Society of Environmental Toxicology and Chemistry (SETAC)

Date: 30 June 1998

To: Whom it May Concern

From: Rodney Parrish

Subject: Application of TIEs/TREs to Whole Effluent Toxicity: Principles and Guidance

The attached report was prepared to assist those individuals and organizations concerned with Whole Effluent Toxicity (WET), and to help clarify the initial steps that lead to a Toxicity Identification Evaluation (TIE) or a Toxicity Reduction Evaluation (TRE).

This report was generated by the WET Expert Advisory Panel on TIE/TRE and peer reviewed by the WET Expert Advisory Panels Steering Committee, all volunteers and all members of the Society of Environmental Toxicology and Chemistry (SETAC). Each person involved is an expert in some aspect of WET, and the information provided here represents the consensus of the Panel on TIE/TRE and the Steering Committee's collective expertise at the time this document was written (June 1998).

The report is intended to stimulate further discussion about WET, WET-related research, and the science underlying WET. The report and information contained therein are not to be construed as representing an official position of SETAC, the SETAC Foundation for Environmental Education, or the U.S. Environmental Protection Agency. [This report was produced under the SETAC Foundation's WET Cooperative Agreement with USEPA, No. CX 824845-01-0.]

Attachment

APPLICATION OF TIEs/TREs TO WHOLE EFFLUENT TOXICITY:
PRINCIPLES AND GUIDANCE

A report of the WET Expert Advisory Panel on TIE/TRE
Society of Environmental Toxicology and Chemistry (SETAC)

and

SETAC Foundation for Environmental Education
1010 North 12th Avenue
Pensacola, FL 32501-3367, USA

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FOREWORD

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The assistance of Barbara Albrecht, WET Technical Assistant, is gratefully acknowledged, as is the volunteer efforts of the SETAC WET Expert Advisory Panels Steering Committee and the TIE/TRE Expert Panel.

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Application of TIEs/TREs to Whole Effluent Toxicity: Principles and Guidance

The Whole Effluent Toxicity Expert Advisory Panels (WET EAP) have been formed by the SETAC Foundation for Environmental Education through a cooperative agreement with the U.S. Environmental Protection Agency (USEPA). This report was generated by the WET EAP on TIE/TRE and peer reviewed by the WET EAP Steering Committee, all recognized WET experts, all volunteers, and all members of the Society of Environmental Toxicology and Chemistry (SETAC). The USEPA tasked the WET EAP Steering Committee to develop the TIE/TRE Panel to assess specific questions about technical issues relating to the use of toxicity identification evaluations (TIEs) and toxicity reduction evaluations (TREs). This report is the synthesis of deliberations of this panel and represents their collective expertise at the time this report was prepared (June 1998).

This report will provide a resource to water quality regulatory agencies, NPDES permittees, consultants, and academic researchers engaged in the complex effluent toxicity identification and reduction processes. While technological experts in the WET field may not find significant new information in this report, they will see a consensus opinion by recognized authorities that may help support actions or directions of the TIE/TRE processes.

Many problems that arise in TIEs/TREs result from misuse and misinterpretation of TIE/TRE methods and results. This report may be most effective if placed in the hands of administrators and policy makers who may not yet have the insights of their technical staffs.

Finally, the Expert Advisory Panel on TIE/TRE recognized a widespread scarcity of expertise in TIE/TRE practice, especially at the administrative, decision-making level. This deficiency can only be changed by commitment on the part of regulated and regulating communities to improve and foster staff experience in this area and to communicate effectively, bottom-to-top, the principles and processes of TIEs/TREs.

The WET EAP Steering Committee invited the following individuals to join the TIE/TRE EAP, based on their experience and expertise:

Academia
Steve Klaine, Clemson University
Tom Waller, University of North Texas

Business
Ray Arnold, Exxon Biomedical Sciences
Bill Goodfellow, EA Engineering, Science, and Technology
Russ Hockett, ENSR Consulting and Engineering
Don Mount, AScI Corporation

Government
Larry Ausley, North Carolina Division of Water Quality
Debra Denton, USEPA
Margarete Heber, USEPA
Teresa Norberg-King, USEPA
Peter Ruffier, City of Eugene, Oregon

The Panel met in Sparks, Maryland, 11 to 13 February 1998, to deliberate the following 7 questions that encompass concepts about TIEs/TREs and are frequently posed by the regulated community, as well as by state/tribal regulatory agencies:

1. Under what circumstances should a permittee conduct a TIE/TRE?
2. How should persistence and magnitude of toxicity events be considered in the TIE/TRE process?
3. How should inconclusive TIEs and incomplete TREs be addressed in the regulatory process?
4. Are schedules of compliance applicable for TIEs and TREs?
5. How should ionic imbalances or total dissolved solids (TDS) be considered as factors contributing to WET?
6. What, if any, distinctions are necessary between acute and chronic toxicity in the TIE process, and what limitations does either pose?
7. What, if any, are the technical limitations of the TIE process?

Calling on the significant experience and insight in the use of TREs and TIEs gained over the last decade, the Panel spent 2½ days discussing these issues and providing the expert opinions presented below. Overall, the Panel concluded that TIE/TRE methods currently in use are sound and have been successful at resolving WET issues in a high percentage of cases when the techniques are applied appropriately and practiced by experienced scientists. The intent of this report is to provide the most current interpretation of technical aspects of the TIE/TRE process and to guide any necessary evolution or modification. The panel emphasized that these deliberations and conclusions are based on science and technical practices only and acknowledged that the regulatory process may require additional or alternative considerations.

Consideration of Questions Posed

1. Under what circumstances should a permittee conduct a TIE/TRE?

The Panel believed that 2 assumptions had to be made prior to discussion of TRE "triggers." The first assumption was that a proper waste load allocation (WLA) was calculated based on either steady-state or dynamic models to protect the toxicity standard for the water body, and the permit limit or monitoring target appropriately represents the exposure predicted to occur in the receiving

stream as reflected in guidance presented by USEPA (USEPA TSD, 1991). USEPA's "Whole Effluent Toxicity (WET) Control Policy,"(USEPA 833-B-94-002, July 1994), outlines the basis for WET controls and specifically states that WET should be evaluated at the edge of the acute mixing zone for acute toxicity and at the edge of the chronic mixing zone for chronic toxicity. The Panel expressed a preference for the use of dynamic modeling in the calculation of permit limits because of increases of accuracy and ability to incorporate site-specific factors. The Panel concluded that many permit limits are written that do not reasonably incorporate mixing considerations or other important exposure factors suggested by the Technical Support Document (USEPA, TSD, 1991). The use of reasonable exposure considerations should be an integral part of permit negotiation and development in which both the permittee and the regulator should be involved.

The possibility of seasonally variable exposures can influence the appropriate exposure factors when establishing WET limits or "triggers." This subject is discussed in a 21 July 1997 memo from Tudor Davies, USEPA Office of Science and Technology, to the USEPA Water Management Directors. Entitled "Clarifications Regarding Whole Effluent Toxicity Test Methods Recently Published at 40 CFR Part 136 and Guidance on Implementation of Whole Effluent Toxicity in Permits," the memo contains guidance for which the development of seasonal limits for WET. One aspect of a TRE may be consideration of the relevant exposure period, and whether determination of a seasonally variable limit or trigger would be appropriate. A variety of models for establishing appropriate design conditions are described by the USEPA Technical Support Document (USEPA TSD, 1991).

The second assumption about "triggers" that has to be made is that the data driving the process represent reasonably valid indications of toxicity. That validity includes an evaluation of the test acceptability criteria, consideration of concentration/response curves, and the significance of the observed effect. As was recommended by the participants in the SETAC sponsored Pellston WET workshop (Grothe et al, 1996), consideration of practical criteria for lower and upper sensitivity ranges should be established for WET endpoints. These ranges would define bounds for the statistical ability of tests either to detect or reject treatment responses versus control populations and limit the effects of alpha and beta errors in these statistical analyzes.

The Panel felt that Question 1 is driven by instances where complex TREs, and more specifically TIEs, have been required by regulatory agencies in cases where there was a single failure of a WET limit or where a trigger occurred. Given the 2 assumptions, there are almost no circumstances where this reaction (a single failure of a WET limit or where a "trigger" occurred) is appropriate in the regulatory context. Almost always, additional information should be generated to demonstrate that further TIE work will have an opportunity for success. The permittee can gain valuable information on preventing future toxic events by immediately initiating a TIE when WET monitoring results indicate that toxicity is present frequently enough to utilize the toxicity characterization methods. Although permittees are frequently reluctant to make this proactive expenditure in response to a toxic event, the Panel emphasized that this may be money well spent toward preventing future costs of ineffective "trial-and-error" activities. Early initiation of a TIE can produce additional information on problem sources and frequency and result in faster resolution of the problem. It was estimated that after only 1 or 2 well-performed Phase I TIEs were accomplished, at an estimated cost of $10,000 to $15,000 (US dollars), the probability of success of a TIE could be judged.

