How to use the Microtox® Acute Toxicity Test to perform an In-House Toxicity Reduction Evaluation (TRE)

Abstract

This guide outlines a practical method for running inexpensive, non-mandated TRE's in wastewater treatment plants. The studies produce information that can be used to hold toxicity levels below regulatory limits, avoiding expensive and disruptive mandated TRE's.

Because every plant is different from every other, it is necessary to first measure toxicity in the influent and effluent streams at the site over a period of 30-60 days. When

charted, this information shows "normal" toxicity baselines at key points, so that the significance of toxicity variations can be determined.

The Microtox® Basic Test is used to measure toxicity in influent streams and in-plant process streams, in which toxicity levels are high enough for calculations of EC50's.

The Microtox® Comparison Test (or Inhibition Test) is used for effluents in which toxicity levels are too low for calculations of EC50's.

With normal baselines and toxicity variations established, it is possible to relate influent and effluent variations, pinpoint sources of toxicity, and develop practical ways to deal with them. After the treatment system has been characterized and optimized, regularly scheduled Microtox® Comparison Tests or should be performed to increase assurance that the final effluent will pass regulatory compliance test.

Preface

Microtox® is an ASTM Standard method (D-5560, 1995) for determining the toxicity of aqueous wastes before and after biological treatment. This is a guide for using Microtox® to conduct an in-house toxicity reduction evaluation (In-House TRE). Since 1979, wastewater treatment plants (WWTP's) have used Microtox® Acute Toxicity Test results to:

- Help assure compliance with NPDES toxicity limits
- Measure toxicity in influent streams
- Determine treatment efficiency in industrial and municipal wastewater treatment plants
- Monitor treatment processes from the raw influent to final effluent

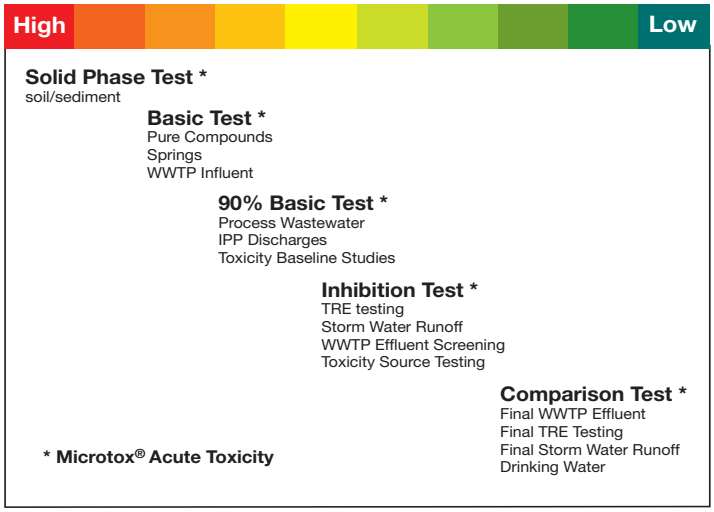
With sufficient historical data from compliance species training, it is also possible to develop a correlation between Microtox® and an NPDES compliance test species. Failure to achieve or maintain compliance with NPDES toxicity limits may trigger a USEPA-ordered TRE to identify the source of the toxics problem, and provide a basis for a recommended solution. Corrective action may be as simple as "improved housekeeping" procedures or as complicated and costly as physical modification of a wastewater treatment plant. In complex facilities with numerous influent streams, the quality and quantity of incoming wastewater may vary unpredictably. Consequently, an official TRE may involve an extensive investigation to identify toxicants and/or cost effective treatment or source remediation options. The cost of non-compliance can be high. This guide suggests which Microtox® protocols are appropriate to:

- Determine the toxicity of individual influent streams and their potential impact on a plant's biomass (How does each individual stream affect the plant?)
- Determine the treatment efficiency at each stage of the water treatment system. (How are the combined streams affecting each stage of the plant's treatment process on a daily basis?)
- Determine the quality of the final effluent. This provides data indicative of NPDES biomontoring test results. (How well did the plant treat the combined waste?)

