

CHAPTER 2.2 - Toxicity Reduction Evaluations

This chapter provides guidance to aid dischargers and their consultants when conducting a TRE. Included is a description of the basic steps usually included in a TRE and the reports usually submitted to the Department.

NOTICE: This document is intended solely as guidance, and does not contain any mandatory requirements except where requirements found in statute or administrative rule are referenced. This guidance does not establish or affect legal rights or obligations, and is not finally determinative of any of the issues addressed. This guidance does not create any rights enforceable by any party in litigation with the State of Wisconsin or the Department of Natural Resources. Any regulatory decisions made by the Department of Natural Resources in any matter addressed by this guidance will be made by applying the governing statutes and administrative rules to the relevant facts.

[Additional TRE/TIE Guidance](#)

The following documents provide guidance on designing and conducting TREs and provide case studies illustrating approaches used successfully by others. They do a good job of describing steps that should be considered when doing a TRE, provide more detail than given here, and are non-technical and easy to understand. These documents are recommended supplemental reading for those doing a TRE on complex wastewaters.

SETAC Publications (available at: <http://www.setac.org> (store) or at www.amazon.com)

- *Toxicity Reduction and Toxicity Identification Evaluations for Effluents, Ambient Waters, and Other Aqueous Media* (2005) Almost 25 years after the WET program was initiated, SETAC invited participants representing universities; government, research, and regulatory agencies; mining and chemical industries; and consulting services to a Pellston Workshop on TRE/TIE to update and advance the understanding of the TRE process and the science of TIEs. What resulted was this comprehensive guide to TRE/TIE, detailing procedures and including more than 30 case studies describing various aspects of the process. 978-1-880611-64-7 , 496 pp. (Order #: SB02-18)
- *Whole Effluent Toxicity CD-ROM* (2002) Offers more than 80 easily searchable state and federal manuals, sources of technical information and guidance on whole effluent toxicity (WET) testing and methods, toxicity reduction, and implementation of aquatic toxicity regulations. Representative of the current status and historical contribution of WET. 978-1-880611-72-2 (Order #: SB02-30)

USEPA Guidance (available at: http://cfpub.epa.gov/npdes/docs.cfm?program_id=2&view=allprog&sort=name)

- *Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures, 2nd Ed.* (1991) Methods to characterize the chemical/physical nature of constituents which cause toxicity. 1 of 3. (EPA-600-R-91-003)
- *Toxicity Identification Evaluation: Characterization of Chronically Toxic Effluents, Phase I* (1992) Methods to characterize the chemical/physical nature of the constituents which chronic toxicity. (EPA 600-6-91-005F).
- *Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity* (1993) Guidance on how to identify the cause of toxicity. 2 of 3. (EPA-600-R-92-080)
- *Methods for Aquatic Toxicity Identification Evaluations: Phase III Toxicity Confirmation Procedures for Samples Exhibiting Acute and Chronic Toxicity* (1993) Guidance on how to confirm the cause of toxicity. 3 of 3. (EPA-600-R-92-081)
- *Toxicity Reduction Evaluation Guidance for Municipal Wastewater Treatment Plants* (1999) Guidance document to

General Discussion - What Is A TRE?

The terms “toxicity reduction evaluation” (TRE) and “toxicity identification evaluation” (TIE) are often used interchangeably, separately, or together (as in "TI/RE"). A TRE is the complete evaluation intended to determine the actions needed to remove toxicity. A TIE is one step in the TRE where effluent samples are taken into a lab and manipulated in various ways in order to identify the chemical(s) causing toxicity. A TRE usually includes steps to identify the source(s) of toxicity and steps designed to identify ways to reduce toxicity. It may identify a simple solution such as improved housekeeping procedures or require a more extensive investigation to identify cost-effective treatment or source reduction options.

At the beginning of a TRE, the review of effluent data and facility-specific information is important in order to define study objectives, identify what is known, and provide clues as to the cause of toxicity. Included should be a review of facility housekeeping practices, treatment plant operation, and the selection and use of process and treatment chemicals, in an attempt to identify/reduce potential sources of toxicity. If none of these practices is identified as the source of toxicity, a TIE is usually the next step. The objective of a TIE is to characterize and identify the chemical(s) causing toxicity so that they can be traced back to their source. Once the chemical compound and its source are identified, the TRE process usually goes in one of two directions. One approach is to evaluate options for treating the final effluent, the other is to remove/reduce the source of toxicity through upstream pretreatment, source reduction, or process modifications. A decision can be made to pursue both approaches, and then to select the most technically and economically attractive option.

Flexibility in the design of a TRE is essential. Each successive step in a TRE can hinge on the results of previous steps. Since every discharge and toxicity situation is different, the approach needed to investigate potential sources is usually different, too. However, there are some investigative techniques and approaches that are common to many TREs. The guidance provided here is intended to describe general approaches which have been used successfully in the past by Wisconsin permittees.

Communication and cooperation between the permittee, their consultant, and the Department is essential in TRE plan development, review, and implementation and will help ensure achievement of the TRE objective. No better way exists to establish this communication than face to face meetings between the permittee, Department staff, and preferably, the consultant hired to perform the TRE. Routine meetings and interim reports during the course of the TRE are beneficial to assess progress and steer future activities. This type of communication insures that there are no surprises to any of the participants and maximizes the efficiency of TRE processes.

Finding A Qualified TRE Consultant. Probably the most important beginning step in any TRE is the identification of a qualified consultant that can help coordinate the toxicity investigations. Since most permittees do not have experience with these types of investigations, they must rely on their consultant to help steer them through the process. Permittees sometimes rely on the lab that conducted previous WET tests to conduct the TRE, which may not be appropriate in all cases. TRE work can be much different than normal WET testing, and not all labs and consultants have similar levels of experience doing successful TRE work.

