Microtox®

Managing the Discharge of Transported Waste with Microtox®

MODERN WATER
A Deepverge Company

Abstract

Effectively managing the discharge of transported waste can reduce operating costs by minimizing plant upsets and other operational problems and hold plant effluent toxicity below mandated limits, avoiding expensive toxicity reduction evaluations, or fines.

This guide presents a practical method for implementing and maintaining a program that measures and controls transported waste discharge to a treatment plant based on Microtox® toxicity data (ASTM D-5660; Standard Methods for the Examination of Water and Wastewater 2009).

Every wastewater treatment plant is different; therefore, it is necessary to first determine the normal toxicity load coming in to the plant by measuring the toxicity of the combined influent. When analyzed, the information shows the normal toxicity of influent. This normal toxicity is the baseline toxicity load going into the plant. This information is then used to determine pass/fail criteria and to calculate safe discharge rates.

When baselines have been established for the normal toxicity loading of the plant, it is then possible to implement programs to measure and control the discharge of hauled wastewater. The Transported Waste Management Programs are related directly to the physical capacities of the plant, and to local/state/federal regulations.

A successful Transported Waste Management Program should be periodically evaluated and updated according to seasonal and/or developmental variations in toxic load. Weekly testing of plant combined influent detects such changes, and allows the operators to modify their program accordingly.

Why Monitor Transported Waste with Microtox®?

A single waste truck of 1000 gallons discharging highly toxic waste can disrupt an entire treatment plant. Problems are all very costly and range from significant raw material consumption, to toxicity pass-throughs, permit violations and irreparable damage to the plant biomass, yet many plants accept such risks on a daily basis. smaller plants are actually at a higher risk than large ones, because a few trucks represent a large proportion of their daily flow. The cost of a pass-through event can be very high, hundreds of man-hours and hundreds of thousands of dollars, but no traditional effective and reliable methods exist for detecting which of the 10 trucks that discharged in one day should have been turned away, or handled in a more specialized manner. The problem is toxicity.

Traditionally, toxicity has been measured with slow bioassays, which take anywhere from hours to days to run. Worse, most bioassays require a highly skilled technician to operate effectively, and considerable effort in maintaining stock cultures.

The Microtox toxicity test can accurately measure the toxicity of a sample in approximately 30 minutes. Microtox is an ASTM standard Method (D-5660; Standard Methods for the Examination of Water and Wastewater, 2009) for determining the toxicity of aqueous wastes before and after biological treatment. It takes only basic laboratory skills to master and even an inexperienced user can expect to generate usable results.

Use of Microtox as a rapid screening procedure can save time and money. Trucks containing high-toxicity loads can be dealt with appropriately. The truck can be discharged slowly in to the plant to avoid upsets, the waste hauler can be fined, or even be refused. Rates for discharge can be set based on Microtox limits. Merely setting up a program to manage discharge of transported waste can discourage some companies from submitting loads that they know, or suspect, will be a problem for the waste treatment facility.

A Realistic Approach

This document is intended as a guide. Waste treatment plants can be complex in design and operation, therefore the approach presented in this document transcends the specific cases we will deal with, and can be used in many different situations.

Two main scenarios exist in the physical parameters of plants receiving transported waste streams. The first is the plant that simply feeds trucked waste in to the waste stream, whether this is at the primary settling tank or the digester. The second type of operation can be described as a batch process. Trucks discharge to a holding tank, and the tank is discharged to the plant. Neither operation is more correct than the other; they simply reflect different physical realities found in a treatment plant.

These two scenarios are covered in this guide.

Program Overview

The program is divided into four distinct phases. Successful completion of each phase is critical for the success of the next.

- **Phase I** – Dedicated to making sure that the user is comfortable with the Microtox system and establishing the plant influent baseline. Phenol and zinc toxicity standards are run daily using the Microtox Basic test to assess user progress. Sampling and analyzing the combined influent is started to establish the baseline, again using the Microtox Basic test.
- **Phase II** – The focus here is on refining the baseline for the plant influent, and for establishing a baseline for transported waste. The Microtox Basic test is used for both purposes. Testing of toxicity standards continues, but on a weekly schedule. As the baselines for the influent and transported waste are developed, the Transported Waste Management Program (see appendix a) is also created. This is the plan of action that will control the fate of transported waste at the plant.
- **Phase III** – Focuses on testing and revising the Transported Waste Management Program created in Phase II. The plan is evaluated and fine-tuned during this phase.
- **Phase IV** – The operating phase. The Transported Waste Management Program is fully implemented at this stage.