This program assumes that the initiative for toxicity control can and should come from within the permitted facility. An In-House TRE makes good economic sense. It is based on an understanding of dynamics of how varying toxicity affects the wastewater treatment plant's performance *even when a mandated TRE is not anticipated.*

Microtox® Test Spectrum

Different Microtox® protocols are appropriate for use with samples in different ranges of toxicity.



Stage I of the In-House TRE

Develop a profile of the plant's daily influent and effluent quality by making and charting key measurements daily. This establishes a baseline of "normal" toxicity levels in the plant, and often reveals unsuspected cycles in toxicity that may strongly influence the results of compliance tests.

To develop the profile

1. Microtox® Basic Test
Perform a Microtox® Basic test, calculate an EC50 on a daily basis on each influent stream.

2. Microtox® Basic Test
Perform a Microtox® Basic test and calculate an EC50 on a daily basis on the whole combined influent. This will help establish the normal levels of toxicity the plant sees on a daily basis.

NOTE: For initial testing and high toxicity influent streams, use the *ASTM Extended (9 dilutions) Basic Test.* For those influent streams that prove to be lower toxicity, use the *81.9% Basic Test.*

3. Inhibition test
Perform a Microtox® Inhibition Test on a daily basis on the whole combined effluent. If toxicity effects greater than 5% are observed (either inhibition or stimulation), and if the compliance bioassay is a chronic test, begin an investigation into possible causes. This Stage II investigation is discussed below. Routine monitoring, after the baselines are established, is best achieved in the Microtox® Comparison Test, which provides more precise data than Inhibition Test.

Comparison Test
With an additional step in the procedure, it provides results in the same 30 minute time period.

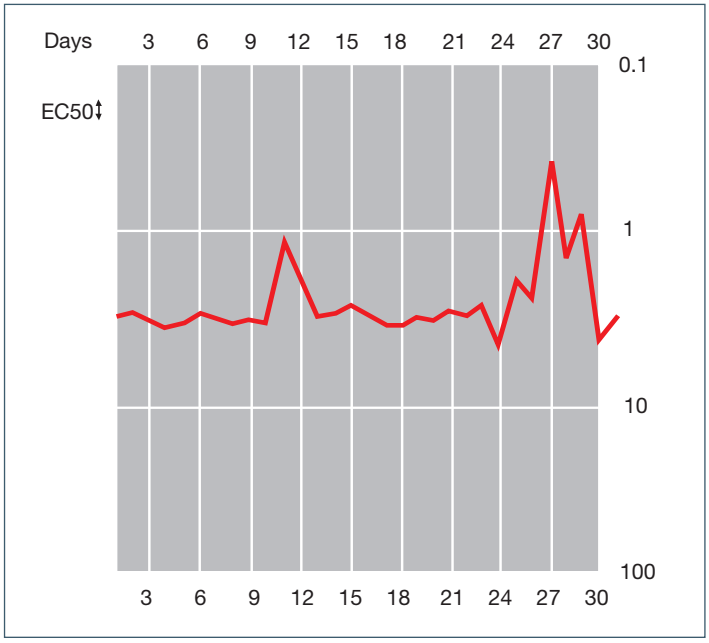
Examples of Daily Profiles

Note that the examples shown here are qualitative, not quantitative. The key factor is change.

What typical influent toxicity profiles look like:
The following three charts show typical examples of WWTP influent toxicity when daily Basic Test EC50's are charted over a month. Your facility may have many individual streams that you will initially test daily. Once their relative toxicity is determined, they can be flow-weighted, and each stream's total effect on the treatment plant can be ranked "best to worst." The profile at your facility will obviously not look exactly like those depicted here, because toxicity in influent streams is always site specific. (The Microtox® user can also test each stage of the WWTP's treatment process to qualify the actual amount of toxicity reduction at each stage and hence the efficiency of the plant's removal of toxic compounds.) Note also that seasonal changes in the baseline are very likely to appear over longer-term charts.