The selection of a consultant requires careful review and evaluation. Potential consultants should be asked to provide references, with special emphasis given to the company’s knowledge, experience and successful resolution of TREs in general, and specifically with the same type of facility and testing requirements. Relevant TRE expertise far outweighs the selection of the lowest bidder because the latter often lack staff experience and do not have a track record of solving similar toxicity reduction challenges. When properly written, requests for bids that include specific capabilities and experience can avoid problems when awarding contracts.

When selecting a consultant to address toxicity problems, the chief criterion for that choice should be a history of success with TREs. A simple ability to conduct select TIE procedures alone does not constitute this experience. A consultant should demonstrate the capacity to put together a multifaceted team of individuals including toxicologists and chemists, and possibly engineers and other professions pertinent to the client's situation so that the results of TIE and TRE activities can be effectively funneled from one expertise to the next.

It is crucial to the success of a TRE that the primary investigator has experience conducting successful TREs. The quality of the work being done is entirely dependent on the experience and knowledge that the lab or consulting firm can bring to the project. A good TRE consultant, whether part of the WET lab's staff or a separate individual, must be a good "detective". He/she should tour the facility, understand treatment plant operations and contributing wastestreams, review process and WET records, and become familiar with staff and operations at the facility. **If you do not get this type of individual attention from the consultant you have hired to coordinate your TRE, then you may not be getting your money's worth.** The best way to choose a lab that is up to the challenge is to talk to peers who have performed TREs, in order to determine which consultants have been the most successful at identifying toxicity sources. You should try to identify labs that have had success identifying the cause of toxicity during these studies, and separate those from investigations that were long and costly without identifying a cause.

TIE costs vary greatly, depending on the amount and type of work needed. Permittees should not limit their search to local labs, since lab work is usually much more expensive than shipping costs. The Department cannot officially endorse or recommend labs or consultants, but can identify those that have performed TREs successfully in the past (see <http://dnr.wi.gov/topic/wastewater/WETlabTRE.html>).

WET Requirements Driven by Limited Data or Intermittent Toxicity. Federal and state regulations sometimes require that a WET limit be given when a small number of WET failures have occurred, even if toxicity has not occurred in the effluent for some time or if toxicity is not always present in the effluent. Although past WET failures suggest that there is reasonable potential for receiving water impacts from the effluent due to toxicity, which requires the imposition of a WET limit, TREs are more difficult to perform when toxicity is not present consistently. In these situations, a successful TRE may include WET monitoring that shows that toxicity in past tests is no longer present (even if the reason for the disappearance is unknown). If no toxicity occurs during monitoring performed during the TRE period, it will not be possible for past sources of toxicity to be identified. If monitoring done during the permit term (during the TRE and after) shows that toxicity is no longer present in the discharge, it may be possible to argue that older WET failures are no longer representative of the discharge (which may mean the limit is not needed in the next reissuance).

When Is A TRE Necessary?

The Department strongly encourages permittees to begin a TRE as soon as their effluent has shown severe or repeated bouts of toxicity. The following is standard language that is included in permits with WET monitoring:

Whole Effluent Toxicity (WET) Identification and Reduction

Within 60 days of a retest which showed positive results, the permittee shall submit a written report to the Biomonitoring Coordinator, Bureau of Watershed Management, 101 S. Webster St., PO Box 7921, Madison, WI 53707-7921, which details the following:

- A description of actions the permittee has taken or will take to remove toxicity and to prevent the recurrence of toxicity;

- A description of toxicity reduction evaluation (TRE) investigations that have been or will be done to identify potential sources of toxicity, including some or all of the following actions:
 - Evaluate the performance of the treatment system to identify deficiencies contributing to effluent toxicity (e.g., operational problems, chemical additives, incomplete treatment)
 - Identify the compound(s) causing toxicity
 - Trace the compound(s) causing toxicity to their sources (e.g., industrial, commercial, domestic)
 - Evaluate, select, and implement methods or technologies to control effluent toxicity (e.g., in-plant or pretreatment controls, source reduction or removal)
- Where corrective actions including a TRE have not been completed, an expeditious schedule under which corrective actions will be implemented;
- If no actions have been taken, the reason for not taking action.

The permittee may also request approval from the Department to postpone additional retests in order to investigate the source(s) of toxicity. Postponed retests must be completed after toxicity is believed to have been removed.

When an effluent has shown a severe or persistent toxicity problem, the Department has the authority to modify or reissue the permit to include additional monitoring and WET limits, since the potential for exceedance of water quality standards exists. If there is evidence that severe or persistent toxicity exists at the time of permit reissuance, the Department will likely reissue that permit with a WET limit and compliance schedule that requires the permittee to find and fix the source(s) of that toxicity. However, if permittees can find and fix the problem on their own before the permit is modified or reissued, they can avoid WET limits, compliance schedules, and potential enforcement.

Whether voluntary or permit-required, TRE studies should be well thought out and study objectives and results well documented and communicated to the Department. This chapter was created in order to provide some guidance regarding the basic information expected in TRE plans and reports. The primary purpose of TRE plans and reports are to inform the Department about work that was done (or is to be done) to identify the source(s) of toxicity. It is important to remember that although TRE plans and reports are intended primarily for the Biomonitoring Coordinator who is the Department's technical expert on WET, others (WDNR permits staff, local government, public, industry, environmental groups, etc.) will need to read and understand them as well. For this reason, TRE plans and reports should be written so that the general public can understand them.

Toxicity Reduction Evaluation Plans and Reports

The submittal and completeness of TRE plans and reports required by WPDES permits is the responsibility of the permittee, even if parts or the whole are written by consultants. The following guidance describes the TRE plans and reports required by WPDES permits, but should also be used by permittees who are doing a TRE voluntarily. In order for everyone to fully understand the goals and intent of the TRE, plans should include specific steps to be taken, specific dates when each step is to be completed, and a description of what each step is meant to accomplish. In order to help permittees and their consultants design plans and reports, some general outlines of information that should be included are given in the following pages.

In order for permit-required TRE plans and reports to be approved by the Department, they should include the information given below for each step. The Department does recognize that each TRE will be facility specific, some of the areas outlined may not apply in some situations, and that in some cases more or different information may be necessary. Communication and cooperation between the permittee and the Department is essential in TRE plan development, review, and implementation and will help ensure achievement of the TRE objective.

