because IU monitoring information could be incomplete, chemical analysis techniques may not be sensitive to low levels of effluent toxics, and the estimated toxicity of individual compounds may not reflect the whole effluent toxicity. Due to these factors, comparisons of suspected toxicants to effluent toxicity may yield false correlations. Whenever possible, results of TIE testing should be used in lieu of PPR results, because the TIE test directly measures the relative toxicity of the effluent constituents.

Refractory Toxicity Assessment

Influent toxicity tracking is necessary when TIE testing is only able to identify a broad class of toxicants, rather than specific compounds, that are causing the POTW effluent toxicity. Toxicity tracking may also be required in situations where there are a large number of effluent toxicants and the occurrence of these toxicants in the POTW effluent is highly variable. In this case toxicity testing may be more cost-effective than chemical analysis.

The toxicity of influent wastewaters is not necessarily the same toxicity that is observed in the POTW effluent, because the POTW is capable of removing or degrading toxic wastewater constituents. The level of toxicity in a sewer discharge which could potentially pass through the POTW must be estimated by treating sewer samples in a simulation of the POTW process prior to toxicity analysis. Based on the experience to date, a simulation of activated sludge treatment has been developed for predicting the potential for a sewer discharge to contribute to the POTW effluent toxicity. This treatment step accounts for the toxicity removal provided by the POTW. A toxicity tracking approach can be applied to IU discharges and sewer line wastewaters to locate the sources contributing either acute or chronic toxicity that is refractory to POTW treatment.

The refractory toxicity assessment (RTA) approach involves treating sewer samples in aerobic batch bioreactors and testing the resulting effluents for toxicity. Batch bioreactors have been used by several researchers to screen wastewaters for activated sludge inhibition (Grady, 1985, Adams et al., 1981, Philbrook and Grady, 1987, and Kang et al., 1983) and non-biodegradable aquatic toxicity (Dague and Hagelstein, 1984, Lankford et al., 1987, and Sullivan et al., 1987). Dague and Hagelstein (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 Patapsco TRE (Botts et al., 1987) to evaluate the potential for indirect dischargers to contribute toxicity that was refractory to treatment provided by the POTW. This protocol involves treating sewer samples in a bench-scale batch reactor that 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). Acute and/or chronic toxicity measurements of the batch effluent indicate the amount of refractory toxicity in the sewer sample. In the protocol, coarse filtration of the decant from the batch reactor is used to produce the batch effluent. Decant-filtration more closely simulates sedimentation in the full scale plant because batch settling alone is not as efficient as the POTW settling process.

A general description of the RTA procedure is presented as follows. A step by step protocol for applying the RTA test to sewer wastewaters is provided in Table 5-1. It is important to note that the RTA procedure was developed based upon the experience to date and additional research is in progress to further refine the protocol. Public works managers should recognize that variations of the RTA protocol can be used to address site-specific circumstances. Best professional judgment will be important in applying the procedures and in interpreting the results.

Biomass Toxicity Measurement

During the Patapsco TRE, filtrate from coarse filtration of the POTW return activated sludge (RAS) was found to be acutely toxic to *Ceriodaphnia* (Botts et al., 1987). The high level of toxicity from residual biomass in the filtrate masked the measurement of batch effluent toxicity, and thereby reduced the effectiveness of the RTA test for monitoring IU refractory toxicity. The existing data on the toxicity of sewage sludges is not sufficient to determine how widespread is the occurrence of biomass filtrate toxicity. The following discussion provides information on how to proceed, if the POTW biomass filtrate toxicity presents an interference in the RTA test.

Additional testing was conducted during the Patapsco TRE to determine if the biomass toxicity interference could be removed. TIE results found that the effluent toxicity was caused mainly by non-polar organic compounds which preferentially adsorb onto sewage solids. Thus, tests were performed to determine if solids removal would reduce the Patapsco RAS toxicity. These tests demonstrated that toxicity in the RAS coarse filtrate could be removed by filtering the coarse filtrate through a 0.2 µm pore size filter to remove colloidal material from the liquid (Botts et.al., 1987). Alternatively, centrifugation of the coarse filtrate at 15,000 xg for 10 minutes also substantially reduced the biomass toxicity. Although biomass toxicity can be removed by applying these treatment steps to RTA test effluents, the resulting effluent toxicity will only indicate the soluble refractory toxicity of the IU wastewater, instead of the total refractory toxicity (i.e., soluble and particulate).

Prior to conducting the RTA, aquatic toxicity tests of the POTW activated sludge should be performed to determine if the sludge is toxic. This testing involves filtering the activated sludge through a coarse glass fiber filter, which is the same type of filter used for SS analysis, and testing the filtrate toxicity (Mount and Anderson-Carnahan, 1988a). If the biomass coarse filtrate is observed to have toxicity that is equal to or less than that of the POTW effluent, the POTW biomass can be used in RTA testing. Small particle filtration or centrifugation of the resulting RTA batch effluents will not be required.

If the biomass coarse filtrate is observed to be more toxic than the POTW effluent, the authors suggest the use of a non-toxic biomass such as another POTW biomass or a commercially available freeze-dried preparation. The non-toxic biomass will not be acclimated to the influent wastewaters of the POTW, but its use may allow an estimate of the non-biodegradable toxicity of the sewer discharge. The authors also recommend that the POTW activated sludge be used in a parallel series of RTA tests to determine the amount of soluble refractory toxicity in the sewer wastewater. The use of toxic POTW biomass is suggested because it is acclimated to the

Table 5-1. Tier I - Refractory Toxicity Assessment

Biomass Toxicity Measurement:

- Collect 5 liters of fresh return activated sludge (RAS) and aerate vigorously for 15 minutes.
- Prepare glass fiber filter [same type used for SS analysis (APHA, 1985)] by rinsing two 50 ml volumes of high purity water through the filter.
- Filter RAS to yield 200 ml of filtrate.*
- Test RAS filtrate for acute toxicity using the procedure described by Mount and Anderson-Carnahan (1988a) or for chronic toxicity using the methods provided by Horning and Weber (1985).
- Repeat above steps on several RAS samples.
- If RAS filtrate is more toxic than the POTW effluent, obtain non-toxic biomass (e.g., another POTW biomass or a freeze-dried preparation)

Sample Collection (volumes based on single sewer sample):

- Obtain 24-hour composite samples of sewer discharge (i.e., IU effluent or sewer line wastewater) and POTW primary effluent. Lag collection of primary effluent sample by the estimated travel time of sewer wastewater to POTW.
- Refrigerate 6 liters of sewer sample at 4°C until use. Determine the maximum holding time by measuring sample toxicity over time using methods described by Mount and Anderson-Carnahan (1988a).
- Refrigerate 5 liters of primary effluent sample at 4°C until use. Determine the maximum holding time as described above.
- Hold 3 liters of tap water for 2 days to dissipate chlorine.
- Collect 10 liters of RAS (and non-toxic biomass) on day of test and aerate vigorously for 15 minutes before use.

Sample Characterization (performed on day of sample collection):

- Analyze sewer wastewater for TKN, TP, TDS, COD, SCOD, pH.
- Use historical ratio of COD/BOD_5 of sewer wastewater, if available, to estimate BOD_5.
- Prepare glass fiber filter as stated above. Filter RAS to yield 200 ml of filtrate.* Test filtrate for acute toxicity (Mount and Anderson-Carnahan, 1988a) or chronic toxicity (Horning and Weber, 1985).
- Determine percent volume of sewer wastewater in POTW influent based on flow data.

Sample Preparation:

- Add nutrients to sewer sample to adjust BOD_5/TKN/TP ratio to 100:5:1.
- Adjust pH of sewer sample to average pH value of POTW influent.
- Test sample toxicity (Mount and Anderson-Carnahan, 1988a) after nutrient addition and pH adjustment to determine if these steps affect the sample toxicity.

Preparation of Batch Test Mixtures (three to six batch tests):

- Warm all refrigerated samples to room temperature using 30°C water bath. Do not overwarm.
- Select volume of RAS (V_B) to yield a MLSS concentration in 1.5 liters of batch mixture that is equal to the average POTW MLSS. If RAS is toxic (i.e., more toxic than POTW effluent), also select appropriate volume of nontoxic biomass (V_{NB}).
- Add RAS volume (V_B) to three 2-liter beakers, add diffused air (use air stone), and gently aerate. If RAS is toxic (i.e., more toxic than POTW effluent), add nontoxic biomass (V_{NB}) to three additional beakers and aerate.
- Prepare 2 liters of synthetic wastewater solution using stock synthetic wastewater (Table 5-2) and the tap water. Add volume of stock that will yield a solution COD equal to the primary effluent COD. Measure acute toxicity of the synthetic solution using the procedure described by Mount and Anderson-Carnahan (1988a). Chronic toxicity can be measured using the methods described by Horning and Weber (1985).
- Measure sewer sample volume (VW) that will yield a percent volume in 1.5 liters equal to 10 times the nominal percent volume of sewer wastewater in the POTW influent.

(continued)

Table 5-1. Continued

Performance of Batch Tests (total batch volume equals 1.5 liters):

- Add V_W and primary effluent to one beaker containing V_B. If RAS is toxic (i.e., more toxic than POTW effluent), also add V_W and primary effluent to one beaker containing V_{NB}.
- Add V_W and synthetic wastewater to one beaker containing V_B. If RAS is toxic (i.e., more toxic than POTW effluent), also add V_W and synthetic wastewater to one beaker containing V_{NB}.
- Add primary effluent to one beaker containing V_B. If RAS is toxic (i.e., more toxic than POTW effluent), also add primary effluent to the remaining beaker containing V_{NB}.
- Adjust aeration rate to allow complete mixing in all batch reactors. Periodically check DO level and maintain DO above 2 mg/l.
- Calculate the required reaction period necessary to achieve a batch F/M ratio (F/M_B) equal to the nominal F/M ratio (based on SCOD) in the POTW.
- Periodically check the batch reactor pH. Adjust pH to 6-9 range, if necessary.
- Note: batch tests should be performed at room temperature.

Effluent Toxicity Analysis:

- Stop aeration after the required reaction period and allow the V_B (and V_{NB}) to settle for 15 minutes.
- Decant 200 ml of clarified batch supernatant from each beaker. Rinse glass fiber filters as stated above. Filter each batch supernatant using separate filters.* Wash filter apparatus between each sample filtration using 10% HNO_3, acetone and high purity water.
- Batch filtrates that were treated with toxic biomass (V_B) must be either filtered through prewashed 0.2 µm glass filters or centrifuged at 10,000 xg for 10 to 15 min to remove colloidal size particles. Viscous mixtures may require faster or longer centrifugation (ASM, 1981).
- Prepare filter blanks for each filter type using dilution water from toxicity test procedure.
- Analyze the batch filtrates, centrates, and filter blanks for acute toxicity using the procedure described by Mount and Anderson-Carnahan (1988a). Chronic toxicity can also be measured (Horning and Weber, 1985)

*Note: Positive pressure filtering is required. Also, note that chronic toxicity measurement will require larger filtrate volumes as described by Horning and Weber (1985).

POTW influent wastewaters and will therefore provide a level of batch treatment that is more similar to the treatment efficiency of the POTW than that of the unacclimated alternate biomass. In this case, small particle filtration or centrifugation is required to remove the interfering biomass particles. By performing RTA tests with POTW biomass in parallel with RTA tests with alternate biomass, both the soluble and total refractory toxicity of the IU wastewater may be estimated.

Sample Collection

Wastewater and activated sludge samples should be collected according to the procedures described in Section 13. Return activated sludge (RAS) is recommended for use in batch testing, because it is in a concentrated form that can be easily diluted to the correct 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 SS concentration as that of the RAS before use.

Sample Characterization and Preparation

The sewer sample should be analyzed for total and soluble COD, total kjeldahl nitrogen (TKN), total phosphorus (TP), total dissolved solids (TDS) and pH on the day of sample collection. An estimate of the sample BOD_5 can be calculated using historical data on the COD/BOD_5 ratio of the wastewater. Based on these results, the BOD_5/TKN/TP concentration ratio of the sewer sample should be adjusted, if necessary, to 100:5:1 which is the typical ratio for municipal sewage. This BOD_5/TKN/TP ratio will ensure that sufficient nutrients are available for consistent batch treatment of the IU wastewaters. Phosphorus should be added in the form of three parts KH_2PO_4 to four parts K_2HPO_4. Nitrogen should be added as urea nitrogen.

