this issue:

Whole Effluent Toxicity Testing

SETAC**TIP***

*technical issue paper

Purpose: SETAC is a professional society with worldwide membership from academia, government, business, and nongovernmental organizations. TIPs provide a credible and balanced scientific discussion of important environmental issues.

What is Whole Effluent Toxicity (WET) testing?

Whole Effluent Toxicity (WET) testing is an important component of the U.S. Environmental Protection Agency's (USEPA's) integrated approach for detecting and addressing toxicity in surface waters. The National Pollutant Discharge Elimination System (NPDES) permit program, authorized by the Clean Water Act, controls water pollution by regulating point source discharges into waters of the United States. Point sources are discrete structures such as pipes or man-made ditches. The wastewater discharges from point sources are commonly called "effluents." Facilities must obtain permits for direct discharges to surface waters and, in many cases, for storm sewer systems. A permit specifies the conditions that must be met to discharge. Permits often include WET tests as a monitoring requirement and sometimes for compliance determination.

"WET" is a term used to describe the adverse effects or toxicity to a population of aquatic organisms caused by exposure to an effluent. This toxicity can be experimentally determined in the laboratory by exposing sensitive organisms (usually surrogate organisms representative of those found in the environment) to effluents using WET tests. Responses assessed usually include survival, growth, and/or reproduction. This type of test can be used to evaluate the toxicity of effluents, storm water, or ambient surface waters. WET testing is used to assess and regulate the combined effects of all constituents of a complex effluent rather than the conventional methods of controlling the toxicity of single chemicals or constituents.

WET testing exposes laboratory populations of aquatic organisms such as fish, invertebrates, and algae to diluted and undiluted effluent samples under controlled conditions in order to estimate the environmental toxicity of that sample. The information is used to prevent the discharge of toxic amounts of pollutants to surface waters. The standardized procedures of WET tests allow one to determine the actual environmental exposure of aquatic life to an effluent or ambient water without knowledge of the chemical, physical, and biological characteristics of that discharge or ambient water.

WET tests can be performed on a variety of commonly used test species under a variety of exposure periods and dilution regimes. Acute tests are conducted for a relatively short period and usually focus on how well an organism survives. Chronic tests are conducted for longer periods relative to certain organisms' life cycle or during a very sensitive life stage to evaluate survival, growth, and/or reproduction. Tests may be conducted on an undiluted sample to answer the question, "Is this sample toxic to this test species?" Often, testing includes the undiluted sample along with a series of dilutions of the sample to answer the question, "How toxic is this sample to this test organism?"

Some regulatory approaches focus on controlling toxic amounts of individual chemicals known to be present in the effluent. In contrast, WET testing actually measures the potential toxicity of all chemicals in a solution. Furthermore, WET testing may show that chemicals known to be toxic to aquatic organisms may be rendered non-toxic by particular characteristics of the effluent matrix and/or receiving stream chemistry. When receiving water from a stream, lake, or river is used as the dilution water for an effluent sample in WET testing, the test can account for changes in quality of a discharge as the discharge dilutes and mixes with the receiving water. The synthesis of WET testing results, along with chemical analyses and other information, can provide a more comprehensive and realistic picture of potential effects of discharges into aquatic systems. No other water quality control tool has this capability.

How did WET testing get started?

Aquatic toxicologists realized a number of years ago that it was not possible to test all chemicals and possible combinations of chemicals that may occur in certain effluents. This, coupled with the fact that there were few toxicity data for the vast majority of chemicals in commerce, led to the testing of toxicity in effluents and streams influenced by industrial and wastewater treatment facilities. These toxicity analyses were conducted in conjunction with chemical analyses to link measurements of toxicity with specific chemicals when possible. As toxicity tests were refined, more formalized laboratory tests were developed that included multiple dilutions and treatments. Methods and species became standardized, allowing for comparison among tests and aqueous samples.

Through these efforts, the science of effluent assessment moved forward. Biomonitoring approaches (i.e., sampling and identification of aquatic populations living in a body of water) were added to chemical-specific and WET regulations to provide additional information on water quality in aquatic ecosystems. Aquatic toxicologists have continued to refine WET tests that are now used for effluent and stormwater quality monitoring. The tools are now widely applied to ambient stream samples to detect not only point source effects but also the cumulative effects of non-point sources of pollution like urban or agricultural runoff.

What are some causes of toxicity?

Toxicity observed in WET testing can be caused by a number of factors that can act independently or jointly. These generally include the following:

- Chemical Factors
  - Inorganic chemicals (e.g., ammonia, chlorine, and heavy metals)
  - Organic chemicals (e.g., dioxins, polychlorinated biphenyls, and surfactants)
  - Pesticides (e.g., chlorpyrifos, diazinon, and heptachlor).
- Physical Factors
  - Dissolved and suspended solids
  - Temperature.
- Biological Factors
  - Bacteria, fungi, and parasitic invertebrates.

