CHAPTER 1.13 – Spills Toxicity Testing: Guidance for Wastewater Engineers & Specialists, Wardens, & Other Field Staff

This chapter is intended to help staff collect samples and make arrangements for toxicity testing at the Wisconsin State Lab of Hygiene (SLH) in response to a spill.

NOTICE: This document is intended solely as guidance, and does not contain any mandatory requirements except where requirements found in statute or administrative rule are referenced. This guidance does not establish or affect legal rights or obligations, and is not finally determinative of any of the issues addressed. This guidance does not create any rights enforceable by any party in litigation with the State of Wisconsin or the Department of Natural Resources. Any regulatory decisions made by the Department of Natural Resources in any matter addressed by this guidance will be made by applying the governing statutes and administrative rules to the relevant facts.

In response to an accidental or intentional spill of potentially toxic materials it may be necessary to conduct toxicity tests on effluent, surface water, and/or sediment samples in order to determine the potential adverse environmental impacts from the spill. Spills can reach the environment through municipal or industrial outfalls (e.g., when spills occur into sanitary sewer systems or factory floor drains), via storm sewers, overflowing storage facilities, or other routes, as well as by dumping directly into receiving waters or on adjacent land. While analyses of specific chemicals can provide an idea of what harm a single chemical may cause, toxicity tests provide a measure of the aggregate effect of chemical mixtures and should be conducted when something has been spilled into the environment that is an unknown chemical mixture or is made up of more than one chemical compound.

What Are Toxicity Tests?

In toxicity tests, organisms are exposed to samples (e.g., effluents, surface waters, sediments, etc.) for a set time period in order to determine the sample's effects on survival, growth, and reproduction. The organisms most commonly used in effluent and surface water toxicity tests are *Pimephales promelas* (fathead minnow), *Ceriodaphnia dubia* (zooplankton), and *Selenastrum capricornutum* (green algae). These species are used as surrogates to represent the three trophic levels of aquatic organisms found in receiving waters.

There are two main types of toxicity tests - acute and chronic. Acute tests last 48 to 96-hr and measure the concentration of sample that causes significant mortality. Acute tests result in an LC_{50}, which is a statistical interpretation of data which predicts the percentage of sample that causes 50% of the population to die. Chronic tests predict the concentration that interferes with the growth, development, or reproductive potential of aquatic organisms. During a chronic test several life stages of the organism are exposed to the test material at various concentrations. Tests last 4-7 days and responses such as growth, reproduction, and survival are measured. Chronic tests result in an IC_{25}, which is a statistical interpretation of data which predicts the percentage of effluent that causes a significant (25%) reduction in growth or reproduction of the population.

Screening vs. Definitive Testing

Toxicity tests can be conducted as screening tests that include 100% sample only or as definitive tests that include a dilution series. A screening test is a single dilution (plus a control) toxicity test on 100% effluent or other sample, and requires a smaller sample volume than a full definitive test. A screening test is completed on the same organisms and under the same test conditions as the definitive test. The biggest difference is that data is provided on 100% sample only and therefore less information is learned about the magnitude or severity of toxicity.

Definitive tests are conducted using a dilution series of at least five test concentrations and a control (usually 100, 50, 25, 12.5, and 6.25% of the sample is tested). These tests provide the best information for evaluating the severity of toxicity and can also provide more information about how the sample behaves as it is mixed in the receiving water (when the receiving water is used for dilution). A full dilution series also allows for a more thorough evaluation of test performance, allowing anomalous test results to be identified more easily.

