Microtox Acute Toxicity Test

PRINCIPLE OF OPERATION

The test exposes luminescent organisms in Microtox Acute Reagent to aqueous samples, and measures the increase or decrease in light output by the test organisms.

Reagent contains living luminescent bacteria that have been grown under optimal conditions, harvested, and then lyophilized (freeze-dried). The lyophilized bacteria are rehydrated with Reconstitution Solution to provide a ready-to-use suspension of organisms.

The test system measures the light output of the luminescent bacteria after they have been challenged by a sample and compares it to the light output of a control (reagent blank) that contains no sample. A difference in light output (between the sample and the control) is attributed to the effect of the sample on the organisms.

PRECISION

Each test cuvette contains roughly a million individual test organisms that are challenged by the test sample. Variations among individual organisms become statistically insignificant. The system measures a single parameter, the simultaneous light output of all of the organisms.

Each batch of Reagent, containing lyophilized test organisms, is prepared under conditions within very rigidly controlled limits.

TIME AND TEMPERATURE

Different chemicals affect living organisms at different rates, reflecting differences in mechanism of action.

For some classes of chemicals, the effect on light output is complete in 5 minutes.

For other classes of chemicals, the light output is still decreasing rapidly at 5 minutes. In these cases, 15-minute data may be more reliable.

We recommend that 5-minute and 15-minute data be taken routinely when dealing with unknown samples. Some laboratories elect to collect data routinely at five, fifteen and thirty minutes. The software will

automatically process data taken at any one, two, or three times you select.

The temperature during exposure to various materials will affect the response of the living organisms. For the Microtox Acute Toxicity Test procedures the Incubator Wells and READ Well temperature is 15°C.

CONTROL (BLANK)

Reagent Control is required for each test and is run concurrently with the test. Light levels normally change with time, for reasons other than the bioreactivity of the test sample.

Use of the Reagent Control allows the operator to compensate for some of these variables when reducing the data. In all variations of the Basic Test procedure, the responses of all the test cuvettes are normalized to that of the Control test response, which compensates for errors up to 20% in the 10 µL pipetting of reagent.

SUPPLIES & ACCESSORIES

Testing requires several special items in addition to those commonly found in testing laboratories.

REAGENT

The Microtox Acute Toxicity Test Reagent is specially formulated for bioreactivity testing with sensitivity to a broad range of toxicants.

The reagent is a freeze-dried preparation of a specially selected strain of the marine bacterium *Vibrio fischeri* (formerly known as *Photobacterium phosphoreum*, NRRL number B-11177). A vial of reagent contains roughly one hundred million test organisms.

Shelf life is one year when stored at minus (-) 20ºC to (-) 25ºC. Do Not store the Reagent below minus (-) 25ºC. Storage at normal refrigerator temperature greatly reduces shelf-life.

Reagent should not be stored in a self-defrosting freezer, which defrosts by warming up periodically.

Use the reagent within one to two hours after

reconstitution. The sensitivity of the reagent is essentially unchanged for 1-2 hours after reconstitution. Changes in sensitivity may become significant after that time for some samples.
Some laboratories reconstitute a vial of Reagent at the beginning of the day, store it in the REAGENT Well, then use the rehydrated reagent as needed through the day until it is consumed.
For many applications, this is acceptable practice, but if Reagent is to be used 90 minutes or more after reconstitution, its performance should be monitored periodically with a standard such as PHENOL, to indicate changing sensitivity.

RECONSTITUTION SOLUTION

Reconstitution Solution is specially prepared nontoxic Ultra Pure Water. Shelf life is one year when stored at room temperature.

DILUENT

The Diluent is a specially prepared nontoxic 2% Sodium chloride (NaCl) solution, used for diluting the sample and the reagent.
The marine bacterium in the reagent requires osmotic protection that is provided by the 2% NaCl.
Shelf life is one year when stored at room temperature.

OAS

OAS (Osmotic Adjusting Solution) is a specially prepared nontoxic 22% Sodium chloride (NaCl) solution, used to adjust the osmotic pressure of the sample to approximately 2% NaCl.
To adjust a sample osmotically, add one part of OAS to ten parts of sample.