Toxicity observed in effluents has been attributed to several chemicals commonly found in or added to wastewaters, including chlorine used for disinfection and ammonia created by the breakdown of organic substances in domestic wastewater. Some chemicals, known as “biocides” (e.g., chlorine), are intentionally added to control nuisance biological growth in plumbing or cooling water systems. When discharged, these chemicals can retain their toxic characteristics and may be harmful to aquatic life in receiving streams. Domestic wastewater systems receive many household chemicals that are improperly disposed of, including organic solvents and pesticides. Some commonly used soaps and detergents, particularly some commercial detergents, have proven highly toxic if inadequately treated prior to discharge. Recently, it has been established that a number of elements and compounds commonly found in aquatic ecosystems can be toxic to aquatic organisms when present in concentrations above or below an organism’s physiological tolerance level. Ions such as potassium, magnesium, and calcium, all of which are essential elements used by organisms, can be toxic when the ions are added or taken out of water during various industrial processes. A separate SETAC Technical Issue Paper (TIP) discusses the ion imbalance problems encountered in WET testing.

How are WET tests conducted?

Several standardized methods are commonly used to conduct WET tests. The specific method selected can be influenced by the exposure regime to be tested, the nature of the receiving water body, the resident species in the stream, and a variety of other factors. Most WET tests consist of the following basic procedures:

Aquatic test organisms are placed in test containers (e.g., beakers for fish and invertebrates or flasks for algae) that usually contain a series of concentrations of a sample. Tests usually include 100% sample and sample mixed with various amounts of dilution water (control water containing no sample) to form a series of sample dilutions. Observations of the organism’s response, such as mortality, reduction in growth, and/or lack of reproduction, are made at specific time intervals. The duration of the test may range from periods as short as 40 minutes up to 7 days depending on the organisms used and whether acute or chronic effects are of interest. At the end of the test, the results are used to estimate the toxicity of the sample.

In addition to the actual conduct of the WET tests, a number of other considerations are also important. These include the following:

- Health and safety of personnel conducting the tests
- Quality assurance to affirm that the tests meet acceptable performance criteria
- Adequate facilities, equipment, and supplies to conduct the tests;
- Proper methods for the tests and organisms being used in the tests
- Proper dilution water for dilutions of the effluent samples
- Correct test endpoints to estimate the sample effect concentration
- Proper statistical analysis of the data to determine the effect concentration for the endpoints being used

- A report that clearly summarizes the results of the WET test.

What organisms are used for WET testing?

Organisms currently used for toxicity testing in the United States are usually cultured under carefully controlled laboratory conditions. It is important to fully understand the environmental requirements and tolerances of the test species. In some cases, test organisms are collected from field sites for WET testing. Consideration of the receiving water characteristics (e.g., salinity) is important in selection of the test species. The sensitivity of an organism to various pollutants is also an important consideration in the selection of the test organisms. WET test organisms include both animals (vertebrates and invertebrates) and plants. The following are examples of organisms frequently used in WET testing:

- Freshwater organisms
  - Fish (e.g., fathead minnow [*Pimephales promelas*])
  - Invertebrates (e.g., daphnids [*Ceriodaphnia dubia*, *Daphnia magna*, and *D. pulex*])
  - Algae (e.g., green alga [*Raphidocelus subcapitata*, formerly *Selenastrum capricornutum*]).
- Saltwater organisms
  - Fish (e.g., sheepshead minnow [*Cyprinodon varigates*] and inland silverside [*Menidia beryllina*])
  - Invertebrates (e.g., mysid [*Americamysis bahia*, formerly *Mysidopsis bahia*] and sea urchin [*Arbacia punctulata*])
  - Algae (e.g., red alga [*Champia parvula*]).

How are WET tests used?

Information gained from WET tests is used to evaluate the impact of the effluent sample on survival, growth, reproductive capacity, and normal development of the test population, and that information is then extrapolated to the receiving system. The data provide an estimate of the concentration above which detrimental impact from the effluent would be predicted to occur in the receiving stream. One of two basic statistical approaches is used to analyze the data. One method compares the results of a test dilution with non-toxic reference water to identify significant reductions in an organism's response in the test dilution. The other approach is to mathematically model the relationship between the sample concentration and the response of the test organisms. The model is then used to identify the sample concentration that causes a pre-selected reduction in response (e.g., 25% reduction in growth or 50% reduction in survival). Both methods are supported by the USEPA, but the regulatory agency responsible for each permit selects which method is to be used to evaluate WET results.

Once the toxicity of a sample is estimated, the result is then compared to the water quality standard established for toxicity to determine if sample quality is acceptable. In general, consistent toxicity observed in samples from the same source over time will result in a need to use a Toxicity Identification Evaluation (TIE) procedure to identify the effluent characteristics causing toxicity. Effective approaches to reduce the toxicity are outlined and evaluated using a Toxicity Reduction Evaluation (TRE) procedure once the discharge characteristics causing toxicity are identified. When an effective toxicity reduction approach is confirmed, it can be implemented to bring the discharge quality back into compliance with the water quality standard.