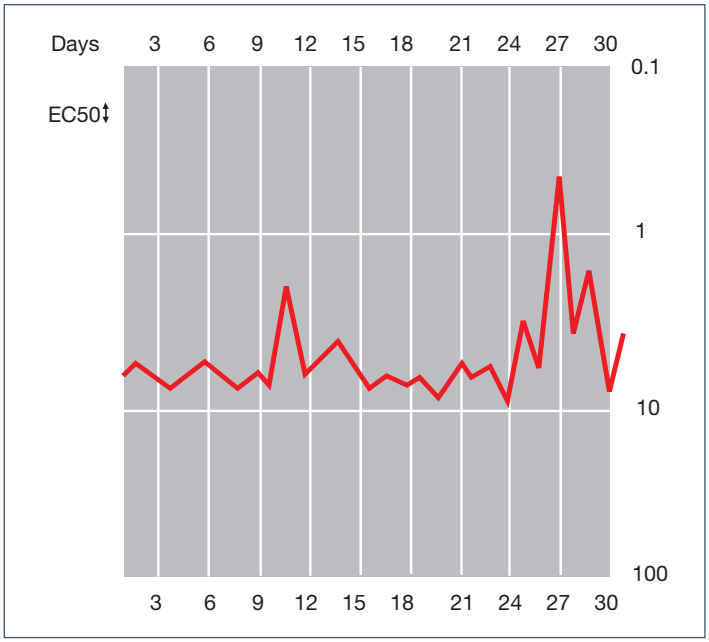
EXAMPLE 1

This is a one-month toxicity profile of an Individual influent stream. The Microtox® Basic Test is appropriate for use with this relatively toxic, untreated stream, easily calculating EC50's.



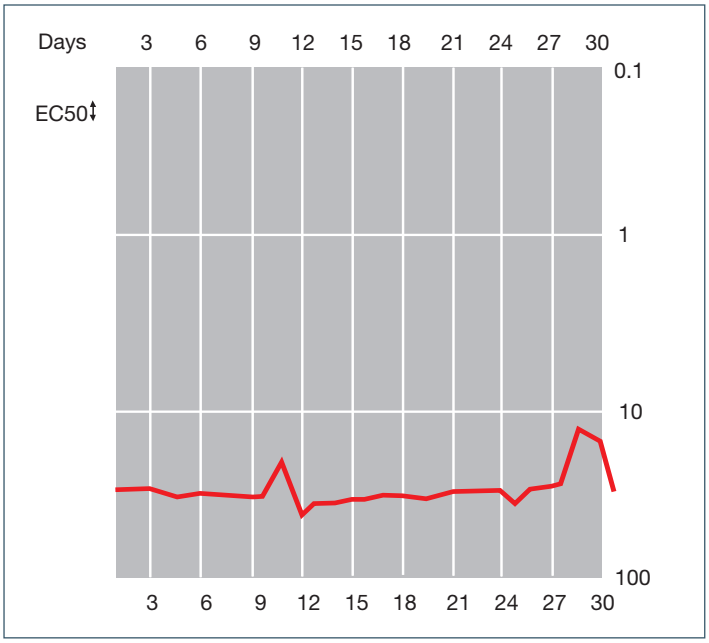
EXAMPLE 2

This is a one-month toxicity profile of the whole combined influent stream for a treatment plant. Again, the Microtox® Basic Test is appropriate because it can readily calculate EC50's from the relatively toxic samples.



EXAMPLE 3

This is a one-month toxicity profile for an intermediate treatment stage, using data from the Microtox® Basic Test.

