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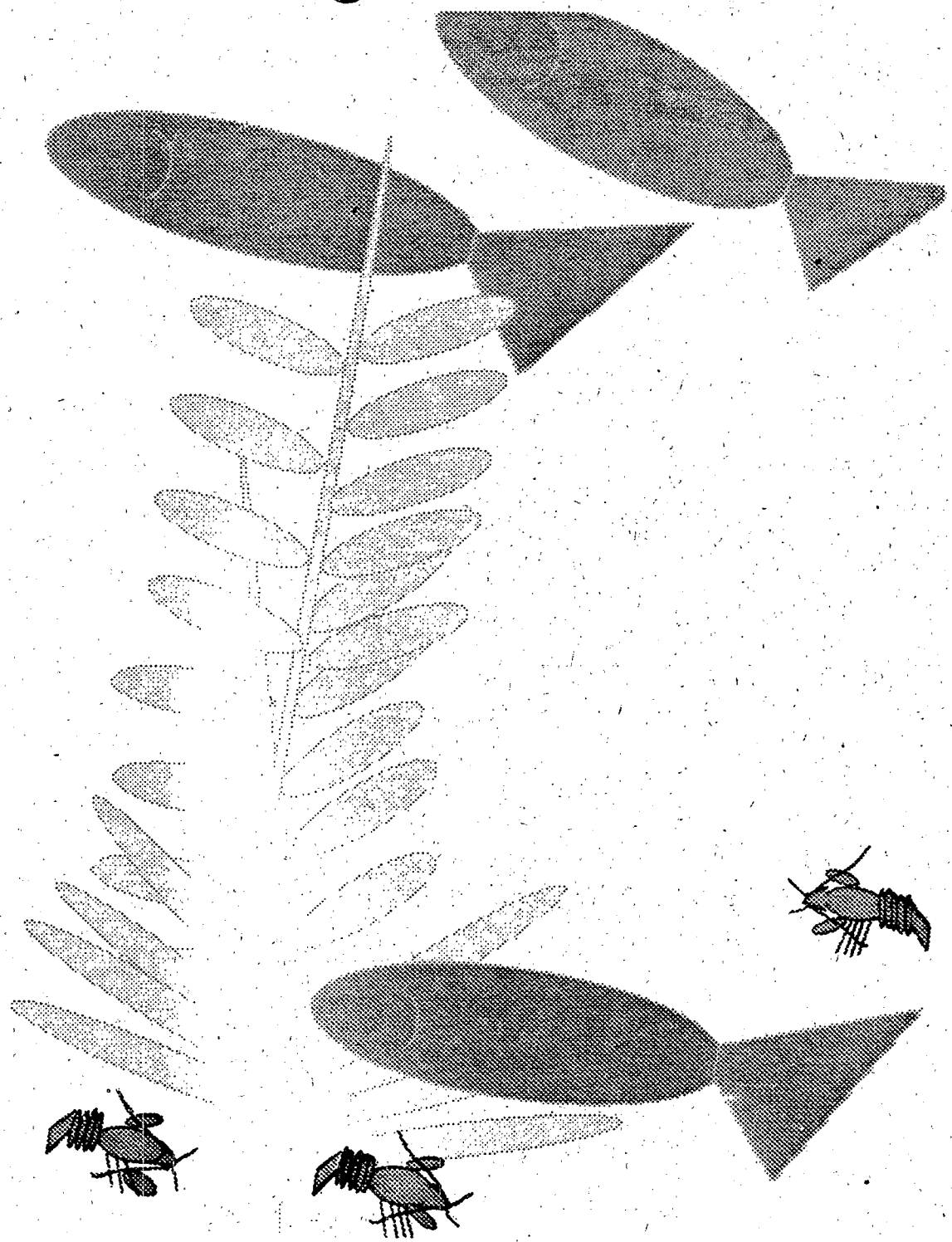
Introduction To Water Quality–Based Toxics Control For The NPDES Program



Introduction to Water Quality-Based Toxics Control for the NPDES Program

U.S. Environmental Protection Agency

Enforcement Division
Office of Wastewater Enforcement and Compliance
Enforcement Branch Support Branch
March, 1992

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
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ACKNOWLEDGEMENTS

The Office of Wastewater Enforcement and Compliance (OWEC), in its constant effort to provide current guidance, training, and methods for program priority areas to Regional and State staff, often finds it necessary to produce "user friendly" versions of its technical materials. In response to the need for these supplementary educational materials, the Enforcement Support Branch (ESB) of OWEC has developed Introduction to Water Quality-Based Toxicity Testing for the NPDES Program.

This document is written for non-biologists and other staff unfamiliar with water quality-based (WQB) permitting and enforcement and whole effluent toxicity (WET) testing. Many thanks must go to those who have reviewed and commented to assure the document's scientific accuracy, consistency with the Technical Support Document for Water Quality-based Toxics Control (TSD), and focus on a layman audience:

Sheila Frace, Industrial Section, Permits Division, OWEC
Mary Reiley, Enforcement Support Branch, Enforcement Division, OWEC
Bill Peltier, Environmental Services Division, Region IV
Margarete Heber, Ecological Risk Assessment Branch, Office of Science and Technology
Ted Coopwood, Enforcement Support Branch, Enforcement Division, OWEC
Jim Pendergast, Water Quality and Industrial Permitting Branch, Permits Division, OWEC

This document is not guidance, policy, or regulation. Its sole function is to provide the necessary introduction to the basic concepts of the WQB - WET program and ease the transition to technical guidance and methods. Comments and questions are welcome. Address comments or questions to Mary Reiley, Enforcement Support Branch (EN-338), 401 M Street, S.W., Washington, D.C. 20460.

Richard G. Kozlowski, Director

This guide was prepared by the Cadmus Group, Inc. for the U.S.Environmental Protection Agency, Office of Wastewater Enforcement and Compliance through Contract No. 68-C9-009 and Work Assignment No. S-3-7(R). The Project Manager was Michael J. Dover, Ph.D., and the author was Joan S. Jolly, Ph.D.

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Introduction to Water Quality-Based Toxics Control for the NPDES Program

The U.S. Environmental Protection Agency (EPA) and designated State agencies are responsible for enforcing the Federal Water Pollution Control Act of 1972, as amended by the Clean Water Act (1977, 1981) and the Water Quality Act of 1987. Collectively, these are usually known as the Clean Water Act, or CWA. The Clean Water Act's overall objective is "to restore and maintain the chemical, physical, and biological integrity of the nation's water." EPA is authorized by the CWA to regulate the discharge of substances into the waters of the United States. To this end, EPA issues permits specifying the conditions under which a facility may discharge effluents into a body of water. The agency also tracks compliance with these permits by requiring permittees to monitor their effluents. A primary goal of this monitoring is to ensure that, in keeping with the law, no discharges contain "toxic pollutants in toxic amounts."

EPA makes use of both chemical and biological monitoring in evaluating compliance with permits. Chemical monitoring checks water for the presence and concentration of substances. Biological monitoring, also called **biomonitoring**,[1] makes use of living organisms to monitor water quality.[2] Biomonitoring includes such monitoring as toxicity testing and bioassessment (which evaluates the condition of a body of water by studying its resident organisms).

This Guide focuses on a principal means of biomonitoring: **whole effluent toxicity (WET)** testing. The legal definition of whole effluent toxicity is the "aggregate toxic effect of an effluent as measured directly by a toxicity test." In plainer language, whole effluent toxicity testing evaluates the toxic effects of effluents on living organisms. The Guide presents whole effluent toxicity in the broader context of the scientific necessity to regulate water quality and EPA's legal authority to do so. The Guide then describes whole effluent toxicity in greater detail and discusses practical aspects of toxicity testing, from collecting samples to reporting results. Finally, the Guide covers the subject of maintaining compliance with a permit that has a limit on whole effluent toxicity.

Whole Effluent Toxicity Testing evaluates the toxic effects of effluents on living organisms.

The Scientific Necessity to Protect Water Quality

What does it mean to say that a pollutant or effluent is toxic? **Toxicity** has two characteristics. First, toxicity is a harmful effect occurring in a human, other animal, plant, or microbe as a result of a chemical substance. This adverse effect can take many forms: disease, deformity, behavioral changes, reproductive malfunction, or genetic damage. For example, certain pesticides contain chemicals called organophosphates. These compounds break down an animal's neurotransmitters—substances that the animal produces to regulate the transmission of impulses along the nerves. In the absence of neurotransmitters, nerves fire continuously, causing the animal to suffer convulsions and death. Second, toxicity is a direct, rather than an indirect, result of a chemical substance or mixture of substances. The organophosphates in pesticides are toxic because they cause direct harm to organisms.[3]

[1] Terms included in Appendix D, the Glossary, are extra bold the first time that they appear in the text.

[2] U.S. Environmental Protection Agency. *Technical Support Document (TSD) for Water Quality-based Toxics Control* (EPA/505/2- 90-001), 1991. p. xix.

[3] Suspended matter in water is an example of a substance that is harmful but not toxic because its effect on organisms is indirect. Suspended matter limits the amount of light that penetrates water, thereby reducing the rate of photosynthesis— the process by which plants convert carbon dioxide, water, and energy from the sun into usable food. The reduced rate of photosynthesis in turn adversely affects algae by lowering their growth rate.

Figure 1 Bioaccumulation and Bioconcentration

BIOACCUMULATION = UPTAKE THROUGH FEEDING + BIOCONCENTRATION

- Absorption Through The Outermost Layer (such as an animal's integument or a plant's cell wall)
- Uptake Through Respiration

When toxic substances occur in water, these substances can adversely affect all the organisms that live there: microorganisms, plants, insects, worms, shrimp, other invertebrates, fish, amphibians, waterfowl, and such mammals as beavers and otters. Toxic substances that settle into the sediment can also damage organisms living there. Some toxic substances cause immediate harm to organisms, but others cause effects over a longer period of time. In some cases a toxic substance brings about a long-term effect as a result of **bioaccumulation**, the passage of a substance from the environment into living tissues. Bioaccumulation occurs by two routes: feeding and bioconcentration. (See Figure 1.) **Bioconcentration** is the passage of a substance from water into an organism. For a fish, then, bioconcentration can occur by absorption through the skin or by uptake through the gills. A substance that bioaccumulates reaches higher concentrations in living tissues than occurs in the surrounding water. The Regional Environmental Services Divisions (ESDs) can advise regulatory personnel as to which substances tend to bioaccumulate and which do not.

If all the members in a **food chain** bioaccumulate a substance, the stage is set for biomagnification. (See Figure 2.) **Biomagnification** is the process of a substance's passing up the food chain and becoming concentrated in the tissues of the organisms toward the top of the food chain. For example, suppose aquatic vegetation in a polluted river bioconcentrates a toxic substance and crayfish then feed on the vegetation. The crayfish too become contaminated but at a higher concentration because each crayfish consumes a quantity of vegetation. A fish that feeds on these crayfish also becomes contaminated, and at a still higher concentration because the fish eats several crayfish. Hence, a chemical that bioaccumulates in each

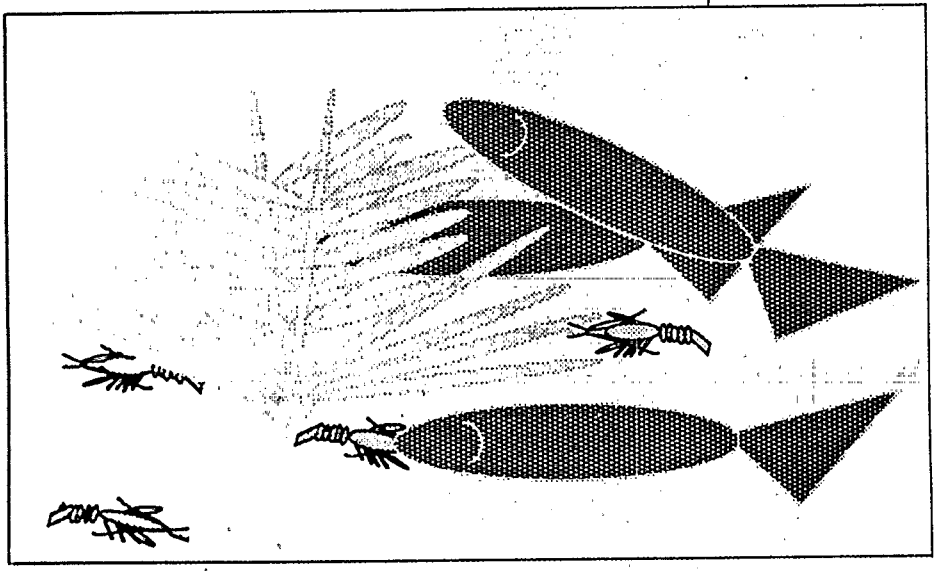

Figure 2 Biomagnification of a Toxic Substance

In this picture the shading of the water represents a toxic substance that vegetation, crayfish, and fish bioaccumulate. These organisms make up a food chain, with the crayfish feeding on the vegetation and the fish feeding on the crayfish. Consequently, biomagnification of this toxic substance—represented by the increasingly darker shading of the vegetation, the crayfish, and the fish—occurs as the substance passes up the food chain.

member of a food chain may pose more of a risk to animals higher in the food chain than to those lower down the chain.

