Microtox® SOLO Manual

1. Introduction

AZUR Environmental (formerly Microbics Corporation) has been in the forefront of developing and applying biosensor-based measurement systems for toxicity testing of water, sediment, and soil samples for over 17 years. The use of living test organisms is the only reliable way to measure the potential biological impact (toxicity) of a water or soil sample. This type of testing, referred to as toxicity testing, has been in use for many years but has suffered from common deficiencies such as cost per test, time to obtain test results (usually days) and the inherent variability of test data. AZUR Environmental has combined the advantages of whole organism toxicity testing and instrumental precision to produce biological test systems which possess the features of instrumental based analytical test systems.

Test Organisms In a Vial

AZUR Environmental scientists published a paper in 1979 entitled " The Use of Luminescent Bacteria for Determining Toxicity in Aquatic Environments" establishing the technology and a commercial basis for a rapid, low cost and standardized aquatic toxicity test system. The technology breakthrough described in this paper utilized a special freeze-drying technique to provide shelf stable toxicity test organisms that could be instantly reconstituted and used whenever toxicity testing was needed. This preservation technology has provided the basis for producing and distributing standardized test organisms in a vial with a shelf life of up to 18 months. This test organism preservation technology is the basis for preparing and manufacturing toxicity test reagents for all Microtox Test Systems, including the Microtox SOLO.

Why Luminescent Bacteria Are Useful for Toxicity Testing

The technology of the Microtox Test Systems is based upon the use of bioluminescent bacteria, specifically the strain *Vibrio fischeri* NRRL B-11177, to measure toxicity from environmental samples. When grown under specific conditions the bioluminescent bacteria produce light as a by-product of their cellular respiration. Cell respiration is fundamental to cellular metabolism and all associated life processes. Bacterial bioluminescence is intimately associated with cell respiration, and any inhibition of cellular activity (toxicity) results in a changed rate of respiration and a corresponding change in the rate of bioluminescence. The more toxic the sample, the greater the percentage light change from the test suspension of luminescent bacteria. Bacterial bioluminescence has proved to be a convenient measure of cellular metabolism and consequently, a reliable sensor for measuring the presence of toxic chemicals in aquatic samples. Strain B-11177 was originally chosen because it displayed a high sensitivity to a broad range of chemicals.

The Microtox SOLO Test

The Microtox SOLO Test measures the light output of bioluminescent bacteria after they have been challenged by a sample and compares it to the light output of a non-toxic control. A difference in the light output between the sample and the control is attributed to the toxic effect of the sample.

The Microtox SOLO Test reagent is a specially prepared preparation of *Vibrio fischeri* NRRL B-11177 that has been grown, harvested and freeze dried under optimal conditions. Each individual vial contains sufficient reagent to carry out one test. One test consists of one control and one sample at one concentration.

Although the bacterial strain used in the Microtox SOLO Test is the same as in the Microtox Acute Test, the method of preparation is different. It should not be expected that this reagent will have the same performance characteristics as Microtox Acute reagent with all chemicals.

2. Supplies and Accessories

2.1 Reagent

The freeze dried reagent should be stored at -20°C to -25°C to maintain activity.

The reagent is stable for 4 weeks when stored at +4°C. It is recommended that if it is necessary to transport the reagent, a thermally insulated container and frozen ice packs are used.

The reagent is stable for 24 hours at +22°C. However, it is recommended that the reagent should, wherever possible, be kept at -20°C or +4°C until required for use.

Once the reagent has been reconstituted, it is recommended that it is used within 30 minutes. The recommended reconstitution time is 15 minutes. It is possible that the sensitivity of the reagent to some samples may change if the reconstitution time is extended beyond 30 minutes.

2.2 Reconstitution Solution, Diluent and Control Solution

The same solution is used to reconstitute the reagent, dilute samples and act as a non-toxic control sample. This solution is Microtox® Diluent.

Diluent is a specially prepared non-toxic 2% sodium chloride solution. It's shelf life is one year when stored at room temperature.

If contamination of the Diluent is suspected, it should be discarded and fresh Diluent used, as the contamination may compromise the performance of the test.