A TRE is usually divided in two parts, identification and removal of toxicity. Decisions on the most appropriate way

to remove toxicity are usually dependent on the cause(s). The standard WET limit compliance schedule (described in Chapter 1.12 and shown below) is set up so that sufficient time is allowed for part one and part two of the TRE to be completed. Descriptions of the steps included in that standard schedule, including descriptions of the TRE plans/reports that must be submitted to the Department, are given below.

STEP ONE

Standard WET Limit Compliance Schedule

Required Action	Date Due
 <p>Submit part one of a Toxicity Reduction Evaluation (TRE) plan describing procedures to be used to identify the source(s) responsible for the effluent toxicity.</p>	See due dates in WPDES permit
Implement part one of the TRE plan, make a reasonable attempt to identify the source(s) of the toxicity, and submit a report to the Department presenting the results of the evaluation.	
Submit part two of the TRE Plan describing actions to be taken to reduce or eliminate the toxicity identified in part one of the TRE and the dates by which those actions will be implemented.	
Submit a progress report identifying the actions taken to date to implement part two of the TRE plan.	
Complete all actions identified in the TRE plan and achieve compliance with the effluent toxicity limitation.	

Step One: Submittal of Part One of the TRE Plan

The first step in any TRE, and the first step in the standard WET limit compliance schedule shown above, usually involves the development of a plan describing actions to be taken to investigate sources of toxicity. Typically, this plan is required to be submitted to the Department within about 3 months of permit reissuance. This plan should be designed to provide the Department with a description of work to be done to identify toxicity, a schedule for conducting specific tasks and reporting results, relevant background information on the facility, and identification of the lab and/or consultants who will be involved in the investigations.

This “part 1 TRE plan” should include: a description of tasks to be done to identify the source of toxicity, a schedule for conducting these tasks, relevant background information, objectives of the study, and scheduled completion dates and milestones. The following is an outline of suggested information to be included in these plans (see the discussion under "*Step Two: Implementation of Part One of the TRE Plan and Submittal of a Report*" for other tasks that may be included in part one):

1. Introduction. (1-2 pages)

- A. Narrative description of past WET Tests, toxicity identification work done to date (if any)
- B. Summary (*tables, graphs, etc.*) of specific WET test results, TIE work done (if any)

2. Outline or flowchart of study. (1-2 pages)

- A. Timelines for when each phase of the work is expected to be done.
- B. Discussion of data gathering & review steps, facility-specific investigations that will be done
 - 1) Review of in-house data (*effluent data, operational records, treatment chemicals, etc.*)
 - 2) Field data collection (*inventory of hauled wastes, user surveys, etc.*)
- C. Discussion of Phase I, II, and II TIE steps planned (*usually more detail regarding Phase I, Phase II & II in general terms, since they're dependent on results of previous Phase*).

3. Toxicity Identification Evaluation (TIE) & other laboratory investigation steps. (4-5 pp)

- A. Species used, type (*acute or chronic, screening vs. dilution series, etc.*) & frequency of tests

B. Description of TIE manipulations - what's done, what results mean if a reduction in toxicity is noted (*1-2 paragraphs on each manipulation*)

- 1) Initial toxicity tests (*if applicable*)
- 2) Baseline tests (*if applicable*)
- 3) pH adjustment (*if applicable*)
- 4) filtration (*if applicable*)
- 5) aeration (*if applicable*)
- 6) C₁₈/SPE (*if applicable*)
- 7) sodium thiosulfate/oxidant reduction (*if applicable*)
- 8) EDTA chelation (*if applicable*)
- 9) any additional selected

4. Reporting timelines for the project (*1-2 pages*)

- A. Description of when progress reports will be made to the Department
- B. Dates when Phase I, II, & III reports will be due (*options include separate dates for each as they are completed or one final report when all work is finished*)
- C. Date when report is expected (*on or before next date specified in compliance schedule*)

5. Summary, potential future work (*1-2 pages*)

Other steps can be taken to identify toxicity sources. The permittee and their lab/consultant may be the best source of ideas for these additional steps. The Biomonitoring Coordinator and other Department staff can assist permittees, where possible. Permittees should provide regular updates during the TRE, in order to insure that studies are proceeding as planned. ***Communication and cooperation between the permittee and the Department is essential in TRE plan development, review, and implementation and will help ensure achievement of the TRE objective.***

Things To Avoid When Planning Or Conducting A TRE:

1. **Reliance on "gut feelings" & "trial and error" studies.** Since TIEs are expensive, some often narrow their search by limiting studies to look for sources they "feel" may be the cause. Substantial modification of the Phase I TIE, or preconceived assumptions about the cause of source of toxicity should be avoided. Trial and error type studies often result in starting over from scratch when preconceived ideas are proven wrong. While toxicity sources may be identified through this type of approach, they often take longer and the cost of doing so may quickly surpass the cost of a more organized TRE.

Guessing the cause or source of toxicity can increase the probability that toxicity resolution will be delayed, continued non-compliance will occur (when a limit is in place), additional cost will be incurred, and regulator and/or public trust will be diminished. **No Phase I tests should be dropped from use on the basis that the toxicant it is designed to target are not likely to be present in the effluent. The investigator should approach effluent characterization without a preconceived notion as to the cause of toxicity.**

2. **Reliance on priority pollutant scans.** Toxicity may be caused by compounds which don't appear on chemical analysis lists or may be caused by a combination of compounds. Priority pollutant scans may provide helpful information to supplement TIE tests, but shouldn't be relied on to provide all of the answers.
3. **Confirmation steps not carried out for identification or treatability studies** Money can be wasted if conclusions are rushed and not confirmed before treatment options are considered.

4. **Quick solutions without modifying ongoing behavior.** Solutions to toxicity should become standard operating procedure. TRE activities should not be once in time, but continuing activities.