**success of the first three phases will dictate the success of the fourth.

Glossary of Terms

- **Continuous discharger** - an industrial facility that discharges waste through a sewerage system to a POTW over a 24-hour period.
- **Batch discharger**
  - (1) an industrial facility that discharges a certain volume of waste over a certain period of time through a sewerage system to a POTW;
  - (2) a transported waste stream that discharges from a tank truck or container into a POTW waste collection system.
- **EC50** – exposure concentration of a sample which has a defined effect upon 50 percent of the test population. This is the same as LC50. In the case of toxicity, it represents the concentration of the sample that kills or impairs 50% of the test population.
- **MGD** – million gallons per day

Microtox Testing Guidelines

1. It is recommended that all samples be tested within 24 - 48 hours after collection.
2. It is recommended that samples be collected in borosilicate glass containers (polycarbonate and polypropylene containers are also acceptable) and sealed with no headspace using a teflon or equivalent lined cap.
3. Always test reference standards (phenol and/or zinc sulfate) on a frequent basis to ensure the accuracy and validity of test results.
4. When testing industrial discharges to establish baselines, determine how many samples can be adequately tested per day using the Microtox test.

Phase I

Duration: 1 to 2 weeks
Influent: Daily with Microtox Basic test
Waste Trucks: none
Standards: Three per week using Microtox Basic Test

The purpose of this phase of the program is to learn the normal toxic load on the plant. It also serves as a ramp-up period for new users. The normal toxicity loading is developed further and used in the next two phases to determine parameters in the Transported Waste Management Program.

The sampling point for the plant influent should be the first point in the flow at which all contributions to the plant combine. Often this is a primary settling tank. Any convenient point in the flow will suffice.

Although we are trying to generate Microtox data on a daily basis, it is not necessary to open a new vial of reagent every day. Instead, samples may be collected in clean borosilicate glass containers and sealed with no headspace using a teflon or equivalent lined cap. Samples may then be stored for up to two days (48 hours) at 5°C±3°C. Actual testing can then take place on only three days of the week, using three vials of reagent per week, shown below in Table 1.