***NOTE**: Do not make up Diluent yourself or use substitutes. Diluent is prepared from the highest grade, quality controlled source materials in a custom built facility. The absolute need for a non-toxic Diluent is a pre-requisite for this test and the performance of the test cannot be guaranteed with solutions other than those supplied by AZUR Environmental.*

2.3 Osmotic Adjusting Solution

Microtox Osmotic Adjusting Solution (OAS) is a specially prepared non-toxic 22% sodium chloride solution. It is used to bring the salinity of the samples to approximately 2%, by adding one part OAS to 10 parts sample.

The OAS has a shelf life of year when stored at room temperature.

***NOTE**: Do not make up OAS yourself or use substitutes. OAS is prepared from the highest grade, quality controlled source materials in a custom built facility. The absolute need for a non-toxic OAS is a pre-requisite for this test, and the performance of the test cannot be guaranteed with solutions other than those supplied by AZUR Environmental.*

2.4 Cuvettes

Microtox cuvettes are used to carry out the tests. Cuvettes supplied by AZUR Environmental are non-toxic and disposable.

Used cuvettes cannot be reliably cleaned for re-use. Traces of detergent or sample contamination may interfere with subsequent tests, compromising the performance of the test.

3. Safety Information

Reagent

The reagent preparation does not contain any hazardous materials. It consists of *Vibrio fischeri* bacteria, sodium chloride, non-toxic carbohydrates and pH stabilizers.

Vibrio fischeri has not been reported to cause disease in mammals.

In case of eye contact, flush with copious amounts of water for 15 minutes. If large quantities are ingested obtain medical attention.

The reagent is not considered to be a fire or explosion hazard and does not require special control measures. There are no known hazardous decomposition products.

Spilled material should be absorbed with paper towels or similar material and disposed of according to local procedures. Excess liquid can be flushed down a sewer drain.

NOTE: Any special handling and disposal requirements for specific toxicants and samples should be observed. This is the responsibility of the operator.

4. Sample Handling

Sample Types

A wide variety of environmental samples may be tested with the Microtox SOLO system. These include raw water, waste water, industrial discharges, influent and effluent, pore water, spills, septage and extracts of soils and sediments.

Sample Collection

Ideally, samples should be collected in new borosilicate glass screw cap containers with Teflon® lined caps. Polycarbonate and polypropylene containers are also suitable. Fill the container to the top, leaving no airspace as this helps to keep volatile material in solution.

Sample Storage

Samples should be tested as soon as possible after collection to prevent unpredictable changes in sample composition. If testing is delayed, store the samples at normal refrigeration temperatures (+2°C to +8°C). Even if stored under refrigeration it is suggested that samples should be tested within 72 hours of collection.

Sample Preparation

Most samples will not require any special preparation before testing other than adjustment to 2% salinity. However, certain samples may require special preparation due to specific sample characteristics.

Salinity Adjustment - For most samples this can be done by adding 0.1 mL Osmotic Adjusting Solution to 1 mL sample and mixing thoroughly. This results in dilution of the sample to 90.9% original concentration.

If it is necessary to test a sample without any prior dilution, the salinity can be adjusted to 2% by dissolving 0.02g solid sodium chloride to 1 mL of the sample.

Turbidity - Samples which are turbid or contain particulate matter that will not settle may require clarification. This can be carried out by centrifugation if such facilities are available or by filtration using a suitable filter. Do not use cellulose acetate or cellulose nitrate filters. It is recommended that polysulphone filters are used. It is important to remember that toxicity may be associated with the turbid components of the sample.

Colored Samples - Samples which are highly colored (particularly red, brown or black) may interfere with the test results by absorbing light.

Samples Containing Chlorine - If chlorine has been used to disinfect the water source that the sample is taken is from, this may mask any other toxicity present in the sample. The sample can be de-chlorinated using 1% w/v sodiium thiosulphate solution. Prepare the 1% w/v sodium thiosulphate solution using distilled or deionized water in a clean glass container. Add 1 part of the solution to 100 parts of the sample e.g. 0.1 mL thiosulphate solution to 10 mL sample. Mix the sample and solution well and test immediately for toxicity.

Sample pH - Ideally, the sample pH should not be modified as the test relevance and sample integrity may become questionable. It is preferable to test each sample at it's native pH. However, if it is necessary to adjust the pH in order to bring it within range for regulatory purposes or comparison with other test results this should be carried out with either sodium hydroxide solution or hydrochloric acid.