How do toxic substances enter bodies of water? Many types of events can introduce toxic substances into ponds, lakes, rivers, and streams. Pollutants can leach from contaminated soils. For example, if a farmer sprays insecticide on a field, rain may wash the insecticide into nearby waters, polluting them. This type of discharge constitutes a non-point discharge, because the discharge's source is broad. In addition, sewage treatment facilities and industrial plants can pollute waters by discharging effluents into them through pipes, ditches, channels, or tunnels. Discharges from such specific locations are called **point source discharges**, which are the primary target of the toxicity testing activities described in this Guide.

However, regulators should keep in mind that CWA's definition of point source discharges (40 CFR 122.2, also section 122.3) includes discharges that do not have such a highly localized source. For example, discharges from a concentrated aquatic animal production facility constitute point source discharges, as do the discharges from combined sewer overflows and discharges of storm water. In deciding whether a discharge is a point source or a non point source, regulators should consult the cited federal regulations.

The Legal Authority to Protect Water Quality

Since EPA's authority to protect water quality originates with the CWA, an understanding of the Act will clarify EPA's role. (See Appendix A for excerpts from the CWA.) The CWA has as one goal the policy "that the discharge of toxic pollutants in toxic amounts be prohibited." The Federal regulations' "free froms" summarize this statement well: water should be free from pollutants that settle to cause objectionable deposits; float, such as debris, scum, and oil; cause objectionable odor, color, taste, or turbidity; cause injury or toxic effects to humans, animals, or plants; and cause population by undesirable or nuisance aquatic life. To make its goal a national one, the CWA requires States to include in their Water Quality Standards narrative statements addressing each of the "free froms."

In addition to describing what constitutes clean water, the CWA states that no one may discharge a pollutant into water unless the discharge is in compliance with the Act. The CWA protects public health, water supplies, and wildlife in part by mandating limitations on point source discharges. Toxicity constitutes one of the parameters that CWA limits in point source discharges.[4]

Federal regulations state that water should be free from pollutants resulting in

- **objectionable deposits;**
- **floating material, such as debris, scum, and oil;**
- **objectionable odors, colors, tastes, and turbidity;**
- **harmful effects to humans, animals, or plants;**
- **undesirable or nuisance aquatic life.**

National Pollutant Discharge Elimination System (NPDES) Permit Program

To accomplish its goals, several sections of the Clean Water Act give EPA the authority to restrict and monitor the discharge of pollutants, including toxic substances, into the nation's waters. To this end, EPA has established the National Pollutant Discharge Elimination System (NPDES) permit program, which sets guidelines for issuing permits to facilities that produce effluents they wish to discharge into national waters.

Any facility discharging effluents into the water must have a permit. Permits limit the concentration of pollutants in effluents. In addition, permits must establish limits to an effluent's toxicity in cases where a discharge may result in a violation of water quality standards. When permit limits are based on existing wastewater treatment technologies, EPA documents refer to them as **technology based**. In some cases a permit limit aims at attaining a specified level of water quality without regard to existing treatment technologies. In such a case EPA documents refer to the permit limit as **water quality based**.

In addition to the technology based limits described above, an NPDES permit issued to a discharging facility aims at safeguarding the State's **Water Quality Standard (WQS)**. This standard establishes for a body of water the maximum in- stream pollutant concentrations compatible with both the water's assimilative capacity and its desig-

[4] In *Natural Resources Defense Council, Inc. v. EPA*, 859 F.2d 156 (D.C. Cir. 1988), the Court concluded that EPA has the authority to express permit limitations in terms of toxicity provided that the limits reflect requirements of CWA, as described in 40 CFR 125.3(c)(4). The Court held that the CWA's broad definition of "pollutant" in section 502(6) authorizes the use of toxicity to regulate effluents, even though toxicity is an attribute of pollutants rather than a pollutant itself.

nated use. To this end, an NPDES permit specifies an effluent's contents, frequency, and site of discharge. (See Appendix B for excerpts from NPDES permits.) It also controls the effluent's concentration and mass (in lbs/day) by setting a maximum daily limit, a monthly average limit, and for publicly owned treatment works a seven-day average limit for the effluent. The **maximum daily limit (MDL)** is the highest value allowable for a discharge during a 24-hour period. The more restrictive **average monthly limit (AML)** consists of the highest value allowable for the average of daily discharges occurring over a one-month period.

An NPDES permit written to protect water quality derives the MDL and the AML from two other models: the wasteload allocation and the long term average. The **wasteload allocation (WLA)** is the maximum amount of effluent that the receiving water can assimilate in a day from a permitted facility without violating the WQS. The WLA is set to achieve the WQS. The **long-term average (LTA)** represents the acceptable mean of an effluent's pollutant concentrations or parameters. The LTA takes into account the variability in a facility's effluent, so that there is 99% probability that the WLA will not be exceeded. Together the five models and model outputs of concern to most NPDES permittees—WQS, WLA, MDL, AML, and LTA—make up a hierarchy that protects the receiving water (Figure 3).

The Integrated Approach to Water Quality-Based Toxics Control

As well as mandating regulation of discharges, the Clean Water Act also specifies that EPA shall measure the health of waters by means of three methods:

- *Analyzing the chemical content of waters.* This type of monitoring, called **chemical-specific testing**, is accomplished by subjecting samples to laboratory tests that identify chemical substances and measure their concentrations.

- *Studying organisms inhabiting the receiving water.* This is achieved through biological assessment, generally called **bioassessment**. Bioassessment evaluates the biological condition of a body of water by studying its biota, which are its resident organisms, and its chemical and physical characteristics. Specific means of bioassessment include surveying biota and measuring biological criteria to determine whether a pollutant has had an adverse effect on the biota.

- *Testing effluents for toxic effects on living organisms.* This is accomplished principally through **whole effluent toxicity testing**.

Figure 3 Relationship Between Daily Concentrations, Long-term Averages, Wasteload Allocations, and Permit Limits

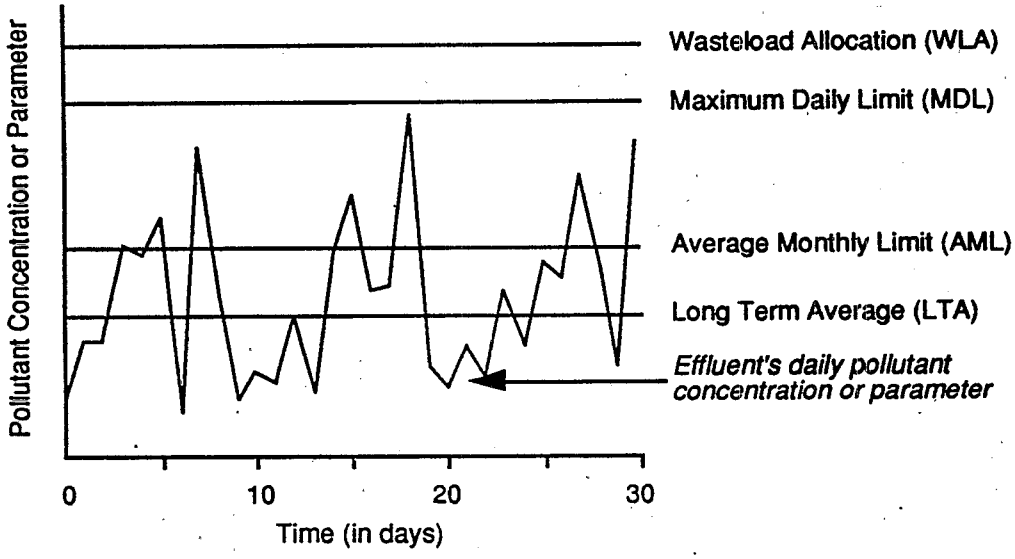

Table 1.
The Integrated Approach:
Advantages and Disadvantages of Each Method

METHOD	ADVANTAGES	DISADVANTAGES
CHEMICAL-SPECIFIC TESTING	• Precise • Complete toxicology • Information about human health • May know fate of substance and/or how to treat it • Prevent impact • Inexpensive where effluent contains only a few toxics	• Expensive where effluent contains many toxics • Bioavailability unknown • May not consider all toxics present • Misses interactions between toxics • Does not measure ecological effects
BIOASSESSMENT	• Measures ecological effects • Shows historical trends	• Impact has already occurred • Does not specify source of damage • Difficult to interpret • May not evaluate effects of variation in flow rate • No information about human health
WHOLE EFFLUENT TOXICITY TESTING	• Total toxicity • Does not require detailed knowledge of chemical nature of substances in an effluent • Only bioavailable toxics measured • Prevent impact	• Specific toxics not known • No information about treatment, persistence, or presence in sediment • Toxicity may differ in ambient conditions • No information about human health

Taken together, these three methods are referred to as the **integrated approach** to monitoring water quality. Each method contributes specific types of information to an evaluation of water quality, and all three are required for a complete evaluation of the biological condition of a body of water. (See Table 1.)

An NPDES permit establishes by means of limits how the permittee will meet WQS. When setting water quality-based limits and determining compliance, EPA uses the principle of **independent application** of WQS. This principle states that no one of the three methods is inherently superior to the other two in evaluating the health of water. Rather, as Table 1 indicates, each method contributes to the analysis, and each also has its limitations. As a result, data collected using one method should not be used to contradict or overrule data obtained with either of the other two. Specifically, if results of any one method show an impairment of water quality, then EPA believes that an impairment may exist.

Capabilities and Limitations of Whole Effluent Toxicity Testing

Whole effluent toxicity testing measures the total toxic effect of an effluent by means of tests that expose living organisms to that effluent and note the effects of the effluent on these organisms. Several studies have demonstrated that results obtained with testing for whole effluent toxicity are reproducible within a laboratory and also between laboratories to the same degree as is found for chemical-specific testing.[5] Studies also demonstrate that whole effluent toxicity correlates well with the observed impact on receiving water.[6]

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[5] A Review of Inter- and Intralaboratory Effluent Toxicity Test Method Variability (W.J. Rue, J.A. Fava, and D.R. Grothe. 1988. *Aquatic Toxicology and Hazard Assessment: 10th Volume.* ASTM STP 971).

A Perspective on Biological Assessments (D.R. Grothe, R.A. Kimerle, and C.D. Malloch. 1990. *Water Environment and Technology*).

Results: Interlaboratory Comparison of Acute Toxicity Tests Using Estuarine Animals (S.C. Schimmel. 1981. EPA-600/4-81-003).

[6] *Biomonitoring to Achieve Control of Toxic Effluents* (U.S. EPA. 1987 EPA 625/8-87/013).

Examining the Relationship Between Ambient Toxicity and Instream Impact (K.L. Dickson, W.T. Waller, L.P. Ammann, and J.H. Kennedy. 1991. Submitted to: *Env. Toxicol. and Chem.*).

Comparison of Measured Instream Biological Responses with Responses Predicted by *Ceriodaphnia* Chronic Toxicity Tests (K.W. Eagleson, D.L. Lenat, L. Ausley, and F. Winborne. 1990. *Env. Toxicol. and Chem.* 9: 1019-28).

A Comparative Ecological and Toxicological Investigation of a Secondary Wastewater Treatment Plant Effluent and its Receiving Stream (W.J. Birge, J.A. Black, T.M. Short, and A.G. Westerman. 1989. *Env. Toxicol. and Chem.* 8: 437-50).

Testing for whole effluent toxicity provides a way to evaluate an effluent in the absence of detailed information about the chemicals it contains. Using this method, then, facility personnel or lab technicians can measure the toxicity of an effluent without knowing all of its components, which ones are toxic, and whether components interact in ways that alter their toxicity. Because whole effluent toxicity testing uses living organisms to detect pollutants, it also has the advantage of measuring the effects of only those toxic substances that are **bioavailable**, or present in a form that can affect organisms. On-going whole effluent toxicity testing can alert regulators should an effluent's effects become more damaging.

Whole effluent toxicity testing provides a way to evaluate an effluent in the absence of detailed information about the chemicals it contains.

Testing for whole effluent toxicity also has its limitations. Because this method does not specify which substances in an effluent are toxic, it gives no indication of how to treat the effluent's toxicity. Furthermore, without knowledge of specific chemical substances, a toxicity test provides no information about protecting human health. Since toxicity tests involve a limited number of species, results give only a partial toxic profile of an effluent. In addition, whole effluent toxicity testing does not indicate how long toxicity persists in water or whether sediment has become toxic as well. Finally, toxicity tests may not take into account changes in toxicity that can result from environmental changes, such as water temperature or acidity.