When to Sample

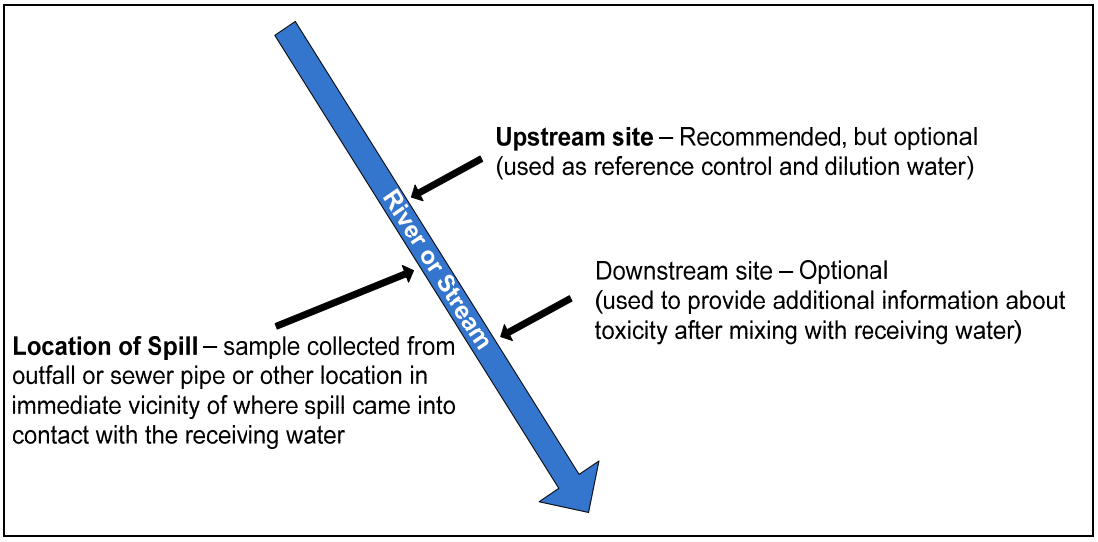
If you believe a spill has caused, or has the potential to cause, lethal or sublethal affects to fish or aquatic life of the waterbody in question, toxicity testing should be conducted. A visual inspection of the site may help determine if toxicity testing is warranted, but keep in mind that toxics can affect macroinvertabrates, not just fish, and can have prolific effects to the ecosystem that may not be immediately observed. As mentioned, toxicity tests should be conducted when something has been spilled into the environment that is an unknown chemical mixture or is made up of more than one chemical compound.

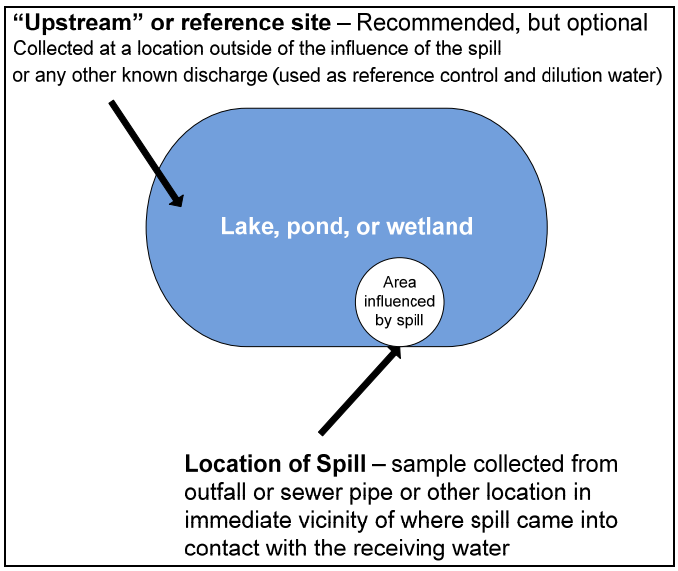
Please note: When responding to spills and illicit discharges, staff should not be serving as a first responder. Remember, safety is the first priority in any spill response. Staff must "stop, look and listen" before attempting to collect field samples to make sure the site is safe and they will not put themselves in danger. If first responders or spill coordinators are on site, staff must have permission from them prior to entering the site. Staff must use personal protective equipment specific to the discharge when collecting samples. Use MSDS and other literature to choose Personal Protective Equipment (PPE) such as gloves and suits. If staff can't identify the liquid or material and its properties, use the highest level of protection.

Sample Location

The amount of toxicity in a sample is determined by comparing that sample to a relevant control. If the intent of the testing is solely to determine the presence/absence of toxicity, then the control can be a standard lab water. However, if it is desirable to better define impacts as the sample entered and mixed with the receiving water, then a better study design might include a receiving water control. In a river or stream, the control sample should be collected upstream and/or outside of the influence of the spill and possibly another sample collected downstream of the spill site. If the spill occurs into a pond, lake, or wetland, collect the control at a spot outside of the influence of the spill. See more descriptions and example diagrams of potential sample sites below.