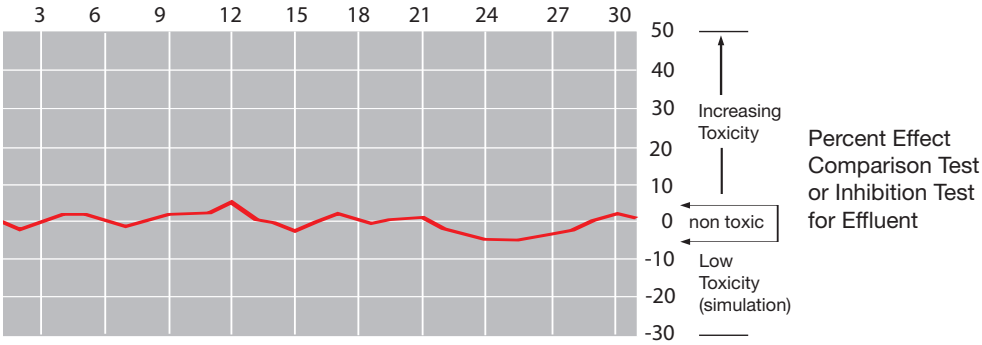


What typical effluent toxicity profiles look like:

The following charts show three different, but typical, examples of charts plotted with data from the Microtox® Inhibition Test.

EXAMPLE 4

A low Toxicity Effluent Profile. (Charted over one month with the Microtox® Inhibition Test.)



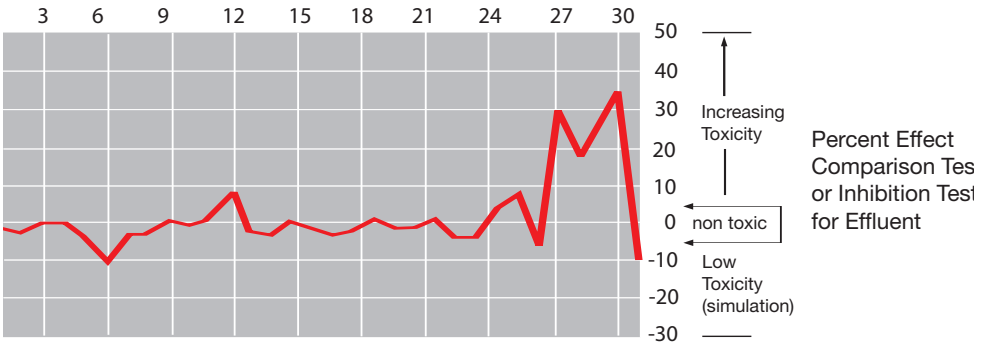
Because the effluent toxicity is too low for calculation of EC50's, the Inhibition Test is used. The data appear at the bottom of the Daily Toxicity Chart. The values indicate "percent difference" of sample bioreactivity (either inhibition or low level stimulation, which is often an expression of low level toxicity) from bioreactivity of a non-toxic control. Differences less than 5% are considered insignificant, indicating likely, though not certain, compliance. Should the facility fail a chronic ceriodaphnia test during the profile depicted here, it would indicate that the plant is experiencing pass-through of chronic toxicity. No acute toxicity was observed.

This is typical of a municipal WWTP with no significant industrial users, or with industry that generates no significant toxic waste. Dischargers with this profile are low-risk candidates to become involved in a TRE.

EXAMPLE 5

An Intermittent Toxicity Effluent Profile (Charted over one month with the Microtox® Inhibition Test.)

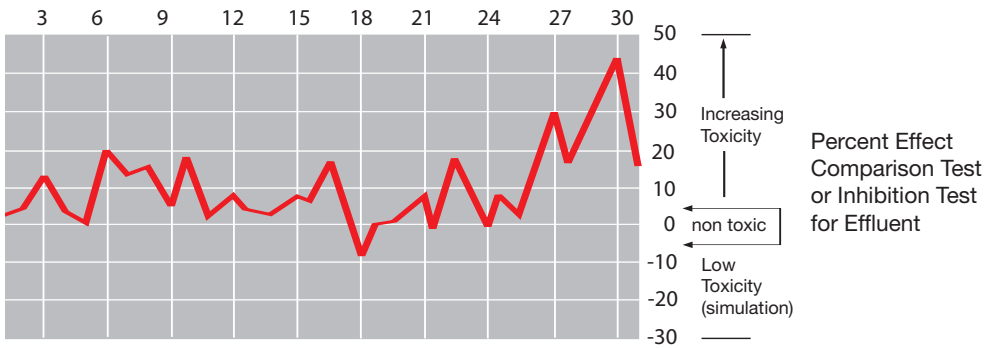
This is typical of a municipal WWTP with some significant industrial contributors, or contributors who generate contaminants that are hard to treat, or a treatment plant that receives toxicity in slugs. Dischargers with this profile are candidates to become involved in a TRE, because of intermittent toxicity excursions in their effluent.