(X mL sample x 0.1 = mL OAS)

Example:

2.5 mL sample x 0.1 = 0.25 mL OAS

The sample can also be osmotically adjusted by the addition of solid AR grade NaCl to a final concentration of 2.0%.
Shelf life is one year when stored at room temperature.

DO NOT PREPARE SOLUTIONS
Do not make Diluent or OAS yourself or use substitutes. The production of uncontaminated solutions is difficult, and some laboratories have created problems using their own Diluent and Osmotic Adjustment Solution.

CUVETTES

Cuvettes are used to contain samples, controls, and Reagent during testing. They are nontoxic and disposable.
Used cuvettes cannot reliably be cleaned for reuse. Traces of detergent or sample contaminants interfere with later tests. The risk of interference from contamination is unacceptably high.

PIPETTORS AND PIPETTOR TIPS

Test protocols require repeated precise transfer of small amounts of liquid, as little as 10µL. High precision adjustable micro-pipettors are necessary. It is highly recommended to have and use the following pipettors:

10 - 100 µL adjustable volume pipettor
0.25 - 2.5 mL adjustable volume pipettor
repeat pipettor

Sample Collection and Preparation

SAMPLE COLLECTION

Use new clean, borosilicate, screw cap containers (30 to 50 mL) with Teflon® lined caps. Fill the container completely to the top with sample, leaving NO airspace.
Completely filling the container helps keep volatile material in solution.

SAMPLE STORAGE

Test the sample as soon as possible after collection. If testing is delayed, store samples at normal refrigerator temperature.

The toxicity of the sample can change with time, so testing the sample within 1-2 hours after collection is best, but this is not always possible. Try to test the refrigerated sample within 24-48 hours after collection.

COLOURED SAMPLE

Perform the test protocol, and determine the sample's IC_{XX} (EC_{XX}). Check the sample concentration at the IC_{XX} (EC_{XX}) for visible colour. If it contains obvious colour, perform the Colour Correction Protocol.

TURBID SAMPLE

If the sample is turbid and this is objectionable, centrifuge the sample at an adequate speed and time to remove the turbidity.

The turbidity is objectionable when the toxicity from the turbid material is not wanted. If the toxicity is desired from the material that causes turbity, do not centrifuge the sample. If the sample is too turbid to perform a Basic Test use the Solid-Phase Test.

CHLORINE CONTENT

The Reagent (organism) is sensitive to chlorine as are all microorganisms.

Municipal Water Treatment (drinking water) or Waste Water Treatment Plants have a common problem with the water they are producing, that is bacterial contamination. Chlorination of the water is generally used to solve this problem. Chlorine is toxic: it is used for killing microorganisms.

Most of the time when either a Municipal Water Treatment or Waste Water Treatment Plant wants to check for acute toxicity, they do not want to know the effect of chlorination.

Collect the samples before chlorination, unless you are testing for the effect of chlorination. Do not collect a chlorinated sample for testing, unless you want to know the effect of chlorination or can not collect a sample that is not chlorinated. If knowing the effect of chlorination is desired, it is

recommended to test the sample just before chlorination and just after chlorination.
When the effect of chlorination is desired or can not be avoided, dechlorinate the sample with Sodium Thiosulfate.

Sodium Thiosulfate Dechlorination

To dechlorinate a sample add 1 part of Sodium Thiosulfate Stock Solution to 100 parts of Sample.
Example: 100 µL Sodium Thiosulfate Stock Solution added to 10 mL Sample.
Final Sodium Thiosulfate concentration in the sample is 100 mg/L.

Sodium Thiosulfate Stock Solution Preparation

1. Weight out 1.0 g Sodium Thiosulfate ($Na_2S_2O_3$).
2. Add 100.0 mL Diluent.
   The Sodium Thiosulfate will last for 1-2 months when stored in the refrigerator.

