

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 and Type

This section is intended to help department 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), the effluent sample location must be identified in the WPDES permit. The DNR person most familiar with the facility should help determine the best WET sampling location. The location used by the permittee to collect other permit-required samples **may** be also be an appropriate location for effluent sampling, however, it should be verified that this location would provide a representative sample for WET testing.

Samples used in WET testing should represent the final effluent that is discharged to the environment. This location should be as near to the "end of pipe" as possible. Whenever possible, effluent should be collected after all chemical addition in the manufacturing process or wastewater treatment system (e.g., after chlorination/dechlorination). This "end of pipe" effluent is necessary whenever possible because one of the goals of WET testing is to determine the effects of the entire "chemical soup" of effluent, as it is discharged. Reasonable attempts should be made to collect this end of pipe sample. For example, if necessary, staff should consider requiring the facility to move a dedicated sampler, use a portable sampler, or use an alternate sample type (e.g., time proportional composite, grab sample) if staff believe it would be representative (see "Representative Sample" below). If Department staff determine that collecting an end of pipe sample is not possible, the sample should be collected as near to this point as possible.

The intake sample line should be 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.

Effluent Sample Adjustment

The goal of WET testing is to simulate the conditions which occur as the discharge enters the environment. Although it may be necessary to take WET test samples prior to dechlorination in some situations, this should be avoided whenever possible. Samples which arrive at the lab with measurable amounts of chlorine will almost certainly cause WET test failures.

The facility is **NOT** allowed to shutdown chlorination during WET test sampling (when the permit requires disinfection). Permittees are responsible for chlorine toxicity, just as they are responsible for toxicity caused by other substances, unless the chlorine which is present is not representative of final effluent or if the discharge is a noncontact cooling water and the chlorine is present due to a water supply source (exemption allowed by s. NR 106.10, Wis. Adm. Code - see Chapter 2.6).

Section 4.15 of the Methods Manual specifies that samples must not be manipulated in any way (e.g., dechlorination, filtration, aeration, pH adjustment, etc.), unless parallel testing is done to demonstrate what, if any, affect the manipulation may have had on the test. If a facility has reason to demonstrate a toxicant is the only cause of toxicity, they may demonstrate this by conducting parallel tests of adjusted and unadjusted effluent. These parallel tests should be similar in every way other than the adjustment being demonstrated (i.e., with the same dilutions and under the same test conditions). Controls should be conducted that show the adjustment itself has not caused toxicity. No more chemical should be introduced into the sample than is absolutely necessary for a successful toxicity test. The adjustment chemicals themselves might be toxic or enhance the toxicity of other substances.

The Department will use data from parallel tests when necessary to determine whether an effluent has passed or failed a toxicity test, to determine the cause(s) of a failure, whether follow up work is necessary, or to determine an effluent's toxicity potential (i.e., when completing the WET Checklist described in Chapter 1.3).

Sampler Care and Cleaning

Sampling equipment should be cleaned appropriately, in order to insure that samples are representative of the effluent and to remove any possible contamination. **Artifactual toxicity may occur when sampling equipment is not cleaned regularly. Microorganisms can colonize surfaces that are in contact with the effluent. Some of these microbes can produce endotoxins that are toxic to the test organisms. Before sampling, we strongly recommend that you replace all tubing and clean any parts that contact effluent and receiving water samples.**

If using automatic samplers, 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 the following requirements, as required by the Methods Manual. All equipment used for grab sampling 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."*

Representative Sample

Samples used in WET tests should be representative of the final effluent as it is discharged to the environment. A representative sample should be obtained under "normal" operating conditions unless there is a specific reason to collect a sample during an atypical situation. Collection of samples for WET testing will usually consist of either two (for acute tests) or three (for chronic tests) 24 hour composites samples. Often times the sample location and type for WET testing will be the same as for other permit-required samples such as BOD₅, suspended solids, nutrients or metals.

Operating conditions may change over the sampling period, however, tests should continue once they are started.

For example, 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 WET Test Report Form.