- One sample from the location where the spill occurred – taken from an outfall pipe if the spill occurred at or upstream of a wastewater treatment plant; or taken from a sewer pipe or manhole if the spill occurred within a storm sewer system; or taken from the waterbody at the site of the spill, if spilled on land near a waterbody or directly into a waterbody.
- A second sample could be taken from an area outside of the influence of the spill, collected upstream in a river or stream or outside of the mixing zone of the spill in a lake, pond, wetland or other non-flowing waterbody. This location should also be outside of any other known discharge such as an outfall or storm sewer. This sample may be used as a control and/or the dilution water in a definitive test.
- Additional samples can be taken downstream of the spill, if more information is required on the toxicity of the spilled substance as it mixes with the receiving water and moves further away from the spill site.





Determining Where a Spill Occurred

Toxicity testing can be used for various purposes. It can be a tool to determine if a known spill is causing toxicity – for example, if toxicity testing is done in an area around a known spill, it can tell you whether toxicity is present even if something obvious like a fish kill did not occur. Sampling for testing around a known spill is described above, where it is possible to sample in and out of the impacted area. In cases such as these, definitive tests might be done to determine whether toxicity is present and how severe it may be.

Toxicity testing can also be used to identify the source of an unknown spill. For example, in a situation where a fish kill or other obvious effect has occurred, but no one knows what caused it or where it came from, toxicity testing might be able to pinpoint the location of the spill. If toxicity is present in the waterbody, sampling could be done to follow the toxicity back to its source. For example, if a fish kill occurs in a river system, screening tests could be conducted on samples collected from various sites upstream of the kill area to determine where/when toxicity is present. The success of this type of testing would of course depend on whether toxicity was still present at measurable levels and might be most useful when a spill/illicit discharge is ongoing.

Similar sampling and screening tests could be done in various spots downstream of a kill area in an attempt to locate "hot spots" or track the progress of toxicity downstream (e.g., locate slugs traveling downstream, slow moving areas where toxics might settle out, etc.). Screening or definitive tests could also be used to assess the presence of toxicity moving through or remaining in a system (e.g., a wetland) after a spill is over.

Determining What Caused Toxicity

In addition to being a useful tool for tracking where/when toxicity is present in a system, toxicity testing can also be used to determine the cause of toxicity. A toxicity identification evaluation (TIE) is an investigation done in the lab to determine the cause of the toxicity. The objective of a TIE is to characterize and identify the compound(s) causing toxicity so that they can be traced back to their source. In a TIE, samples are taken to the toxicity testing lab, where they can be manipulated to remove suspect chemicals (e.g., metals, organics, etc.) and then re-tested to see if toxicity remains. If a specific manipulation removes toxicity, then the researcher has a clue about the chemical causing the toxicity. The evaluation can use both characterization procedures and chemical-specific analyses, therefore, the identifications may range from generic classes of toxicants to specific chemical compounds. Once a specific class or individual compound has been identified as the cause of toxicity, this information can be used to find or confirm the source of the spill.

Minimum Sample Volumes:

Acute Tests:

Sample to be tested (effluent, area of surface water impacted by spill, etc.)

- 100% Screen Only = 4.5 Liters (1.2 Gallons)*
- Full Dilution Series = 8.5 Liters (2.3 Gallons)

Reference site/upstream receiving water control water = 18 Liters (4.75 Gallons)

* Reduced volume acute screening (RVAS) tests can be done with as little as 1 liter of sample, if that is all that is available. More information about RVAS tests can be found in Chapter 1.9 of this document (http://dnr.wi.gov/topic/wastewater/documents/Chap1x9RVASTests.pdf).

Chronic Tests:

Sample to be tested

- 100% Screen Only = 5 Liters (1.4 Gallons)
- Full Dilution Series = 12 Liters (3.2 Gallons)

Reference site/upstream receiving water control water = 15 Liters (4 Gallons)

Toxicity Identification Evaluations (TIE):

- Acute TIE = Minimum of 16L (4.25 gal); 20-25L (5-6.5 gal) preferred to test both species
- Chronic TIE = Minimum of 30L (8 gal) for Ceriodaphnia; 60L (16 gal) for fathead minnow; 11L (3 gal) for algae (~100 L/26.5 gal total)

NOTE: These volumes are in addition to those above; if staff only requested acute toxicity testing, then only need to collect enough volume for an acute TIE, if acute & chronic requested, collect enough for both TIE types, etc. This does **not** cover extra volumes needed to run any inorganic/organic analyses based on what is found in the TIE, these volumes are just to run the TIEs themselves.