RESOLVE™ Software

RESOLVE Software is a PC based Windows® 95/98 computer program that has been designed to take Microtox SOLO's percent effect results for tankered/truck waste (septage) and calculate an extrapolated EC_{50} value. RESOLVE stores the EC_{50} values and calculates the mean EC_{50} value using all provided data. The EC_{50} values and the mean EC_{50} values are plotted on a chart, and the provided information is used to calculate a recommended discharge rate for the disposal of the septage into your treatment process.

The RESOLVE software is simple to use, here are the following steps:

1 Add new account buy clicking the "Create a new site information file" button (first button from the left). Enter the following required site information:
Identifying name for the site.
The flow rate in megagallons per day.
Contact time for the Microtox SOLO test.
Click "Create a new account" button.

2 Open the new file generated above, buy clicking the "Open an existing site file." button (second button from the left).

3 Click on the "Calculate a safe discharge rate" button (top row of buttons, third button from the left).

4 "Enter % Effect" for the sample as provided by the MicrotoxOmni Software or by the DeltaTox analyzer.

5 Enter the "Volume of Waste" (septage) the the truck is disposing.

6 Press the "Calculate" button.

7 The recommended "Safe Minimum Discharge time" is shown in red.

8 To add this data to the chart click "Add Result to the EC50 Data File."

Note: It will take data from three samples before a chart can be displayed.

When a file is open a chart is displayed, and second rows of buttons are available for the chart, from left to right they are:

- Import a chart from a file (CHF)
- Export the chart to a file (CHF)
- Copy to the clipboard as a bitmap
- Print the chart
- Line chart
- Mark (point) chart
- Fit to curve chart
- Show or Hide the legend
- Vertical grid
- Horizontal grid
- Edit titles
- Change text fonts
- Change chart options

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P/N 686305

Microtox® SOLO 2% Test Procedure
Using the Microtox® Model 500 Analyzer; Acute Mode

For Testing High Toxicity Samples such as Septage & Waste Water Treatment Plant Influent Samples.

Required Materials

- Microtox® Model 500 Analyzer
- Microtox® SOLO Reagent
- Microtox® Diluent
- Pipettors:

10 µL pipettor and tips
100-1000 µL pipettor and tips
or
10 µL pipettor and tips
500 µL pipettor and tips

Analyzer Setup

1. Set the Microtox® Model 500 Analyzer in Acute Mode.

Test Preparation

2. Place cuvettes in wells A1 and A2 and the Reagent Well.
3. Add 2.5 mL Microtox® Diluent into the Reagent Well.
4. Wait 5 minutes.

Reagent Preparation

5. Reconstitute a vial of Microtox® SOLO Reagent using the 2.5 mL (2500 µL) of Microtox® Diluent in the Reagent Well.
6. Pour the reconstituted Reagent back into the Reagent Well cuvette.
7. Mix reagent 3-4 times using the 500 µL pipettor.
8. Add 1000µL reconstituted Reagent into cuvettes A1 and A2.
9. Wait 15 minutes.

Test Procedure

10. Call up the Microtox® SOLO 2% Test procedure in the MicrotoxOmni™ Software.
11. Place A1 into Read Well and press the Set button.
12. Touch the computer <space bar> key.
13. Read the I_o light levels as prompted by the computer: A1, A2
14. Immediately add 10µL of Sample (at room temperature) to cuvette A2.
15. Touch the computer <space bar> key.
16. Mix contents of A1 and A2 by swirling.
17. When alarm sounds read I_t light levels as prompted by the computer: A1, A2

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Microtox® SOLO 2% Test Procedure
Using the DeltaTox® Analyzer; B-Tox Mode

For Testing High Toxicity Samples such as Septage & Waste Water Treatment Plant Influent Samples.

Required Materials

- DeltaTox® Analyzer
- Microtox® SOLO Reagent
- Microtox® Diluent
- Pipettors:

10 µL pipettor and tips
100-1000 µL pipettor and tips
or
10 µL pipettor and tips
500 µL pipettor and tips

Analyzer Setup

1. Place the Analyzer on a level and dry surface.
2. Power on the unit by pressing the ON key. The Analyzer will perform a 1 minute self-test.
3. If the 1 minute self-test is successful, the Default Power-Up screen (Default screen) will appear. Verify that the Analyzer temperature (on the display) is within a range of 10-28°C.
4. Press the MODE key until B-Tox appears.
5. Set the DeltaTox for a 5-minute incubation time.