STEP TWO

Standard WET Limit Compliance Schedule

Required Action	Date Due
Submit part one of a Toxicity Reduction Evaluation (TRE) plan describing procedures to be used to identify the source(s) responsible for the effluent toxicity.	See due dates in WPDES permit
Implement part one of the TRE plan, make a reasonable attempt to identify the source(s) of the toxicity, and submit a report to the Department presenting the results of the evaluation.	
Submit part two of the TRE Plan describing actions to be taken to reduce or eliminate the toxicity identified in part one of the TRE and the dates by which those actions will be implemented.	
Submit a progress report identifying the actions taken to date to implement part two of the TRE plan.	
Complete all actions identified in the TRE plan and achieve compliance with the effluent toxicity limitation.	

Step Two: Implementation of Part One of the TRE Plan and Submittal of a Report

Following the plan submitted in step one, the permittee should begin investigating the sources of effluent toxicity. The first step is usually the collection of data and facility-specific information. This step is used to define study objectives, identify what is known, and provide clues as to the cause(s) of toxicity. This information may also suggest immediate actions which may be useful in reducing effluent toxicity. The next step involves optimization of facility operations in an attempt to reduce effluent toxicity. Three areas are usually investigated during this step: facility housekeeping, treatment plant operation, and the selection and use of process and treatment chemicals.

After it has been established that sampling protocols, facility housekeeping, treatment plant operations, and treatment chemicals are not causing toxicity, a toxicity identification evaluation (TIE) is often done. The objective of the TIE is to characterize and identify the compound(s) causing toxicity so that they can be traced back to their source. In a TIE, effluent samples are taken to the WET lab, where they can be manipulated to remove suspect chemicals (e.g., metals, organics, etc.) and then re-tested to see if toxicity remains. If a specific effluent manipulation removes toxicity, then the researcher has a clue about the chemical causing the toxicity. The evaluation can use both characterization procedures and chemical-specific analyses, therefore, the identifications may range from generic classes of toxicants to specific chemical compounds. Multiple samples are usually needed for a TIE and one objective of a TIE may be to determine if and how toxicity varies over time. Once a specific class or individual compound has been identified as a potential cause of toxicity, the investigation turns towards finding the source (e.g., contributing industrial process, commercial source, etc.) of that compound.

Specific tasks that should be done during the first half of a TRE:

- ◆ **Check For Sampler Contamination.** Sampling locations and gear should be checked for possible toxic contributions and to see whether equipment was new and/or properly cleaned prior to testing (see Chapter 1.1 for guidance on sampler cleaning). Sampler contamination and sampling procedures can also be studied by conducting concurrent tests with different samplers or different WET labs.
- ◆ **Data Gathering.** TREs usually begin with information gathering and study of on-site situations. Data gathering steps may include: 1) checking effluent data to see if other limits were exceeded or operational upsets may have occurred at the same time as effluent toxicity; 2) identifying potential sources of

toxicity within the process or wastewater treatment plant, such as cleaning or disinfection agents, process side-streams, or treatment chemicals (e.g. phosphorus removal chemicals, polymers, flocculants, etc.); 3) looking for significant levels of individual chemicals in the combined wastestream (using pretreatment data, Merck index, or other chemical references) and 4) inventorying what is entering the sewer system by industry, commercial, domestic, & batch loads accepted by the headworks or elsewhere.

Specific operating practices can be implemented by permittees that will greatly aid in identifying toxic effluent constituents. A thorough inventory of all contributions to the waste stream is a must. Attachment 1 at the end of this chapter includes an example "User Survey", which has been used by POTWs successfully in the past to collect this type of information.

Industries should identify all possible contributions to the wastewater stream, including floor drains. Unwanted materials may be getting into the discharge without wastewater staff's knowledge. This understanding of all potential constituents and their relative presence (in time and concentration) is most beneficial to resolving toxicity problems. In addition to the obvious process waste streams, side stream such as cooling tower discharges, boiler blowdown, and other potential sources should be reviewed for the presence of toxic chemicals. In many cases, toxicity sources are not process-related, but rather side streams where unintentional releases or unconsidered chemicals (e.g., shift-dependent equipment sterilization, highly ionic wastes, chlorinated tap water, etc.) contribute to observed problems. It is not uncommon to experience a process section of an industrial facility that does not communicate with the wastewater treatment section, leading to unknown treatment needs.

- ◆ **Public and Employee Education.** Public education is becoming more important in toxicity reduction for municipal dischargers. Employee education regarding proper disposal and use of process and treatment chemicals has reduced toxicity in some instances. Commonly used household pesticides and cleaners have been implicated at many POTWs as sources of toxicity. Educating the public and employees about environmental and monetary costs of improper disposal of toxic substances has resulted in reductions in effluent toxicity in some instances. In a few cases, toxicity has disappeared from a municipal effluent after efforts were made to alert the public about toxicity problems.
- ◆ **Facility Housekeeping.** It is important to verify that process chemicals are not overused, housekeeping practices are not contributing wastes directly to the effluent, and other facility practices are not contributing to toxicity (see Attachment 2 "Housekeeping Logic Flow") and the selection and use of process and treatment chemicals. Chemical optimization is a process that can be performed in conjunction with the housekeeping parts of the TRE. The goal of this process is to identify simple solutions to toxicity problems by evaluating and possibly modifying chemical use at the facility.

A general inspection of housekeeping practices by the TRE investigation team may indicate possible problem areas that may be contributing to toxicity. Treatment chemicals used within the facility should be reviewed to determine if any new chemicals (or chemical concentrations) are being discharged by the processes (for an industrial facility) or the treatment plant that could potentially explain the observed effluent toxicity. Common sources of toxicity are chemical additives (surfactants and biocides) applied in production areas, wastewater treatment chemicals (polymers and defoamers) used at the WWTP, and chlorine/dechlorination chemicals.

- ◆ **Optimization Of Facility Operation.** An attempt should be made to see what operational adjustments could be made that might reduce toxicity, such as increasing aeration basin detention time, sludge age, etc. and develop a scheme for testing how such adjustments in process control may reduce toxicity in the

effluent (allowing time at each adjusted setting to ensure several detention times to occur plant-wide). See Attachment 3 "Treatment Plant Optimization Logic Flow".