Due to the need for these larger volumes, staff should collect samples in new 20 L/5 gal Cubitainers® (or other brand name collapsible plastic containers). If DNR staff do not have these on hand , they should be able to find new, collapsible drinking water containers for sale in the camping departments of local hardware/home stores (Farm & Fleet, Wal-Mart, Shopko, Pamida, Menards, etc.). If Cubitainers® are not available, other sampling containers can be used, as long as they are new and/or clean (see guidance on proper cleaning methods referenced below). For guidance on which types of TIEs may be appropriate for a given situation, contact Kari Fleming in the Bureau of Water Quality (contact information shown below).

Sample Collection, Shipping and Holding Requirements

Detailed effluent sampling guidance, including a step by step schedule for use when collecting 24-hr composite samples for whole effluent toxicity (WET) testing, is available in "CHAPTER 1.1 - Samples For WET Testing" of this document (http://dnr.wi.gov/topic/wastewater/documents/Chap1x1Sampling.pdf, see attachment 1 on p. 6).

Collection: All samples used for toxicity testing should be collected with clean equipment (see Chapter 1.1 for suggested cleaning procedures) that has been rinsed once with sample prior to collection. The head space above the sample should be held to a minimum. Air which enters a container should be expelled by compressing the container before reclosing, if possible (i.e., where a Cubitainer® is used), or by using an appropriate discharge valve. Details of sample type, sample temperature, date, time, location, duration, name of collector, type of container, and procedures used for sample collection should be recorded on chain-of-custody forms. When collecting reference site/control

samples from flowing waters, samples should be collected from a point that is well-mixed. For river situations, this could be a mid-stream and mid-depth location which may require a boat or specialized sampling equipment (e.g., horizontal Kemmerer bottle, etc.). Attempts should be made to not collect samples in stagnant areas or near sediment.

Holding Time and Temperature: Efforts should be made to insure that holding time prior to the initial use of a sample for toxicity testing does not exceed 36-h after sample collection. However, if samples cannot be shipped to the lab immediately due to weekend, weather, or other conditions, samples should be stored as close to $\leq 4^{o}C$ (without freezing) as possible and kept in the dark until they can be delivered to the lab. Ship samples on ice in an appropriate container, such as a cooler.

Shipping: Ship samples for next day delivery or hand deliver within 24 hours of collection, whenever possible. If samples will be arriving on a weekend or legal holiday, please call the lab (608-224-6230) to make arrangements for weekend staffing. Also, it can be helpful for lab staff to know how the samples will be delivered (by hand, UPS, FedEx, etc.), so they can prepare for them. If samples will be shipped via FedEx/UPS, the tracking # can help track its shipment to the lab. Be sure to include the word "toxicity" as a test parameter on the labslip or chain-of-custody form or otherwise clearly indicate that you would like to have this type of testing done. This will insure that the sample gets to the right lab at the State Lab of Hygiene.

Other Available Information: Provide Material Safety Data Sheets (MSDS) of spilled substance, if known and available, and any other information known about the substance. Not only is this important for determining potential environmental impacts, but it might also be important for lab staff to know how potentially hazardous the material might be.

Funding for Toxicity Tests:

The DNR provides an annual contract to the Environmental Toxicology Lab at the SLH to perform toxicity tests on effluents, sediments, and receiving waters as requested by DNR staff, without a separate fee for individual tests. While this contract covers all of the costs of toxicity testing (and sampling assistance, when needed), it does not cover the cost of concurrent chemistry analyses or other services. For more information related to funding or conduct of non-toxicity tests, contact Ron Arneson (ronald.arneson@wisconsin.gov; 608-221-6322) or refer to http://intranet.dnr.state.wi.us/int/es/science/ls/Account.htm.

Toxicity Testing Contacts:

If you would like to schedule a toxicity test, or if you are not sure if toxicity testing is applicable to your situation, contact Kari Fleming for advice. Kari is responsible for helping other DNR staff coordinate effluent, surface water, and sediment toxicity tests at the State Lab. Kari also reviews toxicity test results after they have been completed by the lab and then distributes them to the appropriate field staff. Kari and the lab work closely together and will share information provided to each other, so it is also acceptable to contact SLH staff directly when Kari is not available. Contact information for both is provided below.

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