EXAMPLE 6

High Effluent Toxicity Profile (Charted over one month with the Microtox® Inhibition Test.)

This profile is characteristic of an outdated, overworked, or hard-to-operate WWTP. With continuous acute toxicity, this plant is probably already involved in a TRE.

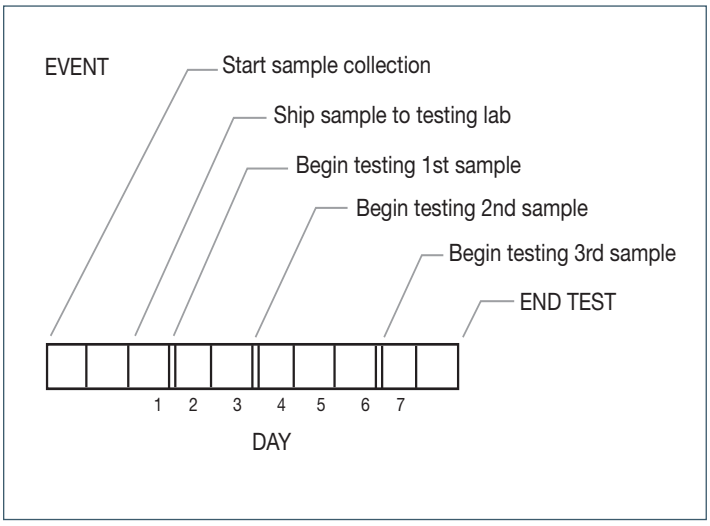


Review of Effluent Toxicity Profiles

Facilities with profiles like that in Example 6 obviously need internal toxicity identification for process control and biomass protection. The profiles of effluent toxicity in Example 4 and 5 present the most challenging problems in identification and reduction of toxicity in effluent. The levels of toxics in the effluent Example 4 are so low at time that their detection and identification are difficult. Pesticides can cause this type of profile. Example 5 is not as difficult, because intermittent acute toxicity, sufficient to cause failure of a chronic *Ceriodaphania* test was easily observed. When a facility fails a *Ceriodaphania* test it is often falsely assumed that the plant is experiencing chronic toxicity pass-through. Chronic toxicity may have played a part in the failure, but it is even more likely in this example that the failure was caused by intermittent acute toxicity.

Review of Compliance Test Procedures

TRE testing reveals how a facility that is experiencing intermittent acute toxicity, as in Example 5, may easily pick an inopportune time to begin a compliance test, fail it, and be out of compliance. A 24-hour composite sample is exposed to the test organism for two days. Then another composite sample is taken and the same organisms are exposed to it for two days. A final composite sample is then taken, and the organisms exposed to the sample for the remainder of the test. Every day between 80-95% of the sample currently being tested is poured off, and fresh sample is added. The organisms are exposed to three separate composite samples over a one-week period. The following example relates to the protocol most commonly used in NPDES effluent testing where multiple samples and renewal are involved. Here is a histograph of a composite sample testing sequence.



Interspecies correlation is not necessary before using Microtox® You can start an in-house toxicity reduction program simply by developing Daily Toxicity Profiles of wastewater plant's influent and effluent. Most Microtox® users never try to determine interspecies correlation. For wastewater plant process control, a reasonable response relationship between Microtox® and biomass is more relevant than expending time and effort to develop an interspecies numerical correlation. Even if Microtox® could tell you that final compliance test results would be, once the test begins, it must continue. A large body of literature documents Microtox® interspecies correlation. For those who feel the need, some useful references and comparison techniques are provided in an appendix.

Stage II of the In-House TRE

If Inhibition Tests on effluent show measurable toxicity, and if the compliance test battery includes chronic testing, it's wise to do a Stage II investigation. You'll want to run a common sense investigation of possible sources of toxic contamination.