The Panel felt that some form of accelerated WET testing is an appropriate required or voluntary initial response to a toxic event. The frequency and duration of this accelerated testing will depend on the frequency of routine monitoring requirements and results of subsequent analyses. The purpose of this testing is to define duration and magnitude of the toxic event and to assess the applicability of TIE procedures to address toxicants (i.e., is toxicity observed consistently enough for TIEs to be conducted efficiently?). Permittees should recognize the benefits of performing this accelerated testing, not only from the benefit of providing additional information to the process of toxicity reduction, but also strictly to define the duration of non-compliance as a consideration of the enforcement process. Logistical constraints such as sampling and test scheduling should play a role

in determination of frequency. The permittee should begin this work proactively and conduct any number of a series of logical steps that could help identify and resolve toxicants. These activities could include concurrent monitoring of chlorine or ammonia or other obvious toxicants, determining the sensitivity of other species and the degradability of toxicity, and performing voluntary TIEs. There should be no constraints (e.g., pre-approval) that prevents permittees from beginning these types of proactive responses.

The Panel believed that testing at least every other week would usually be appropriate until it is determined that either the TIE (or other TRE activities) can be performed with a probable chance for success, or until it is determined that the toxicity has ceased or is of insufficient duration or frequency for successfully conducting a TIE/TRE. This guidance should not be construed to discourage even more frequent analyzes to demonstrate limits of duration of non-compliance.

It is probably not feasible to prepare a generalized flowchart or to define a series of events that should lead directly to specific TRE activities or a TIE. Decisions should be made on specific cases by individuals experienced in the application of toxicity identification and reduction tools and should be goal-oriented toward solution of problems by using techniques likely to produce useful results. The Panel acknowledged that these case-specific decisions may be difficult to implement in a national regulatory program, and the USEPA may need to establish policy recommendations on specific triggers and time frames for conducting TREs. One of the chief difficulties from the regulatory perspective is a deficiency of expertise in WET and TRE issues among local and even national regulatory authorities. The WET training courses currently underway as part of the SETAC Foundation/USEPA Cooperative Agreement should improve this situation if the instructors can reach key state and regional regulatory staff. More advanced training and experience will be needed in the future.

2. How should the persistence and magnitude of toxicity events be considered in the TIE/TRE process?

The Panel felt that this question is asked to address technical considerations of how the persistence (i.e., regular presence) of toxicity should be considered in the TIE/TRE. For the purpose of this discussion, "persistent toxicity" is defined as toxicity that appears consistently in a series of toxicity tests rather than toxicity that is persistent in a single sample over time. Intermittent toxicity in a discharge increases the difficulty of conducting a TIE, and very infrequent toxicity may make a TIE very difficult. Random toxicity events may require analysis of more samples and, in some cases, may even necessitate abandoning TIE work on individual non-toxic samples. The presence of intermittent toxicity events can provide useful information during Phase III toxicity confirmation steps to correlate toxicant concentration with observed responses. While the Panel acknowledged these potential difficulties, the experts felt that by using experienced laboratories for toxicity identification reduction the chances of obtaining poor-quality results are lessened. However, finding qualified laboratories may prolong the process and should be considered when developing compliance schedules (see discussion of laboratory experience in Question 3).

The magnitude, duration, and frequency of toxicity all need to be considered during the accelerated testing process in the context of application of the TIE. The magnitude of the toxic event is important relative to the WET limit or "trigger." If the magnitude is measurable in the compliance monitoring test, consensus of the Panel was that it is sufficient to conduct a TIE characterization. If magnitude exceeds the limit or "trigger", toxicity assessment activities are necessary to determine if toxicity is persistent and if a TRE should proceed. If the magnitude of toxicity is such that environmental harm is observed, then toxicity reduction activities should be further expedited. Assessment of receiving-system biota, conducted either proactively by the permittee or by the regulator, at this point can provide context to decisions about ecological harm from the toxic event. However, the Panel warned that poorly designed bioassessments of receiving-system biota may not provide sufficient statistical power to detect important changes in receiving-system biota. It is reasonable to assume

that the effectiveness of bioassessments will be directly proportional to the degree of toxicity present (magnitude, duration, and frequency). More subtle, sublethal effects are more likely to be missed by bioassessment than are effects caused by acutely lethal concentrations.