SAMPLE pH

1. Measure the pH of the sample, and record the pH as part of the information in the sample Description.
   The bacterial Reagent is sensitive to pH. There is a minimal pH effect between 6.0 and 8.0. When the pH is higher than 8.0 or lower than 6.0, and the sample has buffering capacity the effect can be dramatic. Example: distilled water adjusted to pH 5.0 with HCl, has NO pH effect because there are not enough hydrogen ions to affect the pH of the Reagent. Another sample at the same pH (5.0) may show considerable toxicity if it does have buffering capacity.
2. If the pH of the sample is below 6.0 or above 8.0 and pH adjustment is required, adjust the pH as shown below.
   Adjustment is required when the toxic effect of pH is not wanted.
   A. If the pH is below 6.0, adjust the pH to 6.0 with NaOH. If over-titration occurs, discard sample and start again.
   B. If the pH is above 8.0, adjust the pH to 8.0

with HCl. If over-titration occurs, discard sample and start again.

When the sample is below pH 6.0, titrating the sample above 6.0 may precipitate the sample, thus changing the effective toxicity.

When the sample is above pH 8.0, titrating the sample below 8.0 may precipitate the sample, thus changing the effective toxicity.

When the sample has been over-titrated, back titrating may not resolubilize the sample, if precipitation occurred.

Use a 5 Normal (5N) acid or base for coarse pH adjustment of a sample with a high/strong buffering capacity to minimize sample dilution.

Use a 0.5 Normal (0.5N) acid or base when the sample has a low/weak buffering capacity.

PURPOSE OF A STANDARD

Testing a standard (reference toxicant), whose test results are well characterized, confirms your understanding of a test protocol and checks the performance of the complete test system (e.g. analyzer, Reagent, Diluent and Reconstitution Solution).

The 3-Basic Test Protocol is the best procedure for testing "Standards," as it provides the highest confidence level, precision and flexibility.

When testing "Standards" never except an IC_{50} derived from extrapolated data, always retest using the appropriate "Standard" dilutions.

Microtox Acute Toxicity Test

PHENOL STANDARD

Phenol $IC_{50\ 5\ minutes}$ = 13 to 26 mg/L

1. Weigh out ~50 mg (~0.050 g) of crystalline Phenol, add it to a 500 milliliter (mL) amber volumetric flask.
   Do not try to weigh out the exact amount of sample, calculate sample concentration for the amount weighed out.
   If an amber volumetric flask is not available, cover the entire flask with aluminum foil to protect the Phenol Standard from light.
2. Add Diluent to the 500 mL mark on the volumetric flask.
3. Seal the flask, and mix well by inverting the volumetric flask.
4. Label the flask and store at normal refrigerator temperature (2-8°C).
   The Phenol standard will last for 3-4 months when stored in this manner.

ZINC SULFATE STANDARD

$IC_{50\ 15\ minutes}$

$ZnSO_4 \cdot 7H_2O$ = 3 to 10 mg/L

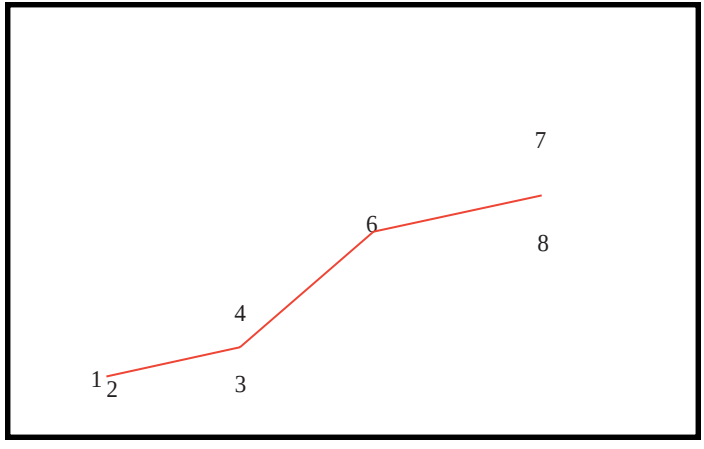
Zn^{++} = 0.6 to 2.2 mg/L

1. Weigh out ~ 50 mg (~ 0.050 g) of ZnSO4·7H2O, add it to a 500 milliliter (mL) volumetric flask.
   Do not try to weigh out the exact amount of sample; calculate sample concentration for the amount weighed out.
2. Add Diluent to the 500 mL mark on the volumetric flask.
3. Seal the flask and mix well by inverting the volumetric flask.
4. Label the flask and store at normal refrigerator temperature (2-8°C).
   The Zinc sulfate standard will last for 3-4 months when stored in this manner.