Most WET test samples are 24 hour flow-proportional composite samples, unless a time proportionate or grab sample is deemed appropriate due to lack of effluent variability. Flow proportional sampling is usually most desirable to best represent effluent quality over a 24 hour 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 sample container and 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 and/or effluent quality is variable over a 24 hour period.

A series of grab samples may be collected to provide 24 hour composite samples. If flow is metered, a flow proportional sample may be composited by collecting a series of grab samples and proportioning based on flow at the end of the collection period. A series of grab samples may be advantageous if it is necessary to maintain chain of custody to guarantee sample integrity in an enforcement situation. One grab sample is appropriate if effluent quality is not expected to vary significantly over a 24 hour period. For example, a single grab sample is usually acceptable for municipal stabilization pond systems. In some situations a grab sample or series of grab samples may be the only type of sample that can be collected (i.e. enforcement case, discharges of short or intermittent periods, some noncontact cooling water discharges, etc.). Sample type should be noted on each lab slip and on the WET report form (see Section 6 of the Methods Manual for reporting requirements).

Sample security should be maintained. This may require a location inside a facility's fence or located in a sample building. Section 2.2.7 of the Methods Manual requires that "***Samples shall be chilled with ice or other means of refrigeration during and immediately after collection. Every effort should be made to achieve a sample temperature of $\leq 4^{\circ}\text{C}$ (without freezing).***" This may require a power source nearby. 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. 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. Freezing conditions should also be considered in winter, when selecting a location for the sampler.

Guidelines for Sample Handling

This section summarizes tips from past experience and procedures found in the Methods Manual for handling samples used in WET tests. By following these suggestions, the most common mistakes that invalidate or compromise samples can be avoided. For additional guidance regarding scheduling and appropriate sample volumes, see attachment 1 at the end of this chapter.

Samplers

Whether using a flow proportional composite sampler or a timed composite sampler, several problems with the sampler can occur that will cause the sample to be missed or cause artifactual (i.e., artificial, method-related) toxicity. Disconnection of tubing or power supplies is probably the single most 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 electric 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 in the middle of the sample period.

Frozen sample lines may occur in winter if using a composite sampler. You should consider minimizing the risk of freezing by selecting a protected site for the sampler, repositioning the tubing, or decreasing the

intervals between sampling. If possible, select a site that is indoors. Otherwise, position the equipment in an area that has a higher temperature due to the surrounding environment. The temperature of most effluents is usually above 40°C. 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 both the inlet and outlet are sloped away from the sampler. Dips in the tubing will collect water and freeze between sampling intervals. Decreasing the sampling intervals may keep the water in the tubing from freezing.

Containers

You may use Cubitainers (1, 2.5 or 5 gallon), carboys or 1 gal milk jugs. Containers that are used for collection should be either new or washed according to the protocols described on page 2 of this chapter.

Cooling

As required by Section 2.2.7 of the Methods Manual, sample must be chilled during collection, through the use of a refrigeration unit or a cooler with at least twenty pounds of 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.

Grab Samples

Any equipment used to collect grab samples should be cleaned according to the protocols described on page 1 of this chapter. You should avoid collecting unrepresentative sediment while retrieving samples.

Documentation

The sample slip/chain of custody should be filled out as thoroughly as possible. The facility name, location, permit number, outfall number and effluent/receiving water sample locations should be included. Also, the names of the collector(s), the site contact person, the beginning and ending dates, and times of the sample should be provided. In addition, the temperature and the pH of the completed sample must be recorded, so that it can later be transferred to the WET Test Report Form, required in Section 6 of the Methods Manual

Packing

You should force out any air that is trapped in collapsible sample containers. Then you should place the sample in a cooler, pack both sides of the sample with ice (20 lbs), and drain any water from the cooler. If the sample is shipped via commercial carrier, you should seal the sample and ice within a large plastic bag, as the carrier may return the sample if it leaks in transit.

Shipping

As specified in the Methods Manual, the holding time is 36 h after the end time of the sample collection. **Therefore, you will have to make sure samples are collected to accommodate shipping schedules.** Plan the sampling period to end as close to the shipping time as is practical. Depending on the situation, you may want to choose different modes of transportation. Sometimes you may want to deliver the sample yourself, in other cases you may choose to ship the sample via a commercial carrier.