Scientific Concepts in Toxicity Testing

Toxicologists test for whole effluent toxicity by means of toxicity tests. These involve exposing a designated species of live organisms, called the **test organisms**, to an effluent and to dilutions of that effluent. Toxicity tests include two types: acute and chronic. (See Table 2.)

Acute Toxicity Tests

Acute toxicity tests last no more than 96 hours and measure an effect occurring in this short time period. Generally, acute toxicity tests measure an effluent's lethality. Results from an acute toxicity test indicate the effluent concentration at which a certain percentage of the organisms died. This concentration, which is referred to as the **Lethal Concentration (LC)**, is generally followed by the percentage of test organisms killed. For example, if a certain concentration of an effluent causes 50 percent of the organisms in the test to die, then this concentration is the effluent's LC_{50}.

To conduct an acute toxicity test, lab technicians expose groups of test organisms to different concentrations of effluent. Lab technicians prepare these concentrations by diluting effluent with uncontaminated water adjusted to meet the test organism's needs (such as for salinity or hardness). An acute toxicity test must also include a group of organisms subjected to the same conditions as the other test organisms but exposed to diluting water only. Since these organisms are not exposed to effluent, any deaths that occur among them do not result from toxic substances that might be in the effluent. Such a group, which receives no exposure to the factor being tested, is called a **control**. For data from an acute toxicity test to be valid, the control group must have at least 90 percent survival. (The Guide discusses quality control more fully in the section entitled "Compliance with Whole Effluent Toxicity Limits.")

EPA has published a manual that presents the Agency's approved protocols for acute toxicity tests.[7]

[7] U.S. EPA. 1991. *Methods for Measuring the Acute Toxicity of Effluents to Aquatic Organisms*, 4th Edition. Office of Research and Development, Cincinnati, OH. EPA-600/4-90-027.

Table 2.
Acute vs. Chronic Toxicity Tests

TRAIT	ACUTE	CHRONIC
Duration	Up to 96 hours	Partial or Full Life Cycle of Test Organism
Measurement	Death, generally	Death or Sublethal Effect (such as decrease in growth or reproduction)

Chronic Toxicity Tests

Unlike acute tests, **chronic toxicity tests** may continue for as long as the entire life cycle of the test organism. Shorter chronic tests last seven days or even less. Such tests, called **short-term chronic toxicity tests**, focus on the period in a test organism's life cycle when it shows the greatest sensitivity to its environment.

In chronic toxicity tests, lab technicians record the death of test organisms but also monitor other effects, such as fertilization, growth, and reproduction. Toxicologists use these tests to determine the lowest tested concentration at which organisms show an adverse effect from their exposure to effluent. This concentration is called the **Lowest Observed Effect Concentration (LOEC)**. Another important concentration is the highest tested concentration displaying no effect on the organism, called the **No Observed Effect Concentration (NOEC)** or the **No Observed Effect Level (NOEL)**. The LOEC or NOEC (NOEL) is determined by a statistical procedure called hypothesis testing and can vary considerably depending on the specific series of dilutions used in the test. Chronic toxicity tests may also report a presumably safe concentration called the **Chronic Value (ChV)**, which lies between the LOEC and the NOEC. Specifically, the ChV represents the geometric mean of the LOEC and the NOEC.

An alternative to an LOEC or an NOEC is a parameter called an **Inhibition Concentration (IC)**. An IC indicates the concentration of effluent that inhibits a biological process, such as reproduction, by a specified percent. Since some studies show that NOECs determined by hypothesis testing are analogues of IC_{25}s, regulatory agencies may want to stipulate that permittees use IC_{25}s rather than LOECs or NOECs.[8]

The basic set-up for a chronic toxicity test is much the same as that for an acute toxicity test. Lab technicians expose groups of test organisms to different effluent concentrations, which are prepared by diluting effluent with an appropriate diluting solution. As with an acute toxicity test, a chronic toxicity test must include a control. In order for a chronic toxicity test to be valid, the organisms in the control group should have a minimum survival of 80 percent and should achieve an acceptable level for the effect being measured, such as growth or reproduction. The EPA manuals that present the approved protocols for short-term chronic toxicity tests specify the acceptable effect levels for each specific test. One of these manuals deals with toxicity tests using freshwater organisms,[9] and the other describes toxicity tests employing marine and estuarine organisms.[10]

Generally, acute toxicity tests measure an effluent's lethality. In chronic toxicity tests, lab technicians record the death of test organisms but also monitor other effects, such as fertilization, growth, and reproduction.

[8] *TSD for Water Quality-based Toxics Control.* p. 6.

[9] U.S. EPA. 1991. *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms* 3rd Edition. Office of Research and Development, Cincinnati, OH. EPA-600/4-91/000.

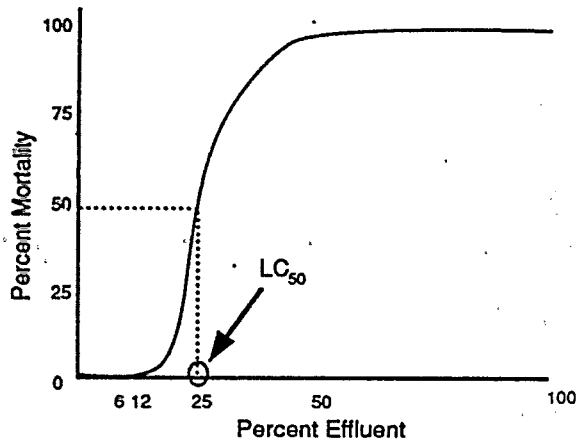
[10] U.S. EPA. 1991. *Short-Term Methods for Estimating Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms.* 2nd Edition. Office of Research and Development, Cincinnati, OH. EPA/600/4-91/003.

Figure 4 Dose-Response Curve for a Hypothetical Acute Toxicity Test

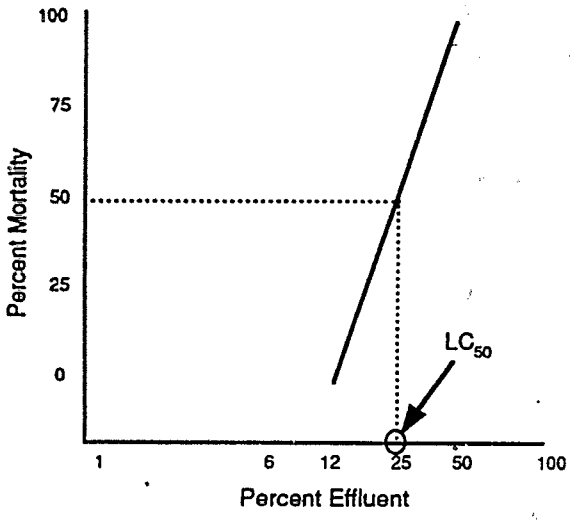
Data

Percent Effluent	Percent Mortality
100	100
50	100
25	50
12	0
6	0
0	0

Regular Plot of Data, showing S-shaped curve



Semi-log Plot of Data, giving a straight line



Dose-Response Curves

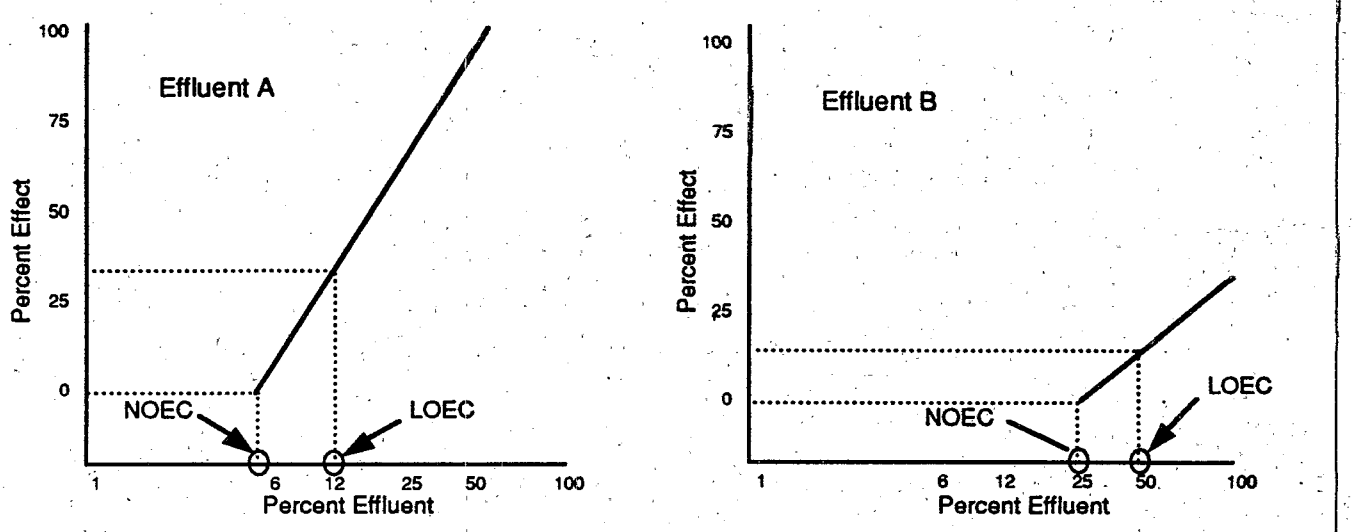
Displaying data graphically often helps in understanding results from a test. To this end, biologists frequently make use of a type of graph called a **dose-response curve**, which plots the concentration of a substance against organisms' response to the substance. In the case of a toxicity test, the dose-response curve plots the concentration of the effluent against the observed effect, giving an S-shaped curve (Figure 4). To make interpretation of such curves easier, biologists often plot the data in such a way as to give a straight line rather than a curve. This straight-line plot is called a semi-log plot. For comparison, Figure 4 shows data from a toxicity test both as an S-shaped curve and as a semi-log plot.

Figure 4 presents data from an acute toxicity test. For this test, organisms were exposed to 6, 12, 25, 50, and 100 percent (undiluted) effluent. The dose-response curve for the data shows that at a 50 percent concentration all of the test organisms died, at a 25 percent concentration half of the organisms died, and at 12 percent none died. For this effluent, the LC_{50} occurs at an effluent concentration of 25 percent.

Data from chronic toxicity tests can also be visualized with a dose-response curve. Figure 5 shows dose-response curves for data collected during a chronic toxicity test using two effluents, A and B. For this test, organisms were exposed to 6, 12, 25, 50, and 100 percent concentrations of each effluent. In order to compare the data for the two effluents, technicians would have had to perform the same chronic toxicity test on both and use the same species to test the two effluents. Other test conditions, such as temperature and oxygen availability, also must be the same for both. According to the dose-response curve, effluent A appears to have 12 percent effluent concentration for its LOEC and 6 percent effluent concentration as its NOEC. For effluent B the LOEC appears to be 50 percent and the NOEC 25 percent.

Comparing dose-response curves indicates the relative toxicities of effluents. The dose-response curves in Figure 5 show effluent B as less toxic than A. Effluent A requires considerably more dilution than effluent B to reach an LOEC or an NOEC. In other words, toxicity is inversely proportional to LOECs and NOECs: the more toxic an effluent, the lower the value of its LOEC or NOEC. The same relationship holds true for LCs.

Figure 5 Dose-Response Curve for a Hypothetical Chronic Toxicity Test



Toxicity Units

To avoid the confusion that tends to accompany inverse relationships, toxicologists have defined toxicity units. The **acute toxicity unit (TU_a)** is the reciprocal of the acute LC_{50} multiplied by 100:

$$TU_a = \frac{1}{LC_{50}} (100).$$

Suppose we perform an acute toxicity test on an effluent and find that its LC_{50} occurs at an effluent concentration of 50 percent. Its toxicity measures (1/50)(100) = 2 TU_a. Similarly, the **chronic toxicity unit (TU_c)** is the reciprocal of the NOEC multiplied by 100:

$$TU_c = \frac{1}{NOEC} (100).$$

In Figure 5, effluent B measures 1/25 x 100 = 4 TU_c, as compared with effluent A: 1/6 x 100 = 17 TU_c. Toxicity units make the greater toxicity of effluent A more readily apparent.

An important point to keep in mind when working with toxicity units is that TU_as and TU_cs measure different parameters and are not equivalent. Consequently, the user must always specify which type of toxicity unit he or she intends.