Microtox Acute Toxicity: True Deviation or Human Error?

At times it is very difficult to tell whether a large confidence range is the product of human error or true deviation. In such a case it is best to retest the sample using a Duplicate Test procedure. If the repeated results show the same type of dose-response curve, it is probably a true deviation. If they do not, it is probably human error. Human error is suggested when the placement of gammas on the dose-response curve of the Microtox Acute Test Data Report is random or skewed. Error is also indicated when the results of tests on duplicate concentrations of a sample do not agree with one another.

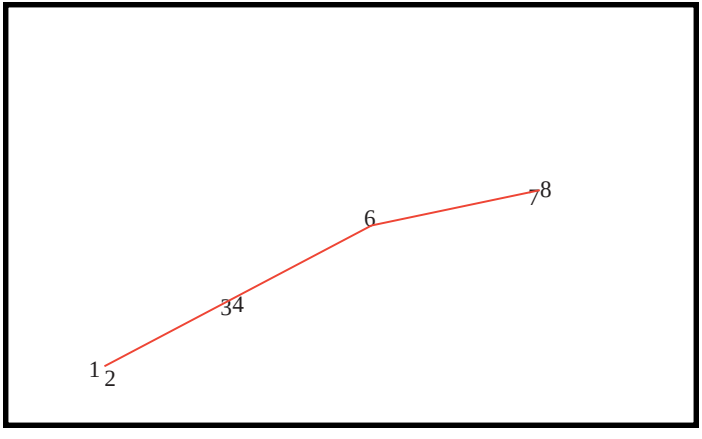
An example of human error is shown below.



In this example it is easy to see that this human error, as the test is in duplicate (1&2, 3&4, 5&6, 7&8), and the duplicates do not agree with each other.

Microtox Acute Toxicity Test

True deviation from theory is suggested when repeated tests show the same curvature. In this case the data are good, but the statistical information (e.g. residual variance, 95% confidence factor, correlation coefficient) will indicate that the fit to a straight line is poor, as in the case of human error.



In this example it is easy to see that this True Deviation, as the test is in duplicate (1&2, 3&4, 5&6, 7&8), and the duplicates closely agree with each other.

Microtox Acute Toxicity: Precision and Bias

Quality data are produced when test procedures are followed as stated. When the highest confidence and precision are required, duplicate testing methods are recommended. In duplicate testing, data quality is improved through cross comparison. The most common source of error will be that due to operator error. Errors are most likely to occur during sample preparation, salinity adjustment, sample dilution, reagent dilution, sample transfer and mixing steps, and data interpretation and resulting calculations. Use of the proper equipment and development of the appropriate skills required for using the test equipment are necessities in producing quality data.

Duplicate test results are unacceptable if the light measurements at any duplicate concentration are different by more than 20% of either value. This is likely to occur when improper transfer (pipetting errors), dilution, and mixing procedures have been used.