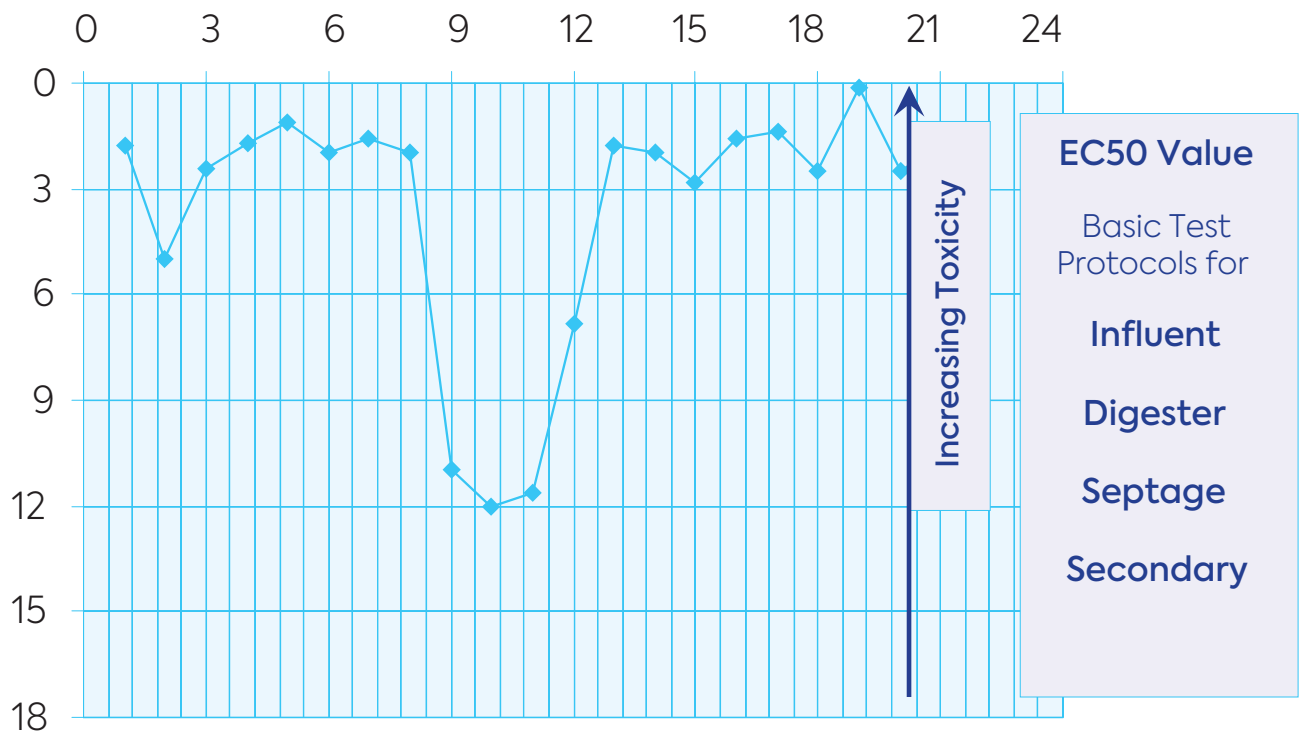
TABLE 1 – Daily Schedule for Testing

Day	Collect Samples Influent Only	Test Sample + Stored	Test Standard
Saturday	Yes	No	No
Sunday	Yes	No	No
Monday	Yes	Yes (Saturday, Sunday)	Yes
Tuesday	Yes	No	No
Wednesday	Yes	Yes (Tuesday)	Yes
Thursday	Yes	No	No
Friday	Yes	Yes (Thursday)	Yes

The daily toxicity chart provided by MWI can be used to plot the EC50 data obtained on your influent. A mean is generated. This represents the average toxicity loading (EC50) coming into the treatment plant. The lower the average EC50, the more toxic your average influent.

During Phase I, data is compiled on the combined flow through the plant. The average daily flow in MGD is used later to generate safe discharge times for transported waste. The flow of the plant is usually tracked as a part of normal plant operations. Data is compiled on the capacity and discharge times of transported waste. It is used in Appendix C to generate initial guidelines for receiving limits.

Example of a Daily Toxicity Chartphase



Phase II

Duration: 2-4 weeks
Influent: Daily with Microtox Basic test
Waste Trucks: Daily or as available using Microtox Basic test
Standards: Three per week using Microtox Basic Test

This phase is designed to find the toxicity of the average load from transported wastes. It also serves as a period in which to add data to the plant influent baseline, and to prepare a rational contingency plan to use in Phase III.

Preparation of the contingency plan, which is called the Transported Waste Management Program is the most complex part of the entire program. In creating the plan, one must remember that the treatment plant has specific limitations and strengths that must be addressed. **Also, legal issues may arise in application.** Issues must be identified and addressed before the program is fully implemented. Suggestions and some specific guidance and examples are covered in Appendix A of this guide.

The baseline for plant influent is updated constantly. Basic tests are run on a daily basis on plant influent. **At least 30 data points on plant influent must be gathered before going on to Phase III.**

Standards should continue to be analyzed three times per week to track performance. These standards can be analyzed simultaneously with the influent samples.

Sample and analyze as many transported waste containers as possible during this period of time. As before, samples should be collected and stored until they can be analyzed in a batch to save both operator time and reagent usage. The average EC50 value obtained from these transported containers is tracked. This helps to illuminate the specific needs of that plant. Knowing what is "normal" for the treatment plant is essential to managing a successful program, in that the main objective is to differentiate the exceptionally toxic loads.

Again, samples may be collected in clean borosilicate glass containers and sealed with no headspace using a Teflon or equivalent lined cap. The samples may then be stored for up to two days at 5°C±3°C. Actual testing can then take place on only three days of the week, using three vials of reagent per week, shown below in Table 2.

TABLE 2 – Daily Schedule for Testing

Day	Collect Samples Influent + Trucked	Test Sample + Stored	Test Standard
Saturday	Yes	No	No
Sunday	Yes	No	No
Monday	Yes	Yes (Saturday, Sunday)	Yes
Tuesday	Yes	No	No
Wednesday	Yes	Yes (Tuesday)	Yes
Thursday	Yes	No	No
Friday	Yes	Yes (Thursday)	Yes

In some cases it may not be possible to gather samples from trucks seven days a week. This should not be viewed as a barrier to proceeding with the program, **but it should be noted that at least 10 trucks should be tested before moving on to Phase III.**

The daily toxicity chart provided by MWI can be used to plot the EC50 data obtained on the transported waste containers. The Daily toxicity chart started on the influent in Phase I can continue to be used for plotting EC50 data from the plant influent samples.

