

CHAPTER 1.1 - Samples For WET Testing

The purpose of this chapter is to provide staff with guidance regarding sample type and location to be specified in WPDES permits and to provide guidance to permittees, consultants, and others taking WET samples. Permittees must follow permit requirements when taking WET samples.

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.

Effluent Sampling Location

This section is intended to help staff identify the appropriate location for WET sampling. According to Section 2.2 of the "State of Wisconsin Aquatic Life Toxicity Testing Methods Manual, 2nd Edition" (Methods Manual; see <http://dnr.wi.gov/topic/wastewater/WET.html>), the effluent sample location must be identified in the WPDES permit. The DNR staff most familiar with the facility should help determine the best WET sampling location to be included in the permit. The location used by the permittee to collect other permit-required samples **may** be an appropriate location for effluent sampling, however, it should be verified that this location would provide a representative sample for WET testing.

Ideally, samples used in WET testing should represent the effluent as it is discharged into the environment. To accomplish this, the sampling location should be as near to the end of pipe as possible. Whenever possible, effluent samples should be collected after all chemical addition in the manufacturing process and/or wastewater treatment system (for example, after chlorination and dechlorination at a municipal wastewater treatment plant). An end of pipe sampling location is desirable because one of the goals of WET testing is to determine the effects of the entire effluent mix as it is discharged into the environment. Reasonable attempts should be made to collect this end of pipe sample - if necessary, staff should consider requiring the facility to install or move a dedicated sampler, use a portable sampler, or use an alternate sample type (for example, time proportional composites or grab samples, instead of flow proportional composites), if staff believe that a new location would be more representative. If Department staff determine that collecting an end of pipe sample is not possible, due to safety concerns or for other reasons, the sample should be collected as near to this point as possible.

Intake sample lines should be set in a location that will provide a well mixed sample away from any quiescent zone. This is often mid-stream and mid-depth in a channel situation. A weighted sieve may be necessary to position the sample line in a well-mixed location. Samples should be collected under normal operating conditions, unless there is a specific reason to collect a sample during an atypical situation. Sample security should be maintained in all situations. This may require a location inside a fence or sample building. Section 2.2.7 of the Methods Manual requires that samples be chilled with ice or another means of refrigeration **during and after** collection. All reasonable steps should be taken to obtain a sample and cool it to $\leq 4^{\circ}\text{C}$ (without freezing) as quickly as possible. If a warm effluent is being collected during hot weather conditions and ice is being used to preserve the sample, additional ice may be needed during or after the collection period to cool the sample. Conversely, freezing conditions should be avoided in winter when selecting a location for the sampler.

Effluent Sample Type

Two samples collected over a three day (72-hr) period are needed to complete an acute test and three samples collected over a six day (144-hr) period are necessary to complete a chronic test, in most cases. Intermittent or seasonal discharges may require deviations from this standard sampling schedule (see Chapter 1.6 for a discussion of intermittent discharges: <http://dnr.wi.gov/topic/wastewater/WETguidance.html>). Operating conditions could change during the sampling period, but tests should continue once they are started (e.g., a test in progress should continue even if the treatment plant enters

an upset condition). Permittees and labs should be careful to note any changes or abnormal conditions on the lab slip and on the WET Test Report Form.

Most WET test samples should be collected as 24-hr flow-proportional composite samples, unless a time proportionate or grab sample is deemed appropriate due to a lack of effluent variability. Flow proportional sampling is usually most desirable to best represent effluent quality over a 24-hr period. If the facility sampler will not provide enough volume, a greater volume may be obtained by attaching a hose to the pump and running it outside of the sampler to a large cooler with a sample container on ice. Time composite samples may be acceptable when flow proportional is not available; however, this type of sample will not be as representative of effluent quality as a flow proportioned sample, if flow or effluent quality is variable over a 24-hr period.

A series of grab samples may be collected to provide a 24-hr composite when flow or time proportional sample collection is not possible. One grab sample may be appropriate if effluent quality is not expected to vary over a 24-hr period. For example, a single grab may be acceptable for municipal stabilization pond systems. In other situations, a grab sample or series of grab samples may be the only type that can be collected (e.g., in an enforcement case where sample security is paramount, where discharges are for short or intermittent periods, etc.). Sample type must be noted on chain of custody forms and on WET test report forms (see Section 6 of the Methods Manual for reporting requirements).