Microtox Acute Toxicity: Data Interpretation

Light Level-Time Response Differences

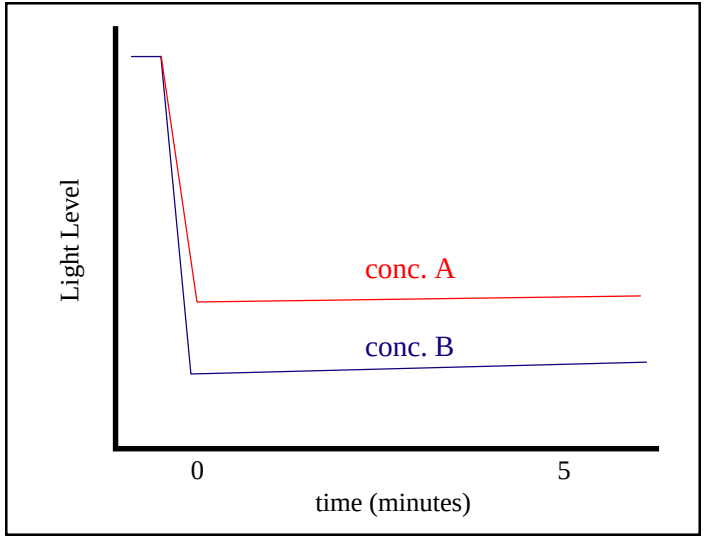
Different chemicals affect the organisms in Microtox Acute Test Reagent at different rates, producing distinctly different Light Level-Time Response curves. Three types of Light Level-Time Response curves for the Microtox Acute Test Reagent typically occur.

The three following Light Level-Time Response curves indicate why multiple light readings (It) at 5, 15 and sometimes 30 minutes, are recommended for testing samples whose average characteristics are not well documented.

Phenol Light Level-Time Response curve.

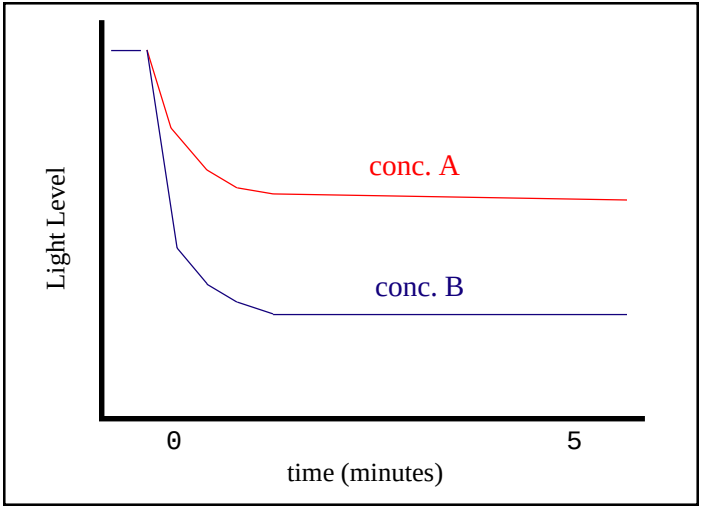
Phenol, for example, completes its action very rapidly. Light output drops sharply, then levels off, or rises slightly over time thereafter.

Phenol Light Level-Time Response curve.



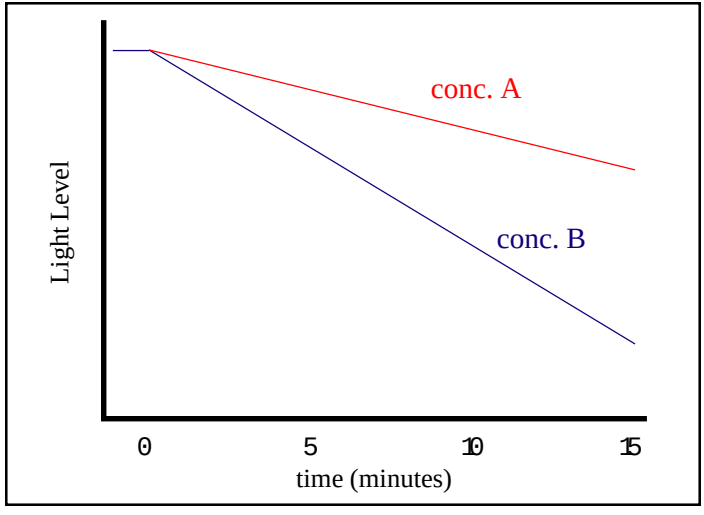
Microtox Acute Toxicity Test

Most Common Light Level-Time Response Curve.