However, by means of a ratio called the **acute-to-chronic ratio (ACR)**, interconversion of TU_a and TU_c becomes possible: $TU_a = (ACR)(TU_c)$. ACR compares the concentration of an effluent or a toxic substance that causes acute toxicity in a species with the concentration of an effluent or a toxic substance that causes chronic toxicity to the same species. To learn more about the interpretation and use of the ACR, consult the *Technical Support Document for Water Quality-based Toxics Control (EPA/505/2-90-001).*

Practical Aspects of Whole Effluent Toxicity Testing

Testing the toxicity of whole effluents involves a number of steps. Permittees must follow acceptable procedures for each of these steps in order to obtain valid results. (See Appendix B for examples of how an NPDES permit specifies details of whole effluent toxicity testing.) Toxicity testing begins with collecting and handling samples. The tests themselves may occur at the facility, requiring it to store samples properly and perform the tests according to accepted protocol. In some cases, the facility may send samples to a laboratory for testing. Acceptable transport to the lab and appropriate handling at the lab then become practical matters to consider. Finally, collecting and analyzing data and reporting results to the regulatory agency complete the testing process. To gain a better understanding of the practical aspects of whole effluent toxicity testing, let us consider each step in greater detail.

Sample Collection

A facility's NPDES permit specifies many aspects concerning the collection of the sample. These may include the location, timing, and method of sample collection. Unless personnel at the facility follow the guidelines in the permit, the data from the sample will not be considered valid.

In most cases, permits specify the outfall (the site of discharge) as the place for collecting samples. For some facilities, however, a location between the final treatment and the outfall may give better access to a sampling point. In the case of a facility that chlorinates its effluent, the regulatory agency may wish to evaluate toxicity before chlorination. Alternatively, a permit may specify sampling before and after chlorination and after dechlorination as well. A third situation that may result in a sampling site other than the outfall occurs when a regulatory agency wishes to assess a wastewater stream before it joins other wastewater streams.

Several factors enter into the timing of sample collection. One of these factors is a facility's schedule for discharging: some facilities discharge continuously and others only intermittently. The purpose of the toxicity test can also affect sample timing. For example, a regulatory agency may want sampling for an acute toxicity test to correspond with the point in a facility's operation when its discharge is most toxic.

The actual collection of samples can occur by one of two methods. The method specified in the permit will depend on the object of the test and the nature of the facility's operation. A **grab sample**, as the name implies, is a single sample. This type of sampling requires little time and a minimum of equipment. It can prove useful for sampling an effluent with toxicity that changes little over time.

The other type of sample, called a **composite sample**, is a mixed sample collected over a specified period of time. Although composite sampling can be performed manually, devices exist to accomplish this task automatically. A composite sample may prove ideal for a chronic toxicity test. However, this sampling method may mask periods of peak toxicity, which are relevant to acute toxicity tests.

Whichever sampling method a facility uses, personnel collecting samples should minimize aeration. Aeration results in the loss of volatile chemicals, which are substances that readily pass from the liquid to the gaseous state. When an effluent sample loses such substances, a toxicity test may indicate less toxicity than the effluent actually contains.

Sample Handling

As with sample collection, permittees must handle samples appropriately in order for toxicity tests to give valid results. First, permittees need to store samples in suitable containers and, as with sample collection, they need to avoid aeration during transfer to storage containers. Because stainless steel is easily decontaminated, it makes good containers for tests conducted on-site. Glass or disposable plastic containers are recommended for samples shipped to laboratories for off-site testing.

Once samples are in suitable containers, permittees must provide proper storage. For on-site tests, permittees should store on ice any sample not used immediately. However, a sample should not be stored for longer than 24 hours before being used for a test. Some tests require such large volumes that storing the sample on ice becomes impractical. Permittees may store such samples at ambient temperatures. During cold weather, personnel should use heat tapes to prevent large samples from freezing.

If a facility sends a sample to a laboratory for testing, personnel at the facility should store the sample on ice and ship it on ice as soon as possible after collection. When the sample reaches the lab, technicians there should store it at refrigerator temperature (4°C). Laboratories are required to initiate tests within 36 hours of receiving a grab sample or upon completion of a composite sample.

In some cases, permittees must also make chemical adjustments to samples. The pH, which indicates how acid or basic a liquid is, may require adjustment before the start of a toxicity test. A permittee may also need to alter a sample's hardness, which is principally the amount of calcium carbonate in water. When a sample requires

adjustment, the permittee should allow a portion of the sample to remain unadjusted. This unadjusted portion is used in a parallel study, which consists of conducting the toxicity test on a full dilution series. The parallel study reveals whether the adjustment contributes to, masks, or has no effect on the observed toxicity.

Designs for Toxicity Tests

To monitor a facility's compliance with its limit, the permit specifies which types of tests a facility needs to perform on its effluent. These may include toxicity tests—acute, chronic, or both. A permit that specifies toxicity tests will also indicate the test design, which varies with the site and operation of the discharging facility.

Static tests are tests that use the same effluent sample throughout the test or in which only limited replacement of the sample effluent occurs. Static tests, which are generally conducted in a laboratory, have the advantage of being simple and inexpensive to perform. They require little space, manpower, and equipment. Generally, they use only small effluent volumes, one to 20 liters. However, static tests do not show changes in the effluent over time. Also, over the course of the test, dissolved oxygen can become depleted. As a result, organisms could suffer adverse effects not caused by toxicity.

Aquatic toxicologists have developed two types of static tests. **Static nonrenewal tests** use the same effluent sample for the duration of the test. The nonrenewal test can provide some measure of how long toxicity persists in an effluent. On the other hand, the adsorption of toxics onto the test chamber and the degradation of toxics may occur, resulting in a decrease in the apparent toxicity. An effluent can also lose some of its volatile toxics over the course of a static nonrenewal test. Finally, organisms release substances, such as wastes and carbon dioxide, as they metabolize. Over time, these accumulating substances, called metabolites, may interact with toxics, resulting in increased, decreased, or otherwise altered toxicity.

In **static renewal tests**, fresh effluent replaces all or part of the effluent in test chambers at specified intervals. For example, a static renewal test lasting 96 hours may specify fresh effluent at 24 hours, 48 hours, and 72 hours. Lab technicians accomplish renewal either by transferring test organisms to fresh effluent at the same dilution or by replacing all or part of the effluent in test chambers. Static renewal tests reduce the loss of toxics associated with the nonrenewal static test.

The **flow-through test** provides a different set-up for a toxicity test by continuously pumping fresh effluent or effluent dilution through test chambers. Fresh effluent can come directly from the outfall. Alternatively, personnel can place grab samples or composite samples in a holding tank to provide fresh effluent for pumping through sample chambers. Flow-through tests are conducted on-site.

The flow-through test has several advantages over the static test. First, it gives information about fluctuations in the toxicity of a facility's effluent. Second, dissolved oxygen levels remain higher in a flow-through test than in a static test. Third, this method of testing reduces the loss of toxic substances to adsorption and volatilization. Finally, the continuous flow method prevents metabolites from building up and interacting with toxics.

Although flow-through tests have many advantages over static tests, they also have some disadvantages. The flow-through test does not provide information about the persistence of toxicity. Such tests require large volumes of effluent and dilution water. They also entail complex and expensive equipment, which requires more maintenance than the equipment used for static tests.

Static tests are tests that use the same effluent sample throughout the test or in which only limited replacement of the sample effluent occurs. The flow-through test provides a different set-up for a toxicity test by continuously pumping fresh effluent or effluent dilution through test chambers.

Test Organisms

Toxicologists generally choose as test organisms species that biologists have studied thoroughly and that are known to be sensitive to many substances. In addition, species used as test organisms must be readily available as well as easy to maintain and culture under laboratory conditions. Table 3 lists the species commonly used in chronic toxicity tests. Acute toxicity tests encompass a larger number of species.

Several factors determine which species a protocol requires for a given test. These factors include both the pollutants present in the effluent and the sensitivity of test organisms to these pollutants. Whether a facility discharges into freshwater or seawater may also influence the choice of test organism. If a permit specifies a toxicity limit for the effluent itself, then toxicity tests may use freshwater organisms even if the facility discharges into salt water. If a permit specifies a facility's toxicity limit in the water that receives the effluent, then tests will use freshwater organisms for a freshwater receiving water and marine organisms for a salt water receiving water.

The species chosen for a test may not be a major inhabitant in the discharging facility's location. In many cases, the test species may not inhabit the facility's locale at all. Such circumstances frequently cause regulators and permittees to wonder why tests do not employ local species. EPA has several reasons for discouraging the use of resident organisms:[11]

- Studies show that species commonly used as test organisms represent the range of sensitivity shown by resident species in ecosystems currently subject to testing.
- Receiving waters may lack sensitive species as a result of previous exposure to pollutants.
- Many States require collecting permits, which may prove difficult and time-consuming for facilities or testing laboratories to obtain.
- Using resident species imposes additional burdens for quality control to ensure that all organisms belong to the same species, fall within the appropriate age range, and do not vary in condition as a result of handling procedures or seasonal environmental changes.
- A facility or laboratory using a resident species would need to develop protocols for culturing and testing the species and for assessing inter- and intra-laboratory variability. Such additional tasks might well prove time-consuming and expensive.

The Discharge Monitoring Report

The report containing the results of tests, including toxicity tests, performed on effluents is called a **discharge monitoring report (DMR)**. For tests performed on-site, the permittee prepares the DMR, following the specifications outlined in the permit. For tests performed off-site, the contracted laboratory prepares the report. The laboratory sends the report to the facility, and in some cases sends a copy directly to the regulatory agency as well.

[11] *TSD for Water Quality-based Toxics Control*. p. 17.

Table 3.
Species Commonly Used as Test Organisms
in Chronic Toxicity Tests

TYPE OF ORGANISMS	FRESHWATER SPECIES	MARINE SPECIES
VERTEBRATE	• fathead minnow, *Pimephales promelas*	• sheepshead minnow, *Cyprinodon variegatus*
INVERTEBRATE	• a cladoceran, or water flea, *Ceriodaphnia dubia*	• mysid, or shrimp, *Mysiodopsis bahia* • sea urchin, *Arbacia punctulata*
ALGA	• a green alga, *Selanastrum capricornutum*	• a red macroalga, *Champia parvula*

What information should a DMR provide? The DMR must show the test result, such as the LC, LOEC, NOEC, or IC. These results should represent appropriate statistical analysis of the "raw data"—the unanalyzed numbers obtained from each test chamber and entered on a data sheet. Many States and EPA Regional Offices require DMRs to include these raw data along with the test results. As an example, the data sheet for an acute toxicity test measuring LC50 would include the number of test organisms alive in each test chamber at different times after the start of the experiment, ending at 96 hours. The data sheet would also report on additional parameters, such as the amount of dissolved oxygen, the pH, and the temperature. Appendix C outlines the information that a DMR should contain. In addition to the test results and, in some States, raw data, the DMR also includes a sheet detailing quality assurance of the test. Quality assurance is discussed fully in the following section.

Compliance with Whole Effluent Toxicity Limits

To determine whether the facility's effluent remains within the limits of the permit, the permit specifies the means for monitoring compliance. These include, but are not limited to, self monitoring reports, a quality assurance (QA) summary of the WET test, and inspections. Regulatory agencies also monitor compliance by investigating citizens' complaints about a facility. Quality assurance has a major role in compliance, assessing the validity of test results.

Self-monitoring Reports

Self-monitoring reports include DMRs and also reports on progress in maintaining compliance schedules. The regulatory agency receives these reports and enters data from them into the **Permit Compliance System (PCS)**, a national computerized database. PCS flags violations of permit limits, compliance schedules, and reporting schedules. PCS has additional functions, including automating the preparation of the **Quarterly Noncompliance Report (QNCR)**. This report lists facilities that have violations requiring attention.

Because the PCS is a computerized system, monitoring compliance involves having the acceptable limits for the DMR accurately reflect the permit writer's intention. To ensure this close correspondence, the permit writer should record the acceptable test limits in a sample DMR. PCS personnel can then enter these limits into the database.

Discharge Monitoring Report/Quality Assurance

Because self-monitoring constitutes such a large part of compliance monitoring, the EPA has instituted a program called **Discharge Monitoring Report/Quality Assurance (DMR/QA)** to track the quality of self-monitoring. This program determines whether a permittee can analyze and report data accurately. The permittee receives a sample containing the substances occurring in the facility's effluent. Using the appropriate tests, the permittee must analyze the sample and report results.

Regulatory agencies note whether these results fall within an acceptable range of the actual pollutant concentration in the sample. The regulatory agency then follows up with the permittee on poor test results or late submittal of test results. In the past, this program has applied only to chemical analyses, but as of 1991 the EPA has extended it to include toxicity testing.

Inspections

A third aspect of compliance monitoring involves onsite inspections conducted by the regulatory agency. These include inspections that focus on records and facility operation and others that collect samples for independent analysis. The Performance Audit Inspection and the Compliance Evaluation Inspection concern records and facility operation. A **Performance Audit Inspection (PAI)** evaluates a permittee's self-monitoring program, verifying reported data and compliance by checking records. A PAI also includes an inspector's observing the self-monitoring process from the collection of samples through laboratory analysis and reporting. Like the PAI, the **Compliance Evaluation Inspection (CEI)** examines records and makes observations, but the emphasis differs from a PAI. The CEI concentrates on records and only briefly observes the facility, its effluent, and its receiving water.