Duration of a non-compliant event can be better defined by continued or accelerated testing. By proactively increasing toxicity monitoring frequency, the permittee can define (to the extent of monitoring conducted) the duration of the event and any ephemeral presence of toxicants. Knowledge of the sources or characteristics of toxicants can even be enhanced by altering sampling design to incorporate shorter duration composite samples or by the use of a series of grab samples. This information is often useful in associating toxicant presence with identifiable waste source events (e.g., industrial process timing, industry operational schedules, changes in wastewater treatment activities, etc.).

3. How should inconclusive TIEs and incomplete TREs be addressed in the regulatory process?

In order to understand the causes of inconclusive TIEs and incomplete TREs, it is necessary to define what constitutes successful identification and reduction. USEPA's TSD (1991) defines a TRE as a site-specific study conducted in a step-wise process designed to identify the agent causing effluent toxicity, isolate the source of toxicity, evaluate the effectiveness of toxicity control options, and then confirm the reduction in effluent toxicity. TIEs are defined as a set of procedures that identify the specific agent responsible for effluent toxicity. Therefore, a TIE can be a subset of tools used in a TRE.

Successful TREs reduce toxicity to an acceptable level. This may be accomplished through the identification and control of the source of the toxicity or identification and implementation of a treatment strategy that reduces toxicity to an acceptable degree.

The Panel recognized that there have been unsuccessful TIEs, but believed that the majority of these cases occur when toxicity was too ephemeral to effectively apply TIE techniques or when the individuals performing the TIEs were unqualified or inexperienced in the techniques (discussed below). Where the requirements for a TIE are justified, competent teams are successful in identifying or at least characterizing the cause(s) of toxicity to allow for selection of treatment options. TIEs should be considered successful, reliable, and powerful tools in TREs. Again, requirements to conduct TIE and TRE work should be predicted on the assumption that limits or monitoring "triggers" have been appropriately established relative to predicted exposures.

Experience of the panel has shown that successful TREs generally contain certain common elements including the following:

- All parties have a working knowledge of the process;
- Communication between the regulated and the regulator is an integral part of the process, occurs early, often, and for the duration of the process, and emphasizes a common goal of reducing toxicity to acceptable levels; and
- The strategy is developed by competent and knowledgeable individuals.
- Success of the TRE process often begins with development of a toxicity reduction plan or in some instances development of a pre-toxic event "fire plan." These plans can prepare for issues like the following:
- Coordination of resources (staff teams, equipment, and finances) that need to be committed quickly to a toxicity investigation;
- Pre-qualification of consultants or contractors for assistance with a TIE/TRE or development of the qualifications and specifications for a RFP for technical assistance;
- Establishment of a communications plan that covers reporting and information exchange with

the regulatory agency, governing bodies, and the public; and

- Development of an outline for a rapid toxicity source identification that addresses likely pollutant contributions (production processes, industrial waste streams), "housekeeping" practices, and wastewater treatment process efficiency and performance.

Experienced and qualified teams can be highly efficient, rarely failing to determine a cause of toxicity or treatment strategy. Disappearance of a particular episode of toxicity obviously will make the TIE/TRE process unnecessary but the analysis should not be considered a lack of success. Often, management attention in earlier stages of TIE/TRE efforts leads to subtle behavioral or operational changes that lead, in turn, to a reduction or elimination of toxicity without a clearly definable cause. The possibility that current identification or resolution methods have not reached the level of need for particular toxicants (i.e., lack of methods) also exists. Pollutants like diazinon, originating from diffuse sources and entering Publicly Owned Treatment Works (POTWs), have proven difficult to resolve. Regulatory innovations like public awareness campaigns may hold promise for resolution, but they take time. The regulatory process must take these considerations into account through avenues like administrative relief-actions that encourage, allow, and eventually require problem resolution.