Following nutrient addition, the pH of the sewer sample should be adjusted to the average pH value of the POTW influent. Sulfuric acid and sodium hydroxide can be used for pH adjustment.

Following nutrient addition and pH adjustment, the sewer sample toxicity should be measured to determine if the 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. A discussion of the use of pH adjustment for toxicity characterization is given by Mount and Anderson-Carnahan (1988a).

Preparation of Batch Test Mixtures

The volume of RAS biomass (V_B), to be used in batch testing, should yield a batch MLSS concentration that is equal to the average MLSS concentration in the POTW aeration basins. The amount of RAS to be added to the total batch volume of 1.5 liters is calculated as follows:

$$V_B = \frac{POTW\ MLSS}{RAS\ SS}\ x\ 1.5\ liters.$$

The same equation is used to determine the alternate (non-toxic) biomass volume (V_{NB}).

A total of three batch influent solutions are prepared for each sewer sample: sewer sample spiked into synthetic sewage, sewer sample spiked into POTW influent (primary effluent) and primary effluent alone. The synthetic wastewater provides a standard non-toxic substrate that will allow consistent batch treatment of the IU wastewaters and will permit a determination of the refractory toxicity of the IU wastewaters. The composition of this synthetic wastewater reflects the soluble COD (SCOD) and nutrient content of typical domestic sewage (Table 5-2). Similar synthetic preparations have been used as supplements in biodegradation studies (Kirsh et.al., 1985) in lieu of domestic sewage.

Table 5-2. Synthetic Wastewater Composition*

Constituent	Concentration (g/l)
Bacto Peptone	32.0
Beef Extract	22.0
Urea	6.0
NaCl	1.4
$CaCl_2 \cdot 2\ H_2O$	0.8
$MgSO_4 \cdot 7\ H_2O$	0.4
KH_2PO_4	3.5
K_2HPO_4	4.5

*SCOD of the stock solution is 64,000 mg/l.

Prior to use in RTA testing, the synthetic wastewater, which has an SCOD of 64,000 mg/l, is diluted in dechlorinated tap water. The synthetic wastewater should be diluted to an SCOD concentration that is equal to the average SCOD concentration of the POTW primary effluent. The required stock synthetic wastewater volume can be calculated as follows:

$$\text{Stock Synthetic Wastewater Volume} = \frac{Primary\ Effluent\ SCOD}{64{,}000\ mg/l\ SCOD}\ x\ 1.5\ liters$$

The amount of sewer sample to be used in batch testing should reflect the percent volume of sewer wastewater in the POTW influent. In some cases, the toxicity in sewer wastewater from small contributors may not be readily observed when the wastewater is mixed by percent volume with synthetic wastewater or POTW influent. Thus, the authors recommend using a volume of sewer wastewater (V_W) equal to ten times the percent volume of sewer wastewater typically found in the POTW influent. For example, if the sewer wastewater flow is 0.5 mgd and the POTW influent flow is 50 mgd, V_W would equal 10% of the batch influent solution. In cases where the sewer wastewater flow is ≥10% of the POTW influent flow, the sewer sample would comprise 100% of the batch influent.

Performance of Batch Tests

In the batch tests, the sample/synthetic solution is used to measure the refractory toxicity of the sewer sample. The sample/primary effluent solution provides an indication of the interactive effects (e.g., additive or antagonistic) that can occur when the sewer wastewater and POTW influent are combined. The third batch solution, primary effluent, serves as a control for the sample/primary effluent test by providing a measure of refractory toxicity in the primary effluent.

The batch influent solutions are mixed with RAS (V_B) to yield a total batch volume of 1.5 liters and diffused air is applied to the mixture. The diffused aeration must be performed in appropriate laboratory fume hoods to prevent exposure of laboratory staff to any toxic vapors stripped from the wastewater samples (Section 11).The aeration rate is adjusted to ensure complete mixing in the batch reactor and to maintain a DO concentration above 2 mg/l.

The organic loading to the batch reactors can vary substantially depending on the type of sewer wastewater being tested. To allow comparable treatment of the various sewer wastewaters, the food-to-microorganism ratio of the batch reactor (F/M_B) can be standardized by varying the time of aeration. F/M_B should be made equal to the F/M (based on SCOD) of the POTW bioreactors. The required batch test period can be calculated as follows:

$$Test\ Period\ (days) = \frac{Batch\ Influent\ SCOD\ (mg/l)}{MLVSS\ (mg/l)\ x\ F/M_B}$$

A typical test period for a sewer sample with COD <1000 will be appropriately 2 to 4 hours.

Toxicity Measurement

Either acute or chronic refractory toxicity can be measured in RTA testing. Procedures for acute toxicity measurement should follow the methods described by Mount and Anderson-Carnahan (1988a). In order to obtain comparable acute toxicity results, RTA testing should utilize the same species that was used for TIE tests. Other acute toxicity analyses such as bacterial bioluminescence tests (e.g., Microtox™) can be used in conjunction with the preferred test species to provide additional information. Chronic toxicity testing should utilize the same species that was used for TIE testing. Chronic toxicity test methods are described by Horning and Weber (1985).

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 the batch settling alone is not as efficient as the POTW settling process. 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 to remove colloidal size particles from the wastewater. Membrane filters such as cellulose nitrate filters are not recommended 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 10. Alternatively, the coarse filtrate can be centrifuged at 10,000 xg for 10 to 15 min to separate colloidal material from the wastewater (ASM, 1981).

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 Non-Toxic --

In cases where the POTW biomass filtrate is determined to have toxicity that is equal to or less than that of the POTW effluent, RTA tests will utilize POTW biomass and wastewater samples. Results for each IU sample analysis will consist of data on three batch tests: one test of sample/synthetic sewage solution, one test of sample/primary effluent solution, and one test of primary effluent. The batch test of the sewer sample/synthetic wastewater solution reveals the amount of refractory toxicity in the sewer wastewater excluding the effects of other influent wastewaters. Perhaps the most important batch test is the analysis of the sewer sample/primary effluent solution, because test data will indicate the toxicity that would realistically occur upon mixture of the sewer wastewater with POTW influent. Results of this combined wastewater test are compared to results of the primary effluent batch test to determine if combining the wastewaters decreases the refractory toxicity (i.e., antagonistic effect) or increases the refractory toxicity (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 test, the sewer wastewater source can be presumed to be a contributor of refractory toxicity. A list of toxic sewer sources should be prepared for further evaluation. In situations where sewer line tracking is being conducted, this list can be compared to a sewer collection system map to identify possible toxic IU dischargers on the sewer lines.

Results of RTA Tests if POTW Biomass is Toxic --

In situations where the POTW biomass filtrate is found to be more toxic than the POTW effluent, RTA tests will utilize alternate (nontoxic) biomass and wastewater in addition to tests with POTW biomass and wastewater. The data on each IU sample analysis will consist of results of three batch tests using alternate biomass (i.e., one test of sample/synthetic sewage, one test of sample/primary effluent, and one test of primary effluent); and results of three batch tests using POTW (toxic) biomass (i.e., using same wastewaters as above). The results of tests that use alternate biomass may provide an estimate of non-biodegradable toxicity of the sewer wastewater. The disadvantage of these tests is that the biomass is not acclimated to the POTW influent wastewaters, therefore the level of toxicity reduction may not reflect the treatment efficiency of batch tests using the POTW acclimated biomass. Nonetheless, the non-toxic biomass tests can give an indication of the relative level of refractory toxicity being contributed by the sewer wastewater sources.

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 causes artificial changes in the batch effluent toxicity. The advantage of toxic biomass tests is that the soluble refractory toxicity of source wastewaters can be determined. The nontoxic biomass tests cannot provide as good an estimate of soluble toxicity, because this biomass is not acclimated to the POTW influent wastewaters.

RTA tests which utilize POTW toxic biomass and alternate biomass can provide information on both the soluble and total refractory toxicity of the IU wastewater. Further studies are in progress to improve the utility of the RTA test for toxicity source evaluation. These studies will focus on the development of procedures to account for the toxicity interferences caused by toxic activated sludge.

RTA Conclusions

If the RTA testing is successful in locating the sources of refractory toxicity, further testing is required in Tier II (Section 6) to confirm the suspected toxic sources. Tier II testing is necessary because Tier I information is not sufficient to proceed to the evaluation and selection of pretreatment control options (Section 8).

In situations where RTA testing proves to be a prodigious task, the permittee may elect to evaluate alternatives for in-plant toxicity control (Section 7). This choice may be determined by assessing the best use of the resources that are available for the TRE. In this regard the POTW has the option to recover costs associated with toxicity source evaluation through the process of local limits development.

Section 6
Toxicity Source Evaluation - Tier II

Introduction

A Tier II evaluation is conducted to gather additional information on the indirect dischargers to the POTW to confirm the suspected sources of toxicity identified in the Tier I evaluation. Based on the Tier I data, potentially toxic indirect dischargers are identified and their wastewaters are sampled and analyzed using the Tier II Refractory Toxicity Assessment procedure. Like the RTA step used in the Tier I evaluation, the Tier II RTA estimates the toxicity that is refractory to POTW treatment and would therefore be expected to pass through the POTW. The Tier II RTA, however, includes several additional procedures for toxicity assessment including:

- a series of wastewater dilutions to determine the concentration of wastewater at which toxicity pass-through would be expected to occur at the POTW;
- an optional series of control tests to allow a comparison of the relative levels of refractory toxicity and inhibition effects for the various sewer wastewaters; and
- TIE Phase I tests of toxic batch effluents to indicate the components of the refractory toxicity.

A diagram of the Tier II RTA is presented in Figure 6-1. The step by step procedures for implementing RTA tests are shown in Table 6-1.

The results of the Tier II testing are used to rank indirect dischargers in terms of their potential to contribute inhibitory material or refractory toxicity to the POTW. Results of the optional TIE Phase I analysis may also provide important information on the toxic components of the sewer wastewater. The Tier II data are used to identify the major toxic dischargers and to evaluate pretreatment options for controlling the discharge of toxics or toxicity by these dischargers. In situations where existing pretreatment regulations (i.e., general, specific or categorical) are insufficient for the POTW to achieve an acceptable level of effluent toxicity, the municipality is encouraged to develop local limits for its sewer users (USEPA, 1987a).

Refractory Toxicity Assessment

The procedures for biomass toxicity measurement, collection, characterization and preparation of samples, preparation of batch test mixtures, performance of the batch test, and batch test toxicity measurement are similar to those described for Tier I RTA testing (Section 5). A description of the unique elements and optional steps in the Tier II RTA procedures is provided as follows.

Biomass Toxicity Measurement

Results of the biomass toxicity measurement conducted in Tier I (Section 5) will dictate whether or not an alternative (nontoxic) biomass will be required for Tier II testing. If the POTW biomass filtrate was found to be less toxic than the POTW effluent in Tier I, only POTW biomass will be used in Tier II tests. If the POTW biomass filtrate was observed to be more toxic than the POTW effluent in Tier I, both the POTW biomass and a non-toxic alternative biomass will be needed for RTA testing in Tier II.

Sample Collection, Characterization, and Preparation

The procedures for collection, characterization, and preparation of samples are the same as those described in Section 5. Sewer sampling locations for Tier II testing are the points of discharge of the IU effluent to the sewer collection system. If sewer line sampling was conducted in Tier I, sufficient data should have been collected to identify the IUs to be sampled for Tier II.