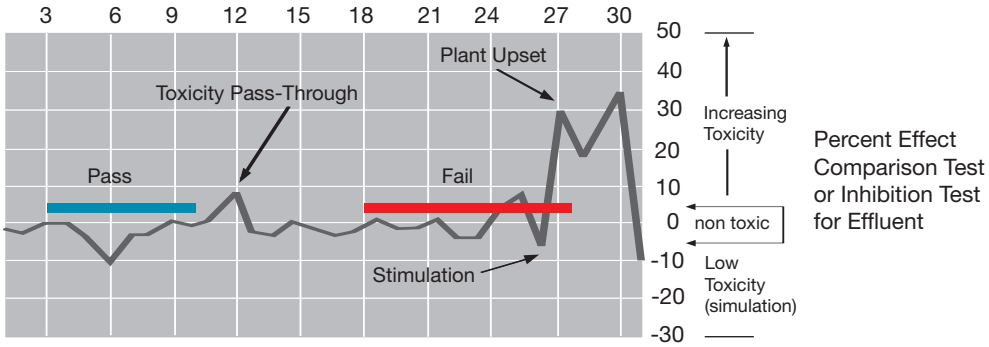
- Was any equipment being cleaned in the facility?
- Was painting taking place?
- Was there an unreported spill that got into the wastewater system?
- Has some process been changed in the wastewater plant?
- Is the dechlorinator out of service?
- Was rain a factor?
- What did the other water quality and chemical specific tests look like?
- Is the elevated toxicity related to unusual activity upstream; e.g. a contributor releasing materials that pass through treatment without toxicity reduction?

Stage III of the In-House TRE

With an investigation that answers such questions, with problems pinpointed, and solutions developed, a routine monitoring program should be initiated to catch future problems while they are still minor. The routine monitoring program will differ by facility, industry, and city. It should be designed to monitor for situations that can be anticipated from the plant's previously developed influent and effluent profiles.

It is not uncommon to observe toxicity in one of the samples but not the others. There are many other steps, such as storage, shipping and renewal, and in theory their execution is controlled by strict guidelines. In practice, the guidelines cannot always be followed, adding an element of variability to the results.

EXAMPLE 5 with Ceriodaphnia test histographs overlaid



Final results may depend upon which day the test begins. The test easily in the month would have shown a pass, and the later test would have shown failure. To make sense of the compliance test results, especially when dealing with intermittent toxicity, it's clearly important to know what the overall situation is.

Look at test results in context WWTP's typically run a number of different tests such as pH, TSS, total nitrogen, ammonia, etc… generating useful data. When the results of these tests are tabulated and displayed in a matrix that includes the results of the compliance tests, like the one below, patterns may appear to the experienced eye that correlate with compliance test results.

Effluent Toxicity Characteristics

Site-specific Tests	Samples									
pH	●	●	○	○	○	○	○	○	○	○
TDS	●	○	○	○	●	●	○	○	○	○
COD	●	○	○	○	○	○	○	○	●	○
Total Nitrates	○	○	○	○	○	○	○	●	○	○
Ammonia	○	○	○	○	○	○	○	○	○	○
Salinity	●	○	●	○	○	○	○	○	○	○
BOD	●	○	○	○	○	○	●	○	●	●
Microtox®	●	○	○	○	○	●	●	○	●	●
Results of Chronic Test	fail	pass	pass	pass	pass	pass	fail	pass	fail	pass

○ = Within predetermined limits
● = Outside predetermined limits

Step by Step Summary for Conducting an In-House TRE Daily Toxicity Profile Plan

Stage I

1. Begin testing both influent and effluent at least once per day.

   a) Perform a daily Microtox® Basic Test on the combined influent. Use this data to begin a daily influent profile.

   b) Test each individual influent stream with Basic Test if time allows.

   c) Perform a daily Inhibition Test on effluent. Chart the data to create the Effluent profile. Elevated effluent toxicity will usually be preceded by elevated influent toxicity. If not, the treatment process may need scrutiny.