The performance of successful TREs requires teams of individuals with a variety of expertise. The team should include experts from within the permittee's organization who are required to prepare and participate in the TRE, or outside experts may be used. An integrated team approach is considered an important characteristic of a successful TRE effort. The team should include treatment engineers, plant operators, and process engineers/pretreatment staff. In addition, chemists, aquatic toxicologists, data analysts, and individuals with regulatory experience should be part of the team. The team should be able to develop equal partnerships containing regulators, dischargers, and external experts and should demonstrate that it has experience successfully completing TREs, analyzing the data, and applying the findings to the elimination or control of the toxicity. The ability to communicate the results to non-scientists is also an important attribute of the team. Choosing a team based on a low bid does not necessarily provide the best product. Permittees should design bid proposals for TREs that favor teams who have experience with successful completion of TIEs/TREs. Because proposal requests frequently occur under the pressure of enforcement liability, this information should be developed before it is needed.

TIEs should also be performed by teams of experienced individuals. These teams may be similar to the one performing TREs and should include chemists, regulatory specialists, aquatic toxicologists, and statisticians/data analysts. If the team is from outside the organization, it should provide references from satisfied customers and provide evidence of personnel who have performed relevant TIEs in a timely manner. The team should provide evidence of and ability to interpret TIE data. If the team does not have analytical capabilities (trace organics, trace metals, chlorine, ammonia, pH, etc.) they must have ready access to these capabilities.

4. Are schedules of compliance applicable for TIEs and TREs?

The 1994 USEPA WET Control Policy states, "In a few highly unusual cases where the permittee has implemented an exhaustive TRE plan, applied appropriate influent and effluent controls, maintained compliance with all other effluent limits, compliance schedules, monitoring, and other permit requirements, but is still unable to attain or maintain compliance with toxicity-based limits, special technical evaluation may be warranted and civil penalty relief granted. Solutions in these cases could be pursued jointly with the expertise from U.S. EPA and/or the States as well as the permittee." The Panel felt that compliance schedules provide a useful structure for resolving WET noncompliance by describing milestones and desired endpoints of the TRE process, if implemented with realistic goals and flexibility. These schedules should be used as a resolution tool rather than as an enforcement deadline. Compliance schedules should be developed incrementally to cover different stages of the TRE process (e.g., the initial toxicity assessment, TIEs, treatment evaluations, and corrective actions). Such sequential construction of compliance schedules allows for the schedules to be

appropriately defined for each stage of the investigation based upon the increasing knowledge of the cause of the toxicity and the corrective actions that may be effective in controlling it. Because of their varied wastewater sources and complex composition, effluents from POTWs may present more difficult problems in TREs. TIE and TRE activities may therefore need to include more or different expertise and possibly longer time frames when assessing these effluents.

There must be frequent and complete information exchanges between the permittee and the regulator during the conduct of any toxicity evaluation. Schedules may also be impacted by the time required to complete a TIE, conduct a treatability or source-control assessment, develop and build any additional treatment capability, and assess whether there has been a prior study or control strategy that was successfully implemented that could be applied to the current situation. The Panel noted that additional treatment has rarely been necessitated by results of TIEs where causes of toxicity have been identified.

5. How should ionic imbalances or total dissolved solids (TDS) be considered as factors contributing to WET?

Toxicity to a test organism that results from ionic imbalances has caused confusion in technical assessments and permitting/compliance. Specifically, some effluents have been toxic because of ionic imbalances in the receiving system environment in which the test organisms are exposed. Such imbalances may be the result of mixing effluents and receiving waters that yield a test solution to which the test species is physiologically intolerant. There is currently a debate about whether salinity should be considered a toxicant in all situations. It should be noted that ionic imbalances that cause toxicity usually encompass high concentrations of ions, but ionic imbalances can also occur in situations where the effluent does not contain sufficient ions (e.g., condensate discharges) that are functionally deionized. Thus, ionic imbalance occurs when ion concentrations and compounds are not within the physiological tolerance of the selected test organism. The SETAC WET EAP Steering Committee is evaluating this technical issue of inorganic ion imbalance as a result of national interest and based upon information submitted from numerous trade associations, scientific organizations, and permitted facilities. A report will be published under the SETAC Foundation/USEPA Cooperative Agreement later this year or in early 1999.