Test Preparation

6. Place cuvettes in wells A1 and A2 of the cuvette rack.

Reagent Preparation

7. Reconstitute a vial of Microtox® SOLO Reagent using 2.5 mL (2500 µL) of Microtox® Diluent.
8. Pour the reconstituted Reagent into a cuvette.
9. Mix reagent 3-4 times using the 500 µL pipettor.
10. Add 1000µL reconstituted Reagent into two (2) cuvettes A1 and A2.
11. Wait 15 minutes.

Test Procedure

12. **<u>Without</u>** a cuvette in the Sample Chamber (make sure that the lid is down and the latch is completely closed), press the START key. The instrument will display in sequence: "Close lid, and latch," "Waiting for PMT to warm up," "Reading dark current. Please wait." During this time the instrument creates and opens a record.

DeltaTox Prompt – "Insert Control cuvette"

13. Insert the A1 (Control cuvette) into the Sample Chamber. Close the lid and latch. Press the READ key.
14. Remove A1 from the DeltaTox and place it back into the cuvette rack.

DeltaTox Prompt – "Insert cuvette"

15. Insert the A2 (Sample cuvette) into the Sample Chamber. Close the lid and latch. Press the READ key.
16. Press the STOP key, remove A2 from the DeltaTox and place it back into the cuvette rack.

DeltaTox Prompt –" Waiting for next time to read"

(DeltaTox begins a 5 minute countdown this is the test time.)

17. Immediately add 10µL of Sample (at room temperature) to cuvette A2.
18. Mix contents of A2 by swirling.

When alarm sounds there will be the following prompt:

DeltaTox Prompt – "Insert Control cuvette"

19. Insert the A1 (Control cuvette) into the Sample Chamber. Close the lid and latch. Press the READ key.
20. Remove A1 from the DeltaTox and place it back into the cuvette rack.

DeltaTox Prompt – "Insert cuvette"

21. Insert the A2 (Sample cuvette) into the Sample Chamber. Close the lid and latch. Press the READ key.

DeltaTox Prompt – % Light (Loss or Gain) of the Sample

22. The DeltaTox Analyzer will then automatically display the % Light Loss or Gain.

Microtox® SOLO 45% Test Procedure
Using the Microtox® Model 500 Analyzer; Acute Mode

For Testing Medium Toxicity Samples such as Storm Water Samples.

Required Materials

- Microtox® Model 500 Analyzer
- Microtox® SOLO Reagent
- Microtox® Diluent
- Microtox® OAS
- Pipettors:

  100-1000 µL pipettor and tips

  or

  100 µL pipettor and tips

  500 µL pipettor and tips

Analyzer Setup

1. Set the Microtox® Model 500 Analyzer in Acute Mode.

Test Preparation

2. Place cuvettes in wells A1, A2, B1, B2 and the Reagent Well.
3. Add 1.5 mL (1.500 µL) of Microtox® Diluent to Reagent Well.
4. Add 1000µL Microtox® Diluent to cuvette A1.
5. Add 1000µL Sample to cuvette A2.
6. Add 100µL Microtox® OAS to cuvette A2, and mix using the pipettor.
7. Wait 5 minutes.

Reagent Preparation

8. Reconstitute a vial of Microtox® SOLO Reagent using the 1.5 mL (1.500 µL) of Microtox® Diluent in the Reagent Well.
9. Pour the reconstituted Reagent back into the Reagent Well cuvette.
10. Mix reagent 3-4 times using the 500 µL pipettor.
11. Add 500µL reconstituted Reagent into cuvettes B1 and B2.
12. Wait 15 minutes.

Test Procedure

13. Call up the Microtox®-SOLO 45% Test procedure in the MicrotoxOmni™ Software.
14. Place B1 into Read Well and press the Set button.
15. Touch the computer <space bar> key.
16. Read the I_o light levels as prompted by the computer: B1, B2
17. Immediately transfer 500µL from:
    A1 to B1
    A2 to B2
18. Touch the computer <space bar> key.
19. Mix contents of B1 and B2 by swirling.
20. When alarm sounds I_t light levels as prompted by the computer: A1, A2

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Microtox® SOLO 45% Test Procedure
Using the DeltaTox® Analyzer; B-Tox Mode

For Testing Medium Toxicity Samples such as Storm Water Samples.