Preparation of Batch Test Mixtures

The steps for determining the volume of biomass (V_B) and the synthetic wastewater concentration to be used in batch tests are the same as those described for Tier I RTA testing (Section 5). Because additional dilutions are performed in Tier II RTA testing, sufficient biomass and synthetic wastewater should be prepared for seven batch tests (if the POTW biomass is non-toxic) or fourteen batch tests

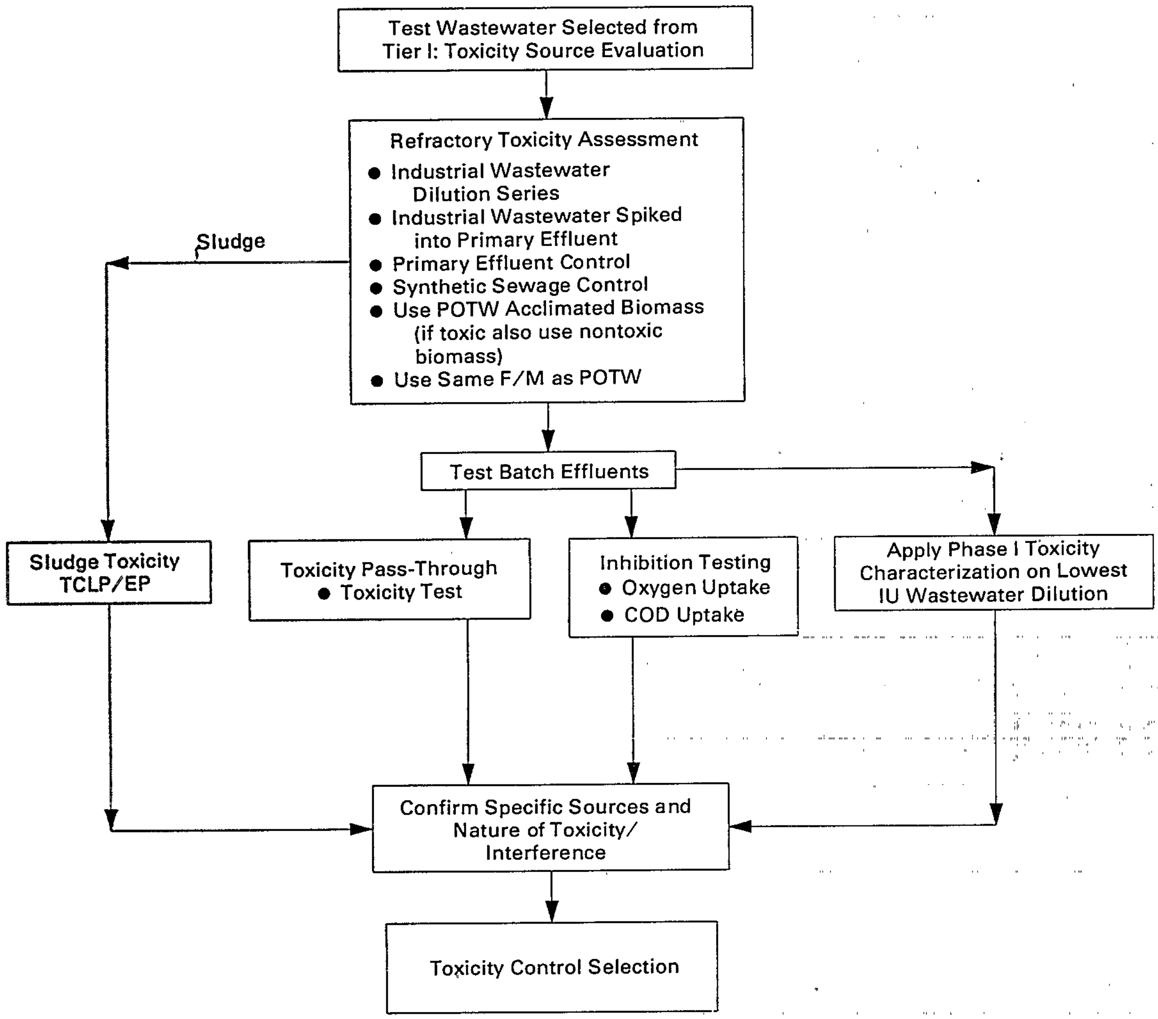

Figure 6-1. **Tier II: toxicity source evaluation (source ranking/pretreatment evaluation).**

(if both toxic POTW biomass and non-toxic biomass must be used).

Additional IU sample dilutions are performed to determine the wastewater concentration that causes refractory toxicity to become apparent in the batch test effluent. Three IU sample volumes are used in RTA tests: one equivalent to two times the percent volume of IU wastewater (V_{W2}) typically found in the POTW influent; another equal to five times the percent volume of IU wastewater (V_{W5}) in POTW influent; and a third equal to ten times the proportion of IU wastewater (V_{W10}) in POTW influent. In cases where the IU discharge flow is ≥20% of the POTW influent flow, the V_{W5} and V_{W10} will be 100% of the batch influent. If this occurs, the sample volumes can be reduced to allow an appropriate range of sample dilutions to be tested.

Performance of Batch Tests

Tier II testing utilizes the same three types of influent solutions applied in Tier I RTA tests: IU sample spiked into synthetic sewage, IU sample spiked into POTW primary effluent, and primary effluent alone. The difference between Tier II and Tier I RTA testing is that additional dilutions of IU sample are prepared in Tier II using the synthetic sewage and primary effluent wastewaters. Also, extra volumes of the batch influent solutions are prepared and held for subsequent toxicity measurement.

The batch tests of the three types of influent solutions provide data on the level of refractory toxicity in the IU wastewater. The sample/synthetic solution test indicates the refractory toxicity of the IU wastewater by itself. The sample/primary effluent solution

Table 6-1. Tier II - Refractory Toxicity Assessment

Sample Collection (volumes based on single sewer sample):

- Obtain 24-hour composite samples of IU discharge and POTW primary effluent. Lag collection of primary effluent sample by the estimated travel time of IU wastewater to POTW.
- Refrigerate 18 liters of sewer sample at 4°C until use. Determine the maximum holding time by measuring sample toxicity over time using methods described by Mount and Anderson-Carnahan (1988a).
- Refrigerate 12 liters of primary effluent sample at 4°C until use. Determine the maximum holding time as described above.
- Hold 9 liters of tap water for 2 days to dissipate chlorine.
- Collect 10 liter grab sample of fresh return activated sludge (RAS) (and non-toxic biomass) on day of test and aerate vigorously for 15 minutes before use.

Sample Characterization (performed on day of sample collection):

- Analyze sewer wastewater for TKN, TP, TDS, COD, SCOD, and pH.
- Use historical ratio of COD/BOD_5 of sewer wastewater, if available, to estimate BOD_5.
- Determine percent volume of sewer wastewater in POTW influent based on flow data.
- Prepare glass fiber filters [same type used for SS analysis (APHA, 1985)] by rinsing two 50 ml volumes of high purity water through each filter. Filter 200 ml aliquots of samples of IU wastewater, primary effluent and RAS.* Wash filter apparatus between each sample filtration using 10% HNO_3, acetone, and high purity water.
- Test the filtrates of the IU wastewater, primary effluent and RAS for acute toxicity (Mount and Anderson-Carnahan, 1988a) or chronic toxicity (Horning and Weber, 1985).

Sample Preparation:

- Add nutrients to IU sample to adjust BOD_5/TKN/TP ratio to 100:5:1.
- Adjust pH of IU sample to average pH value of POTW influent.
- Test sample toxicity (Mount and Anderson-Carnahan, 1988a) after nutrient addition and pH adjustment to determine if these steps affect the sample toxicity.

Preparation of Batch Test Mixtures (seven to fourteen batch tests):

- Warm all refrigerated samples to room temperature using 35°C water bath. Do not overwarm.
- Select volume of RAS (V_B) to yield a MLSS concentration in 1.5 liters of batch mixture that is equal to the average POTW MLSS. If RAS is toxic (i.e., more toxic than POTW effluent), also select appropriate volume of non-toxic biomass (V_{NB}).
- Add RAS volume (V_B) to seven 2-liter beakers, add diffused air (use air stone), and gently aerate. If RAS is toxic (i.e., more toxic than POTW effluent), add non-toxic biomass (V_{NB}) to seven additional beakers and aerate.
- Prepare 2 liters of synthetic wastewater (Table 5-2) using the tap water. Add volume of stock that will yield a solution COD equal to the average primary effluent COD. Measure acute toxicity of synthetic solution (Mount and Anderson-Carnahan 1988a). Chronic toxicity (Horning and Weber, 1985) can also be measured.
- Measure IU sample volumes that will yield a percent volume in 1.5 liters equal to 2, 5, and 10 times the nominal percent volume of IU wastewater in the POTW influent (V_{W2}, V_{W5}, V_{W10}, respectively)

Performance of Batch Tests (total batch volume equals 1.5 liters):

- Mix V_{W2} and primary effluent (allow for excess of 200 ml). Add this mixture to one beaker containing V_B. Keep the remaining 200 ml. If RAS is toxic (i.e., more toxic than POTW effluent), also add additional V_{W2} and primary effluent mixture to one beaker containing V_{NB}. Repeat these steps for V_{W5} and V_{W10}.
- Mix V_{W2} and synthetic wastewater (allow for excess of 200 ml). Add this mixture to one beaker containing V_B. Keep the remaining 200 ml. If RAS is toxic (i.e., more toxic than POTW effluent), also add additional V_{W2} and synthetic wastewater mixture to one beaker containing V_{NB}. Repeat these steps for V_{W5} and V_{W10}.
- Add primary effluent to one beaker containing V_B. If RAS is toxic (i.e., more toxic than POTW effluent), also add primary effluent to remaining beaker containing V_{NB}.
- Adjust aeration rate to allow complete mixing in all batch reactors. Periodically check DO level and maintain DO above 2 mg/l.
- Calculate the required reaction period necessary to achieve a batch F/M ratio (FM_B) equal to the nominal F/M ratio (based on SCOD) in the POTW.
- Periodically check batch reactor pH. Adjust pH to 6-9 range, if necessary.
- Note: batch tests should be performed at room temperature.

(continued)

Table 6-1. Continued

Inhibition Testing (optional - two additional batch tests).

- Prepare two synthetic wastewater solutions using stock synthetic wastewater (Table 5-2) and tap water: one with an SCOD concentration equal to the average primary effluent SCOD and another with an SCOD level equal to that of the lowest sample dilution (i.e., V_{W10} spiked into primary effluent).
- Add synthetic wastewater solutions to beakers containing V_B. If RAS is toxic (i.e., more toxic than POTW effluent), also add synthetic solutions to beakers containing V_{NB}.
- Perform batch tests in parallel with above batch tests.
- Subsample 300 ml from *all* batch reactors at 30 min. and every 2 hours following test initiation, and at the completion of the test. Upon subsample collection, immediately measure OUR using the BOD bottle method (APHA, 1985). Return the subsamples to the reactors immediately following use.
- Subsample 50 ml from *all* batch reactors at 5 minutes and ever 2 hours following test initiation, and at the completion of the test. Subsample 50 ml of the original undiluted biomass stock. Filter the subsamples through a 0.45 µm pore size filter. Measure the SCOD of the filtrates.

Toxicity Analysis:

- Stop aeration after the required reaction period and allow the V_B (and V_{NB}) to settle for 15 minutes.
- Decant 200 ml of clarified batch supernatant from each beaker. Prepare filters as described above. Filter the batch supernatants.* Rinse filtration equipment between sample filtrations as stated above.
- Batch filtrates that were treated with toxic biomass (V_B) must be either filtered through prewashed 0.2 µm glass filters or centrifuged at 10,000 xg for 10 to 15 min to remove colloidal size particles. Viscous mixtures may require faster or longer centrifugation (ASM, 1981).
- Prepare filter blanks for each filter type using dilution water from toxicity test procedure.
- Analyze the untreated solutions (batch influent), batch effluent and filter blanks for acute toxicity (Mount and Anderson-Carnahan, 1988a) or chronic toxicity (Horning and Weber, 1985).

Phase I Toxicity Characterization (Optional)

- The batch effluent of the V_{W10}/synthetic wastewater test should be used for Phase I analysis. Phase I testing requires 3.5 liters for analysis, therefore the volume used in batch testing should be increased to a minimum of 7 liters.
- Following batch treatment, allow the V_B (and V_{NB}) to settle for 1 hour. Decant 3.5 liters of supernatant.
- Prepare coarse glass filters (SS type only) as described above. Filter the batch supernatant.*
- Analyze the batch effluent using the Phase I protocol (Mount and Anderson-Carnahan, 1988a).

*Note: Positive pressure filtering is required. Also note that chronic toxicity measurement will require larger sample volumes as described by Horning and Weber (1985).

provides information on the possible interactive effects (e.g., antagonism, additivity) that may occur upon mixture of the IU wastewater and the POTW influent. The third batch influent, primary effluent, serves as a control for the sample/primary effluent test by providing data on the refractory toxicity of the primary effluent alone.

Following mixture of the batch influents with the biomass, the mixed liquors are aerated for a period of time that will allow comparable treatment of the sample dilutions. Because the organic loading to the batch reactors can vary depending on the sample concentration, the F/M_B should be standardized by varying the time of aeration for each sample dilution. The procedure for calculating the required reaction period based on F/M_B is described in Section 5.

Inhibition Testing (Optional)

Biological treatment inhibition can be assessed by monitoring substrate (COD) removal and oxygen uptake rates in the batch tests and comparing the results of the IU sample batch tests to the results of the synthetic wastewater batch tests. If COD or oxygen removal rates in IU sample tests are lower than those in synthetic wastewater tests, biological inhibition is indicated.