Effluent Sample Adjustments

Since the goal of WET testing is to simulate the conditions which occur as the discharge enters the environment, Section 4.15 of the Methods Manual requires that WET samples not be manipulated in any way (e.g., no dechlorination, filtration, aeration, pH adjustment, etc.), unless parallel testing is done to demonstrate what, if any, affect the manipulation had on the test. If a facility has reason to demonstrate that a chemical is the cause of toxicity, they may choose to demonstrate this by conducting parallel tests of adjusted and unadjusted effluent. For example, if it is necessary to collect WET test samples after chlorination but prior to dechlorination, it may be desirable to conduct side-by-side tests to show that any chlorine present in the sample is the only cause of toxicity. Side-by-side tests may also be desirable when deficiency toxicity is suspected (see Chapter 1.3, for more discussion).

Parallel tests should be similar in every way other than the adjustment being demonstrated. Controls should be included to show that the adjustment itself has not caused toxicity. No more chemical should be introduced into the sample than is absolutely necessary for a successful test; the adjustment chemicals themselves might be toxic or enhance the toxicity of other substances. The Department may use data from parallel tests to determine what has caused an effluent to fail a toxicity test, to determine whether follow up work is necessary, or to help determine an effluent's toxicity potential.

Wastewater Treatment Plant Conditions

As stated in standard permit language, permittees are ***NOT*** allowed to shut down chlorination, chemical addition, or other wastewater treatment processes during WET test sampling. Testing must be done under normal operating conditions. Permittees are not allowed to turn off or modify treatment systems, change production or waste treatment schedules, or change other normal operating or wastewater conditions during WET tests. Standard permit language in the WET footnote addresses this in the following manner:

WET testing shall be performed during normal operating conditions. Permittees are not allowed to turn off or otherwise modify treatment systems, production processes, or change other operating or treatment conditions during WET tests.

If something unusual is occurring within the distribution or treatment system before testing begins, permittees should contact the Department's Biomonitoring Coordinator (Kari.Fleming@wisconsin.gov; 608-267-7663) to discuss the situation and decide whether testing should be rescheduled. If conditions change during testing, tests should continue.

Permittees and labs should note any changes or abnormal conditions on the lab slip and WET Test Report Form.

Sampler Care and Cleaning

Equipment used to collect effluent and receiving water samples for WET testing should be cleaned appropriately before use, in order to remove any potential sources of sample contamination. Artifactual toxicity can occur if equipment is not properly cleaned before sampling, due to chemicals that may be present. Microorganisms that colonize on dirty surfaces can cause biological interference in WET tests or produce endotoxins that are toxic to the WET test organisms.

It is strongly recommended that permittees replace all tubing and clean any parts that come in contact with effluent or receiving water samples, before sampling. If using automatic samplers, used tubing should be replaced with new tubing (including the pump head tubing) prior to every WET test. If this is not possible, all tubing should be cleaned and rinsed according to procedures outlined in the Methods Manual. All equipment used for collecting grab samples of effluent or receiving water should be cleaned in this manner, as well. The following is an excerpt from cleaning requirements found in Section 3.12 of the Methods Manual:

"All...sample containers...that are reused shall be cleaned according to the following procedures, except where sampling equipment may not be compatible with acids or acetone, in which case the manufacturer's recommended cleaning procedures should be followed:

1. Soak 15 minutes and scrub with detergent in tap water, or clean in an automatic dishwasher.
2. Rinse twice with tap water.
3. Rinse with 10% HCl or 10% HNO₃ (v:v) to remove scale, metals, and bases. **Caution:** HNO₃ is a strong oxidizer and may react and combust with acetone.
4. Rinse twice with tap water.
5. Rinse once with liberal amounts of fresh, full-strength, reagent grade acetone (or an alternate solvent approved for use by the Department) to remove organic compounds. Use a fume hood or canopy.
6. Rinse three times with distilled or deionized water."

Sample Acceptability

Samples must meet the following criteria, according to the Methods Manual (Section 2.4), in order to be acceptable for permit compliance. If samples do not meet these criteria and are rejected, tests may need to be restarted at the cost of the permittee.