Required Materials

- DeltaTox® Analyzer
- Microtox® SOLO Reagent
- Microtox® Diluent
- Microtox® OAS
- Pipettors:

100-1000 µL pipettor and tips
or
100 µL pipettor and tips
500 µL pipettor and tips

Analyzer Setup

1. Place the Analyzer on a level and dry surface.
2. Power on the unit by pressing the ON key. The Analyzer will perform a 1 minute self-test.
3. If the 1 minute self-test is successful, the Default Power-Up screen (Default screen) will appear. Verify that the Analyzer temperature (on the display) is within a range of 10-28°C.
4. Press the MODE key until B-Tox appears.
5. Set the DeltaTox for a 5-minute incubation time.

Test Preparation

6. Place cuvettes in wells A1, A2, B1 and B2 of the cuvette rack.
7. Add 1000µL Microtox® Diluent to cuvette A1.
8. Add 1000µL Sample to cuvette A2.
9. Add 100µL Microtox® OAS to cuvette A2, and mix using the pipettor.

Reagent Preparation

10. Reconstitute a vial of Microtox® SOLO Reagent using 1.5 mL (1.500 µL) of Microtox® Diluent.
11. Pour the reconstituted Reagent into a cuvette.
12. Mix reagent 3-4 times using the 500 µL pipettor.
13. Add 500µL reconstituted Reagent into two (2) cuvettes B1 and B2.
14. Wait 15 minutes.

Test Procedure

15. **Without** a cuvette in the Sample Chamber (make sure that the lid is down and the latch is completely closed), press the START key. The instrument will display in sequence: "Close lid, and latch," "Waiting for PMT to warm up," "Reading dark current. Please wait." During this time the instrument creates and opens a record.

DeltaTox Prompt – "Insert Control cuvette"

16. Insert the B1 (Control cuvette) into the Sample Chamber. Close the lid and latch. Press the READ key.
17. Remove B1 from the DeltaTox and place it back into the cuvette rack.

DeltaTox Prompt – "Insert cuvette"

18. Insert the B2 (Sample cuvette) into the Sample Chamber. Close the lid and latch. Press the READ key.
19. Press the STOP key, remove B2 from the DeltaTox and place it back into the cuvette rack.

DeltaTox Prompt –" Waiting for next time to read"

(DeltaTox begins a 5 minute countdown this is the test time.)

20. Immediately transfer 500µL from A1 to B1 (Control at room temperature)
21. Transfer 500µL from A2 to B2 (Sample at room temperature)
22. Mix contents of B1 and B2 by swirling.

When alarm sounds there will be the following prompt:

DeltaTox Prompt – "Insert Control cuvette"

23. Insert the B1 (Control cuvette) into the Sample Chamber. Close the lid and latch. Press the READ key.
24. Remove B1 from the DeltaTox and place it back into the cuvette rack.

DeltaTox Prompt – "Insert cuvette"

25. Insert the B2 (Sample cuvette) into the Sample Chamber. Close the lid and latch. Press the READ key.

DeltaTox Prompt – % Light (Loss or Gain) of the Sample

26. The DeltaTox Analyzer will then automatically display the % Light Loss or Gain.

Microtox® SOLO 81.9% Test Procedure
Using the Microtox® Model 500 Analyzer; Acute Mode

For Testing Low Toxicity Samples such as Waste Water Treatment Effluent Samples.

Required Materials

- Microtox® Model 500 Analyzer
- Microtox®-SOLO Reagent
- Microtox® Diluent
- Microtox® OAS
- Pipettors:

  100-1000 µL pipettor and tips

Analyzer Setup

1. Set the Microtox® Model 500 Analyzer in Acute Mode.

Test Preparation

2. Place cuvettes in wells A1, A2, B1, B2 and Reagent Well.
3. Add 300µL Microtox® Diluent to cuvette in the Reagent Well.
4. Add 1000µL Microtox® Diluent to cuvette A1.
5. Add 1000µL Sample to cuvette A2.
6. Add 100µL Microtox® OAS to cuvette A2, and mix using the pipettor.
7. Wait 5 minutes.