Inhibition testing requires two additional batch reactors consisting of synthetic sewage and biomass: one with a synthetic sewage SCOD concentration equal to the average primary effluent SCOD and another with a synthetic sewage SCOD concentration equal to the SCOD level of the lowest IU sample dilution (i.e., V_{W10} spiked into primary effluent). The two synthetic wastewater concentrations effectively bracket the highest and lowest expected SCOD loadings to the batch reactors. This range of synthetic sewage concentrations is necessary to compare COD and oxygen removal rates for the wastewater dilutions, because COD and oxygen utilization usually increase with increasing wastewater strength. At high soluble substrate concentrations (i.e., 1 mg/l SCOD to 4 mg/l MLVSS) the biomass activity generally reaches a maximum rate. At soluble substrate concentrations below this "plateau," biomass activity rates vary with substrate concentration. Thus, the soluble substrate concentrations of the IU sample tests and the synthetic sewage tests must be similar to allow an accurate comparison of COD and oxygen utilization rates for inhibition measurement.

Soluble COD removal can be used as an indicator of specific substrate removal rate (SSUR). SSUR is reported in units of mg/l SCOD/g MLVSS/min, and is calculated as shown in an equation at the end of this page.

The POTW biomass used in batch testing contains nonbiodegradable SCOD remaining from biological treatment which must be accounted for when calculating the SCOD of the batch effluent. This correction for biomass SCOD is calculated as shown in an equation at the top of the next page.

Specific oxygen uptake rate (SOUR) is reported in units of mg O_2/l/g MLVSS/min and is calculated as follows:

$$SOUR = \frac{Oxygen\ Consumed}{MLVSS\ x\ D.O.\ Measurement\ Period\ (min)}$$

The SSUR and SOUR data for the IU sample dilution series and the two synthetic sewage tests are plotted against the SCOD of the batch influent solutions. A reduction in the SSUR and SOUR rates of the IU sample tests relative to the SSUR and SOUR rates of the synthetic control tests (for samples with equivalent batch influent COD concentrations) indicates the presence of inhibitory material in the IU wastewater. The degree of inhibition can be inferred by the amount of deviation in the biomass activity rates for IU sample tests compared to the biomass activity rates for the control rates.

Toxicity Analysis

The procedures for acute and chronic toxicity measurement of the RTA samples are the same as those described for Tier I (Section 5). In addition to the batch effluent samples, the batch influent solutions are measured for toxicity.

Phase I Toxicity Characterization

Phase I tests can be applied to the batch effluent of the lowest dilution of the sewer wastewater in synthetic sewage (i.e., highest wastewater strength). The purpose of this analysis is to determine the types of toxicants causing refractory toxicity in the IU wastewater. Results of Phase I testing can be compared to TIE results on the POTW effluent to indicate whether or not the IU wastewater contains refractory toxicants that were also observed in the POTW effluent. A description of the Phase I procedure is given in Section 4.

Data Evaluation

Results of the Tier II evaluation are used to confirm the sources of the refractory toxicity identified in Tier I. Tests of the series of dilutions of the IU wastewater samples can indicate the wastewater concentration at which toxicity passthrough would be expected to occur at the POTW. This information can be used to identify the major contributors of refractory toxicity to the POTW.

Results of RTA Tests if POTW Biomass is Non-Toxic --

In cases where the POTW biomass filtrate is determined to be less toxic than the POTW effluent, RTA tests will utilize POTW biomass and wastewater samples. Results for each IU sample analysis will consist of data on seven batch tests: three tests of sample/synthetic sewage dilutions, three tests of sample/primary effluent dilutions, and one test of primary effluent.

Sample/synthetic sewage test--The batch tests of the series of IU sample/synthetic sewage dilutions will reveal the IU wastewater concentration that causes toxicity to occur in the batch effluent. For example, batch effluent toxicity may become apparent in tests of IU wastewater at sample concentrations five times the wastewater concentration (V_{W5}) typically observed in the POTW influent, but not at sample concentrations two times the typical wastewater concentration (V_{W2}). In this case toxicity pass-through occurred somewhere between two and five times the IU wastewater concentration typically found in the POTW influent.

$$SSUR = \frac{Batch\ Influent\ SCOD\ (mg/l) - Batch\ Effluent\ SCOD\ (mg/l)}{MLVSS\ (mg/l)\ x\ Test\ Period\ (min)}$$

$$SCOD = \frac{[(V_R) \times (SCOD\ Batch\ Reactor\ Effluent)] - [(V_B) \times SCOD\ biomass)]}{V_R}$$

where V_R is the total volume in the batch reactor, and V_B is the volume of RAS added to the reactor.

The relative amounts of refractory toxicity contributed by the IUs can be determined by accounting for the discharge flow of each of the tested IUs. The first step is to convert the 48-hour LC_{50} values for each sample dilution to toxic units (TU) by multiplying the reciprocal of each LC_{50} value by 100 (i.e., $100/LC_{50}$). The toxic units for each sample dilution series are then summed and the total toxic units are multiplied by the flow rate of the IU discharge. An example calculation is described below.

	Sample Dilution (Times Percent Flow in POTW Influent)		
	2X	5X	10X
Batch effluent LC50 (as percent effluent):	50	30	10
Batch effluent toxic units	2	3.3	10

Relative Score = Sum of TUs x IU Discharge Flow Rate,
where sum TU = 15.3, and
IU Discharge Flow Rate = 1 mgd.

Thus, the IU relative score is:

15.3 TU x 1 mgd = 15.3 TU - million gal/day.

The relative scores of the IU wastewaters are ranked to determine the major contributors of refractory toxicity. Although the relative score is in units of TU-million gal/day, it does not represent the actual toxicity loading of the IU wastewater. Instead, the relative score is an estimate for comparing the relative levels of refractory toxicity contributed by the IUs.

Sample/primary effluent tests--Results of the batch tests of the series of IU sample/primary effluent dilutions will indicate the IU wastewater concentration that causes refractory toxicity to occur upon mixture of the wastewater with POTW influent. These tests are an attempt to measure the effects of mixing the IU wastewater with the POTW influent wastewater, as would realistically take place in the POTW influent. Combining the wastewaters may decrease the refractory toxicity (i.e., antagonistic effect) or increase the refractory toxicity (i.e., additive effect) of the IU wastewater.

The relative level of refractory toxicity contributed by the IUs can be determined by calculating a relative score using the batch effluent LC_{50} for each IU sample as described above. This relative score will account for the antagonistic or additive toxic effects that may occur upon mixture of the IU wastewater with the POTW influent. Relative scores for the IUs can be ranked to identify the major contributors of refractory toxicity.

The ranking of IUs based on the IU sample/primary effluent tests is preferred over the IU ranking based on the IU sample/synthetic sewage tests, because the IU wastewater/primary effluent mixture better reflects the composition of the POTW influent. The IU ranking can be used to determine which major toxic dischargers should be targeted for pretreatment control.

Results of RTA Tests if POTW Biomass is Toxic --

In situations where the POTW biomass filtrate is found to be more toxic than the POTW effluent, RTA tests will utilize alternate (non-toxic) biomass and wastewater in addition to tests with POTW biomass and wastewater. The data on each IU sample analysis will consist of results of seven batch tests using alternate biomass (i.e., three tests of sample/synthetic sewage dilutions, three tests of sample/primary effluent dilutions, and one test of primary effluent) and results of seven batch tests using POTW biomass (i.e., using same wastewaters as above). The batch effluent results of tests using POTW biomass will indicate soluble refractory toxicity, because the toxic biomass particles are removed (via filtration or centrifugation) prior to toxicity analysis. Batch effluents of tests using alternate biomass do not require treatment for toxic particle removal, therefore, the batch effluent results will reveal the total refractory toxicity of the IU wastewater.

Batch tests using POTW biomass--Batch tests which utilize POTW (toxic) biomass will provide an indication of the soluble refractory toxicity of the IU wastewater. The relative contribution of soluble refractory toxicity from the IUs can be determined by calculating relative scores for the IU sample/synthetic sewage dilution tests and the IU sample/primary effluent dilution tests.

Batch tests using alternate biomass--Batch tests that utilize an alternate (non-toxic) biomass provide data on the total refractory toxicity (i.e., soluble and particulate) of the IU wastewater. These tests are an indirect measure of refractory toxicity because an alternate biomass is not acclimated to the POTW influent wastewaters and the level of toxicity reduction that can be achieved by an unacclimated biomass will not be the same as that achieved by POTW acclimated biomass. In some cases, the batch influent may partially or completely inhibit toxicity degradation when unacclimated biomass is used. Nonetheless, batch tests utilizing alternate biomass may give a relative estimate of the level of total refractory toxicity contributed by IUs. The relative contribution of refractory toxicity from the IUs can be determined by calculating relative scores for the IU sample/synthetic sewage dilution tests and the IU sample/ primary effluent dilution tests.

Toxicity measurement of batch influent solutions--The batch influent solutions are also measured for toxicity to provide information on the toxicity of the raw (untreated) IU wastewater. This information can be used together with the batch effluent data on soluble and total refractory toxicity to identify the major toxic IUs.

Results of Optional Inhibition Testing --

The optional analyses for wastewater inhibition can also provide useful data. RTA information on the potential for a wastewater to inhibit biomass activity can be used to identify IU wastewaters that may interfere with the POTW biological treatment process. This information is important in situations where inhibitory wastewater may interfere with the normal operation of the biological treatment process to the degree that it causes toxicity passthrough.

Results of Optional TIE Phase I Testing --

Phase I analysis of the batch treated wastewater can indicate the types of toxicants responsible for the refractory toxicity in the IU wastewater. A comparison of these Phase I results with the results of the TIE analysis of the POTW effluent will indicate whether or not the IU wastewater toxicants are the same types of toxicants observed in the POTW effluent. IUs which discharge the same types of toxicants as those found in the POTW effluent should be candidates for pretreatment control evaluation.

Pretreatment Control Evaluation

Pretreatment control options can be developed by the POTW to prevent the pass-through of toxics and toxicity, and treatment interferences, which have been traced to indirect dischargers. In cases where current pretreatment regulations are insufficient for the POTW to achieve an acceptable level of effluent toxicity, the municipality should develop local limits for its sewer users (USEPA, 1987a).

The EPA Local Limits Guidance Manual (USEPA, 1987a) describes several technical approaches to the development of local limits. These approaches are outlined as follows:

- *Allowable Headworks Loading Method*: Numeric limits are defined based on the maximum loadings of pollutants that will allow compliance with receiving water quality criteria or sludge quality criteria, or protection against treatment interferences.
- *Industrial User Management Method*: Based on an in-depth review of IU practices the municipality 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 comparable industries.

Some of the local limits approaches address specific issues of concern related to toxics or toxicity. For example, the allowable headworks loading method is well-suited for developing limits to prevent the pass-through of toxic compounds identified by Tier I chemical-specific analysis or by POTW effluent TIE tests. This method can be used to establish the maximum level of the toxic pollutant that can be safely received by the POTW without exceeding the effluent toxicity limit.

The industrial user management method provides a framework for implementing chemical management practices including slug discharge control. In cases where IU slug loadings contribute to POTW effluent toxicity, spill prevention or load equilization can be implemented at the IU facility to moderate the slug loadings. EPA's Slug Loading Control Manual (1988b) describes methods for the development of slug loading control programs.

The case by case permitting method can be used when the POTW effluent toxicity cannot be traced to specific IU chemicals, but information on the general classes of toxicants is available based on TIE results. In this case an engineering decision can be made in selecting a pretreatment technology for removing general types of toxicants (i.e., non-polar organic compounds). In situations where the sources of toxicity have been identified, the POTW has the authority to require the IU to take steps to limit the discharge of refractory or inhibitory toxicity.

Although EPA and the states have overview authority, the choice of which technical approach to use for local limits development is the POTW's decision. The information to be used in the development of local limits will include the data gathered in the TIE and toxicity source evaluations (Tier I and II). Methods for calculating numerical limitations and preparing narrative limitations are described in the EPA Local Limits Guidance Manual (USEPA, 1987a). The goal in developing local limits is to implement pretreatment regulations that are technically and legally defensible. The local limits can include provisions for equitable recovery of costs associated with the toxicity source evaluations and local limits development.