Another inspection, the **Compliance Biomonitoring Inspection (CBI)** requires the collection of effluent samples for analysis by acute and chronic toxicity tests. In addition, the CBI includes the examination of records and the brief observations of the CEI.

Major facilities receive annual inspections. These inspections serve five functions:

- Verification of permittee compliance,
- Development of enforcement information,
- Response to citizen complaints,
- Collection of information for permit development, and
- Maintenance of a regulatory presence.

The *NPDES Compliance Inspection Manual* (May 1988) details different types of inspections and procedures for carrying them out.

Citizen Complaints

Citizens' concerns form part of the monitoring process. When citizens make complaints about a facility's operation, these are registered with a State or EPA Regional Office. The regulatory agency then follows up the complaints with a review of the facility's self-monitoring data, an inspection, or both.

The Role of Quality Assurance in Compliance

NPDES permits place responsibility for quality assurance with the permittee. In fact, some States and Regions have developed QA forms, which permittees must submit along with their self-monitoring reports. To monitor quality assurance, then, regulators can examine self-monitoring reports and QA forms to determine whether a permittee has had tests performed and data analyzed according to the terms and schedule set out in the permit and whether controls for these tests fall within acceptable limits. Appendix C contains a sample of this form.

Only valid testing can give valid results.

If the permittee has met QA standards, then test results and analyses are considered valid. If the permittee has failed to meet these standards, the test results and analyses are unacceptable. The regulator should spend no more time studying them, and the permittee must repeat the test. Only valid testing can give valid results. Invalid tests constitute permit violations and leave a permittee open to enforcement action.

Documents called **chain of custody** form an important part of quality assurance. On these documents the permittee and the laboratory record details about the collection, handling, transport, and analysis of a sample. Regulators can study the chain of custody to determine whether permittees and contracting laboratories have followed acceptable protocol. When a facility violates its permit, the chain of custody may provide valuable information about the source of the problem. Appendix C contains a sample chain of custody form.

Violations of Permits Having Whole Effluent Toxicity Limits

What happens when a facility violates a permit limit or requirement that relates to whole effluent toxicity? For permits with water-quality based limits, regulators must review any violation for potential impact on water quality. The EPA guidelines set out in the *Enforcement Management System for the National Pollutant Discharge Elimination System* (September 1986) suggest that regulators gear responses to the level of the violation.

Minor violations may require no more than a telephone call or a letter. A violation that results from improper analytical methods may result in a warning letter, called a **Notice of Violation (NOV)** or a Section 308 letter, which requires the permittee to repeat the tests using acceptable procedures. In tracking a permit that has "monitor only" requirements, qualified regulatory personnel must determine whether to establish a discharge limit.

Types of Violations

As mentioned earlier, the QNRC generates a list of violations that require attention. These include, but are not limited to, violations regarding compliance schedule milestones, DMRs, and effluent limits. Compliance schedule milestones concern actions required of a facility to meet or return to compliance standards. When a permittee misses one of these milestones by more than 90 days, the permittee is in violation. Failing to submit a DMR withing 30 Days of its due date also constitutes a violation. In addition, effluent violations constitute permit violations. Any effluent violation, but particularly one with a potential for adversely affecting water quality, requires review or action. Regulators should consult their regional Environmental Services Division to determine whether a particular violation has such a potential.

When a regulator becomes aware of an effluent violation, he or she should gather as much information as possible about the violation. The regulator should examine the DMR to determine whether the test was run according to protocol. Next the regulator will send a Section 308 letter, requesting information about processing at the facility and inquiring about any problems occurring at the time of the violation. The regulator may also request additional testing. The regulator should remind the permittee of the responsibility to report within 24 hours any violation that may endanger health or the environment and to submit in writing within five days a noncompliance report of the circumstances of such a violation. If the violation does not pose a threat to health or the environment, the permittee can submit the report at the time of the next DMR. A noncompliance report should address the cause of noncompliance, the anticipated duration, and the steps taken or planned to correct the situation. When the violation concerns a water quality-based toxicity limit, the permittee must also examine plant management to see whether changes will reduce the impact on the environment.

When a permittee realizes that the facility is going to violate a permit limit, he or she must take steps to prevent or minimize the violation and its potential impact. These include additional monitoring, a review of in-house processes, and a review of self-monitoring QA to determine whether the facility's personnel can solve the problem.

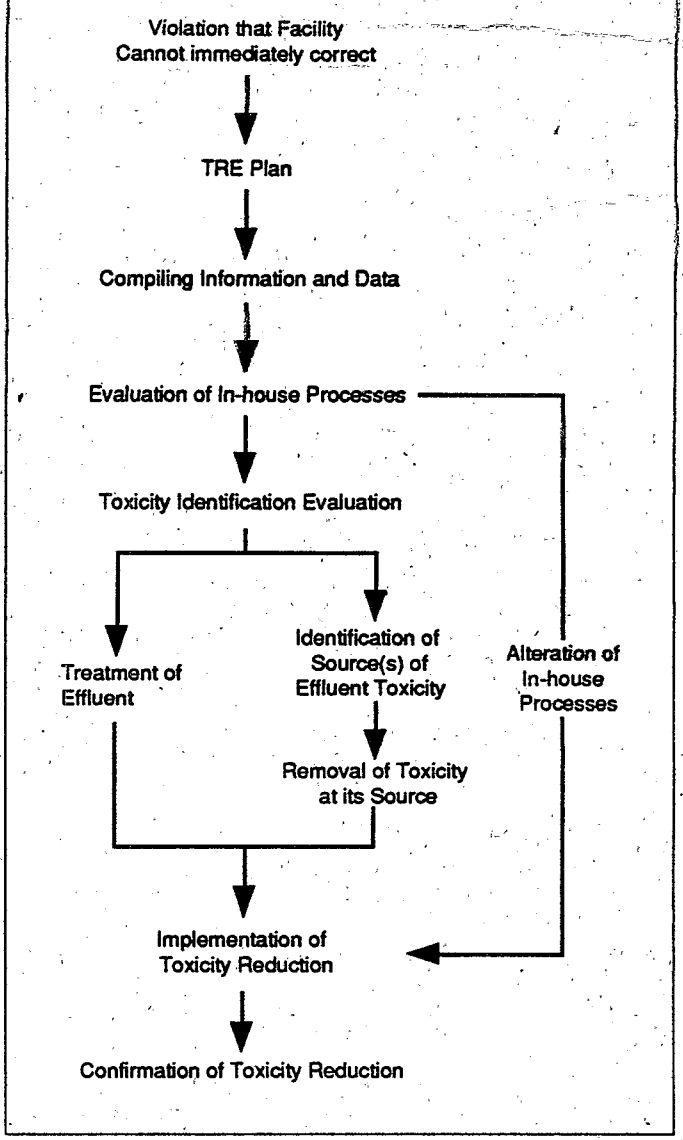
The Role of the Toxicity Reduction Evaluation

If the permittee cannot correct the violation immediately or if such violations occur frequently, a study called a **Toxicity Reduction Evaluation (TRE)** follows. A TRE investigates a specific site in a stepwise fashion to discover the cause of the toxicity, locate its source, determine effective measures for reducing the toxicity, and evaluate these measures. (See Figure 6.) The ultimate goal of the TRE is to return the facility to compliance with its effluent limit. Some NPDES permits require a TRE when a violation of the limit occurs. If a permit has no such requirement, the regulatory agency will seek a formal enforcement action to initiate a TRE and to establish a schedule for implementing the plan.

While regulators should provide oversight during the TRE, the permittee has responsibility for carrying out the study. To assist permittees in this task, the regulatory agency may wish to refer them to the relevant EPA publications:

- *Generalized Methodology for Conducting Industrial Toxicity Reduction Evaluations* (EPA/600/2-88/070)

Figure 6 Flowchart for a Toxicity Reduction Evaluation (TRE)



- *Toxicity Reduction Evaluation Protocol for Municipal Wastewater Treatment Plants* (EPA/600/2-88/062)
- *Methods for Aquatic Toxicity Identification Evaluations:*
  - Phase 1 Toxicity Characterization Procedures (EPA/600/6-91/003)
  - Phase 2 Toxicity Identification Procedures (EPA/600/3-88/035)

- Phase 3 Toxicity Confirmation Procedures (EPA/600/3-88/036)

- *Toxicity Identification Evaluation: Characterization of Chronically Toxic Effluents, Phase I* (EPA/600/6-91/005).

Preparing a TRE plan constitutes the first step in conducting a TRE. This plan needs to include a description of the study, the contractor who will perform the study, and relevant background information on the facility. The TRE plan also includes a schedule for conducting specific tasks, such as final toxicity reduction and confirmation, and for reporting the results of these tasks to the regulator. The regulator then evaluates the TRE plan and notifies the permittee of any shortcomings. Regulators should evaluate the schedule as well as the plan. Though a state may approve TIE/TRE plans, EPA does not recommend doing so. Approval of a plan could imply that the regulator accepts liability if carrying out the plan fails to return the facility to compliance.

When the facility has submitted the plan to the regulator, the facility must then put the plan into action. This entails compiling all the available data on the plant's processes, self- monitoring results, and operating guidance. With this information, the facility conducts an evaluation of its in-house processes to determine whether these contribute to the violation. In-house processes include cleanup of spills, maintenance of machinery, and the operation of treatment systems. If any of these has contributed to the violation, the facility should institute steps to correct these areas. Note that these "self- examination" tasks are among the steps that a facility should automatically follow when it has violated its permit. In addition, a facility should include results of these tasks in its noncompliance report, which becomes due at the latest with the next DMR.

If these areas have not caused the violation, the facility then may begin a **Toxicity Identification Evaluation** (TIE), an evaluation of the waste product at each step in production or waste treatment. The first phase of a TIE consists of additional testing, which starts as soon as possible after a violation has occurred and even before it is known whether a TIE will be necessary. The additional testing establishes the effluent's variability and indicates the severity, frequency, and duration of toxic events. Separation of the constituents of the waste stream into volatile compounds, metals, pesticides, biocides, and other potentially toxic components should identify the class of substances causing toxicity.

With the toxics' classes identified, the permittee can determine a means for resolving the problem. The permittee may decide to treat the waste so as to render it nontoxic. Alternatively, the permittee can identify the source of toxicity and eliminate it at the source. An industrial facility may elect to accomplish this by substituting a different chemical in a particular process, substituting the process, or eliminating the process altogether. To bring about a reduction at the source of toxicity, a municipal facility can impose local limits or a pretreatment requirement. Public education may also provide a solution for a municipal facility.

The ultimate goal of a TRE is to return the facility to compliance with its effluent and limit.

Once a permittee has determined a method for reducing toxicity, he or she must implement the method and confirm to the regulatory agency that this method reduces toxicity to the limits set forth in the permit. When the permittee has achieved this, the facility has returned to compliance, and the TRE is complete.

If tests do not confirm an adequate reduction in toxicity, the TRE process continues until it achieves its goal. In some cases, the permittee does not succeed in achieving source identification and reduction. In such an instance the permittee must apply the other means for returning to compliance: treating its waste to eliminate toxicity or ceasing to discharge.

Case Histories of TREs

The following cases provide a brief glimpse of the TRE process.

Municipal

In New Jersey, a small community of 100 homes had an unmanned treatment plant. When regulatory authorities inspected the plant, they discovered a toxicity problem. Since the plant had no industrial input and its in-house processes were running according to design standards, the toxicity had to enter the system from a household. Concentration and separation of the waste stream revealed high levels of silver. The TRE traced the entry point of the silver to a householder's basement darkroom. The owners of the home were asked either to limit the quantity of film that they developed or to pay for an upgrade to the plant so that it could treat the toxicity.

Industrial

A facility that manufactured pantyhose violated its whole effluent toxicity limit. As with the waste-treatment plant in New Jersey, in-house operations and processes functioned adequately. The TIE revealed high levels of copper and other metals as well. Rather than treat the

wastestream with chelators to tie up the metals, the facility opted to reduce the source of toxicity. To this end they limited the volume and altered the mix ratio of dyes in their vats.

Municipal

When a municipal treatment plant violated its whole effluent toxicity limits, personnel cleaned up in-house processes. This effort reduced the level of toxicity but did not eliminate it. A TIE revealed high levels of pesticides in the effluent, and a pretreatment review showed that a pesticide manufacturer on the influent wastestream was not operating at full efficiency. The industrial plant had two problems. First, while its settling pond was designed to have a retention time of 4 to 7 days, dye studies determined the actual retention time as only 1.5 hours. Second, when workers rinsed the storage barrels for the pesticides, they dumped the rinse water directly into the wastestream. From the wastestream the rinse water flowed into the pond without pretreatment. Large slugs of pesticide passed through the settling pond and entered the municipal treatment plant's influent. The TRE established two control strategies. First, the pesticide manufacturing facility dredged its settling pond and installed a baffling system in it to increase retention time. Second, workers rinsed storage barrels at a different site.