Effluent toxicity that results from inorganic ionic imbalance is pervasive in permitted discharges from many industrial processes and municipal discharges where process streams are concentrated, adjusted, or modified. Procedures to identify ionic imbalance toxicity include direct measurement, predictive toxicity models for freshwater systems, ion exchange resins, "mock effluents," and tolerant/sensitive test-species comparisons. Deviations in concentration or proportions of dominant ions usually indicate a potential ionic imbalance in solutions to which the organisms have been physiologically accustomed. The ionic composition of freshwater varies greatly relative to seawater. However, there are certain cations (Ca_2^+, Mg_2^+, Na^+, and K^+) and anions (HCO_3^-, CO_2^-, SO_4^{2-}, and Cl^-) that are typically present. Deviations in concentrations of the major ions are usually indicative of ion imbalance and may lead to challenges for WET testing procedures.

Estuarine and brackish waters also pose challenges for many discharge situations (e.g., low-salinity effluent entering a high-salinity receiving system or conversely, high-salinity effluent entering a low-salinity receiving system). The environmental requirements and tolerance of aquatic organisms to salinity or other ionic compositions vary and should be recognized. Ion regulation problems may arise when marine organisms are moved rapidly from full-strength seawater to lower salinity. WET testing requirements for marine and freshwater organisms vary because of different osmoregulatory requirements that must be considered when selecting test species to identify sources of toxicity caused by ions. In some cases, this relative sensitivity or tolerance can be useful in the toxicant characterization process.

Cost-effective waste-treatment control options for a facility whose effluent is toxic because of TDS or

ion content are scarce, at best. Depending on the discharge situation, TDS toxicity may not be viewed with the same level of concern as are other toxicants because of situations like natural occurrence of high-salinity inland systems or the rapid dilution and balance of these ubiquitous ions (e.g., Ca, Na, Mg, K, SO_4, Cl) in estuarine and marine systems. These discharge situations may often not require the conservative safety factors of other toxicants. Regulatory options for ionic imbalance toxicity (when no other toxicants are identified or characterized as part of the TIE process) may include modifications to site-specific exposure through discharge modification, use of alternative models (e.g., dynamic models), exposure-specific toxicity tests, or alternative mixing zones. These options could prove to be more reasonable than potentially expensive or high energy-dependent treatment options that may be ineffective for the removal or addition of ions that do not pose human health concerns or significant ecological hazards and are rapidly diluted or assimilated in the receiving water system.

Other concerns about TDS are the complications that may be created in the TIE process. In freshwater TIEs, pH modifications and ion exchanges during treatments need to be watched carefully. The performance of system blanks, while strongly encouraged, may not be sufficient to characterize the effects of sample manipulations and chemical additions on observed toxicity. Interpretation of these changes in results must be made carefully unless supporting analytical data yield a broader understanding of effects. Simply measuring conductivity gives a prediction of the electrically conductive ions in the solution only and does not describe the individual ions. In many treatments, the solution will be monoionic with regard to a dominant anion and cation, and in some instances necessary ions may have been removed or replaced. Depending on the organisms tested, it may be more useful to perform the TIE manipulations before salinity adjustment if information on treatment control options is part of the TRE objectives. This is important because the wastewater will never be augmented with salinity before the incorporation of an additional treatment option at the waste-water treatment plant (WWTP) or before enhancement of the existing treatment.

In the TIE, it is very important that pH modifications be managed carefully with minimal acid and base solution addition in the treatment and neutralization processes. The investigator should have a broad understanding of what is happening ionically as part of the TIE manipulation. Methods using "mock effluent" meant to mimic only the TDS composition of the effluent without other toxicants are also useful in the TIE process where TDS toxicity is expected or anticipated.

6. What, if any, distinctions are necessary between acute and chronic toxicity in the TIE process and what limitations does either pose?

While specific TIE technical procedures have evolved over the last decade, the Panel felt there are no substantial technical distinctions between the acute and chronic TIEs that would prohibit successful use of either procedure. Though the chronic procedure can be assumed somewhat more difficult, time-consuming, and thus more expensive than the acute, these differences are manageable when compared to the usefulness of the chronic analysis.