This Light Level-Time Response curve is typical of organic compounds. The light output drops somewhat more gradually than for phenol, then levels off.

Heavy metals Light Level-Time Response Curve



This Light Level-Time Response curve is typical of many heavy metal compounds. The decay rate of the light is essentially constant for an extended period of time, and depends on concentration. Response curves of this type indicate why multiple light readings (It) at 5, 15 and 30 minutes, are recommended.

Which Incubation Time to Report for Microtox Acute Toxicity Tests

Assume that two assay times have been used for a single sample, one 5 minute, 15 minute. Both times will always be available for reference, but for simplicity you probably want to select a single assay time to represent the results of testing this sample. Which assay time is appropriate to report?

For most samples, the 5 and 15 minute IC_{50}s and the quality of data will be about equal. In such cases the 5 minute data are usually used in reports.

When the sample contains certain metal compounds, the 15 minute data will show considerable increase in bioreactivity response. If this is the case, and if the 95% confidence range is comparable to that for the 5 minute data, report the 15 minute data.

Do whatever satisfies your report requirements. Some organizations require that all data be reported. In any case, it is appropriate to denote the time (5 or 15 minutes) with the data actually presented. In some instances inhibition can occur at 15 minutes which may not be seen at 5 minutes.

Microtox Acute Toxicity: Difficult Samples Made Easy

In several situations, samples may require special handling.

1. SAMPLES THAT HAVE KNOWN SPECIAL REQUIREMENTS

   Some samples are already saline, requiring omission of the osmotic adjustment step; others are coloured, others have unusual physical characteristics. Such samples are characteristic of certain industries and activities and various manufacturing situations

   This manual contains a number of special protocols (variations on the fundamental Microtox Acute Test protocols) designed specifically for dealing with samples known to require special handling. Examine these special protocols carefully, and select one that serves your particular need.

2. SAMPLES THAT ARE EXCEPTIONALLY TOXIC

   Some samples are so bioreactive that the tests you normally run show complete light loss at all concentrations.

   Retest the sample, using the Extended (13 Dilution) Basic Test Protocol. With adequate dilution, the ECXX of any highly toxic sample can be found.

3. SAMPLES THAT ARE OF EXCEPTIONALLY LOW TOXICITY

   The toxicity of some samples is extremely low, but important to know. When the toxicity levels are very low, they may be confused with the background “noise” produced by irregularities in pipetting.

   Run a 90% Basic Test protocol, in duplicate, using two controls in each set. When the 90% Basic Test protocol does not provide ECxx, run a Comparison Test or Inhibition Test.

4. SAMPLES THAT HAVE AN EXTREMELY STEEP DOSE-RESPONSE CURVE

Some test samples show a dose-response curve so steep that the light level drops from very high to very low from one sample concentration to the next, providing too few useful data points for calculation of ECXX.

Complete light loss may occur at all concentrations in an Abbreviated Basic Test Protocol. After a second test with a primary dilution, all concentrations may show no light loss at all. A trial-and-error search for the narrow range in which the change occurs can be time-consuming.

This sort of data has a dose-response curve so steep at times that it cannot be plotted by the system.

Use narrower (1:1.5) serial dilutions.

5. SAMPLES THAT HAVE A NEGATIVE SLOPE

Some test samples show a negative slope (reverse dose-response curve). The light level appears to increase with increase in sample concentration. This condition may be caused by one or more of the following:

(1) The sample dilutions were made in reverse sequence.

(2) The I_t light readings were read in reverse sequence.

(3) The most common cause is background noise (pipetting error) which by chance forms a negative slope. Background noise can be more noticeable with low toxicity samples and the 90% Basic Test protocol.

Run the test protocol used, in duplicate, using two controls with higher sample concentration if possible. During the test be very careful in pipetting, making the sample dilutions and reading the light levels.

6. SAMPLES THAT HAVE A FLAT DOSE-RESPONSE CURVE

Some samples show dose-response curves so flat (at 1:2 dilution ratio) that the data points may be confused with background (pipetting) noise, and calculation of IC_{XX} may require extrapolation to a distant point, widening the confidence range beyond acceptable range limits.