Where is there more information about WET testing?

Many sources are available for information on WET testing. The SETAC website http://www.setac.org/wetindex.html can be used to obtain additional information including Frequently Asked Questions on WET issues. SETAC also provides training opportunities on WET testing. The training course schedule is posted on the website listed above. The literature listed below will help with further understanding of WET testing from a scientific and regulatory perspective:

Bergman HL, Kimerle RA, Maki AW. 1986. Environmental hazard assessment of effluents. Elmsford NY, USA: Pergamon. 390 p.

Grothe DR, Dickson KL, Reed-Judkins DK, editors. 1996. Whole effluent toxicity testing: An evaluation of methods and prediction of receiving system impacts. Pensacola FL, USA: SETAC. 372 p.

Oris J, Klaine S, editors. 2000. Whole effluent toxicity testing. *Environ Toxicol Chem* 19:1-255.

[SETAC] Society of Environmental Toxicology and Chemistry. Applications of TIEs/TREs to whole effluent toxicity: Principals and guidance [online document]. 1998. Pensacola FL, USA: SETAC. Available from: http://www.setac.org/wettre.html. Accessed 1 Mar 2004.

[SETAC] Society of Environmental Toxicology and Chemistry. 2002. Whole Effluent Toxicity (WET) Technical Reference CD-ROM [CD-ROM]. Pensacola FL, USA: SETAC.

[SETAC] Society of Environmental Toxicology and Chemistry. 2004. Technical issue paper: Whole effluent toxicity testing: Ion imbalance. Pensacola FL, USA: SETAC. 4 p.

Sprague JB. 1973. The ABC's of pollutant bioassay using fish. In: Cairns Jr J, Dickson KL, editors. Biological methods for the assessment of water quality. Philadelphia PA, USA: American Society for Testing and Materials (ASTM). STP 528. p 6–30.

[USEPA] U.S. Environmental Protection Agency. 1985. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. 3rd ed. Washington DC, USA: USEPA. EPA-600-4-8-5-013. 216 p.

[USEPA] U.S. Environmental Protection Agency. 1993. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. 4th ed. Washington DC, USA: USEPA. EPA-600-4-90-027F. 273 p.

[USEPA] U.S. Environmental Protection Agency. 1994. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 3rd ed. Washington DC, USA: USEPA. EPA-600-4-91-002. 334 p.

[USEPA] U.S. Environmental Protection Agency. 1995. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to west coast marine and estuarine organisms. 1st ed. Washington DC, USA: USEPA. EPA-600-R-95-136. 15 p.

[USEPA] U.S. Environmental Protection Agency. 1999. Errata for effluent and receiving water toxicity test manuals: Acute toxicity of effluents and receiving waters to freshwater and marine organisms; Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms; and Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. Washington DC, USA: USEPA. EPA-600-R-98-182. 17 p.

Information on other selected WET documents from the Society of Environmental Toxicology and Chemistry (SETAC), including articles from SETAC's scientific journal *Environmental Toxicology & Chemistry* (*ET&C*), volumes 16 through 18, is available on SETAC's webpage (http://www.setac.org).

Acknowledgments

SETAC is grateful for the efforts and contributions of everyone involved in the publication of this TIP: Larry Ausley, Dennis Burton, Debra Denton, Phil Dorn, Bill Goodfellow, Joe Gully, Scott Hall, Phil Jennings, Matt Matthews, Teresa Norberg-King, Jim Pletl, John Rodgers, Tom Waller, and Barbara Albrecht.

About this document

When referring to this text, please use the following citation: [SETAC] Society of Environmental Toxicology and Chemistry. 2004. Technical issue paper: Whole effluent toxicity testing. Pensacola FL, USA: SETAC. 4 p.

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Society of Environmental Toxicology and Chemistry

In the 1970s, no forum existed for interdisciplinary communication among environmental scientists—biologists, chemists, toxicologists—and others interested in environmental issues such as managers and engineers. The Society of Environmental Toxicology and Chemistry (SETAC) was founded in 1979 to fill the void. Based on the growth in membership, annual meeting attendance, and publications, the forum was needed.

Like many other professional societies, SETAC publishes an esteemed scientific journal (*Environmental Toxicology & Chemistry*) and convenes an annual meeting replete with state-of-the-science poster and platform presentations. Because of its multidisciplinary approach, however, the scope of the science of SETAC is much broader in concept and application than that of many other societies.

SETAC is concerned about global environmental issues. Its members are committed to good science worldwide, to timely and effective communication of research, and to interactions among professionals so that enhanced knowledge and increased personal exchanges occur. Sister organizations in Europe (1989), Asia/Pacific (1997), and Latin America (1999) have been formed, and the nonprofit SETAC Foundation for Environmental Education was founded in North America in 1990. International acceptance of the SETAC model continues with widespread interest in Russia and Africa.

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