7. What, if any, are the technical limitations of the TIE process?

The Panel believed that TIE effectiveness is limited primarily by the qualifications and experience of the practitioners and by technical limitations related to the current state of the science of TIE methodologies. TIEs can be successfully performed for either acute or chronic toxicity when toxicity can be reliably measured by using the relevant tests and species. One must distinguish between actual toxicity and "apparent" toxicity resulting from unusual data sets (e.g., statistical differences detected from unusually low variance or inverted exposure-response curves) that would indicate a biologically unimportant effect or an effect that would not translate from the laboratory into the field.

Much of the current knowledge about TIEs has come from the conduct of lethality tests of both freshwater- and marine-effluent TIEs. Toxicity identification evaluation techniques have been used with chronic estimator methods (short-term) though few changes or additions have been made to

even the growth or reproduction endpoint tests since USEPA provided the first TIE guidance. The application of TIE methods to marine and estuarine situations has been less common than freshwater, which is probably reflective of the vastly greater number of National Pollution Discharge Elimination System (NPDES) permits written for *Ceriodaphnia* and other freshwater species. It is logical, then, that the database on marine and estuarine TIEs is somewhat less comprehensive.

There are areas where TIE methods have not proven successful in identifying specific causative toxicants in effluents. These limitations can be broken down into 2 areas: (1.) limitations associated with inexperience, and (2.) limitations associated with the state-of-the science (i.e., the current methods are not effective.

Inexperience of laboratory staff is most often the cause of unsuccessful TIE studies. Laboratories frequently perform "abbreviated" Phase I TIEs and lose valuable and necessary information, thus leading to inconclusive results or erroneous conclusions. By excluding various manipulations, insight into the characteristics and effects of the toxicant can be missed and misdirect the TIE. The Phase I characterization was designed to gain as much information as possible about the nature of the toxicant. Because data interpretation is the most critical part of the TIE, expertise needed for data interpretation and analysis should be sought to improve the probability of success.

On the technical side, isolation of toxicants for characterization and chemical analysis becomes difficult with some types of effluents (e.g., petroleum refineries and pulp and paper mills with complex organic toxicants). Often, toxicity is lost or markedly reduced through the Solid Phase Extraction (SPE) and High Performance Liquid Chromatography (HPLC) steps described in the Phase II manual. In cases where toxicity is not completely lost, analysis with Gas Chromatograph/Mass Spectrometer (GC/MS) may result in the detection of numerous compounds, none of which can be considered a strong candidate for toxicity. This creates an often laborious task of chemical manipulation of samples to further extract or chemically separate observed peaks from the background "noise" of samples. There may be little or no literature describing the effects of individually isolated toxicants, and data may have to be developed ad hoc in order to interpret effluent test results.

This inability to separate and isolate causative toxicants is associated with analytical methods that are simply not sufficient to separate properly and to isolate toxicants. The separation chemistry problem is also encountered when dealing with other toxicants with complex speciation and toxicity. To complete TIE studies successfully, scientists must have a good environmental chemistry background or at least have access to individuals who do.

With the experience that has been gained since the updated USEPA guidance documents were published (USEPA 1991-1993), researchers have developed additional tools that can be used in the TIE process. Tools characterizing the toxicity associated with major ions (e.g., models and "mock effluent" studies) have been developed and have proven successful. Other issues have been investigated including interpretation of toxicity expressed in analytical blanks during chronic TIEs, comparing TIE with WET test methods, and the use of dual endpoints to gain better insight into the nature and cause of effluent toxicity. Knowledge gained should be incorporated into the TIE guidance to further assist the regulator and the regulated. Another effort of the SETAC Foundation/USEPA Cooperative Agreement is to develop an expert panel to highlight and summarize the current state of knowledge in these developing methods.

For more than 3,000 NPDES permits issued with WET requirements, *Ceriodaphnia dubia* is the most widely required species used in freshwater acute and chronic testing. Because there are far fewer marine dischargers, marine methods have lagged in comparison to those methods for freshwater. There is also a wider range of marine and estuarine species being broadly utilized, including different species for east and west coast tests. Additional insight on marine TIEs has been gained since the USEPA manuals were published. For example, we now have a better understanding that modifying

effluent salinity before or after TIE manipulation impacts the success of marine TIE methods and the development of control options. Additional research is needed to continue to improve TIE methodologies for marine and estuarine discharges. This relative difference in information on marine and estuarine TIEs may need to be considered when developing compliance schedules for marine and estuarine TREs.