Rerun the test, using the Extended (9 dilutions) Basic Test.

7. THE ECXX IS DETERMINED BY EXTRAPOLATION

Some samples are so bioreactive, or so slightly bioreactive, at the range of concentrations tested that all of the data points fall on one side of the IC_{XX}, which can only be determined by extrapolation. This is always less desirable than an IC_{XX} bracketed by data points.

If the gammas are all larger than that corresponding to the IC_{XX} of interest, all gammas greater than 1.0 for IC_{50}, retest the sample using a primary dilution testing lower concentrations. The problem with extrapolation to determine IC_{XX} is that the sample may be nonlinear at lower or higher concentrations.

If the gammas are all smaller than that corresponding to IC_{XX} (gammas less than 1.0 for IC_{50}) retest using a more concentrated sample.

Microtox Acute Toxicity:

Common Questions and Answers

These common Questions & Answers provide insight into the decisions and practices that produce high quality data.

Which Test Procedure Should I use?

The MicrotoxOmni Wizard is the best source for selecting which is the best test procedure to use by sample type.

What Effect Does the Samples pH, Free Chlorine and Colour/Turbidity and Have on the Test Results?

All of the above may have an effect on the test results. Please see Microtox Acute Toxicity Fundamentals pages 5 through 7 for further information.

The Light Level Readings Did Not Enter Into the Software, What is Wrong?

There is a communications problem between the analyzer and the computer. Check the following:

- Make sure you have good cable connection between the analyzer and computer, this is the most common problem.
- Make sure the cable is good, a bad cable is the second most common problem.

If this is the first time the analyzer was connected to a computer also check:

- Make sure the computer cable is connected to a computer communications port (COM1, COM2 etc).
- If the computer has more than one communication port make sure the software is set to the correct communications port. The software default setting is set for COM1.

Microtox Acute Toxicity Test

The Microtox Acute Toxicity Test Work Just Fine for the Reference Toxicant (Phenol or Zinc sulfate) and for Influent Samples but for Effluent Sample the Microtox Did Not Work, What is the Problem?

When first getting started in performing toxicity tests it is generally more difficult to understand the data from low to nontoxic samples. The information below should help.

- The MicrotoxOmni software calculates an ICxx (ECxx) with minimum and maximum effects when three usable gamma's are calculated from the data.
- The MicrotoxOmni software calculates an ICxx (ECxx) without minimum and maximum effects when two usable gamma's are calculated from the data. A minimum of three usable data points is required to calculate statistical data.
- The MicrotoxOmni software calculates Percent when only one usable gamma is calculated from the data.
- When no results are provided the sample showed no toxicity at the concentrations tested. If the initial sample concentration can be increased, retest the sample using a higher sample concentration. If the highest sample concentration tested was 81.9% to 100% (using the 81.9% Basic Test, Comparison Test Inhibition Test or the Whole Effluent Test) the sample is just nontoxic.

What is Toxicity Units (TU)?

$$\text{Toxicity Units (TU)} = 100 \div IC_{50}\ (EC_{50})$$

When Should I Use Toxicity Units?

It is easier to detect a deviation from normal when trend monitoring Toxicity Units of a sample site with high toxicity such as Wastewater Treatment Plant influent. And the Windows 95 MicrotoxOmni software has Trend Monitoring capabilities.

What is Trend Monitoring and When Should I Use It?

Windows 95 MicrotoxOmni software has Trend Monitoring capabilities that will determine the normal variation of a sample site and will detect and indicate (reveal) any deviation from this norm.

Duplicate tests. What are they, and when would they be used?

The use of duplicates provides additional data to check the quality of the test (operator pipetting), and provides a larger context in which to evaluate the data.

How many sample dilutions should be used?

When dealing with well-characterized samples, four sample dilutions that have at least one dilution on either side of the IC_{XX} are enough. In that case, you will typically be testing samples from the same source on a regular basis, looking for significant changes in toxicity levels.