At the end of Phase II, at least 30 data points must be available on plant influent, and at least 10 data points on trucked waste. Mean EC50 values should be generated for each set of data. Determination of a pass/fail point can be based on this data, as well as an initial toxicity limit to use in the first draft of the Transported Waste Management Program. This pass/fail point may be expressed as an EC50 (for use with the Basic test), or as a % light loss at a specific concentration to be used with the Microtox screening test.

Phase III

Duration: 1-2 weeks
Influent: Weekly with Microtox Basic Test
Waste Trucks: As defined in the Transported Waste Management Program developed in Phase II.
Standards: Weekly, using Microtox Basic Test

The purpose of this phase is to test and fine-tune the Transported Waste Management Program developed in Phase II. Essentially, all of the testing included in the Transported Waste Management program is performed during Phase III to expose any gaps in what was developed. As many trucks as possible are tested during this time period with the preferred Microtox test that has been decided upon for the program. The number of trucks which passed and failed these screens is tracked. This lets the operator fine-tune the parameters of the plan, such as pass/fail points, to the specific needs of the application. The consequences of the program, such as rejecting transported waste containers, applying a high toxicity surcharge, and so on, are applied at the discretion of the manager of the program. This is a trial phase for the Transported Waste Management Program itself, and its performance should be evaluated in relation to the success of the plant as a whole.

The baseline for the influent to the plant is checked constantly. Basic tests are run weekly on plant influent. This enables detection of seasonal variation and other changes in the composition of the influent.

Standards are run weekly to track performance. these tests can be run at the same time as the influent tests.

Phase IV

Duration: Indefinite
Influent: Weekly with Microtox Basic Test
Waste Trucks: As defined in the Transported Waste Management Program (developed in phases II & III)
Standards: Weekly, using Microtox Basic Test

This is the steady state phase of the Transported Waste Management Program.

The baseline for the influent to the plant is checked constantly. Basic tests are run at least weekly on plant influent. This enables detection of seasonal variation and other changes in the composition of the influent.

Standards are run at least weekly to track performance. These tests can be run at the same time as the influent tests.

Appendix A

Creating a Realistic Transported Waste Management Program

The major area to be addressed in deciding what is possible for a Transported Waste Management Program is the physical limitation imposed upon the program by factors such as plant design and number of waste containers.

The type of receiving facility will dictate how much, if any, pretreatment can be done on a load of waste. Many smaller plants take waste discharge directly into the influent stream. Holding tanks are perhaps the most common option available, and can be used to discharge the waste at an acceptable rate into the plant's influent stream.

The size of the treatment works and the frequency of waste dischargers should also influence program design. If, for example, a plant has a normal flow of 50 million gallons per day (MGD), and only receives one 2000 gallon waste truck once every two weeks, the program will be straightforward to implement and manage. Smaller capacity plants, with more frequent trucked waste, will have a more pressing need for creating a very detailed Transported Waste Management Program.

The general structure of a Transported Waste Management Program can take two forms. These forms are dictated by the physical facilities at the plant. If a plant has not dedicated a holding tank for trucked waste, the options are quite limited. Either a screening test or a Basic test is used to find the toxicity of trucks as they come into the plant. Every truck, or only some trucks can be spot-tested for toxicity. If the EC50 obtained by the Basic test is below a set pass/fail point, or the light loss obtained by a screening test is above a pass/fail point, the truck is said to "fail."

At this point, further Microtox testing may be warranted. Failed trucks can have many fates. They may be rejected or surcharged before off loading at a very slow rate. It may also be possible to discharge the load in stages to avoid overloading the plant. The consequences of a plan of action should be carefully considered before it is carried out.

Some programs include sliding fees in addition to surcharges for particularly toxic loads to defray the cost of additional time and materials consumed in treating the load.

The second form of a Transported Waste Management Program is a batching type. Trucks discharge to a holding tank. Grab samples are taken from the trucks and stored. At the end of the day, the EC50 of the holding tank is determined. From this number, a safe discharge rate is calculated (see Appendix E). If the EC50 is below a set pass/fail point, one can go back and test the retained samples with a screening test to find their relative toxicity. The most toxic hauler(s) could be summarily surcharged for the event, warned, rejected in the future, or whatever is most appropriate for that particular treatment plant.