Though these accounts provide only simplified versions of the actual handling of the toxicity violations involved, they do suggest the scope of problems that occur and the methods that resolve them.

Appendix A

EPA'S Authority to Regulate Toxicity

Authority to Regulate Toxic Pollutants

Several sections of the Clean Water Act give the EPA authority to regulate for toxic chemicals:

Sec. 101(a)

"The objective of this Act is to restore and maintain the chemical, physical and biological integrity of the Nation's waters."

Sec. 101(a)(3)

Declaration of Goals and Policy - "it is the national policy that the discharge of toxic pollutants in toxic amounts be prohibited...."

Sec. 301(a)

"Except as in compliance with this section and sections 302, 306, 307, 318, 402, and 404 of this Act, the discharge of any pollutant by any person shall be unlawful."

Sec. 301(b)(1)(C)

"In order to carry out the objective of this Act there shall be achieved not later than July 1, 1977, any more stringent limitation, including those necessary to meet water quality standards...."

Sec. 302(a)

provides the authority to establish water quality-based effluent limitations on discharges that interfere with the attainment or maintenance of that water quality which shall assure protection of public health, public water supplies, and the protection and propagation of a balanced population of shellfish, fish and wildlife.

Sec. 303(c)(2)(B)

authorizes the adoption of numeric water quality criteria that are based upon biological monitoring or assessment methods and the use of effluent limitations or other permit conditions based on or involving biological monitoring or assessment methods or previously adopted numeric criteria. "Nothing in the section shall be construed to limit or delay the use of effluent limitations or other permit conditions based on or involving biological monitoring or assessment methods...."

Sec. 304(a)(8)

requires EPA to develop and publish information on methods for establishing and measuring water quality criteria for toxic pollutants including biological monitoring and assessment methods.

Sec. 308(a)

authorizes the installation, use and maintenance of biological monitoring methods by point sources, where appropriate, for the development of effluent limitations or the determination of compliance with such limitations, prohibitions, or effluent standards.

authorizes issuance of a permit for the discharge of any pollutant, or combinations of pollutants upon the condition that the discharge meet all applicable requirements and provisions of the CWA.

40 CFR 122.44(d)

"In addition... each NPDES permit shall include conditions meeting the following requirements when applicable... Water Quality Standards and State requirements: any requirements in addition to or more stringent than promulgated effluent limitations guidelines or standards... necessary to: (1) achieve water quality standards established under section 303 of the CWA, including State narrative criteria for water quality."

(i) "Limitations must control all pollutants or pollutant parameters... which... may be discharged at a level which will cause, have the reasonable potential to cause, or contribute to an excursion above any State water quality standard, including State narrative criteria for water quality...."
(iv) (Numeric criterion for whole effluent toxicity)
(v) "...has the reasonable potential to cause, or contributes to an in-stream excursion above a narrative criterion..., the permit must contain effluent limits for whole effluent toxicity...[except] where chemical-specific limits for the effluent are sufficient to attain and maintain applicable numeric and narrative State water quality standards."

Definition of the Regulated Community

The Clean Water Act (CWA) requires that every point source discharger have a permit. The regulated community, then, could include the entire NPDES program. The Water Quality Act of 1987 provides criteria to reduce the regulated community for toxicity limits to a more manageable level.

40 CFR 122.44(d)(1)(iii)

NPDES permits must include effluent limitations for every pollutant that causes, has the reasonable potential to cause, or contributes to an excursion above a numeric water quality criterion.

40 CFR 122.44(d)(1)(iv)

NPDES permits must include whole effluent toxicity limitations when a discharge causes, has the reasonable potential to cause, or contributes to an excursion above a State numeric criterion for whole effluent toxicity.

40 CFR 122.44(d)(1)(v)

When a discharge causes, has reasonable potential to cause, or contributes to an excursion above a State narrative water quality criterion, the permit must contain limitations on whole effluent toxicity. An exception exists where chemical-specific limitations achieve all applicable water quality standards.

40 CFR 122.44(d)(1)(vi)

Where an actual or projected excursion above a water quality criterion is attributable to a particular pollutant for which the State has not adopted water quality criterion, the permit must contain water quality-based effluent limitations to control the pollutant of concern.

Appendix B

Excerpts Taken From Sample NPDES Permits and Relevant to Whole Effluent Toxicity Testing

Excerpts from an NPDES Permit Issued to a Wastewater Treatment Facility and Requiring Acute Toxicity Limits

PERMIT NO. AB00XXXXX
Major POTW

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION IV

AUTHORIZATION TO DISCHARGE UNDER THE
NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM

In compliance with the provisions of the Clean Water Act, as amended (33 U.S.C. 1251 et seq.; the "Act"),

_____ County, Any State
Public Works Department
Office of Environmental Services
_____ Road
_____, Any State

is authorized to discharge from a facility located at

_____ Wastewater Treatment Plant
_____ Road
_____, Any State

to receiving waters named

The Atlantic Ocean

in accordance with effluent limitations, monitoring requirements and other conditions set forth herein. The permit consists of this cover sheet, Part I 6 pages, Part II 16 pages, Part III 5 pages, and Part IV 2 pages.

This permit shall become effective on January 1, 1991

This permit and the authorization to discharge shall expire at midnight, March 31, 1994

September 26, 1990
Date Issued

John Doe, Director
Water Management Division

Part I

EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS - Final

During the period beginning on the effective date and lasting through the expiration date of this permit, the permittee is authorized to discharge from outfall(s) 001, sanitary wastewater.
Such discharges shall be limited and monitored by the permittee as specified below:

PARAMETERS	DISCHARGE LIMITATIONS			MONITORING REQUIREMENTS		
	Annual Average	Monthly Average	Weekly Average	Measurement Frequency	Sample Type	Sampling Point
Flow, MGD	———	Report	Report	Continuous	Recording flowmeter & totalizer	Effluent
Carbonaceous Biochemical Oxygen Demand (5-Day)	25.0 mg/l	25.0 mg/l	40.0 mg/l	7 days/week	24 hr. composite	Influent & Effluent
Total Suspended Solids	30.0 mg/l	30.0 mg/l	45.0 mg/l	7 days/week	24 hr. composite	Influent & Effluent
Fecal Coliform Bacteria, N/100 ml	See Item 3			7 days/week	Grab	Effluent
Total Residual Chlorine	See Item 9			7 days/week	Grab	Effluent
pH (standard units)	See Item 5			Continuous	Recorder	Effluent
Acute Whole Effluent Toxicity	See Item 12			See Part IV	Grab	Effluent
Total Nitrogen, as N (mg/l)	———	Report	———	1/month	24 hr. composite	Effluent
Total Phosphorus, as P (mg/l)	———	Report	———	1/month	24 hr. composite	Effluent

12. Lethality to more than 50% of any test species in 100% effluent in a test of 96 hours duration or less will constitute a violation of Any State's Administrative Code and the terms of this permit. The testing for this requirement must conform with Part IV of this permit.

Part IV

Whole Effluent Toxicity Testing Program

As required by Part _ of this permit, the permittee shall initiate the series of tests described below beginning in January, 1991 to evaluate whole effluent toxicity of the discharge from outfall ___. All test species, procedures and quality assurance criteria used shall be in accordance with *Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms*, EPA/600/4-90/027, or the most current edition. The control water and effluent used will be adjusted to a salinity of 20 parts per thousand using artificial sea salts as described in EPA/600/4-90/027, Section 5 (or the most current edition). In addition, for the inland silverside test, feeding and solution renewal shall be done at 48 hours with a portion of the original sample that has been kept refrigerated. A standard reference toxicant quality assurance test shall be conducted concurrently with each species used in the toxicity tests and the results submitted with the discharge monitoring report (DMR). Alternatively, if monthly QA/QC reference toxicant tests are conducted, these results must be submitted with the DMR.

1. a. The permittee shall conduct 48-hour acute static toxicity tests using the mysid shrimp (*Mysidopsis bahia*) and 96-hour acute static-renewal toxicity tests using the inland silverside (*Menidia beryllina*). All tests will be conducted on four separate grab samples collected at evenly-spaced (6- hr) intervals over a 24-hour period and used in four separate tests (under full dilution series) in order to catch any peaks of toxicity and to account for daily variations in effluent quality.

   b. If control mortality exceeds 10% for either species in any test, the test(s) for that species (including the control) shall be repeated. A test will be considered valid only if control mortality does not exceed 10% for either species. If, in any separate grab sample test, 100% mortality occurs prior to the end of the test, and control mortality is less than 10% at that time, that test (including the control) shall be terminated with the conclusion that the sample demonstrates unacceptable acute toxicity.

2. a. The toxicity tests specified above shall be conducted once every two months until 6 valid bimonthly tests have been completed, and once every 6 months thereafter for the duration of the permit, unless notified otherwise by EPA. These tests are referred to as "routine" tests.

   b. Results from "routine" tests shall be reported according to EPA/600/4-90/027, section 12, Report Preparation (or the most current edition), and shall be submitted as an attachment to the DMR. Such results are to be entered on the DMR in the following manner: the LC_{50} shall be reported as the % effluent that killed or would kill 50% of the test organisms.

3. a. If unacceptable acute toxicity (greater than 50% lethality of either test species in any of the four separate grab sample tests within the specified time) is found in "routine" test, the permittee shall conduct three additional acute toxicity tests on the species indicating unacceptable toxicity. For each additional test, the sample collection requirements and test acceptability criteria specified in Section 1 above must be met for the test to be considered valid. The first test shall begin within two weeks of the end of the "routine" tests, and shall be conducted weekly thereafter until three additional, valid tests are completed. The additional tests will be used to determine if the toxicity found in the "routine" test is still present.

   b. Results from additional tests, required due to unacceptable acute toxicity in the "routine" tests, shall be submitted in a single report prepared according to EPA/600/4-90/027, Section 12, Report Preparation (or the most current edition) and submitted within 45 days of completion of the third additional, valid test.

4. All tests shall be conducted using a full dilution series. For those tests conducted prior to the effective date of the total residual chlorine limit, samples of effluent which have been artificially dechlorinated must be used. For those tests conducted after this date, samples of final effluent must be used.

Excerpts from an NPDES Permit Issued to a Wastewater Treatment Facility and Requiring Both Acute and Chronic Toxicity Limits

PERMIT NO. AB00YYYYY
Major POTW

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION IV

AUTHORIZATION TO DISCHARGE UNDER THE
NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM

In compliance with the provisions of the Clean Water Act, as amended (33 U.S.C. 1251 et seq.; the "Act"),

_____ City, Any State
P.O. Box 00000
_____, Any State

is authorized to discharge from a facility located at

_____ Water Reclamation Plant
_____ Parkway
_____, Any State

to receiving waters named

_____ River

in accordance with effluent limitations, monitoring requirements and other conditions set forth herein. The permit consists of this cover sheet, Part I 6 pages, Part II 16 pages, Part III 3 pages, and Part IV 3 pages.

This permit shall become effective on January 1, 1992

This permit and the authorization to discharge shall expire at midnight, September 30, 1996

September 26, 1990
Date Issued

John Doe, Director
Water Management Division

Part I

EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS - Final

During the period beginning on the effective date and lasting through the expiration date of this permit, the permittee is authorized to discharge from outfall(s) 001, sanitary wastewater.
Such discharges shall be limited and monitored by the permittee as specified below:

PARAMETERS	DISCHARGE LIMITATIONS			MONITORING REQUIREMENTS		
	Annual Average	Monthly Average	Weekly Average	Measurement Frequency	Sample Type	Sampling Point
Flow, MGD	———	Report	Report	Continuous	Recording Flowmeter & Totalizer	Effluent
Biochemical Oxygen Demand (5-Day)	20.0 mg/l	30.0 mg/l Report	45.0 mg/l Report	5 days/week 5 days/week	24 hr. composite	Effluent & Influent
Total Suspended Solids	20.0 mg/l	30.0 mg/l Report	45.0 mg/l Report	5 days/week 5 days/week	24 hr. composite	Effluent & Influent
Fecal Coliform Bacteria, N/100 ml		See Item 3		5 days/week	Grab	Effluent
Total Residual Chlorine		See Item 8		7 days/week	Grab	Effluent
pH (standard units)		See Item 4		Continuous	Recorder	Effluent
Acute Whole Effluent Toxicity		See Item 10 (a)		See Part IV	24 hr. composite	Effluent
Chronic Whole Effluent Toxicity		See Item 10 (b)		See Part IV	24 hr. composite	Effluent

10 a. Lethality to more than 50% of any test species in any percentage effluent in a test of 96 hours duration or less will constitute a violation of Any State's Administrative Code and the terms of this permit. The testing for this requirement shall conform with Part IV of this permit.

b. The effluent shall not be chronically toxic to, or produce adverse physiological or behavioral responses in, aquatic animals. An effluent no observable effect concentration (NOEC) of less than 15% for any test species will constitute a violation of the terms of this permit. The testing for this requirement shall conform with Part IV of this permit.