- ◆ **Additional Monitoring To Demonstrate Absence Of Toxicity.** In some cases, toxicity may be infrequent, disappear inexplicably, or have been removed by actions taken prior to initiation of the TRE. In these cases, permittees may wish to demonstrate that toxicity is no longer present by conducting additional WET tests. These tests should be performed using the procedures specified in the permit. The number of tests required to make this demonstration will depend on factors such as the seasonality, severity, and cause(s) of toxicity. If toxicity does not occur during this monitoring, the permittee may ask the Department to modify the permit to remove or change the remaining steps in the compliance schedule, the WET limit, and/or subsequent WET monitoring requirements.

- ◆ **Toxicity Identification Evaluations (TIE).** TIE methods include bench-top treatment steps designed to indicate the general types of compounds that are causing effluent toxicity. Initial toxicity tests are performed to determine if samples are toxic, then manipulations for removal or alteration of effluent toxicity are performed and the resulting treated samples are tested for toxicity. The physical/chemical characteristics are indicated by the treatment steps that reduce toxicity relative to a baseline test. It is recommended that the full suite of Phase I-III procedures be performed on effluent samples, in order to characterize, identify, and confirm the cause of toxicity. As information is obtained on the nature and variability of toxicity, additional tests may focus on the steps that are successful in affecting toxicity.
 - ◆ **Three Major Parts to a TIE**
 - ⇒ **Phase I, Characterization:** Intended to characterize the physical/chemical properties of the compounds causing toxicity. Such characteristics as solubility, volatility, pH sensitivity, polarity, and filterability are determined without specifically identifying toxicants. Usually a 1st step in identifying toxicity but can also be used to develop treatment methods to remove toxicity without specific identification of toxicants.

 - ⇒ **Phase II, Identification:** Specifically identifies toxicants if non-polar organics, ammonia, or metals.

 - ⇒ **Phase III, Confirmation:** Confirmation of suspected toxicants.

- No Phase I TIE characterization test should be dropped from use on the basis that the toxicant it is designed to target are not likely to be present in the effluent. The investigator should approach effluent characterization without a preconceived notion as to the cause of toxicity.** The Phase I TIE should be completed in its entirety to minimize the chance of overlooking a toxicant. It is possible to overlook classes of compounds that contribute to toxicity if the Phase I process is substantially cut short. Abbreviated Phase I TIEs may result in the loss of valuable and necessary information on the characteristics of the substances responsible for toxicity, which may lead to inconclusive results or erroneous conclusions. It cannot be overemphasized that in the early stages of a TIE, shortcuts should be avoided. The single most common cause for “failed” TIEs was because of shortcuts being taken by an investigator that thought they already knew the answer before the start of the TIE.

- ◆ **Test Frequency.** TIEs require that toxicity be present so that toxicants can be characterized and identified. Enough testing should be done to assure consistent presence of toxicants for

characterization. (Usually monthly or bimonthly testing is recommended.) In order to reduce costs, it may be wise to do more frequent "screening" tests (100% effluent only) to determine if toxicity is present, rather than full dilution series less frequently.

- ◆ **Most Sensitive Species.** Tests may be limited to the species shown to be the most sensitive in previous WET tests. It is assumed that by removing toxicity to the most sensitive species, toxicity to others is removed as well.
- ◆ **Alternative Toxicity Tests.** At times it may be desirable to do less costly testing before starting complex TIE studies. For example, some investigations have included alternative toxicity tests (e.g., Microtox®, respirometry, etc.), to determine when toxicity was present (e.g., night-time hours, only when an industry was discharging, etc.) or to find out which wastestreams were more toxic.
- ◆ **Toxicity Source Evaluation.** Once effluent toxicants have been identified, steps are taken to locate their source. This evaluation may include a review of existing pretreatment data or data from the collection and analysis of samples from industrial users. Information gathered from an "Industrial and Commercial User Survey" (see Attachment 1) can be invaluable at this point in the TRE.

Part 1 Report

The following is an outline of suggested information to be included in a part 1 TRE Report. In order to comply with permit-required compliance schedules, this report should include: a description of specific tasks done to identify the source of toxicity (e.g., industry, commercial, domestic) and any relevant background information.

1. Introduction. (1-2 pages)

- A. Description of data gathering/analyses, education efforts, and facility-specific investigations done
- B. Summary of specific toxicity test results, TIE work done
- C. General discussion of Phase I, II, and III steps completed and conclusions reached
- D. Any deviations from "normal" WET test procedures (e.g., *feeding schedules, temperatures, pH control, aeration, etc.*) should be highlighted

2. Results of Toxicity Identification Evaluation & other laboratory investigation steps. (4-5 pp.)

- A. Discussion of test type used (*acute/ chronic, screening vs. dilution series, etc.*) and frequency of tests
- B. Description of specific TIE manipulations - what was done, what results were and what they mean (1-2 paragraphs on each manipulation - should contain tables showing survival and growth/reproduction results from each manipulation, when appropriate)

3. Toxicity Source Evaluation.

- A. review of existing pretreatment data or data from the collection and analysis (chemical-specific and/or toxicity) of additional samples from industrial users. Information gathered from an "Industrial and Commercial User Survey" should be reported. Conclusions reached as to the specific industrial, commercial, or other source(s) of effluent toxicity should be discussed.

4. Summary & Conclusions (1-2 pages)

- A. Summary of work done to identify source(s) responsible for toxicity and what results indicate.
- B. Identification of potential source(s) (e.g., *industrial, commercial, internal to WWTP*) of toxicity, based on data review and collection, frequency & duration of toxicity, TIE work.