Holding Time

The maximum holding time prior to the initial use of an effluent or receiving water sample for WET testing is 36 hours after the completion of sample collection. Sample holding time starts when a grab sample is collected or when a composite sampling period is completed and ends when organisms have been introduced into test chambers for all tests.

Temperature

The effluent sample temperature at the time of arrival at the lab must be $\leq 10^{\circ}\text{C}$ and there must be evidence that the sample was packed with ice during shipping. The sample temperature at the time of arrival at the lab may exceed 10°C only if the time elapsed from the end of the sample period is < 4 hrs.

Guidelines for Sample Shipping and Handling

The following tips are from past experience and requirements in the Methods Manual regarding WET sample handling. By following these suggestions, the most common mistakes that invalidate or compromise samples can be avoided. Additional guidance regarding sample scheduling and volume can be found in Attachment 1 at the end of this chapter.

Samplers

When using a composite sampler, several problems can occur that can cause the sample to be missed or cause artifactual toxicity. Disconnection of tubing or power supplies is a common cause for missed samples. All tubing and cords should be secured to a surrounding structure to prevent accidental disconnection. Tubing connectors should be forced on as firmly as possible. Securing the connections with duct tape or nylon ties is recommended. Power supplies can be secured by tying or taping electrical cords together at junctions. If wet conditions are expected, the junctions should be wrapped with waterproof tape to avoid short circuits. If using batteries, make sure they are fully charged. In cases where frequent pumping is required or the temperature is very cold, the battery could be replaced during the sampling period.

Frozen lines can occur in winter when using a composite sampler. Freezing risk can be minimized by selecting a protected site for the sampler, repositioning tubing, or decreasing the intervals between sampling. If possible, select a site that is indoors or in an area that has a higher temperature due to the surrounding environment. The temperature of most effluents is usually above 40°C, so areas nearer to the effluent should be warmer than those further away. Final contact troughs or wells may have a place to set the sampling equipment close to this warmer temperature (**Caution:** do not enter confined spaces unless trained to do so). Tubing should be positioned so that the inlet and outlet are sloped away from the sampler. Dips in tubing will collect water and freeze between sampling intervals. Decreasing the sampling intervals might keep tubing from freezing.

Containers

Samples are usually collected and shipped in Cubitainers[®] (1, 2.5 or 5 gallon), carboys, or similar containers. Containers that are used for collection should be new or washed according to the protocols described above.

Cooling

As required by Section 2.2.7 of the Methods Manual, WET samples must be chilled during collection, through the use of a refrigeration unit or in a cooler on ice. During hot weather or when collecting very warm effluents, it may be necessary to add more ice before the end of the sample period.

Documentation

The sample slip/chain of custody should be filled out as thoroughly as possible. The Methods Manual (Sections 2 and 6) requires that the facility name and outfall, sample temperature and pH, date of collection, time of collection, name of collector, and procedures used for effluent and receiving water sample collection be noted on chain of custody forms and WET Test Report Forms. Any unusual conditions (e.g., plant upsets, slug loads, weather conditions, flooding, algae blooms, etc.) in the WWTP or receiving water must also be noted on these forms.

Packing

Air must be forced out of collapsible sample containers. Samples should be shipped in a cooler, surrounded by ice (20 lbs), with all water drained from the cooler. If the sample is shipped via commercial carrier, samples and ice must be sealed within a large plastic bag, because the carrier will return the sample if it leaks in transit.

Shipping

Sampling schedules will need to accommodate shipping schedules (sampling periods should end as close to the shipping time as is practical) in order to insure that the < 36-hr holding time is met. Samples may be hand delivered by the permittee or shipped via a commercial carrier. Courier services may guarantee delivery within certain time

periods, with the purchase of shipping insurance.

In cases where a sample is very warm after collection, permittees may want to deliver the sample to the lab within the 4 hr time limit (described below) or add more ice during transit in order to meet $\leq 10^{\circ}\text{C}$ arrival temperature criteria. If a very warm sample is shipped by a commercial carrier, samples should be pre-cooled prior to shipping.