Pass/fail limits are set according to the initial EC50 Limit (see Appendix D) and the results of the study in Phase III. At this concentration, how many trucks passed? How many failed? If most trucks would fail given a pass/fail point, it seems self-defeating to use that particular point. Instead, a lower EC50 limit for Basic tests, or a higher % light loss limit for screening tests may be more appropriate.

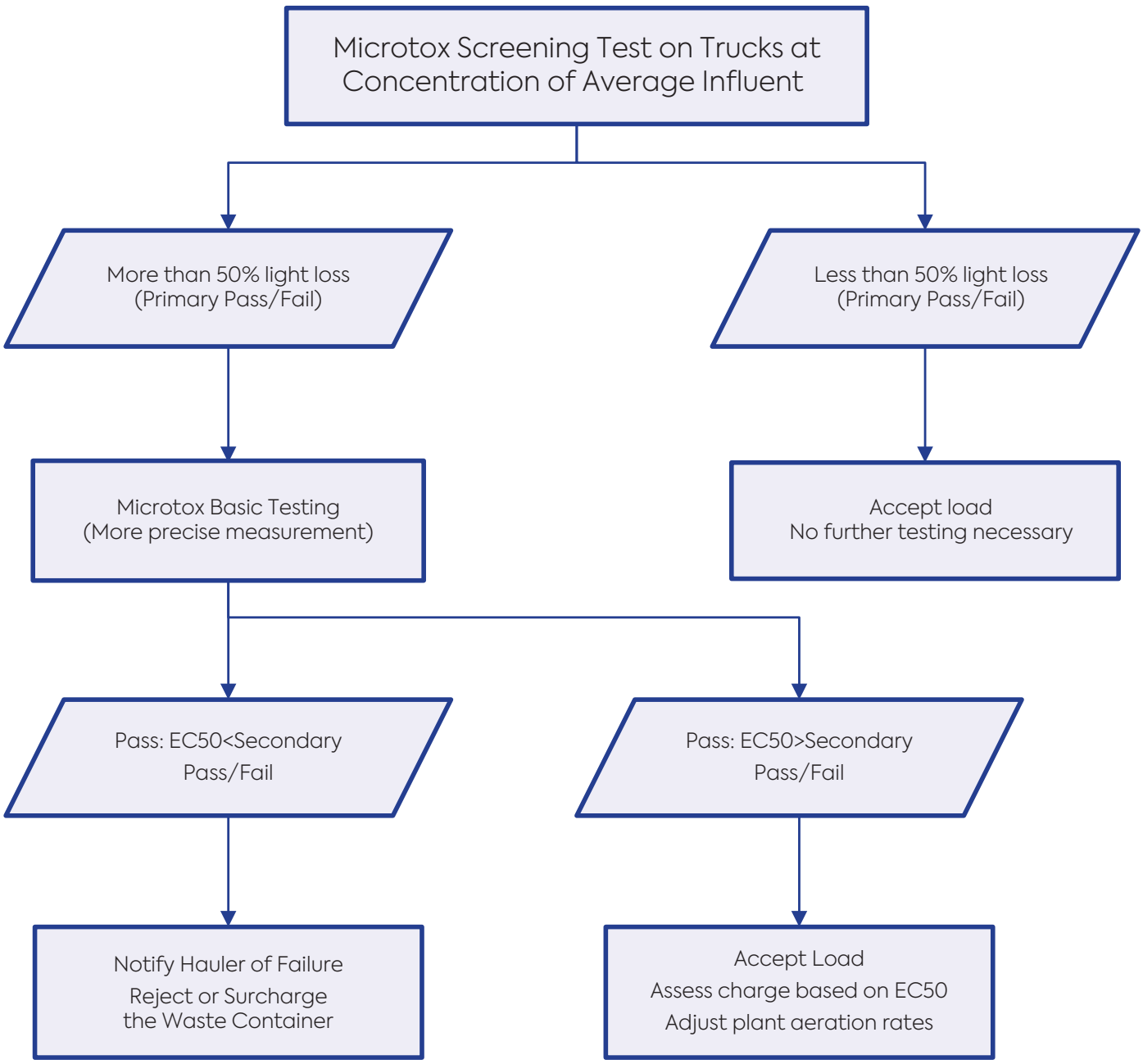
Part of developing the Transported Waste Management Program will be to decide what testing protocol will be performed. As suggested before, the Microtox Screening Test is appropriate where many samples must be processed on a daily basis. The Microtox Basic test is more labor-intensive, but provides more detailed information and is the test of choice for batch type operations, enabling the calculation of safe discharge rates for batches.