In cases where a sample is very warm after collection, you may want to deliver the sample to the laboratory within the 4 hr time limit (described below) or add more ice during transit in order to meet the $\leq 10^{\circ}\text{C}$ arrival temperature criteria. If a very warm sample must be shipped by a commercial carrier, you should pre-cool the sample prior to shipping. Commercial carriers include courier services, USPS, and bus lines. Courier services may provide the least expensive service during the work week and deliver the sample to the laboratory before 2:00 pm the following day. Bus lines can deliver on the same day and may be the least expensive service available on the weekends.

NOTE: Please be aware that Section 2.4 of the Methods Manual requires that samples be rejected by the

laboratory if they do not meet the ≤ 36 hour holding time. If samples are rejected, tests may have to be restarted or repeated, at the cost of the permittee. Courier services will usually guarantee delivery within certain time periods, with the purchase of additional shipping insurance. **Shipping insurance may cost from \$5 - \$30 per sample, but may prevent costs associated with test restarts or repeats.**

Laboratory Receiving

When the sample arrives at the laboratory, a record of the receipt must be produced (Section 2, part 2.4.4, Methods Manual). In most cases, the sample slip has areas where the laboratory receiving information is documented. The laboratory is required by the Methods Manual to include the date and time the sample was received, the signature or name of the person receiving the sample, and the laboratory number assigned to the sample. The Methods Manual also requires the laboratory to 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.) when it arrives at the laboratory. **Because of Methods Manual requirements, if the sample arrives at the laboratory warmer than 10°C, it must be rejected unless the amount of time elapsed from the end of collection is less than 4 h.**

Sample Acceptability

The following acceptability requirements are specified in the Methods Manual (Section 2.4). Samples must meet the following criteria, according to the Methods Manual, in order to be acceptable for permit compliance. If samples do not meet these criteria and are rejected, tests must be restarted or repeated.

Temperature

The sample temperature at the time of arrival at the laboratory must be $\leq 10^{\circ}\text{C}$ and must have evidence that the sample was packed with ice during shipping. The following exception to this criteria is allowed. The sample temperature at the time of arrival at the laboratory may exceed 10°C if the time elapsed from the end of the sample period is less than 4 h.

Holding Time

The amount of time from the end of the sampling period to the time the organisms are initially placed into test treatments prepared from that sample must not exceed 36 h. Other holding time requirements (e.g. maximum time prior to final use of sample, holding time extensions, etc.) are included in the Methods Manual (Section 2.4).

WET Report Form and Chain of Custody

The Methods Manual (Sections 2 and 6) requires that sample temperature, date of collection, time of collection, name of collector, and procedures used for effluent and receiving water samples be noted on the WET report form sent to the Department and on laboratory chain of custody forms. Any unusual conditions (e.g., plant upsets, slug loads, weather conditions, flooding, algae blooms, etc.) in the WWTP or receiving water should also be noted on these forms.

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 laboratory 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 2) 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 laboratory. 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 Number	Activity
1	Set up automatic sampler. Set controls to collect sufficient amount (approximately 1.5 gal for acute; 3.0 gal for chronic). Begin 24 hour composite (or other appropriate composite, as required by the permit). This is not necessary if a grab sample is used.
2	Collect composite sample #1 (or grab, if appropriate). Also collect receiving water (grab sample - approximate volume is 10 gal). Send sample #1 and receiving water to lab. Repeat Day 1 - set up sampler, set controls to collect sufficient amount, begin composite.
3	Collect composite sample #2 (or grab, if appropriate). Send sample #2 to lab. For acute tests, this completes sampling. If doing chronic, continue with Days 4-6.
4	No activity.
5	Repeat Day 1 - set up sampler, set controls to collect sufficient amount, begin composite.
6	Complete sampling for chronic analysis. Collect composite sample #3 (or grab, if appropriate). Send sample #3 to lab.

The activities and dates on this checklist are very general and should be used in consultation with your 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 $> 36\text{h}$ (see preceding chapter for discussion).