2. After performing a sufficient number (30-60 days) of daily Inhibition Tests on effluent, and Basic Test on the influent, chart the results and review them, looking for patterns and phenomena that may cause change in toxicity. At this point, it will probably be apparent which streams introduce the most toxicity to your plant and whether or not your effluent has toxicity. SDI can advise you in this initial data interpretation.

3. After screening the effluent and plotting inhibition for 30 to 60 days, review the results. The inhibition data alone may be all you need to determine how often and how much toxicity is present in the effluent. Spikes in effluent toxicity should directly correspond to effects detected in compliance tests using another species. A large amount of toxicity shown by Microtox® may correlate to lethality in other species. A small amount of toxicity shown by Microtox® may correlate with reproduction or growth problems in the other species. If the object of effluent testing is to determine an interspecies correlation (not always the case), it will be necessary to perform a full Microtox® bioassay, using one of the protocols designed for testing samples with low toxicity each day.

Stage II

4. Continue testing and investigation if elevated toxicity is observed. Continue performing Basic Microtox® Tests on the combined influent and any individual streams that may contribute high levels of toxicity, until problem sources can be identified, and solutions developed.

Stage III

5. Initiate a maintenance and early warning program of regularly scheduled Comparison Tests and/or Microtox® Chronic Tests to monitor the final effluent once toxicity is under control.

Appendix

References on Microtox® Interspecies Correlation and Methods of Interspecies Test Data Comparison

References

The following items are found in publications referenced in the Microtox® Bibliography. They are found in TRE protocol guides published by the USEPA. They place Microtox® in perspective as a surrogate organism and hence a screening test to certain species the USEPA currently recommends NPDES permits.

Toxicity Reduction Evaluation Protocol for Municipal Wastewater Treatment Plant. EPA Doc. EPA/600/2-88/062, pg.12-2.

> "Depending on the species to be used, it may be more economical to culture the test organisms than purchase them. In some cases it may be necessary to use a rapid screening test such as a bacterial bioluminescent test, e.g. Microtox®."

Generalized Methodology for Conducting Industrial Toxicity Reduction Evaluations, EPA, Doc. EPA/600/2-88/070. pg.A-10.

> "Although the Microtox® test endpoint (20-minute) was not an exact predictor of the fish bioassay endpoint (96-hour), it was felt that Microtox® was adequate for cost-effective screening effluent toxicity for the following reasons: In all cases tested, if toxicity was identified by the fish bioassay, the Microtox® also identified toxicity. Microtox® always indicated at least as much toxicity as the fish bioassay, and often more eliminating the possibility of a false-negative result."

The Microtox® Bibliography contains other references to interspecies comparative studies. The data indicate that the more complex the sample, the higher the rate of correlation between the common test species. It also indicates that there is something fundamental about toxicity. Except for some expected variability in dose effect, none of the common test species found in the food chain tolerate toxicants as very well. The Microtox® organism, *Vibrio fischeri (P.phosphoreum)*, was selected from about 70 other bioluminescent organisms by SDI because it demonstrated the highest sensitivity across a broad range of toxicants. The following comparisons emphasize these observations:

Organism Compared with Microtox®	Results of Study	Author, Date and Microbics Reference No.
Fathead Minnow EC50	r = 1.0 r = 0.9	Chang et al, 1987 #5
Selenastrum Rainbow trout	90% agreement 84% agreement	Blaise et al, 1987, #75
Oyster Embryo Amphipod	r = 0.62 r = 0.48	Williams et al, 1986, #63
Daphnia Magna	86% agreement	Vassuer et al, 1984, #24
Daphnia Magna	Good agreement for 162 wastewater samples	Vassuer et al, 1983, #127
Guppies, Brown trout, Sheephead minnow, Bluegill, Rainbow trout, Daphnia Magna and Shrimp	Respective R = 0.89, 0.92, 0.80, 0.77 0.74, 0.87, and 0.68	Ribo et al, 1983, #20
Fathead Minnow	r = 0.91	Indorato et al, 1983, #10

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