Saturday Delivery

When chronic WET tests are being conducted, it may be necessary for the lab to receive an effluent sample on Saturday. When shipping samples to arrive on Saturday, **coolers/shipping containers MUST be Labeled for "Saturday Delivery"** (not "Overnight Delivery") or the shipping company will not deliver overnight samples until Monday morning. Saturday delivery is often a separate option that must be specified. "Overnight" or "Next Day Air" on Fridays usually means the next business day, which is Monday. In many cases, the shipping company may have "Saturday delivery" stickers that need to be affixed to the container.

It is the permittee's responsibility to see that their chosen courier understands that samples must arrive on Saturday in order for successful completion of the test. If samples are shipped from a courier "substation" (e.g., a hardware store or shopping plaza), permittees should make sure that shipments are marked for Saturday delivery (do not assume that store clerks understand the importance of this step). If samples do not arrive within the required < 36-hr holding time, samples could be rejected and tests restarted (see Section 2.4 of the Methods Manual), at additional cost to the permittee.

There may be circumstances outside of the permittee's control (e.g., winter weather) that result in late sample delivery. In these cases, individual samples may be conditionally acceptable if holding times fall outside specifications, depending on the degree of the departure and the objectives of the test. When this occurs, permittees (or their lab) should contact the Department's Biomonitoring Coordinator (Kari.Fleming@wisconsin.gov or 608-267-7663) for permission to continue the test. Any deviation from holding time requirements must also be clearly described on the "WET Test Report Form" (see Section 6 of the Methods Manual). If Saturday delivery does not occur due to shipping company error, permittees should discuss this with shipping company management so that they can address the problem within their system and avoid future occurrences.

Lab Receiving

When the sample arrives at the lab, a record of the receipt must be produced by the lab (Section 2.4.4, Methods Manual). The Methods Manual requires documentation of the date and time the sample was received, the name of the person receiving the sample, and the lab number assigned to the sample. The lab also must measure and record the temperature and the pH of the sample, presence or absence of ice, and any abnormalities of the sample (i.e., open container, leakage, etc.) as soon as it arrives at the lab.

ATTACHMENT 1 - WET TEST SAMPLING CHECKLIST

This checklist is guidance for use when collecting samples for WET testing. Details are provided in the preceding chapter. Additional guidance may be provided by the lab completing the analysis. **Permittees should discuss sampling schedules and volumes with their lab before testing.**

Pre-sampling Preparation:

- 1) Verify effluent sampling location is representative and according to permit requirements. Find a location to obtain receiving water that is accessible and safe (if receiving water is to be used).
- 2) Clean all sampling equipment (see preceding chapter, page 3) including any buckets or funnels used to collect receiving water. Replace the tubing in the pump head and have enough new tubing to run from the sampler to the sample point, if using an automatic sampler.
- 3) Verify shipping schedule with lab. NOTE: Courier services will usually guarantee delivery within certain time periods, with the purchase of shipping insurance. **Shipping insurance may cost from \$5 - \$30 per sample, but may prevent costs associated with test restarts or repeats.**
- 4) Make sure sufficient ice is available for sampler and shipping containers, especially in hot weather.

Sampling Schedule

Day	Activity
1	Set up automatic sampler to begin 24 hr composite period. Set controls to collect sufficient amount (usually 1.5 gal for acute; 3.0 gal for chronic). This step is not necessary if grab samples are used.
2	Collect effluent composite sample #1 (or grab, if appropriate). Collect receiving water grab sample. Send effluent #1 and receiving water to lab. Repeat Day 1 - set up sampler, set controls to collect sufficient amount, begin composite.
3	Collect effluent composite sample #2 (or grab, if appropriate). Send effluent sample #2 to lab. For an acute test, this completes sampling. If doing a chronic test, continue through days 4-6.
4	No activity.
5	Repeat Day 1 - set up sampler, set controls to collect sufficient amount, begin composite.
6	Collect effluent composite sample #3 (or grab, if appropriate). Send sample #3 to lab. Coolers MUST be Labeled for "Saturday Delivery" NOT "Overnight Delivery" or the shipping company will not deliver overnight samples until Monday morning.

The activities and dates on this checklist are general and should be used in consultation with specific lab instructions.

SPECIAL NOTES: Sample temperature upon arrival at the lab must be $\leq 10^{\circ}\text{C}$ and there must be evidence that the sample was packed with ice during shipping. The amount of time from the end of the sampling period to the beginning of the test must not be $> 36\text{-hr}$ (see preceding chapter for discussion).