Section 7
POTW In-Plant Control Evaluation

Introduction

The objective of the in-plant control evaluation is to select and evaluate feasible treatment options for the reduction of refractory toxicity and/or toxic interferences at the POTW. Treatment options are selected using the data gathered in the PPE (Section 3) and TIE (Section 4) investigations and best professional judgement. Following this selection process, treatability testing is conducted to determine the toxicity removal effectiveness and operating characteristics of the treatment options. The resulting test data provide a basis for the final selection and conceptual design of feasible POTW process modifications or additions.

A schematic of the POTW in-plant control evaluation is presented in Figure 7-1. Toxicity control options are selected based on evidence that the options are technically feasible and can be integrated into the overall design of the treatment facilities. Following selection, the options are evaluated in bench-scale tests which utilize acute or chronic toxicity tests or toxic chemical analyses to evaluate the feasibility of the option for toxicity and/or toxics reduction. In some cases, pilot-scale testing may be conducted to provide information for the design of the treatment option. An optional TIE Phase I analysis may be performed to evaluate the removal of selected toxicants by the treatment option.

It is important to consider that major changes in treatment plant facilities or operations may not be feasible due to the cost of new facilities or the complexity of additional process operations. In these situations, pretreatment control of toxicity may be preferred to in-plant control.

Selection of Treatment Options for Testing

The information collected in the PPE and TIE stages of the TRE is reviewed and evaluated to select possible feasible in-plant options to be tested in treatability studies. The first step in the selection process is to review the PPE data on the POTW design to establish the physical space available for

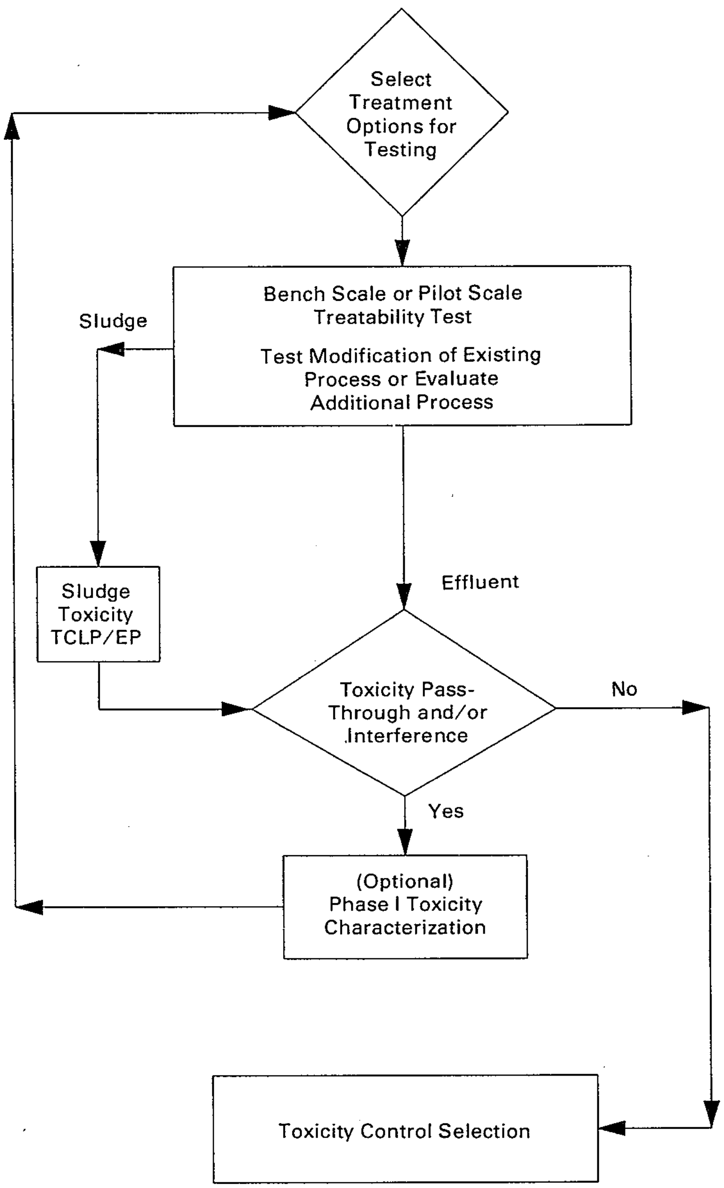

Figure 7-1. POTW in-plant control evaluation.

new process additions and to determine the idle facilities and equipment that could be used for toxicity control. In addition, information on POTW operations and maintenance should be reviewed to determine if the POTW is capable of meeting the increased operational control that may be required with process modifications or additions. This PPE information can be compared to the TIE data to identify potential control options, and to determine how the control options can be integrated into the overall treatment system design.

Secondly, TIE Phase I data on the classes of effluent toxicants can be used to select options to be examined. For example, if non-polar organic compounds are frequently observed to be the principal effluent toxicants, possible options would include granular media filtration, activated carbon adsorption, or coagulation and precipitation. TIE results on specific effluent toxicants, which have been identified and confirmed in Phases II and III, can also be used to determine possible in-plant control options. Although results on specific toxicants are well suited for the application of pretreatment control limitations, the municipality may choose to evaluate in-plant control of these toxicants. An example of this case is the treatment of ammonia by optimizing the POTW activated sludge process (e.g., increase MCRT) to achieve nitrification. 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.

In-plant toxicity control may be achieved by enhancement of the existing treatment system or by the implementation of additional treatment processes. Possible in-plant control alternatives for different categories of toxic compounds are summarized in Table 7-1. A description of these control alternatives is provided as follows.

Process Enhancement

Biological Process Control

Biological process control is generally limited to activated sludge systems, although some modifications to fixed film processes (e.g., trickling filters and rotating biological contactors) may be feasible. The performance of activated sludge systems is generally controlled by adjusting one or more of three process parameters: mean cell residence time, mixed liquor suspended solids and food-to-microorganism ratio. The treatment efficiency of the activated sludge system, and the activated sludge characteristics, are controlled by varying these interrelated process parameters. A description of the use of these parameters for toxics control is provided as follows.

Mean Cell Residence Time --

Removal of biodegradable toxic compounds in activated sludge treatment may be improved by increasing the MCRT (Adams et.al., 1981). MCRT can be increased by lowering the excess sludge wasting rate. Longer MCRTs result in an increased sludge age which can be beneficial for the biodegradation of some types of organic compounds.

Mixed Liquor Suspended Solids --

High MLSS concentrations have been shown to minimize the effects of inhibitory pollutants on activated sludge treatment systems (WPCF, 1976). High MLSS concentrations increase the potential for biodegradation and sorption of toxic wastewater constituents, and can aid in protecting the treatment process from shock loadings.

Food-to-Microorganism Ratio --

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 toxic compounds (Adams et.al., 1981). The F/M ratio is inversely related to MCRT and is an alternative parameter for controlling activated sludge treatment.

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 increase the removal of toxic pollutants. 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 wastestreams in existing POTW treatment processes can be used to improve pollutant removal. Nutrients can be added to influent wastewaters, which have low nutrient levels relative to their carbonaceous content, to improve biological treatment. Lime or caustic 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 toxic pollutants 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 wastewater treatment additives is provided as follows.

Table 7-1. POTW In-Plant Control Technologies for Categories of Toxic Compounds

Biodegradable Organic Compounds and Ammonia	Non-Biodegradable Organic Compounds	Volative Organic Compounds	Heavy Metals and Cationic Compounds
Biological Process Control	Filtration	Biological Process Control	Filtration
Nutrient Addition	Activated Carbon	Aeration	Coagulation/ Precipitation
	Coagulation/ Precipitation		pH Adjustment

Nutrient Addition --

Addition of phosphorus, nitrogen or sulfur may in some cases improve biological treatment of industrial wastewaters with low nutrient concentrations. Improved treatment is attributed to correcting a nutrient deficient condition resulting from a high industrial to domestic wastewater ratio. The optimal $BOD_5/N/P$ ratio for municipal activated sludge treatment is 100:5:1.

pH Adjustment --

Lime and caustic addition can be used to increase influent wastewater pH prior to primary sedimentation to enhance the precipitation of heavy metals. 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 can also be used to provide the alkalinity necessary for efficient biological treatment.

Coagulant Addition --

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

Coagulants can adversely affect the characteristics of sewage sludges and could thereby alter ultimate disposal methods. Coagulants may increase the toxicity of the sludge (as measured by TCLP) as a result of the removal of toxic wastewater constitutents or as a result of the toxicity of the coagulant itself. Thus, coagulants should be carefully evaluated prior to use.

Activated Carbon --

The addition of powdered activated carbon (PAC) to an activated sludge unit can 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 dense nuclei onto which sludge flocs can agglomerate. The PAC process has been used in municipal wastewater treatment; however, recent studies have shown (Deeny et.al., 1988) 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 and thereby improve the stability of the activated sludge system.

Additional Treatment

Where process enhancement is not feasible or will not provide adequate toxics removal, physical addition to or modification of the POTW can be undertaken. Additional treatment processes could include equalization prior to treatment, instrumentation control, and advanced wastewater treatment processes such as coagulation/flocculation, granular media filtration, and granular activated carbon treatment.

Equalization

Equalization can be used prior to biological treatment units 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, and thus 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 controlled by continuously monitoring the pH and conductivity of the influent wastewater. A significant decrease in pH or an increase in conductivity may indicate a slug loading of toxic material (e.g., heavy metals). If this situation occurs, the influent flow can be manually or automatically diverted to a holding basin until the pH and conductivity in the influent return to normal. At that time, the diverted wastewater can be slowly added to the influent wastestream in such a manner that the pollutant concentrations are diluted prior to treatment.

Advanced Wastewater Treatment

POTWs that only utilize primary and secondary wastewater treatment may achieve toxicity or toxics reduction by the addition of advanced wastewater treatment process such as coagulation/flocculation, sedimentation, granular media filtration, and granular activated carbon. Each of these processes can provide enhanced removal of constituents which may be causing effluent toxicity.

Treatability Testing

Bench-scale and pilot-scale treatability tests are commonly used to simulate treatment options selected for wastewater testing. Bench-scale or pilot-scale tests offer several advantages compared with full-scale monitoring, including a more manageable 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 biodegradation kinetics of wastewater treatment.

During treatability testing, influent, effluent and sidestream wastewaters of the treatment process are tested for acute or chronic toxicity using methods described by Mount and Anderson-Carnahan (1988a) and Horning and Weber (1985), respectively. Toxicity testing is used to assess the effectiveness of the treatment process in reducing the wastewater toxicity and to determine the fate of toxicity in the treatment process. Definitive acute or chronic toxicity tests (Peltier and Weber, 1985 and Horning and Weber, 1985, respectively) should be used at the completion of the treatability testing to verify the option's capability to meet the NPDES permit limit.

Activated Sludge

The basic parameters of interest in the design of activated sludge systems include organic loading, oxygen requirements, nutrient requirements, sludge production, and sludge settleability and return rate. Continuous flow systems are most useful for evaluation of activated sludge systems; however, in some cases batch systems may provide sufficient treatability information (Adams et al., 1981).

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 flocculant settling rates for removal of solids and flocculant suspensions (Adams et al., 1981). Results of these tests are used to devise sedimentation treatability tests for evaluating full-scale coagulation/flocculation processes.

Sedimentation

Sedimentation involves removal of suspended solids or flocculant suspensions by gravity settling. Sedimentation is evaluated by conducting a series of settling column tests which measure the settling rates of solids or flocculant suspensions (Adams et al., 1981). Test results are used to calculate a settling profile which 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 operation criteria. Although the process scale is reduced, the bed gradation and thickness should be equivalent to full-scale 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 which are used to evaluate carbon exhaustion rates and the effect of carbon regeneration on toxicity removal performance.

Section 8
Toxicity Control Selection

Introduction

The goal of the TRE is to select and implement toxicity control methods and technologies that will achieve the TRE objective of meeting the permit limits for effluent toxicity. The process of toxicity control selection involves an assessment of the potential control options and the selection of the best option(s) for toxicity reduction based on technical and cost considerations. In the first step of the selection process, information from each stage of the TRE is collected and reviewed to ensure that sufficient data are available. These data should include information on the magnitude and variability of the effluent toxicity and toxicants over time. Following the information review, the data are evaluated to identify feasible toxicity control options. Finally, the feasible options are compared to determine the preferred control option(s).