Tests using more dilutions are important if you are testing samples with which you have no previous experience, and you don't know what to expect. For instance, if you run a test with just a few dilutions of a very toxic sample, every concentration may cause complete loss of light output from the reagent. This indicates that the sample has detectable toxicity, but does not allow calculation of IC_{XX}, because the few sample dilutions used were in the wrong range.

Examples of tests in this manual use 1:2 serial dilutions of the sample. Wouldn't a higher ratio provide a greater range of concentrations?

Yes, but it reduces the resolution of the test. The 1:2 dilutions are a good standard compromise between range and resolution, and work well for most samples.

How long can the frozen Microtox Acute Test Reagent be stored before it is used?

The shelf-life of the reagent after it leaves the factory is at least one year under proper storage conditions. The expiration date of the reagent is printed on the label on the reagent vial. Make a point of using up the reagent with earlier expiration dates before opening new boxes of reagent with later expiration dates. First in/first out.

The M500 Analyzer normally does not show the absolute amount of light emitted by test reagent. It shows relative values. How can I tell if the fresh reagent always produces the same amount of light?

The reagent does not always produce the same amount of light, and the question is of little practical importance. The system measures the percent of light lost in response to change in toxicity, which is consistent, no matter what the original light level. Light levels may be different for several reasons:

a) The light output of different batches of reagent varies.
b) Poor reconstitution technique reduces the potential light output of the reagent.
c) The light output will change with the age (shelf life) of the reagent.

These variations are unimportant, because you calibrate the analyzer to the reagent at the beginning of each test (when you press SET), and the gradual light loss or gain during a test is measured, and accounted for in data reduction calculations of the Basic Test Procedure.

In general, light output is not the primary consideration in evaluating the quality of the reagent. More important is the response of the reagent to various toxic materials.

How long can the reagent be used after reconstitution?

For at least an 2 hours after reconstitution, the organisms respond consistently to a wide spectrum of toxic materials. After that time, the response may begin to change. The reagent does not just become generally “less sensitive.” Its response to different chemicals may either increase or decrease, changing the response spectrum.

The useful life of the reagent after reconstitution is nominally one hour, but in practice, the useful life of the reagent may be several hours. Many laboratories reconstitute a vial of reagent for use all morning or afternoon. The conditions under which you use Microtox Acute Test Reagent beyond the initial hour will depend on your need for precision and verifiability of results.

For instance, if you intend to use the data for routine monitoring, and data quality requirements may not be critical, the use of reagent over several hours may be perfectly acceptable. If unusual or suspicious results are seen, these results could be confirmed with tests using a “fresh” bottle of reagent.

Well-characterized samples can be tested repeatedly with reagent of increasing age, so that the “normal” response of the reagent at any age is well known. The Phenol Standard is useful for this. It is also helpful to run several tests on one or more well characterized stable samples of known toxicity.

To establish baseline information, you may also elect to test each new lot of Microtox Acute Test Reagent with a standard such as Phenol over a period of hours after reconstitution, so you know what the normal response curve is over time.

Once again, the decision on how long the reconstituted reagent may be used is based on what information the test is intended to produce.

The protocols call for transfer of only 10 µl of reagent mixture to the test cuvettes. Can't we increase that to 20 µl or 30µl, as long as we do it every time?

No, don't increase the amount of reagent.

How can we measure and improve pipetting skills?

The Microtox Acute Test requires transfer of small amounts of liquid, as little as 10µl at a time, with an assortment of micropipettors. Pipetting is an ordinary laboratory skill and usually improves with repetition.

To check your reagent pipetting consistency:

Using an analytical balance, transfer 10 µL of water into a teared cuvette and weigh the amount of water pipetted. Repeat this 10 times and calculate the mean.

How can we calibrate a pipettor?

Using an analytical balance, weigh the amount of water transfered into a tared cuvette. Repeat this 10 times and calculate the mean.

Sometimes several or all of the sample cuvettes produce negative gammas. Does that always indicate a stimulatory effect of the sample?

Not always. The effect may also be produced by pipetting error.