Above all, the final plan must specifically deal with the various issues that will arise. If it is decided to turn the hauler away, where would the hauler go? Probably the single biggest issue faced in trying to set up a program such as this will be that of cost. Decide who will pay for the testing, and write it into the Transported Waste Management Program. Many users mitigate the cost by charging a $100 fee for testing. This covers consumables, amortization of equipment and operator time.

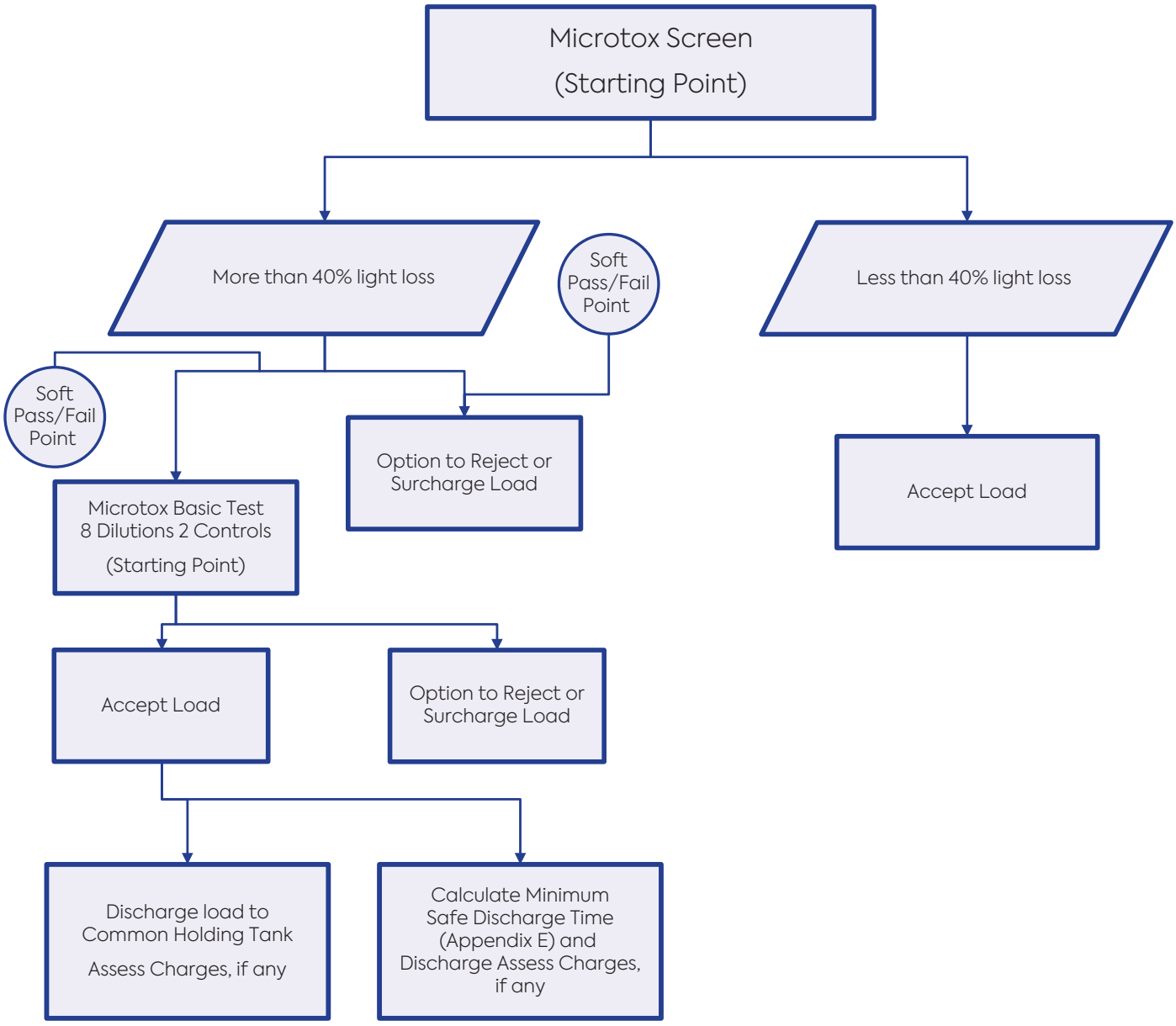
An example plan of the first type follows. It uses the Microtox screening test as a primary pass/fail test. It also features a Microtox Basic test as a highly precise determination of toxicity for surcharge calculation. There are primary and secondary pass/fail points in this plan. If a container is too toxic, it is not accepted. If it is only a little more toxic than the norm, the container is accepted, but a surcharge is assessed based on the Microtox Basic test EC50. The flow chart below shows the decision tree for waste haulers.