The choice of in-plant toxicity control or pretreatment toxicity control will depend largely on the technical and economical feasibility of POTW treatment modifications, and the quality of the pretreatment data on IUs that contribute refractory toxicity or toxicants to the POTW. 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 toxics in the IU discharge. If these conditions are satisfied, the municipality can set local limits using the methods outlined in Section 6. In-plant control will be preferred in cases where the implementation of feasible treatment modifications or additions is more cost-effective than pretreatment control. In-plant options provide the POTW a direct method of controlling effluent toxicity; however, in-plant modifications or additions may result in substantial increases in process operation requirements and operating costs.

Evaluation of Control Options

The TRE protocol is designed to identify possible methods for toxicity reduction at the earliest possible stage in the TRE. As shown in Figure 1-1, sufficient information may be available for toxicity control evaluation at several stages in the TRE: at the completion of the PPE conventional pollutant treatability tests, following the TIE, after Tier I chemical-specific testing, at the end of the Tier II pretreatment evaluation, and at the completion of the POTW in-plant control evaluation. The identified control options must be based on ample data that clearly demonstrates the technical feasibility of each option. Toxicity control options that are appropriate for each of these stages are described as follows.

PPE Treatability Tests

Treatability testing in the PPE may identify options for conventional pollutant treatment which also reduce effluent toxicity to acceptable levels. In addition the optional TIE Phase I tests may provide information on the presence of in-plant toxicants such as suspended solids or chlorine which is corroborated in the operations and performance review. The Phase I information can be used to identify 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 dechlorination treatment to eliminate toxic levels of TRC and biological treatment optimization (e.g., increased MCRT) to improve conventional pollutant removal which also reduces effluent toxicity.

TIE Tests

Results of Phase I testing may indicate the classes of compounds causing effluent toxicity (e.g., non-polar organic compounds) which may be amenable to certain types of treatment (e.g., granular media filtration). The feasibility of options for removal of broad classes of toxicants can be evaluated in the POTW in-plant control evaluation (Section 7). Feasible options developed from this evaluation are described in a following subsection.

Alternatively, results of Phases II and III may identify and confirm the specific toxic compounds in the effluent. If the pretreatment program data are adequate to determine the sources of the toxicants, local limits can be developed and evaluated as noted in Section 6. In this case, pretreatment control would be preferred over in-plant control because of the lower costs of implementation. If sufficient pretreatment program data on the toxicants is not available, chemical-specific testing will be necessary to track the sources of the toxicants.

Tier I Chemical-Specific Testing

Chemical-specific tracking in the Tier I evaluation may locate the sources of the POTW effluent toxicants. Once the sources have been identified, local limits options can be developed and evaluated as discussed in Section 6.

Tier II RTA Testing

Results of the Tier II testing are used to identify the IUs contributing refractory toxicity to the POTW. A ranking system indicates the major toxic contributors. Based on these results, the POTW can require the IU to limit the discharge of IU wastewater toxicity even though the toxic constituents of the wastewater have not been identified. In some cases, the municipality may elect to perform optional TIE Phase I analyses in the RTA to provide information on the toxic IU wastewater constituents. This additional testing may be conducted so that the municipality can set numerical limits on a case by case basis.

POTW Treatability Testing

POTW treatability testing may indicate the in-plant treatment options that can be applied to achieve efficient toxicity reduction. These options may include process enhancements such as biological process control and nutrient addition, or additional treatment processes such as equalization, coagulation/flocculation, granular media filtration and granular activated carbon. The treatability data should include information on the variability of toxicity treatment performance and the design criteria for implementing the treatment option.

Selection of Toxicity Control Options

To aid in the final selection of toxicity control alternatives, a list of pertinent selection criteria should be prepared as shown in Table 8-1. Appropriate selection criteria include the ability of each option to reduce effluent toxicity to acceptable levels; the ability to comply with other NPDES permit limits; capital, operational, and maintenance costs; ease of implementation; reliability; and the environmental consequence of the remedy. Each alternative should be rated in terms of these selection criteria and the alternative with the least points based on environmental, technical, and economic criteria should be selected.

Table 8-1. Comparison of Selection Criteria for Toxicity Control Operations*

	Alternative			
Selection Criteria	A	B	C	D
Ability to achieve effluent toxicity limits				
Ability to comply with other permits				
Capital Cost				
Operational Cost				
Maintenance Cost				
Ease of Implementation				
Reliability				
Environmental Impact				

*Rating criteria is 1 to 10, with 10 being the least favorable situation.

Section 9
Toxicity Control Implementation

Once the evaluation and selection of toxicity control options has been completed, the final steps in the TRE are the implementation of the selected source and in-plant control options, and follow up monitoring to ensure permit compliance. The extent of the implementation step will depend on the severity of the effluent toxicity and on the complexity of the selected control approaches. Depending on the findings of the TRE, implementation may be as simple as modification of POTW operating procedures or as complex as expansion of the POTW's Pretreatment Program or the design and construction of 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 detail the results of the TRE and should specify the control options for reducing toxicity to allowable levels. For in-plant control options, the TCIP should provide the basis of design for the selected control options, provide capital and operating costs, and define the time required for design and construction. For source control options, the selected pretreatment approach should be detailed in the TCIP, and should specify the basis of selection and technical justification of the local limits and IU monitoring methods. In addition, the procedure for implementing the revised pretreatment regulations should be defined.

Follow Up Monitoring

Once a control technology has been implemented, a follow up monitoring program should be prepared and implemented to ensure the effectiveness of the selected control options. In most cases, the conditions and frequencies of this program will be set by the regulatory agency. Additional monitoring requirements set by the POTW for monitoring or reporting by the IUs may also be required. This program may include verification of statements from industries that the required reduction of toxicity has been made. Intensive effluent toxicity monitoring should be performed on the POTW effluent to ensure that toxicity has been reduced to acceptable levels and that the TRE objectives have been met. Any specific toxicants that were determined to be present prior to implementation of control technology should be monitored to ensure that there is no excursion from the effluent limitations.

Section 10
Quality Assurance/Quality Control

Introduction

A Quality Assurance/Quality Control (QA/QC) program for the TRE should be developed and implemented to insure the reliability of the collected data. The QA/QC program should address 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 document 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; and
- Corrective action protocols.

Sampling Collection and Preservation

To ensure quality control in sample collection activities, the TRE Sampling Plan (Section 13) 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 EPA's Methods for Chemical Analysis of Water and Waste (USEPA, 1979) and Standard Methods for the Examination of Water and Wastewater (APHA, 1985). Sampling requirements for acute toxicity tests are provided by Peltier and Weber (1985).

It is important to routinely assess the effects of sampling holding times on wastewater toxicity to predict how long samples can be kept before changes in toxicity occur. The TIE Phase I manual (Mount and Anderson-Carnahan, 1988a) describes how testing the sample toxicity on the day of collection and comparing this initial toxicity to its baseline toxicity (tested one day later) can provide information on appropriate sampling holding times for toxicity analysis.

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 the collection of duplicate samples and field blanks. Samples that are to be used for toxicity and chemical analyses require sample containers that are both toxicologically and analytically clean. Toxicity tests are sensitive to even slight sample contamination, thus equipment and containers used for toxicity test samples require special cleaning according to procedures outlined in Peltier and Weber (1985).

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, and name of the person taking the sample. A field book should also 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. This sample custodian should acknowledge receipt of the sample by signing the COC form and noting the date and time of sample receipt, the sample identification number, and the laboratory assession code. An additional notation should be made each time aliquots of the sample are tested for toxicity by noting the analysis date and time, the analyst, and any changes in the nature of the sample toxicity over time. All COC forms should be maintained in a permanent file so that information on specific samples can be easily traced.

Analytical QA/QC

Analytical tests should provide data of an acceptable quality for characterizing wastewater toxicity and for evaluating methods and technologies for toxicity reduction. Several of the test methods described in this document are new and require careful attention to unique QA/QC procedures. The special QA/QC procedures for each TRE analytical 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 (Mount and Anderson-Carnahan, 1988a, 1988b and Mount, 1988). In general strict adherence to standard quality control practices is not required in 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. Still greater attention to quality control should be provided in Phase III. Sample manipulation should be minimized in Phase III to prevent analytical interferences and toxicity artifacts, and 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 sample 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 which generates 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 (Mount and Anderson-Carnahan, 1988a).

Filtration --

High purity water, which has been adjusted to a specified pH, should be used to rinse filters between filtration steps (Mount and Anderson-Carnahan, 1988a). Filtration equipment should be rinsed with 10% HNO_3, acetone and high purity water between sample aliquots. Filter toxicity can be checked by testing filtered dilution water.

pH Adjustments --

Two concerns in the pH adjustment step involve artificial toxicity caused by excessive ion concentrations from the addition of acid and base and silver contamination from some pH probes. The baseline toxicity test acts as a control for indicating whether addition of acid and base increase the wastewater toxicity. Because toxic concentrations of silver can leach from refillable calomel electrodes, only solid state pH probes should be used.

Methanol/C_{18} Column --

HPLC-grade methanol is required for 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 SPE column to check for resin-related toxicity.

Oxidant Reduction --

Thiosulfate used in oxidant reduction tests may be toxic at high concentrations. This potential interference can be checked by adding increasing quantities of thiosulfate to aliquots of the wastewater sample, testing the resulting toxicity, and comparing this toxicity to the sample's baseline toxicity.

EDTA Chelation --

Toxicity caused by the addition of EDTA can be identified by observing increases in toxicity, relative to the baseline toxicity, when increasing amounts of EDTA are added to the wastewater sample.

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 (Mount and Anderson-Carnahan, 1988a). 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 (Peltier and Weber, 1985). These tests should be performed monthly and should coincide with the implementation of TIE tests. If test organism cultures are not maintained in the laboratory, standard reference toxicant tests should be performed with each group of test organisms received. Information on obtaining and culturing species for toxicity testing is provided by Peltier and Weber (1985).

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 as an initial characterization (Phases I and II) or for toxicant confirmation (Phase III). Because much of Phase I and parts of Phase II rely on relative toxicity measurement, water which is of consistent quality and will support growth and reproduction of the test species is suitable for these phases of the TIE (Mount and Anderson-Carnahan, 1988a). The objective of Phase III, however, is to confirm the true cause of toxicity, thus artifacts are to be excluded and the choice of dilution water should follow standard toxicological practices (Peltier and Weber, 1985). In general the physical/chemical characteristics of the dilution water should reflect those of the receiving water. Synthetic freshwater can be used for TIE tests; however, laboratory deionized water should not be used, because it may lack essential minerals such as calcium and magnesium and may introduce toxic levels of other cations.

Most species used in standard acute toxicity testing do not require feeding during the test. The Phase I manual (Mount and Anderson-Carnahan, 1988a), however, recommends feeding the organisms in the TIE test solutions at the beginning of TIE toxicity tests. Feeding requirements for selected species are described by Peltier and Weber (1985).

Dissolved oxygen measurements are generally not made during TIE toxicity tests because the exposure chambers have a surface to volume ratio that is large enough for adequate oxygen diffusion. In cases where low DO is a problem, DO adjustment should be performed at a rate that will not unintentionally 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. In performing toxicity tests for 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 expressed above for TIE tests. Selection and use of test species and dilution water should follow procedures addressed by Mount and Anderson-Carnahan (1988a).

Potential sources of toxicity contamination should be identified through the use of system blanks. As with TIE testing, the filters used in RTA testing can be tested to determine if toxicity is added during filtration. Each of the solutions used in RTA testing including synthetic wastewater and activated sludge should be checked for toxicity. In the Patapsco TRE, the return activated sludge used in the RTA batch tests was found to be acutely toxic to *Ceriodaphnia*. Similarly, the reagents used in treatability testing such as chemical coagulants should be screened for toxicity.

To ensure precise and accurate results, 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 method.

Chemical Analyses

Quality control for chemical analyses includes the use of calibration standards, replicate analyses, spiked sample analyses, synthetic unknown analyses, and performance standards. The detection limits and the recommended reagents for method calibration and spiking are discussed in EPA's Methods for Chemical Analysis of Water and Wastes (1979) and Standard Methods for Examination of Water and Wastewater (1985). General information on laboratory quality control for chemical analyses is provided in EPA's Handbook for Analytical Quality Control in Water and Wastewater Laboratories (1972).

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. A maintenance log book should be used 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 which 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 TIE toxicity tests for the first time should be soaked for three days in 10% nitric acid (Mount and Anderson-Carnahan, 1988a).

For subsequent use in TIE tests, the glassware should be washed with detergent, and sequentially rinsed with 10% HNO_3, acetone and finally high purity water.

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 pollution control agencies.