Reagent Preparation

8. Reconstitute a vial of Microtox® SOLO Reagent using 300 µL of Microtox® Diluent in the Reagent Well.
9. Mix reagent 3-4 times using the 100 µL pipettor.
10. Add 100µL reconstituted Reagent into cuvettes B1 and B2.
11. Wait 15 minutes.

Test Procedure

12. Call up the Microtox® SOLO 81.9% Test procedure in the MicrotoxOmni Software.
13. Place B1 into Read Well and press the Set button.
14. Touch the computer <space bar> key.
15. Read the I_o light levels as prompted by the computer: B1, B2
16. Immediately transfer 900µL from:
    A1 to B1
    A2 to B2
17. Touch the computer <space bar> key.
18. Mix contents of B1 and B2 by swirling.
19. When alarm sounds I_t light levels as prompted by the computer: A1, A2

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Microtox® SOLO 81.9% Test Procedure
Using the DeltaTox® Analyzer; B-Tox mode

For Testing Low Toxicity Samples such as Waste Water Treatment Effluent Samples.

Required Materials

- DeltaTox® Analyzer
- Microtox® SOLO Reagent
- Microtox® Diluent
- Microtox® OAS
- Pipettors:

  100-1000 µL pipettor and tips

Analyzer Setup

1. Place the Analyzer on a level and dry surface.
2. Power on the unit by pressing the ON key. The Analyzer will perform a 1 minute self-test.
3. If the 1 minute self-test is successful, the Default Power-Up screen (Default screen) will appear. Verify that the Analyzer temperature (on the display) is within a range of 10-28°C.
4. Press the MODE key until B-Tox appears.
5. Set the DeltaTox for a 5-minute incubation time.

Test Preparation

6. Place cuvettes in wells A1, A2, B1 and B2 of the cuvette rack.
7. Add 1000µL Microtox® Diluent to cuvette A1.
8. Add 1000µL Sample to cuvette A2.
9. Add 100µL Microtox® OAS to cuvette A2, and mix using the pipettor.

Reagent Preparation

10. Reconstitute a vial of Microtox® SOLO Reagent using 300 µL of Microtox® Diluent.
11. Mix reagent 3-4 times using the 100 µL pipettor.
12. Add 100µL reconstituted Reagent into two (2) cuvettes B1 and B2.
13. Wait 15 minutes.

Test Procedure

14. **<u>Without</u>** a cuvette in the Sample Chamber (make sure that the lid is down and the latch is completely closed), press the START key. The instrument will display in sequence: "Close lid, and latch," "Waiting for PMT to warm up," "Reading dark current. Please wait." During this time the instrument creates and opens a record.

    DeltaTox Prompt – "Insert Control cuvette"
15. Insert the B1 (Control cuvette) into the Sample Chamber. Close the lid and latch. Press the READ key.
16. Remove B1 from the DeltaTox and place it back into the cuvette rack.

    DeltaTox Prompt – "Insert cuvette"
17. Insert the B2 (Sample cuvette) into the Sample Chamber. Close the lid and latch. Press the READ key.
18. Press the STOP key, remove B2 from the DeltaTox and place it back into the cuvette rack.

    DeltaTox Prompt –" Waiting for next time to read"

    (DeltaTox begins a 5 minute countdown this is the test time.)
19. Immediately transfer 900µL from A1 to B1 (Control at room temperature)
20. Transfer 900µL from A2 to B2 (Sample at room temperature)
21. Mix contents of B1 and B2 by swirling.

    When alarm sounds there will be the following prompt:

    DeltaTox Prompt – "Insert Control cuvette"
22. Insert the B1 (Control cuvette) into the Sample Chamber. Close the lid and latch. Press the READ key.
23. Remove B1 from the DeltaTox and place it back into the cuvette rack.

    DeltaTox Prompt – "Insert cuvette"
24. Insert the B2 (Sample cuvette) into the Sample Chamber. Close the lid and latch. Press the READ key.

    DeltaTox Prompt – % Light (Loss or Gain) of the Sample
25. The DeltaTox Analyzer will then automatically display the % Light Loss or Gain.