A second flow chart diagrams a second generic approach, similar to the first, in which there are primary and secondary tests used to determine the fate of waste containers.

A Sample Trucked Waste Control Program Decision Tree (#1)



A Sample Trucked Waste Control Program Decision Tree (#2)



Appendix B

Sample Preparation for Septage and Influent Samples

The contents of a waste container are not solid, but not quite liquid. A waste stream like this presents some special sample handling challenges for the plant and the Microtox user.

A major concern is that there are often three distinct phases of the sample. The first is a layer of oil or grease, the second is mostly aqueous material, and the third layer is solid. Only the aqueous layer needs to be tested for toxicity.

The oil or grease should be avoided or removed and used for its fuel value. Avoid as many solids as possible. The sample will still be turbid, but this will not affect the Microtox results appreciably. Septage tends to be so toxic that turbidity and color are only minor considerations at the concentration of septage used for the Microtox test. The same rule of thumb holds true for influent samples, at turbid concentrations the sample is usually extremely toxic.

If the transported waste is neutralized before it is introduced into the system (if there is a neutralization tank between the waste receiver and the rest of your plant), the pH of the sample must also be neutralized. Samples with a low pH should be adjusted to pH 6.0 with NaOH, samples with a high pH should be adjusted to pH 8.0 using HCl. Do not pass through pH 7 when adjusting; if you over adjust and pass through pH 7, begin again with a fresh sample. If waste is not neutralized, then the sample should be tested as it is. High or low pH can have a profoundly toxic effect on the biomass of a waste treatment plant or the organisms like the Microtox bacteria. The same rules apply to influents. If influents are neutralized before introduction to the system at large, then neutralize before testing.

Septage, being extremely concentrated, usually requires an initial dilution, outside the Microtox test itself. This dilution can be made with distilled water. The initial concentration line of the "Define test parameters" screen of the Microtox program must be modified to reflect any initial dilution. If a 1:100 dilution is made of the trucked waste or influent before it is tested, the initial concentration should be changed from 45% to 0.45%. Plant influent is normally less toxic, but still may require an initial dilution.

The single most important rule of thumb in sample preparation is this: Whatever is done, do it consistently. There are very few absolutes, and almost every approach to a problem is valid to some extent. To assure that the data gathered now is compatible with the data gathered six months from now, make sure that the protocols followed for preparing the sample do not vary.

Appendix C

Developing a Screening Test for Testing Trucked Waste

If your Transported Waste Management Program calls for a fast toxicity test to screen many samples per day, a Microtox screening test is the best choice.

The screening test only finds the toxicity of one concentration of the sample. This concentration should be determined by the initial limit for the toxicity of transported waste (see Appendix D). This equation generates an EC50 value (in % sample). The screening test should test a concentration close to this value.

Some initial dilutions are given in the screening protocol section of the LX manual. These are for factors of 1:10 only. You may find it necessary to use a concentration which does not fit neatly into one of these categories. There are a few easy rules of thumb to follow for odd concentrations.

First, never dilute more than 1:10 in a single step. The chance for error is too great with bigger dilution steps.

Second, use the following equation:

$$\text{Diluent Volume} = \frac{(\text{Initial Concentration} \times \text{Sample Volume})}{\text{Final Concentration}} - \text{Sample Volume}$$

Assigning Sample Volume as 1mL, initial concentration and final concentration appropriately, the volume of diluent necessary to get an exact concentration is found.