Recommendations

From the experience acquired over the last 10 years by the members of the Panel, the following are recommendations that summarize discussions and conclusions of the February 1998 meeting:

1. Clear guidance should be developed for TREs and for the utilization of TIEs in that context (i.e., the utilization of TIEs conducted as parts of TREs). USEPA should promote broad educational opportunities for its regional staff and for state and tribal regulatory agencies so that nationally consistent TRE goals and practices are encouraged and supported. The Panel unanimously agreed that scarcity of experience among the regulatory community and the subsequent inconsistency of approaches creates many of the difficulties experienced by permittees. The WET training currently being conducted under the SETAC Foundation/USEPA Cooperative Agreement will be a substantial beginning for this educational process but should be further fostered by enhanced financial support for more widespread availability of the curriculum for permittees and regulators and subsequent development of advanced training opportunities.

2. Permittees and regulators should develop interactive TIE/TRE plans that outline expectations and include milestones that incorporate frequent communication between parties. Permittees should develop proactively contingency or "fire plans" that describe how to proceed with toxicity reduction activities in event of noncompliance. These plans should include outlines of proposals to select and retain experienced teams to resolve toxicity.

3. Competence and experience in conducting TREs and TIEs should be examined thoroughly by permittees who are seeking consultants to resolve toxicity problems. A large percentage of ineffective TRE work and wasted resources results from inexperienced individuals/firms attempting work for which they are not appropriately qualified or experienced.

4. USEPA should direct the compilation and dissemination of updated knowledge about TIE and TRE methods and develop procedures to provide such updates regularly. Through the cooperative agreement between USEPA and the SETAC Foundation, the compilation of current knowledge through development of a WET EAP is ongoing. USEPA should utilize this product as the foundation of its efforts.

It is not always important to identify specific toxicants in the TRE in order to develop adequate control options for dealing with effluent toxicity. Regulators and permittees should be aware that the TIE guidance documents are not meant to be an exhaustive list of TIE methods and that other procedures have been successfully utilized.

5. USEPA should develop and maintain some type of center and pool of expertise on WET and TIE/TRE issues, similar to its National Effluent Toxicity Assessment Center (NETAC) model of the past, where problem issues could be addressed. While the SETAC WET EAP program has and continues to prove its value with technical issues, national regulatory assistance is direly needed by regulatory agencies and permittees alike. The SETAC program is restricted to issues of science and cannot resolve policy or enforcement issues. Even more specifically, USEPA should provide, either internally or through contract, a limited number of experienced individuals tasked with review of difficult or unresolved TIEs/TREs who could provide direct guidance to regulatory agencies and permittees alike on individual cases.

6. The development of an "expert system" to assist with the interpretation of TIE/TRE results to assist

permittees, consultants, and regulators with evaluation of observations should be considered by USEPA.

7. TIEs are effective tools for characterizing aqueous toxicants when used by experienced scientists. The Panel emphasized that the procedures should be utilized in their entirety to retain best effectiveness and not abbreviated to look for preconceived toxicants. There is an apparent reluctance to utilize TIEs by many facilities because of their perceived expense or because they are considered as more advanced tools. TIEs are often cost-effective and should be utilized early in the TRE to guide additional work. TIE costs should be assessed as total program costs and not only as per test costs. Repeating the cheapest test may result in the most expensive overall program because of misdirected or wasted efforts. USEPA should consider documenting and disseminating information about TIE costs and effectiveness to help resolve misconceptions.

8. USEPA should support further development of methods to identify and resolve problem toxicants (e.g., non-pesticide organics) and ubiquitous toxicants (e.g., TDS, diazinon, and ammonia) and consider publication and evolution of TIE or treatment methods specific to these situations.

9. USEPA should rapidly make available all published USEPA WET, TIE, and TRE methods documents and policies via the world wide web and should develop a list-server for WET and TIE/TRE discussion to make current knowledge most widely available. Downloadable copies of the USEPA documents listed below should be placed on that web site. Other bibliographies and literature should be included as copyright allows.

Acronyms

TRE-Toxicity Reduction Evaluation
TIE -Toxicity Identification Evaluation
WET -Whole Effluent Toxicity
EAP -Expert Advisory Panel
SETAC -Society of Environmental Toxicology and Chemistry
SETAC Foundation -SETAC Foundation for Environmental Education
USEPA-United States Environmental Protection Agency

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