Part IV

Whole Effluent Toxicity Testing Program

As required by Part _ of this permit, the permittee shall initiate the series of tests described below beginning in January, 1992 to evaluate whole effluent toxicity of the discharge from outfall__. All test species, procedures and quality assurance criteria used shall be in accordance with *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms*, EPA/600/4-87/028, and *Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms*, EPA/600/4-90/027, or the most current edition(s), as appropriate. The dilution/control water and effluent used will be adjusted to a salinity of 20 parts per thousand using artificial sea salts (e.g., Forty Fathoms) as described in EPA/600/4-87/028, Section 7 (or the most recent edition). A standard reference toxicant quality assurance test shall be conducted concurrently with each species used in the toxicity tests and the results submitted with the discharge monitoring report (DMR). Alternatively, if monthly QA/QC reference toxicant tests are conducted, these results must be submitted with the DMR.

1. a The permittee shall conduct a 7-day Mysid shrimp *(Mysidopsis bahia)* Growth and fecundity test and an Inland silverside *(Menidia beryllina)* Larval Survival and Growth test. These tests shall be conducted using a full dilution series including one which is equivalent to the Receiving Water Concentration (RWC) of the effluent in the receiving water at critical conditions. Unacceptable chronic toxicity will be demonstrated if either test results in a no observable effect concentration (NOEC) less than 15% effluent. All test results shall be statistically analyzed according to Appendix H,EPA/600/4-91/000 (or the most current edition).

b. For each set of tests conducted, a minimum of three different 24-hour composite samples of final effluent shall be collected and used per the sampling schedule of section 8.1.4.2, EPA/600/4-91/000, or the most current edition. All test solutions shall be renewed daily. If test results do not meet the acceptability criteria of either Section 13.12 or Section 14.12, EPA/600/4-87/028 (or the most current edition), that test shall be repeated. A chronic test will be considered valid only if the acceptability criteria referenced above are met.

c. If 100% mortality occurs in the RWC test concentration prior to the end of the test and control mortality is acceptable at that time, that test (including the control) shall be terminated with the conclusion that the sample demonstrates unacceptable chronic toxicity.

2. a. The permittee shall also conduct a 48-hour acute static test on the Mysid shrimp (*Mysidopsis bahia*) and a 96-hour acute static-renewal test on the inland silverside (*Menidia beryllina*) using a full dilution series. Unacceptable acute toxicity will be demonstrated if more than 50% lethality of either test species occurs in any dilution of the effluent sample prior to the end of the test. All test results shall be statistically analyzed according to Appendix H, EPA/600/4-91/000, or the most current edition.

b. For each set of acute tests conducted, a fresh 24-hour composite sample of final effluent shall be used at Day 1

of both the Mysid shrimp and the inland silverside tests and at Day 3 of the inland silverside test. The composite samples collected under Section 1(b) above shall be used in the acute tests. For the inland silverside test, feeding and solution renewal shall be done at 48 hours with a portion of the original sample that has been kept refrigerated. If control mortality exceeds 10% for either species, the test for that species (including the control) shall be repeated. An acute test will be considered valid only if control mortality does not exceed 10% for either species. If 100% mortality occurs prior to the end of the test, and control mortality is less than 10% at that time, that test (including the control) shall be terminated with the conclusion that the sample demonstrates unacceptable acute toxicity.

3. a. The toxicity tests specified above shall be conducted once every two months until 6 valid bimonthly tests have been completed, and once every 6 months thereafter for the duration of the permit, unless notified otherwise by EPA. These tests are referred to as "routine" tests.

   b. Results from "routine" tests shall be reported according to EPA/600/4-87/028, Section 10, Report Preparation (or the most current edition), and shall be submitted as an attachment to the DMR. Such results are to be entered on the DMR in the following manner:

      1. For the acute test results, if less than 50% survival of a test species occurs, the LC should be entered on the DMR for that species. If 50% or greater survival occurs, the LC that would have less than 50% survival should be entered.

      2. For the chronic test results, the NOEC should be entered on the DMR for that species.

4. a. If unacceptable chronic toxicity (a NOEC less than 15% effluent in either test) and/or unacceptable acute toxicity (greater than 50% lethality of either test species in 100% effluent) is found in a "routine" test, the permittee shall conduct two additional toxicity tests based on the type of unacceptable toxicity found (i.e., chronic and/or acute tests, as appropriate), on the species indicating unacceptable toxicity. For each additional test, the test acceptability criteria specified in section 1(b) and/or 2(b) above, as appropriate, must be met for the test to be considered valid. The first test shall begin within two weeks of the end of the "routine" test and the second test shall be conducted two weeks later. If either or both of these tests are invalid, additional test(s) are to be conducted every two weeks until two valid tests are completed (e.g., if the first test is valid and the second is not, the permittee shall continue to conduct tests until one more test is valid). The additional tests will be used to determine if the toxicity found in the "routine' test is still present.

      1. For "routine" tests with unacceptable chronic toxicity, the permittee shall conduct additional Mysid shrimp (*Mysidopsis bahia*) Growth and Fecundity and/or inland silverside (*Menidia beryllina*) Survival and Growth multi-concentration tests, as appropriate. The tests will be conducted on a control, 100% effluent, and the following % effluent concentrations: 0.25 x the RWC, 0.5 x the RWC, the RWC, and [the RWC + 100]/2. The sample collection requirements specified in section 1(b) above shall be met.

      2. For "routine" tests with unacceptable acute toxicity, the permittee shall conduct additional 48-hour acute static toxicity tests using the Mysid shrimp (*Mysidopsis bahia*) and/or 96-hour acute static-renewal toxicity tests using the inland silverside (*Menidia beryllina*) with a full dilution series, per EPA/600/4-90/027. Four separate grab samples shall be collected at evenly-spaced (6-hr) intervals over a 24-hour period and used in four separate tests.

   b. Results from additional tests, required due to unacceptable acute and/or chronic toxicity in the "routine" test, shall be submitted in a single report prepared according to EPA/600/4-91/000, Section 9, Report Preparation (or the most current edition) and submitted within 45 days of completion of the second additional, valid test.

Excerpt from an NPDES Permit Requiring Acute Toxicity Testing

D. ACUTE BIOASSAY REQUIREMENTS

The Water Quality Standards of Any State require that all waters be free from substances in concentrations or combinations which are harmful to humans, animals, or aquatic life (Any State, Water Quality Criteria for Intrastate, Interstate, and Coastal Waters, Section II.4. Minimum Conditions Applicable to All Waters, page 3, adopted March 22, 1990) In accordance with such requirements, the permittee is authorized to discharge from the combined outfall(s) 001 and 002 only in accordance with the following conditions:

1. The permittee shall perform 48-hour static definitive toxicity tests in accordance with *Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms* Fourth Edition (EPA/6004-90/027). Static tests will be conducted on a 24-hour composite sample of effluent. Less than 36 hours will elapse between sampling and the use of the sample.

   a. The permittee must use both the following organisms:
      (1) *Pimephales promelas* (fathead minnows)
      (2) *Ceriodaphnia dubia* (water fleas)

   b. Dilution water used for these tests shall consist of reagent grade water, defined as distilled or deionized water that does not contain substances which are toxic to the test organisms. Dilution water shall consist of reagent grade water to which the appropriate reagent grade salts have been added to make moderately hard dilution water according to *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms* Third Edition (EPA/600/4-91/000). These dilution waters will be deemed acceptable if the control organisms in the toxicity tests meet the minimum EPA criteria for mortality.

2. The permittee shall conduct the first series of tests specified in part I above within 90 days of the issuance of the permit. The test shall be conducted at a frequency of once per quarter for the first two years from the date of issuance. Based on results of the eight tests, the Permit may be modified to include further testing requirements. Following the first year of testing, the permittee may petition the State Environmental Quality Permit Board for permission to use the most sensitive organism for the toxicity tests to be performed for the remainder of the permit life, if a clear trend in the toxic response exists in the test data. The results of the 48-hour static definitive bioassay tests shall be reported to the State Environmental Quality Permit Board on the next monthly discharge monitoring report.

3. If a 48-hour definitive toxicity test results in an LC_{50} value of less than 10.7%, the permittee shall immediately after the first 48-hour definitive toxicity test results are finalized perform a second 48-hour definitive toxicity test. The LC_{50} determinations from these tests shall be reported to the State Environmental Quality Permit Board within 10 working days after finalization of the results of each test.

4. In the event that the results of any 48-hour definitive toxicity test reveal that the LC_{50} of the permittee's effluent is less than 10.7%, than this finding will constitute a violation of Part I of this permit, and the permittee shall:

   a. Provide a schedule for the implementation of a Toxicity Reduction Evaluation Plan to reduce the toxicity of the waste discharge to safe levels. (Safe levels will be determined by the State Pollution Control Permit Board).

In addition to the specific conditions of this permit, the permittee shall comply with all applicable conditions of 40 CFR 122.7 and 40 122.61 (2/5/90).

Excerpt from an NPDES Permit Requiring Chronic Toxicity Testing

E. CHRONIC BIOASSAY REQUIREMENTS

The Water Quality Standards of Any State require that all waters be free from substances in concentrations or combinations which are harmful to humans, animal, or aquatic life (Any State, Water Quality Criteria for Intrastate and Coastal Waters, section II.4., Minimum Conditions Applicable to All Waters, page 3, adopted March 22, 1990). In accordance with such requirements, the permittee is authorized to discharge from outfall(s) 001 only in accordance with the following conditions:

1. The permittee shall submit any existing toxicity data for review by the State Office of Pollution Control within 30 days of the effective date of this permit.

2. The permittee shall perform 7-day chronic toxicity tests in accordance with *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms* (EPA/600/4-91/000). These chronic toxicity tests shall be initiated within 60 days of the effective date of issuance of the permit to evaluate wastewater toxicity.

   a. Dilution water used for these tests shall consist of reagent grade water, defined as distilled or deionized water that does not contain substances which are toxic to the test organisms. Dilution water shall consist of reagent grade water to which the appropriate reagent grade salts have been added to make moderately hard dilution water according to *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms* (EPA/600/4- 91/000). These dilution waters will be deemed acceptable if the control organisms in the toxicity tests meet the minimum EPA criteria for mortality, growth, and fecundity.

   b. The permittee shall conduct a *Ceriodaphnia dubia* Survival and Reproduction Test, and a *Pimephales promelas* Larval Survival and Growth Test on serial dilutions of effluent to determine if the discharge from outfall(s) 001 is chronically toxic. Such testing will determine if the water affects the survival, growth, and fecundity of the test organisms. Static renewal tests will be conducted on three 24-hour composite samples of effluent. The first of these composite samples will be used to set up the tests for the day 1 and day 2 renewals, the second of these composite samples will be used to renew the tests on days 3 and 4, and the third composite sample will be used to renew the tests on days 5 and 6. Not more than 36 hours will elapse between sampling and the first use of any of the composite samples. Chronic toxicity will be demonstrated if: 1) there is a 20% or more difference in survival between test organisms exposed to appropriate control water and any serial dilution of effluent; or 2) there is a statistically significant difference at the 95% confidence level in reproduction between *Ceriodaphnia* exposed to an appropriate control water and any serial dilution of the effluent; 3) there is a statistically significant difference at the 95% confidence level in growth between *Pimephales promelas* exposed to an appropriate control water and any serial dilution of the effluent.

   c. Such chronic toxicity tests shall be conducted once per quarter for the life of the permit, provided that each 48-hour LC_{50} is greater than or equal to 100%, and each chronic value is greater or equal to 100%. The permittee may petition the State Environmental Quality Permit Board to use only the most sensitive species if a clear trend is demonstrated in the toxic response of the test organisms.

d. If any one chronic toxicity test indicates the 48-hour LC_{50} is less than 100% or that the chronic value is less than 100%, the provisions in section 2(e) shall apply, and the permittee shall conduct another set of the two chronic toxicity tests within two weeks. The results of each toxicity test shall be submitted to the State Office of Pollution Control within 2 weeks of completion of testing.

e. If the chronic value of any test is less than 100%, or if the acute 48-hour LC_{50} of any test is less than 100%, then the effluent will be considered unacceptably chronically toxic and this result will constitute a violation of Part I of this permit. The permittee will then be subject to the provisions of section 3.