Corrective Action

Procedures should be established to ensure that QA/QC problems such as improper sampling techniques, inadequate chain-of-custody 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 11
Health and Safety

Overview

A health and safety (H&S) plan may be necessary in performing TREs in order 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 health and safety 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 specific conditions under which the TRE is being conducted. For example, if the POTW operates under a RCRA permit by rule, then health and safety must be addressed when collecting and analyzing hazardous wastes.

Important considerations for health and safety for TRE studies include:

- Identification of personnel responsible for health and safety matters;
- Health and safety training activities;
- Protective equipment required for TRE activities;
- Materials cleanup and disposal procedures; and
- Emergency response contingencies.

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

Sample Collection and Handling

Working with wastestreams 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, mutagens and teratogens) 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. Upon sample storage, the sample custodian should indicate the health and safety considerations for handling and disposal of the sample.

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, pipetting aids, 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 is also 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 potentially contain hazardous materials should be decontaminated and/or disposed of in accordance with hazardous waste regulations (NIOSH, 1977).

Analytical Methods

Specific precautions that should be followed for selected TRE analytical techniques are described below.

Toxicity Identification Procedures (TIE)

EPA's Phase I manual (Mount and Anderson-Carnahan, 1988a) addresses the general health and safety concerns involved in performing the Phase I-III analyses. Ventilation is a specific concern when performing the Phase I air-stripping tests. These tests should be performed in laboratory hoods to prevent the inhalation of toxic volatile compounds resulting from air-stripping.

Health and safety considerations for aquatic toxicity testing are addressed by Peltier and Weber (1985). 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 is also important when conducting refractory toxicity assessments 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.

Physico-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

A number of 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 (Miller, 1985). After use, the waste chemicals should be converted into a less hazardous form in the laboratory before disposal (NRC, 1983) or disposed of by a commercial disposal specialist.

General Precautions

Additional laboratory safety procedures (USEPA, 1977 and ACS, 1979) that should be followed in TRE studies include: 1) the 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); 2) protocols for emergency response and materials cleanup; and 3) personnel training in health and safety procedures.

Section 12
Facilities and Equipment

Introduction

Laboratories used for a TRE study 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 site-specific factors involved in the TRE. In general, the minimum facilities and equipment for initiating a TRE will include the equipment used in the TIE Phase I characterization tests. As additional information becomes necessary, the facility and equipment needs will be more site-specific and will depend both on the physical/ chemical characteristics of the causative toxicants and on 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 (i.e., physical/chemical processes such as filtration or biological processes such as increased SRT control).

The choice of whether to work on-site or off-site will depend on the stage of the TRE, the methods used for tracing toxicity to its sources, 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 less cost than on-site testing. If toxicity tracking using RTA tests is required, on-site testing is mandatory, because fresh samples of the POTW acclimated biomass must be used. In addition, treatability tests which require continuous supplies of POTW influent or process wastewaters and/or activated sludge (i.e., flow-through bioreactor tests) can 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 the manufacturing facility.

The general equipment requirements for each of the main TRE methods are summarized below.

Toxicity Identification Evaluations

Laboratories should be stocked with all of the equipment necessary to conduct Phase I characterization tests including filtration and air stripping equipment, pH meter, C_{18} solid phase extraction columns, fluid metering pumps, and the required reagents. Because of the large number of toxicity tests to be performed for Phase I testing, it may be more cost-effective to culture the test species than purchase them. Equipment needs for culturing standard test species are described by Peltier and Weber (1985).

More sophisticated analytical equipment is required for the Phase II causative toxicant identification and Phase III causative toxicant confirmation procedures. The choice of analytical instruments for these procedures will depend on the compound to be measured. Equipment that may be required includes: gas chromatograph-mass spectrometer (GS/MS), high pressure liquid chromatograph (HPLC), atomic absorption spectrometer (AA), inductively coupled plasma spectrometer (ICP), UV-VIS spectrophotometer, ion chromatograph, ion specific electrodes, pH meter, conductivity meter and salinometer. Use of inert materials such as perfluorocarbon plastics for Phase II and III are recommended to protect against toxicity artifacts (Mount and Anderson-Carnahan, 1988a).

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, air diffusers, and a laboratory hood. Instruments for measuring inhibition include respirometer and/or oxygen meters, total organic carbon (TOC) analyzer, spectrophotometers for COD and nutrient analyses, drying oven, muffle furnace, and analytical balance. 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 purchase them. In some cases it may be necessary to use a rapid screening test such as a bacterial bioluminescence test (e.g., Microtox TM).

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.

Section 13
Sample Collection and Handling

Introduction

The most important criterion in sampling is to obtain a sample that is representative of the discharge. To ensure that a sample represents the typical toxicological and chemical quality of the wastewater, several samples will need to be collected. Guidelines for determining the number and frequency of samples required for characterization are presented in EPA's Handbook for Sampling and Sample Preservation of Water and Wastewater (Berg, 1982). This handbook should only be used as a guide, however, because it does not specifically address the requirements for TRE sampling.

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

- Description of sampling locations;
- Sampling equipment and methodology; and
- Sample delivery requirements.

These elements are discussed in the following subsections. QA/QC procedures for sampling, which include identifying the minimum volume requirements, holding times and preservation techniques for samples, preparing chain-of-custody forms, and maintaining sampling equipment, are addressed in Section 10.

Sampling Location

Sampling locations should be established where the wastestream is readily accessible and well-mixed. When sampling wastestreams within the POTW, care should be taken in excluding unwanted wastestreams and in selecting 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 sampling point stated in the NPDES permit for biomonitoring. 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. Sampling before and after chlorination/dechlorination may be needed to differentiate between toxicity caused by pass-through and toxicity resulting from chlorination (i.e., TRC).

Wastewater sampling for toxicity source evaluations requires knowledge of sewer discharge locations. In some cases IUs may have multiple sewer discharges which need to be accounted for. Sampling may be conducted at the point of sewer discharge or in areas within the municipal sewer collection system. The choice of sampling locations for sewer line tracking will be based on existing pretreatment program data indicating the probable toxic sewer lines within the collection system. If these data are not available, a sampling scheme can be devised to locate toxic sources through the process of elimination of segments of the collection system.

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 return activated sludge pipelines.

POTW Sampling

The choice of grab or composite samples of POTW wastestreams (i.e., effluent and influent wastewater and process wastestreams) will depend on the physical/chemical characteristics and variability of the toxicants. Initial effluent toxicity characterization (Phase I) should utilize 24-hour composite samples in order to ascertain the variability of the causative agents over time. If acute 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 are recommended to determine the variability in the type and concentration of effluent toxicants (Mount and Anderson-Carnahan, 1988a). A discussion of the use of grab versus composite sampling for toxicity tests is provided by Peltier and Weber (1985). The choice of a sampling technique 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 detention time of the treatment process in order to get comparable samples. In addition, samples should be collected during representative discharge periods. An evaluation of the condition of the facility's treatment system at the time of sampling can be made by comparing the effluent sample concentrations of BOD, TSS, and other pollutants to long-term historical averages and/or permitted values for those parameters. Such a comparison will provide an indication of the operational status of the treatment system on the day of sampling.

The sample volume requirements for Phase I tests are provided in the Phase I manual (Mount and Anderson-Carnahan, 1988a). Volume requirements for POTW samples used in RTA tests are given in Sections 5 and 6.

If TIE or physical/chemical treatability testing is being conducted off-site, samples should be shipped on ice to the testing facility. RTA and some biological treatability tests require fresh or continuous samples of POTW wastestreams, which requires testing to be conducted on-site. Samples of 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

As with POTW 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 will also 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 the Tier II evaluation 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 14

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Appendix A
Case Histories

A. Baltimore, Maryland

In January 1986 the EPA, in cooperation with the City of Baltimore, began a research study to develop a pragmatic approach and methods for conducting TREs at municipal wastewater treatment plants (Botts et al., 1987). The City's Patapsco Wastewater Treatment Plant (WWTP) was selected for this study because of substantive influent toxicity and history of intermittent pass-through of toxicity to receiving waters. In addition, EPA was interested in conducting a TRE at an urban wastewater treatment plant, like the Patapsco WWTP, which receives its influent from a wide range of industrial discharges. The objectives of the TRE were to characterize the WWTP's capability for treatment of conventional pollutants and toxicity, evaluate techniques to identify the specific components of the toxicity, and assess methods to trace toxicity to its source(s).

The study results demonstrated that the WWTP influent had significant acute and chronic toxicity and substantial amounts of this toxicity remained following secondary treatment even though the WWTP achieved consistent conventional pollutant removal. An evaluation of the WWTP operations indicated that the treatment performance was not the major cause of the effluent toxicity. *Ceriodaphnia dubia* was a more sensitive indicator of acute effluent toxicity than *Mysidopsis bahia* or Microtox™.

A toxicity identification evaluation identified non-polar organic compound(s) as the main cause(s) of effluent toxicity; however, GC/MS analysis of the non-polar organic fractions of the wastewater did not lead to definitive determination of the specific non-polar compounds. The TIE results did show, however, that the non-polar organic compounds have high octanol to water partition coefficients. The compounds sorbed onto solids in the plant effluent. Further testing found that solids (>0.2 μm) were the major toxic fraction.

An evaluation of toxic industrial wastewater samples from selected candidate industries was performed to determine the major contributors of refractory toxicity to the WWTP. The results of this evaluation were used to rank contributors with respect to their refractory toxicity loading.

B. Akron, Ohio

A survey of six Ohio municipal wastewater treatment plants was conducted to determine the level of wastewater toxicity reduction that occurs in municipal treatment plants (Neiheisel et.al., 1988). Of the six WWTPs, the City of Akron wastewater treatment plant (Botzum WWTP) received the most toxic influent wastewater. Although the Botzum WWTP achieved significant toxicity reduction, the effluent discharge comprised a large proportion of the Cuyahoga River flow. A biological impact assessment study of the Cuyahoga River in 1984 revealed a severe impact on aquatic communities downstream of the plant discharge. A preliminary review of the plant's operating records also revealed intermittent bypassing of raw wastewater during storm events.

On the basis of the preliminary survey results, the Botzum WWTP was selected as a site for a toxicity reduction evaluation. The TRE involved conducting toxicity tests of the plant discharge, including the bypass streams, to characterize the variability and source(s) of toxicity that may have an effect on the river water quality. In addition, an attempt was made to identify the compounds causing the toxicity through physico-chemical fractionation and toxicological examination (TIE) of the effluent.

The TIE testing revealed that the toxicity was largely in the eighty-five percent methanol eluate of the SPE column, which indicates that the toxicant(s) is a non-polar organic compound(s). Metals were also identified as possible effluent toxicants. Results of acute toxicity tests of the effluent and the CSO indicated that although bypassed wastewater may contribute intermittently to the poor river quality, the continuous discharge of toxic materials in the effluent was probably the major cause of the observed impact.

Continued toxicity monitoring indicated that the acute effluent toxicity ended abruptly in the summer of 1986. The cause of this abatement is not known, but may have been related to one or more of the following events: an increase in the concentration of activated sludge solids in the plant bioreactors; cessation of discharge by a large chemical manufacturing plant; reduction in the frequency of wastewater bypassing; or the cumulative effects of plant process and pretreatment program improvements. Biosurveys of the Cuyahoga River in 1986, however, continued to show poor water quality despite the decrease in effluent toxicity. It is possible that either not all of the toxic wastewater sources were identified, or the recovery rate of the river is slower than anticipated.

C. Billerica, Massachusetts

A toxicity source evaluation study was conducted at the Billerica WWTP to evaluate the usefulness of the Microtox toxicity test in tracing the source(s) of toxicity in the WWTP's collection system (Durkin et.al., 1987). Billerica WWTP was selected for this study because its influent was found to be toxic as measured by Microtox™.

The Billerica study was conducted in five stages: first, an initial screening of the WWTP influent was conducted; next, toxicity tests were performed on samples from pumping stations in the sewer collection area; the time of day when toxicity was found in the most toxic pump station sewers was determined; toxicity screening was performed on the main sewer lines above the toxic pump stations; and finally, testing of the tributaries to the main sewer lines was initiated.

Of the eleven pump station sewers tested, two were found to have very toxic wastewaters. In one of the two toxic pump station sewers, high toxicity levels occurred only during a daily 8:00 am to 2:00 pm time period. Further investigation of this pump station sewer isolated the principal source(s) of toxicity to an industrial park.