STEP THREE

Standard WET Limit Compliance Schedule

Required Action	Date Due
Submit part one of a Toxicity Reduction Evaluation (TRE) plan describing procedures to be used to identify the source(s) responsible for the effluent toxicity.	See due dates in WPDES permit
Implement part one of the TRE plan, make a reasonable attempt to identify the source(s) of the toxicity, and submit a report to the Department presenting the results of the evaluation.	
Submit part two of the TRE Plan describing actions to be taken to reduce or eliminate the toxicity identified in part one of the TRE and the dates by which those actions will be implemented.	
Submit a progress report identifying the actions taken to date to implement part two of the TRE plan.	
Complete all actions identified in the TRE plan and achieve compliance with the effluent toxicity limitation.	

Step Three: Submittal of Part Two of the TRE Plan

The third step of the standard compliance schedule usually requires the permittee to develop a plan describing actions to be taken to investigate ways to remove the source(s) of toxicity that were identified in previous steps. In order to comply with the third step of the compliance schedule, part two of the TRE plan should include: a description of tasks to be done to identify alternatives for removing/reducing toxicity, a schedule for conducting these tasks, background information from part one of the TRE, identification of those conducting the evaluation (e.g., lab, consultants, etc.), objectives of the study, and scheduled completion dates and milestones. The following is an outline of suggested information to be included in these plans:

1. Introduction. (1-2 pages)

- A. Narrative description of toxicity identification work done & results shown
- B. Summary (*tables, graphs, etc.*) showing specific WET test results, TIE work done (*optional*)

2. Outline or flowchart of study. (1-2 pages)

- A. General description of plan to investigate ways of reducing or eliminating toxicity, including an evaluation of options for treating the final effluent and an evaluation of upstream pretreatment options, source reduction, and/or process modifications.
- B. Timelines for when each phase of the work is expected to be done.

3. Toxicity Reduction Evaluation (TRE) & other investigation steps. (4-5 pages)

- A. Discussion of studies to be done to identify toxicity removal alternatives, which may include:
 - 1) Source reduction alternatives
 - 2) Assessment of the treatment options: trials of modified treatment procedures in existing works or the evaluation of different procedures or works through bench or pilot scale simulation
 - 3) Cost/benefit analysis (*factors such as cost effectiveness and long term effects should be considered when choosing the best alternative*)
- B. Tests to confirm toxicity removal
 - 1) Tests should be done according to procedures specified in the permit.
 - 2) Number of tests will depend on seasonality, severity, and cause of original toxicity (*usually 3 or 4*)

tests, performed at least 60 days apart, is sufficient).

4. Reporting timelines for the project (1-2 pages)

- A. Description of when progress reports will be made to the permittee and the Department
- B. Compliance schedule for installment of pretreatment (*when applicable*)
- C. Date when final report is expected (*on or before date specified in last step of compliance schedule*)

5. Summary, potential future work (1-2 pages)

STEPS FOUR AND FIVE

Standard WET Limit Compliance Schedule

Required Action	Date Due
Submit part one of a Toxicity Reduction Evaluation (TRE) plan describing procedures to be used to identify the source(s) responsible for the effluent toxicity.	See due dates in WPDES permit
Implement part one of the TRE plan, make a reasonable attempt to identify the source(s) of the toxicity, and submit a report to the Department presenting the results of the evaluation.	
Submit part two of the TRE Plan describing actions to be taken to reduce or eliminate the toxicity identified in part one of the TRE and the dates by which those actions will be implemented.	
Submit a progress report identifying actions taken to date to implement part two of the TRE plan.	
Complete all actions identified in the TRE plan and achieve compliance with the WET limit.	

Steps Four & Five:

Implementation of Part 2 of the TRE and Submittal of Progress & Final Reports

Once the cause (i.e., chemical compound) and/or source of toxicity (e.g., industry, commercial, domestic, etc.) have been identified, decisions on the most appropriate approach for removing toxicity need to be made. To do this, the TRE process usually goes in one of two directions. One approach is to evaluate options for treating the final effluent, the other is to evaluate upstream pretreatment options, source reduction, or process modifications. A decision can also be made to pursue both approaches, and then to select the most technically and economically attractive option. This work was planned during the third step of the compliance schedule and must be completed in order to meet the WET limit which will become effective at the end of the compliance schedule.

A progress report is usually required about ½ way through part 2 of the TRE, in order to allow for mid-course corrections, as needed. This report should contain what has been learned to date regarding treatability, reduction, or removal options. If an alternative has been selected, the report should include a detailed schedule for when implementation is expected. This report should also include information regarding whether the TRE is on track to meet final compliance schedule dates.

Specific tasks that may be done during part two of a TRE may include:

- ◆ **Investigating Alternatives For Removing Toxicity.** Once the source has been identified, it is necessary to investigate ways of reducing or eliminating the toxicity. While source reduction of toxicants should be the preferred method of toxicity reduction, a concurrent or alternative approach is the assessment of the treatment of toxicity. Cost/benefit decisions often drive decisions between source reduction or treatment. Paths taken to

address treatability most often take the form of either trials of modified treatment procedures in existing works or the evaluation of different procedures or works through bench or pilot scale simulation.

- ◆ **Choosing The Best Toxicity Control Alternative.** Criteria for the selection of preferred toxicity control options should include: 1) compliance with WET limits, 2) compliance with other regulations (air, solid & hazardous waste, etc.), 3) capital, operational, and maintenance costs, 4) ease of implementation, 5) reliability, and 6) environmental impact (not necessarily in that order). Cost may often be the driving factor, however, the selected option should offer the best potential for consistent, reliable toxicity reduction with the least environmental impact.
- ◆ **Performing Tests To Confirm Toxicity Removal.** Once appropriate toxicity control options have been implemented, it will be necessary to perform WET tests to demonstrate that toxicity has been reduced to an acceptable level. WET tests should be performed using procedures specified in the permit. The number of tests required to make this demonstration will depend on factors such as seasonality, severity, and cause(s) of toxicity. Usually, at least four tests (performed at least 60 days apart) are recommended. WET tests required in the permit after the TRE compliance schedule end date may be used to demonstrate that a TRE was successful, however, the permittee risks permit violations if failures occur after the WET limit becomes effective.