For instance, if the initial limit generated is 0.43%, two serial 1:10 dilutions are made first, giving a concentration of 0.9% (according to the table in the LX manual). The equation is used to generate the volume of diluent to be added. The volume is 1.09mL. 1.09mL of diluent is added to a test tube, along with 1mL of the sample (which had undergone two previous 1:10 dilutions). This mixture is then used as "sample" according to the screening protocol in the LX manual, and it is at approximately 0.43% in the test.

An alternative screening protocol which is used successfully is the 2% screening protocol. This protocol is much faster to set up than the screening test referred to above, but it is less precise. If many trucks per day are run, or a few trucks need to be processed very rapidly, the 2% screening protocol is applicable. Although it has a set concentration of 2%, this concentration may be varied by adding different amounts of sample, or pre-diluting the sample with distilled water.

A table of a few possible concentrations is given on the following page.

Table of Dilutions/Volumes for screening test based on a 2% Screening Test Protocol

Initial EC50 Limit (v/v) % (Calc in Appendix D)	Front-end Dilution	Volume added to test
0.02%	1:100	10µL
0.04%	1:100	20µL
0.055%	1:100	30µL
0.07%	1:100	40µL
0.09%	1:100	50µL
0.2%	1:10	10µL
0.4%	1:10	20µL
0.55%	1:10	30µL
0.7%	1:10	40µL
0.9%	1:10	50µL
2%	None	10µL
4%	None	20µL
5.5%	None	30µL
7%	None	40µL
9%	None	50µL

A 1:10 dilution is 0.25mL of sample plus 2.25mL of distilled water (or diluent).

A 1:100 dilution is two serial 1:10 dilutions.

The volume in the third column is substituted for the standard 10µL of sample defined in the 2% Screening Test protocol.

With these simple dilutions, and the 2% Screening Test, one can run a quick screen on a wide variety of concentrations. Bear in mind that once a particular concentration of sample is determined, that concentration is tested for all waste trucks. This means that the initial dilutions and volumes added to the screening test are figured once, and not changed unless some large variation in the plant occurs.

Appendix D

Calculating an Initial Limit for Toxicity of Trucked Waste

The purpose of this section is to generate an EC50 limit for toxicity of waste, whether it is in a truck, or in a holding tank pending batch discharge. This EC50 limit can then be used to make individual determinations, or to develop a Screening Test Limit.

The equation for this limit is:

$$\textbf{EC50} = \frac{\textbf{(Avg. Influent EC50)} *(0.00144)*\textbf{(Volume of Batch Discharge, gallons)}}{[\textbf{(Total Plant Flow, MGD)}*\textbf{(Duration of Batch Discharge, minutes)}]}$$

Where:

Avg. Influent EC50, is the average EC50 for influent samples tested in Phase I-III.

Volume of Batch Discharge, gallons, is the total volume discharged from the holding tank or average waste container, in gallons.

Total Plant Flow, MGD, is the total flow of the plant, including the batch discharge if appreciable. An acceptable formula is (average Daily flow, MGD + Batch Discharge, MGD).

Duration of Batch Discharge, minutes, is the time it takes the batch (truck, tank or rail car) to discharge. For either, this may be an average value if it is not specifically known.

Appendix E

Calculating a Safe Discharge Duration for Waste Containers

The formula presented here can be used as a first approximation for a safe discharge duration. It uses the toxicity of the average influent, and the toxicity of the batch (truck, tank or rail car) along with some physical parameters, to determine a duration that will not overload the plant's tolerance for toxicity.

$$\textbf{MSD, min} = \frac{\textbf{(Avg. Influent EC50)} *(0.00144)*\textbf{(Volume of Batch Discharge, gallons)}}{[\textbf{(Total Plant Flow, MGD)}*\textbf{(EC50 of Batch Discharge)}]}$$

Where:

MSD, min, is the Minimum Safe Discharge Duration in minutes.

Avg. Influent EC50 is the average EC50 for influent samples tested in Phase I-III.

Volume of Batch Discharge, gallons, is the total volume discharged from the holding tank or waste container, in gallons.

Total Plant Flow, MGD, is the total flow of the plant, including the batch discharge if appreciable. An acceptable formula is (Average Daily Flow, MGD + Batch Discharge, MGD).

Duration of Batch Discharge, minutes, is the time it takes the batch (truck, tank or rail car) to discharge. For either, this may be an average value if it is not specifically known.

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