This study was successful in screening for possible sewer collection areas contributing toxicity to the WWTP. This screening method appears to be a useful initial technique for tracing WWTP influent toxicity. However, a final determination of the sources of toxicity that are refractory to treatment provided by the municipal WWTP would require treating the sewer samples in a simulation of the WWTP prior to toxicity analysis.

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Appendix B
Pretreatment Program Review

Introduction

The objective of the pretreatment program review (PPR) is to gather manufacturing, pretreatment, and discharge data on the industrial dischargers to the municipal treatment plant (Table 2-2). These data together with the results of POTW effluent toxicity tests can be used to identify possible sources of toxicity and provide insight as to probable control technology options. The pretreatment program information should include flow and chemical monitoring data on the IUs, descriptions and schedules of industrial production campaigns, and inventories of chemicals used in production. The final outcome of this review should be an improved understanding of the industries' processes and chemical usage, and the possible identification of sources of toxicity. Source identification through the pretreatment program review has been successful in reducing effluent toxicity at POTWs with a limited number and type of industrial inputs (Diehl and Moore, 1987).

General Procedure

The main steps in a PPR are to: 1) gather the pertinent data; 2) compare the data to POTW effluent toxicity results and/or TIE data; 3) identify potential influent source(s) of toxicity; and 4) evaluate and recommend a toxicity control option(s). A brief description of each of these steps is as follows.

Collect Data on Individual Dischargers to POTW

Data on all categorical, significant non-categorical and other potential toxic dischargers (e.g., IUs with local limits, and RCRA and CERCLA inputs) should be collected. A list of pertinent information that should be considered in a PPR is presented in Table 2-2. The data collection effort should include a survey of each IU, using the example checklist shown in Table B-1.

Information on chemicals that may be used in manufacturing processes can be obtained from the Encyclopedia of Chemical Technology (Kirk-Othmer). Although OSHA regulations require that information on hazardous chemicals is to be made available to the public on Material Safety Data Sheets (MSDS), information on various "specialty" chemicals can be difficult to obtain. When data on a "specialty" chemical are not disclosed, a literature review can be performed to determine the chemical's acute toxicity and biodegradability. This information allows assumptions to be made concerning the biodegradability of the chemical at the POTW and the potential for the chemical to cause effluent toxicity. An initial indication of the possible toxics causing effluent toxicity can be made by comparing expected effluent toxics concentrations to water quality criteria or toxicity values provided in the literature.

Compare PPR Data to POTW Effluent Toxicity Results

Information on the magnitude, variability, and nature of the POTW effluent toxicity can be compared with the PPR data to determine the sources(s) of possible problem chemicals. This comparison can be made using statistical analyses to determine if the variability in the source characteristics can be related to the variability in the POTW effluent toxicity. A description of data analysis techniques for identification of potential sources of toxicity follows.

Data Analysis Techniques for Comparing POTW and Industry Pretreatment Data

Two types of statistical analyses can be used to compare the pretreatment program and POTW effluent toxicity data: linear regression (Draper and Smith, 1966) and cluster analysis (Pielou, 1984 and Romesburg, 1984). Linear regression analysis is used to find correlations among the variables in the database and to relate changes in POTW effluent toxicity to the variables. A cluster analysis using pattern recognition software can weigh and evaluate the significance of toxics/toxicity correlations. The determination of concentration/response relationships through statistical analysis should not be considered as a definitive answer to toxicity tracking because of the complexity of the factors contributing to toxicity in POTW effluents.

The following example illustrates how a stepwise linear regression technique can be used in PPR assessment. The technique is used to identify how changes in several variables can impact the presence and variability of effluent toxicity. Table B-2 presents

Table B-1. PPR Data Sheet

1. Industry Name
   Notes:

2. Address
   Notes:

3. Industrial Category (SIC Code)
   Notes:

4. TRE Objectives
   Notes:

5. Manufactured Products
   Notes:

6. Chemicals Used
   Notes:

   a. Amounts (write on MSDS)
      Notes:

   b. MSDS ________ All Attached ________ Part. Available

   c. Process in which chemical is used (write on each MSDS)
      Notes:

   d. Aquatic toxicity/bio degradability information on all chemical used. Review MSDS, supplier information and literature ________ None ________ Some
      Notes:

7. Engineering drawings of facility
   Notes:

   a. Production flowchart and line schematic ________ Available ________ No
      Notes:

   b. All floor and process drains with schematic ________ Available ________ No
      Notes:

   c. Wastewater pretreatment system schematic ________ Available ________ No
      Notes:

Table B-1. Continued

8. Facility Records
   Notes:

   a. Water usage, water bills ______ Available ______ No
      Notes:

   b. Discharge monitoring reports for 24 months ______ Available ______ No
      Notes:

   c. Pretreatment system operations data ______ Available ______ No
      Notes:

   d. Pretreatment system operator interview ______ Available ______ No
      Notes:

   e. Spill prevention control plan ______ Available ______ No
      Notes:

   f. RCRA reports, hazardous waste manifests ______ Available ______ No
      Notes:

Table B-2. Data Sheet for Regression Analysis

Month	1 LBS	2 INFLOW	3 OFLOW	4 COD	5 BOD_5	6 CU	7 CR	8 ZN	9 LC_{50}
JAN	0.80	1.2	1.0	30	10	0.73	0.02	1.6	20
FEB	1.01	1.5	1.2	33	11	0.61	0.02	1.9	20
MAR	1.20	1.7	1.4	41	15	0.78	0.02	2.0	18
APR	1.25	1.7	1.5	39	14	0.65	0.02	1.6	18
MAY	1.16	1.6	1.4	30	12	0.66	0.02	1.5	22
JUN	0.90	1.2	1.0	28	11	0.68	0.02	1.4	30
JUL	0.90	1.2	0.9	25	10	0.71	0.02	1.8	40
AUG	1.20	1.6	1.4	23	9	0.72	0.02	1.9	38
SEP	1.30	1.8	1.6	25	15	0.69	0.02	2.0	40
OCT	1.27	1.7	1.4	26	18	0.72	0.02	2.1	33
NOV	1.10	1.6	1.4	30	17	0.71	0.02	1.9	28
DEC	0.90	1.2	1.0	40	21	0.75	0.02	2.0	22

an example data sheet for a POTW serving one manufacturing plant. In this example, only a few POTW effluent industry variables were used in the linear regression analysis; however, additional variables could also be added in the regression analysis.

The following variables are the "X" variables:

Industry variables:

LBS	=	Manufactured product per month (millions of pounds)
INFLOW	=	Discharge flow based on water usage (mgd)

POTW effluent variables:

OFLOW	=	Recorded effluent flow (mgd)
COD	=	Chemical Oxygen Demand (mg/l)
BOD_5	=	Biochemical Oxygen Demand (mg/l)
CU	=	Copper (mg/l)
CR	=	Chromium (mg/l)
ZN	=	Zinc (mg/l)

The following variable is the "Y" variable:

LC_{50} = Acute LC_{50} as % effluent

By applying standard stepwise linear regression, the variables OFLOW, BOD_5, CR and CU were eliminated because they were insignificant to toxicity. Stepwise linear regression showed that the remaining (X) variables were significant as regressed versus (Y) LC_{50}. This analysis indicated that ZN, COD, LBS, and INFLOW were correlated with POTW effluent toxicity.

Identify Source(s) of Toxicity

Based on the data analysis, a list can be developed of the possible contributors to effluent toxicity at the POTW. Of the potential toxicity control options, toxic chemical substitution or elimination is usually the most pragmatic approach. Thus, a followup interview with the toxic discharger(s) should be conducted to develop information concerning techniques for the preferred use of problem chemicals. A list of useful interview questions is shown in Table B-3. These questions may enable the industry to identify problem areas and possible corrective actions in the use of toxic chemicals in manufacturing.

Recommend Toxicity Control Option(s)

Based on the results, it may be possible to recommend several conceptual approaches to controlling toxicity. Toxicity control may be practiced at the industrial facility or at the POTW. Source control may include substitution or elimination of problem chemicals, flow reduction, equalization, spill control, and manufacturing process changes. If modifications in the POTW are recommended, treatability studies will be required.

Table B-3. Summary of the PPR Chemical Optimization Procedure

I. Objectives

A. Optimize chemical usage amounts in production and water treatment processes.

B. Optimize chemical structures in process chemicals insuring biodegradability or detoxification is possible.

C. Establish process controls over incoming raw materials, measuring possible toxic components. Example, corrosion-resistant finish put on steel by manufacturer which must be removed prior to part fabrication.

II. Strategy

A. Determine what the role of each chemical is in the process. This is done by supplier interviews and review of data gathered during the initial survey. Ask the questions:

Can less of this chemical be used?

Has the optimum amount been determined for each process?

Do other suppliers offer compounds that will perform as well at lesser concentrations?

Is the compound in reality a part of the manufacturer's water treatment system and independent of product production?

OBJECTIVE: Use less chemicals per pound of product produced.

(continued)

Table B-3. Continued

B. Discover the biodegradability and toxicity of the process chemical. This is done by supplier interview, review of MSDS information, and literature search. Suppliers may not want to supply exact chemical formulations. In this case, ask industry to request supplier to perform tests to develop needed data. Questions to ask:

What are the components in the product?

What is its aquatic toxicity?

Is the product biodegradable?

Are there other component chemicals on the market that meet manufacturing requirements, but are low in toxicity and highly biodegradable?

OBJECTIVE: Use chemicals that will not create toxicity problems.

C. Establish process controls over incoming raw materials. Many raw materials have chemicals used in their manufacturing which are removed in the production of the final product. Many raw materials may have trace contaminants which may cause toxic problems. Questions to ask:

What chemicals are used in the manufacturing of the raw material?

What are the residual amounts of these raw material contaminants or by-products?

Are there quality control procedures that measure the amounts of these chemicals?

What are the statistical process measures used in the monitoring of these chemicals in the raw materials?

If these chemicals are required to be removed before the raw materials can be used in manufacturing the final product what purpose do the chemicals serve in raw material manufacturing?

Can they be eliminated?

Can they be made less toxic or more biodegradable?

OBJECTIVE: Understand all raw materials being used and encourage development of QA procedures to monitor toxic chemicals removed during processing.

III. Outcome of Investigations

A. A list of all chemicals used in processing and manufacturing of products. Included will be the amounts used, why the chemicals are used, and if optimization has been taken.

B. MSDS sheets for all chemicals used will be on file

C. A list of chemicals applied or used in the manufacturing of all raw materials will be on file under that raw material with the residual amounts noted if possible.

D. A list of all chemicals and raw materials purchased on a monthly basis and the amount of product produced.

OBJECTIVE: Hard information to be used in data analysis.

IV. Use of opportunities available due to past experience

A. With experience in various industries, certain chemicals will become "known" as typically used in some process of manufacturing.

B. These known compounds can be categorized and toxicity determinations made. Once found toxic, the first information the industry must supply to the municipality conducting the TRE is whether or not these chemicals are used in its manufacturing process, in raw materials, or in water treatment processes.

C. Letters also are sent to raw material suppliers asking if these compounds are used in raw material production. If they are, the supplier is asked to submit prototype alternative raw materials that do not contain these compounds.

D. This can be done at the beginning of the TRE as past experience identifies the "typical" chemicals. Indeed control regulations also usually involve establishing limits for selected known toxics in industrial operations.

E. What is accomplished by this process can be remarkable. First, the supplier is alerted that these compounds can cause his customers problems. This makes him search for an alternative raw material source that is free of these objectionable chemicals. A successful market search reduces the market demand for contaminated or objectionable raw material. Example: Total brominated toxic organics (TBTO) limits in North Carolina have eliminated TBTO biocides from being applied to hosiery.

(continued)

Table B-3. Continued

V. Tests to help assess toxicity/biodegradability on specialty formulated chemicals and mixtures and to help evaluate competitive products.

A. BOD_5, BOD_{20}

B. BOD_5, BOD_{20} performed at LC_{50} concentration with ET_{50} or LC_{50} concentration with ET_{50} or LC_{50} performed on settled effluent from test.

C. COD before and after BOD_{50} and BOD_{20} at LC_{50}, ET_{50} concentrations.

D. Estimate biodegradability by using BOD_5 and COD tests and the calculation (BOD_5 - COD)/COD x 100 of a 10 or 20 mg/l solutions of chemical. This can be repeated at a 20-day BOD.

E. Biomass Inhibition tests (detailed procedures in Section 6).

F. LC_{50} on products. Screening dilutions 1-10,000 ppm.

OBJECTIVE: Help industry determine relative biodegradability and toxicity of various raw materials, products, and by-products.

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