3. In the event that after review of the above studies, the State Environmental Quality Permit Board determines the waste stream is toxic to the receiving stream, the permittee shall provide a schedule for the implementation of a Toxicity Reduction Evaluation Plan to reduce the toxicity of the effluent to safe levels. Safe levels will be determined by the State Environmental Quality Permit Board.

Appendix C

Documents Relevant to Toxicity Testing

Report Preparation

as described in *Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms*, EPA/600/4-90/027

The following general format and content are recommended for the report:

12.1 INTRODUCTION

1. Permit number
2. Toxicity testing requirements of permit
3. Plant location
4. Name of receiving water body
5. Contractor (if contracted)
   a. Name of firm
   b. Phone number
   c. Address

12.2 PLANT OPERATIONS

1. Product(s)
2. Raw materials
3. Operating schedule
4. Description of waste treatment
5. Schematic of waste treatment
6. Retention time (if applicable)
7. Volume of discharge (MGD, CFS, GPM)
8. Design flow of treatment facility at time of sampling

12.3 SOURCE OF EFFLUENT, RECEIVING WATER, AND DILUTION WATER

1. Effluent Samples
   a. Sampling point
   b. Sample collection method
   c. Collection dates and times
   d. Mean daily discharge on sample collection date
   e. Lapsed time from sample collection to delivery
   f. Sample temperature when received at the laboratory
   g. Physical and chemical data

2. Receiving Water Samples
   a. Sampling point
   b. Sampling collection method
   c. Collection dates and times
   d. Streamflow at time of sampling and 7Q10
   e. Lapsed time from sample collection to delivery
   f. Sample temperature when received at the laboratory
   g. Physical and chemical data

3. Dilution Water Samples
   a. Source
   b. Collection date(s) and time(s) (where applicable)
   c. Pretreatment
   d. Physical and chemical characteristics (pH, hardness, salinity, etc.)

12.4 TEST CONDITIONS
1. Toxicity test method used (title, number, source)
2. Endpoint(s) of test
3. Deviations from reference method, if any, and reason(s)
4. Date and time test started
5. Date and time test terminated
6. Type and volume of test chambers
7. Volume of solution used per chamber
8. Number of organisms per test chamber
9. Number of replicate test chambers per treatment
10. Feeding frequency, and amount and type of food
11. Acclimation temperature of test organisms (mean and range)
12. Test temperature (mean and range)

12.5 TEST ORGANISMS
1. Scientific name
2. Age
3. Life stage
4. Mean length and weight (where applicable)
5. Source
6. Diseases and treatment (where applicable)

12.6 QUALITY ASSURANCE
1. Reference toxicant used routinely; source
2. Date and time of most recent reference toxicant test; test results and current cusum chart
3. Dilution water used in reference toxicant test
4. Physical and chemical methods used

12.7 RESULTS
1. Provide raw toxicity data in tabular form, including daily records of affected organisms in each concentration (including controls)
2. Provide table of endpoints: LC50, NOEC, Pass/Fail
3. Indicate statistical methods used to calculate endpoints
4. Provide summary table of physical and chemical data
5. Tabulate QA data

12.8 CONCLUSIONS AND RECOMMENDATIONS
1. Relationship between test endpoints and permit limits
2. Action to be taken

CHAIN OF CUSTODY RECORD

Client Name_____ Priority Number_____

Address_____

_____

Attention_____

Telephone_____ Fax_____

Page_____ of_____

Dish No.	Sample I.D.	Collection				Container			Preservative	Sample Type	Analysis required/Remarks
		Date	Time	Grab	Comp	Vol	# of	Type			

Analysis List 1:

Analysis List 2:

Analysis List 3:

Collected and relinquished by: Date/Time:	Received by: Date/Time	Hazards Associated with Samples: Comments:
Relinquished by: Date/Time:	Received by: Date/Time	
Method of Shipment: Date/Time:	Received by: Date/Time	pH____ Temp____ Res.Chlorine ____ Flow_____
Relinquished by: Date/Time:	Received for Laboratory: Date/Time	DO____ Compositer Start/Stop ____/____ Other_____

Quality Control Fact Sheet for Self-Biomonitoring Acute/Chronic Toxicity Test Data

Permit No. _____

Facility Name _____

Facility Location _____

Laboratory Investigator _____

Permit Requirement

Sampling Location _____ Type of Sample _____

Limit _____ Test Duration _____

Type of Test _____ Test Organism Age _____

Test Results

$LC_{50}/EC_{50}/NOEC/IC_{25}$ _____ 95% Confidence Interval _____

Statistical Method _____

Quality Control Summary

Date of sample _____ Dates of Test _____

Control Mortality _____ % Control Mean Dry Weight _____

Temperature Maintained within +/- 1° of test temperature?
Yes____ No____

Dissolved oxygen levels always greater than 4 0 mg/L?
Yes____ No____

Loading factor for all exposure chambers less than or equal to maximum allowed for the test type and temperature?
Yes____ No____

Do the test results indicate a direct relationship between effluent concentration and response of the test organism (i.e., more deaths occur at the highest effluent concentrations)?
Yes____ No____

Appendix D

Glossary

Acute-to-chronic ratio (ACR)
A ratio that compares the concentration of an effluent or a toxic substance that causes acute toxicity in a species with the concentration of an effluent or a toxic substance that causes chronic toxicity to the same species. The ACR makes possible interconversion of acute toxicity units and chronic toxicity units: $TU_a = (ACR)(TU_c)$.

Acute toxicity test
A test that uses living organisms and measures an effect that occurs within 96 hours. Acute toxicity tests commonly, but not exclusively, measure the death of organisms.

Acute toxicity unit (TU_a)
The reciprocal of the LC_{50} multiplied by 100. That is, $TU_a = (1/LC_{50})(100)$.

Average monthly limit (AML)
In an NPDES permit, the highest value allowable for the average of daily discharges occurring over a one-month period.

Bioaccumulation
The passage of substances from the environment into a living organism's tissues by means of absorption, respiration, or feeding. Also called biological accumulation.

Bioassessment
Evaluation of the biological condition of a body of water based on studies of the organisms living there and on the chemical and physical characteristics of the body of water. Also called biological assessment.

Bioavailability
The presence of a substance in a form that can affect organisms. Also called biological availability.

Bioconcentration
The passage of a substance from water into an organism by absorption through its skin or during respiration.

Biomagnification
The process of a substance's passing up the food chain and becoming more concentrated in organisms toward the top of the food chain. Also called biological magnification.

Biomonitoring
The use of living organisms to monitor water quality by such means as toxicity testing and bioassessment. Also called biological monitoring.

Biota
The organisms occurring in a specified area or during a specified period.

Chemical-specific
Concerning a limit or test that involves specific chemical substances.

Chain of custody
A document for recording information concerning the collection, handling, transfer, and analysis of an effluent sample.

Chronic toxicity test
A test that measures a sublethal effect on test organisms over an extended period of time, generally the life cycle of the test organisms.

Chronic toxicity unit (TU_c)
The reciprocal of the NOEC multiplied by 100. That is, $TU_c = (1/NOEC)(100)$.

Chronic Value (ChV)
In a chronic toxicity test, a presumably safe concentration between the LOEC and the NOEC. The ChV is a point estimation calculated as the geometric mean of the LOEC and NOEC.

Compliance Biomonitoring Inspection (CBI)
At a facility with an NPDES permit, an inspection conducted by the regulatory agency or its contractor for the purpose of collecting effluent samples for analysis by acute and chronic toxicity tests. In addition, the CBI involves the examination of records and the brief observations of the CEI.

Compliance Evaluation Inspection (CEI)
At a facility with an NPDES permit, an inspection conducted by the regulatory agency or its contractor to evaluate a permittee's self- monitoring by examining records and making brief observations of the facility, its effluent, and its receiving water.

Composite sample
A mixed sample collected over a specified period of time.

Control
In a toxicity test, a group of organisms exposed to the same conditions as other groups of organisms but not exposed to effluent.

CWA Clean Water Act.

Discharge monitoring report (DMR)
A report of the results of tests that have been conducted on an effluent.

Discharge monitoring report quality assurance (DMR/QA)
An EPA program that tracks the quality of self-monitoring.

Dose-response curve
A graph that plots the concentration of a substance against the test organisms' level of response.

Flow-through test
A test in which fresh effluent or freshly diluted effluent is pumped through test chambers continuously.

Food Chain
A series of organisms that sequentially feed on one another.

Grab sample
A sample collected at one time.

Independent Application
The principle that no one of the three methods of monitoring water quality—chemical analysis, biomonitoring, or bioassessment—is inherently superior to the other two.

Inhibition Concentration (IC)
In a chronic toxicity test of an effluent or substance, the concentration causing an inhibition of a biological function, such as reproduction, in test organisms. ICs are reported as the concentration at which test organisms show a specified level of inhibition: IC_{25} is the concentration at which the biological function shows 25 percent inhibition in test organisms.

Integrated Approach
In monitoring water quality, a threefold approach that consists of chemical analysis, biomonitoring, and bioassessment.

Lethal Concentration (LC)
In an acute toxicity test of an effluent or substance, the concentration causing death in test organisms. LCs are reported as the concentration proving lethal to a percentage of the test organisms: LC_{50} is the concentration at which 50 percent of the organisms die.

Long-term average (LTA)
In an NPDES permit, the acceptable mean of an effluent's pollutant concentrations or parameters over the life of the permit or facility.

Lowest Observed Effect Concentration (LOEC)
In a chronic toxicity test of an effluent or substance, the lowest concentration causing an observable effect in test organisms.

Major facility
Either 1) a municipal permittee that has a design flow of one million gallons per day or greater, a service population of 10,000 or greater, or significant impact on water quality, 2) any non-municipal permittee that has an industrial rating of 80 or higher, or 3) any discretionary permittee evaluated as necessary to be declared a major.

Maximum daily limit (MDL)
In an NPDES permit, the highest value allowable for a discharge during a 24-hour period.

No Observable Effect Concentration (NOEC)
In a chronic toxicity test of an effluent or substance, the highest concentration at which no observable effect occurs in test organisms.

No Observable Effect Level (NOEL)
Same as No Observable Effect Concentration.

Notice of Violation (NOV)
A notice from the EPA region to the delegated State stating that a violation of a facility's NPDES permit has occurred and that if the State does not take action, the Federal government will. The permittee receives a copy of the NOV.

Performance Audit Inspection (PAI)
At a facility with an NPDES permit, an inspection conducted by the regulatory agency or its contractor to evaluate the permittee's self-monitoring program. Specifically, the inspection verifies reported data and compliance by checking records and in addition observes the self-monitoring process from the collection of samples through laboratory analysis and reporting.

Permit Compliance System (PCS)
A national computerized database that contains information about NPDES permits—including limits, monitoring schedules, frequency of discharge, inspections, permit date, and self-monitoring reports—and that flags violations of permit limits, compliance schedules, and compliance reporting.

Point source discharge
Generally, a discharge that originates at a specific, identifiable location, such as a pipe, ditch, channel, or tunnel. Point source discharges also include discharges having less localized sources. CWA (40 CFR 122.2) defines point source discharge, and section 122.3 provides further clarification.

QA Quality assurance.

Quarterly Noncompliance Report (QNCR)
A report issued quarterly and listing facilities that have violations requiring attention.

Short-term chronic toxicity test
A chronic toxicity test that focusses on the period in an organism's life cycle when it shows the greatest sensitivity to its environment. Short-term chronic toxicity tests, which must include at least one-tenth of an organism's life cycle, generally last 7 days or less.

Static nonrenewal test
A static test that uses the same effluent for the duration of the test.

Static renewal test
A static test in which fresh effluent replaces all or part of the effluent at specified intervals.

Static test
A test that uses the same effluent for the duration of the test or in which only limited replacement of the effluent occurs.

Technology based
Concerning a standard, requirement, or method having existing technologies as its basis.

Test organism
A species of organism used in a biological test.

Toxicity
Harmful effects occurring in a human, other animal, or plant as a direct result of a chemical substance.

Toxicity Identification Evaluation (TIE)
An evaluation of the waste product at each step in production or waste treatment.

Toxicity Reduction Evaluation (TRE)
A study that investigates a specific site in a stepwise fashion in order to uncover effective measures for controlling effluent toxicity and for resolving a toxicity problem.

Wasteload allocation (WLA)
The maximum amount of pollutants that a body of water can assimilate in a day from a specific facility without violating the State's Water Quality Standards.

Water quality based
Concerning a standard, requirement, or method aimed at attaining a specified level of water quality without regard to what technologies exist to achieve this level.

Water Quality Standard (WQS)
A law or regulation that states the use of a body of water, the numeric and narrative water quality criteria necessary to protect this use, and an antidegradation statement.

Whole effluent toxicity (WET)
A form of biomonitoring that considers the total toxic effect of an effluent as measured by a test that uses living organisms.