Final Report & WET Limit Compliance

The following is an outline of suggested information to be included in the final TRE Report. In order to comply with permit-required compliance schedules, this final report should include: a description of work that was done to remove the source(s) of toxicity and any relevant background information.

1. Introduction. (1-2 pages)

- A. Narrative description of toxicity identification & reduction work done
- B. Summary of specific WET test results, TIE work done (*optional*)

2. Results of Toxicity Reduction Evaluation (TRE) & other investigation steps. (4-5 pages)

- A. Discussion of studies done to find most cost effective toxicity removal alternative(s)
- B. Results of WET Tests completed to confirm toxicity removal

3. Summary & Conclusions (1-2 pages)

- A. Report of which toxicity removal alternatives were chosen, why they were chosen, and whether or not they were successful (*based on results of WET Tests completed to confirm toxicity removal*).

ATTACHMENT 1: Example Industrial/Commercial User Survey

(City/village/town) is performing a toxicity reduction evaluation, since toxicity test results have indicated the presence of substances in the effluent that are potentially harmful to aquatic life. In order to reduce or eliminate effluent toxicity, (city/village/town) must identify and locate its source. As a part of this investigation, (city/village/town) is conducting a survey of industrial and commercial dischargers that may contribute toxic substances to the wastewater treatment facility. This questionnaire has been prepared to assist (city/village/town) in gathering information for this purpose. Please complete the form by filling in answers to the following questions, and provide a copy to (city/village/town). Use additional sheets as necessary.

1. Name and Address of Business:
2. Who should be contacted at your facility for additional information?

Name: _____ Telephone No.: _____

3. Product(s) manufactured or service(s) performed:
4. What is your average volume discharge to the sanitary sewer system in gallons per day?
5. Does your discharge to the sanitary sewer system include process or cleaning-sanitizing wastewater other than normal sanitary wastewater from rest rooms and employee facilities? If yes, please provide the average and peak daily volumes of process wastewater discharged to the wastewater treatment facility. Include any discharge other than restroom and employee facility wastewater. YES () NO ()

*****IF NO, YOU MAY DISREGARD THE REMAINING QUESTIONS ON THIS FORM**

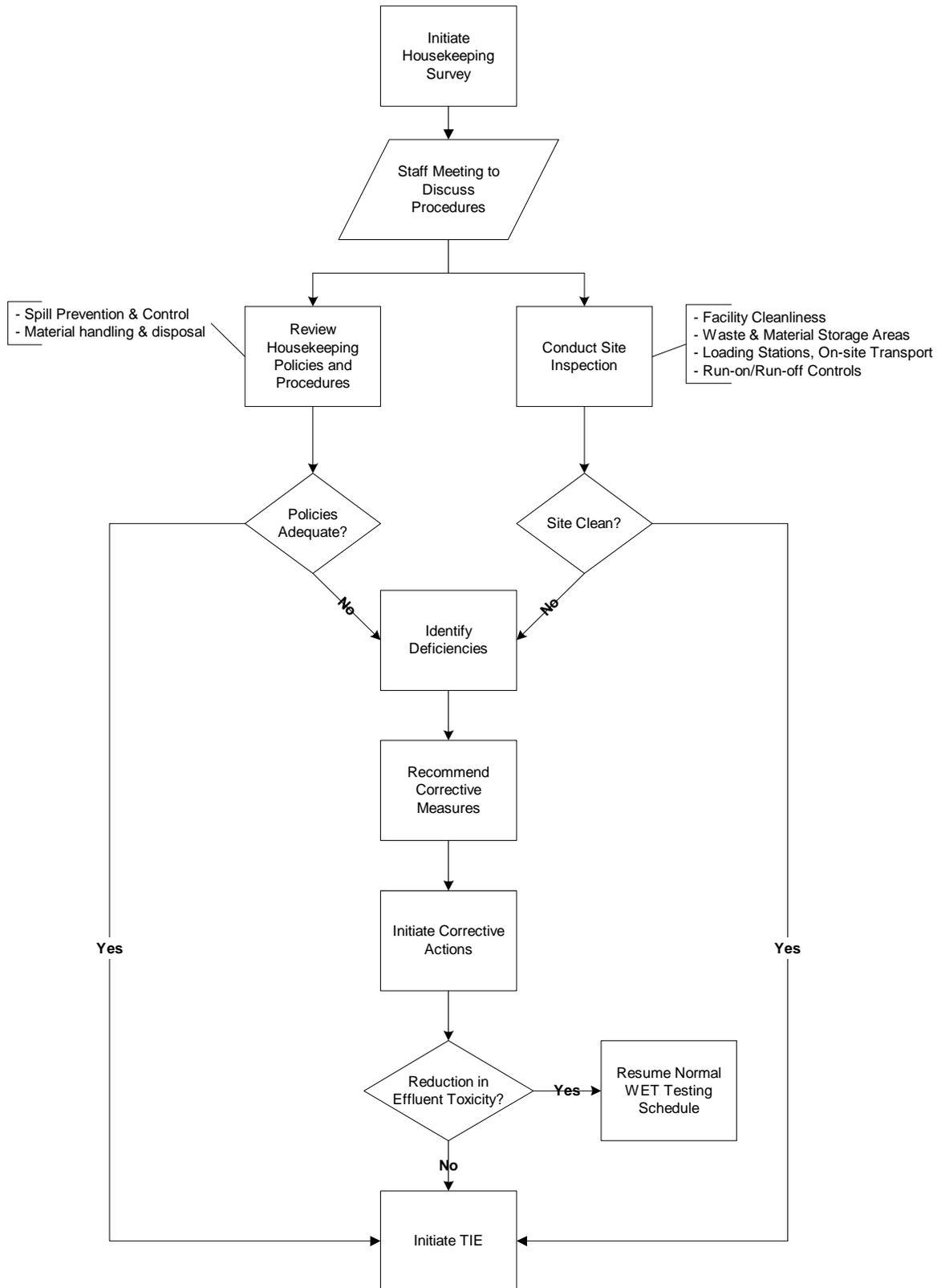
6. Attach copies of any wastewater analytical data from your facility which has been collected in the last five years. Identify whether such data is for your entire discharge or for samples from your process wastewater flow(s) only (specify which flows).
7. Does your facility store any raw materials, cleaners, etc., used in your production processes, which contain any of the pollutants listed on the attached toxic pollutant list? If yes, what specific compounds or formulations (indicate volumes stored)? YES () NO ()
8. Do you use any substances containing any of the substances on the attached sheet in production or cleanup activities (e.g., sanitizers, cleaning agents, cooling water additives, etc.) If yes, please provide a list of all substances used and a copy of the Material Safety Data Sheets (MSDS) for each substance. Also, please provide the following information for any compounds or formulations identified: name of pollutant and containing compound, process compound used in, and amount of compound used per month. YES () NO ()
9. Are there particular days of the week and/or times of the day when potentially toxic discharges from your facility may be highest? If so, please describe by providing peak day to average ratios, peak hourly concentrations and flow rates, etc., to the extent this information may be available. YES () NO ()

Your assistance with this survey is appreciated. If you have questions, or need additional information, please call _____ at the (name and phone no. of facility).

TOXIC SUBSTANCES LIST
INDUSTRIAL/COMMERCIAL USER SURVEY
(sorted by priority pollutant category)

Metals:	Toluene	Dimethyl Phthalate	Diazinon
Antimony	Toxaphene	Di-n-butyl Phthalate	2,4-Dichlorophenoxyacetic acid
Arsenic	1,1,1-Trichloroethane	2,4-Dinitrotoluene	Endosulfan
Beryllium	1,1,2-Trichloroethane	2,6-Dinitrotoluene	Endosulfan Sulfate
Cadmium	Trichloroethylene	Di-n-octyl Phthalate	Endrin Aldehyde
Chromium	Vinyl Chloride	1,2-Diphenylhydrazine	Guthion
Copper		Fluoranthene	Heptachlor
Cyanide	Acid-Extractable Compounds:	Fluorene	Heptachlor Epoxide
Lead	P-Chloro-M-Cresol	Hexachlorobenzene	Malathion
Mercury	2-Chlorophenol	Hexachlorobutadiene	Methoxychlor
Nickel	2,4-Dichlorophenol	Hexachlorocyclopentadiene	PCBs
Selenium	2,4-Dimethylphenol	Hexachloroethane	
Silver	4,6-Dinitro-O-Cresol	Indeno(1,2,3-cd)pyrene	Dioxin:
Thallium	2,4-Dinitrophenol	Isophorone	2,3,7,8-TCDD (dioxin)
Zinc	2-Nitrophenol	Naphthalene	
	4-Nitrophenol	Nitrobenzene	Other Pollutants:
Volatile Organic Compounds:	Phenol	N-Nitrosodimethylamine	Aluminum
Acrolein	2,4,6-Trichlorophenol	N-Nitrosodiphenylamine	Ammonia
Acrylonitrile		N-Nitrosodipropylamine	Asbestos
Benzene	Base-Neutral Compounds:	N-Nitrosodiethylamine	BHC-tech. grade
Bromoform	Acenaphthene	N-Nitrosodi-n-butylamine	Bis(2-chloromethyl)ether
Carbon Tetrachloride	Acenaphthylene	N-Nitrosopyrrolidine	Chloride
Chlorobenzene	Anthracene	Octachlorostyrene	Chlorine
Chlorodibromomethane	Benzidine	Pentachlorobenzene	3-Chlorophenol
Chloroethane	Benzo(a)anthracene	Phenanthrene	4-Chlorophenol
2-Chloroethyl vinyl ether	Benzo(a)pyrene	Pyrene	Dichlorodifluoromethane
Chloroform	3,4-Benzofluoranthene	1,2,3,4-Tetrachlorobenzene	2,3-Dichlorophenol
1,2-Cisdichloroethylene	Benzo(ghi)perylene	1,2,4,5-Tetrachlorobenzene	2,5-Dichlorophenol
Dichlorobromomethane	Benzo(k)fluoranthene	1,2,4-Trichlorobenzene	2,6-Dichlorophenol
1,1-Dichloroethane	Bis(2-chloroethoxy)methane		3,4-Dichlorophenol
1,2-Dichloroethane	Bis(2-chloroethyl)ether	Pesticides:	1,3-Dichloropropane
1,1-Dichloroethylene	Bis(2-chlorisopropyl)ether	Aldrin	2,3-Dinitrophenol
(vinylidene chloride)	Di(2-ethylhexyl)phthalate (DEHP)	Alpha-BHC	Fluoride
1,2-Transdichloroethylene	4-Bromophenyl Phenyl Ether	Beta-BHC	Formalin
1,2-Dichloropropane	Butyl benzyl phthalate	Delta-BHC	Gamma-BHC
1,1-Dichloropropene	2-Chloronaphthalene	Chlordane	Iron
2,3-Dichloropropene	4-Chlorophenyl Phenyl Ether	Chlorpyrifos	2-Methyl-4-Chlorophenol
1,3-Dichloropropene	Chrysene	Dieldrin	3-Methyl-6-Chlorophenol
Ethylbenzene	Dibenzo(a,h)anthracene	4,4'-DDD	Mirex
Methyl Bromide	1,2-Dichlorobenzene	4,4'-DDE	Photomirex
Methyl Chloride	1,3-Dichlorobenzene	4,4'-DDT	2,3,4,6-Tetrachlorophenol
Methylene Chloride	1,4-Dichlorobenzene	Endrin	Trichlorofluoromethane
Pentachlorophenol	3,3'-Dichlorobenzidine	Parathion	2,4,5-Trichlorophenol
1,1,2,2-Tetrachloroethane	Diethyl Phthalate		
Tetrachloroethylene			

ATTACHMENT 2: Housekeeping Logic Flow



ATTACHMENT 3: Treatment Plant Optimization Logic Flow

