BUREAU OF WATER QUALITY
PROGRAM GUIDANCE

WASTEWATER POLICY & MANAGEMENT TEAM

Whole Effluent Toxicity (WET) Program Guidance Document

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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. 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.

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Date

Note: there are missing chapter numbers, due to the rescission of chapters over the years since this document was created. The "State of Wisconsin Aquatic Life Toxicity Testing Methods Manual" (s. NR 219.04, Wis. Adm. Code) refers to specific guidance chapters. Chapters have not been renumbered to keep those Methods Manual references intact.

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Introduction

Whole effluent toxicity (WET) measures the combined toxic effect to aquatic organisms from all pollutants contained in a facility's wastewater effluent. WET testing is one tool used by the USEPA and the Department to implement the Clean Water Act's (33 U.S.C. §1251 et seq. 1972) prohibition of the discharge of toxic pollutants in toxic amounts (ss. NR 102.04 (1) (d) and 102.04 (4) (d), Wis. Adm. Code). WET is used in addition to chemical-specific analyses in an integrated approach to controlling toxic pollutants and protecting aquatic life. The use of WET testing is beneficial in addition to chemical-specific testing for many reasons, including: 1) the limitations of chemical testing (for example, limits of detection may not be low enough to show if criteria are being met), 2) inadequate toxicity data for individual chemicals, and 3) the inability to predict the toxicity of chemicals when combined in an effluent (criteria for individual toxics provide protection against those chemicals individually, but do not account for the effects that chemicals may have when combined).

In Wisconsin, WET testing has been a part of the Wastewater program since the 1980s. Department staff evaluate wastewater effluent surface water discharges when reissuing their individual Wisconsin Pollution Discharge Elimination System (WPDES) permits, to determine if WET testing should be conducted during the permit term. Site-specific factors considered when determining whether WET monitoring should be required may include: 1) designated uses of the receiving water, 2) dilution considerations, 3) facility type, 4) effluent variability, 5) chemical-specific effluent data, and 6) historical WET data. (See Chapter 1.3 for guidance related to WET monitoring and limits evaluations.)

WET tests measure an effluent's effect on the test organisms' ability to survive, grow and reproduce. WET test exposures consist of different effluent concentrations, usually diluted with the receiving water that it is discharged into. The WET test organisms used in Wisconsin are *Pimephales promelas* (fathead minnow), *Ceriodaphnia dubia* (a zooplankton), and sometimes *Selenastrum capricornutum* (a green algae). These species are known to be sensitive to toxic substances and are representative of aquatic populations found in Wisconsin waters. These so called "indicator organisms" represent the three trophic levels (invertebrate, vertebrate, and plant) and are used to predict what may be happening in the environment as the effluent is introduced. They have been used in toxicity tests with effluents and chemicals for many decades and are well suited to a laboratory environment.

There are two types of WET tests - acute and chronic. WPDES permit-required acute tests are conducted with *Ceriodaphnia dubia* and the fathead minnow; they last 48 and 96 hours, respectively. The objective of an acute WET test is to find the concentration of effluent that causes death during a short-term exposure. The second type of WET tests are chronic tests. WPDES permit-required chronic tests are usually conducted using *Ceriodaphnia dubia* and the fathead minnow; both tests last 7 days. Wisconsin's WET test methods also include a chronic test with *Selenastrum capricornutum* (a 4 day test); this test may be used to measure the potential for adverse impacts to aquatic plants. The objective of a chronic WET test is to find the concentration of effluent that causes sublethal effects (reductions in reproduction or growth) during a longer-term exposure.

WET Guidance Document Organization

This WET Guidance Document is intended for use in conjunction with the "*State of Wisconsin Aquatic Life Toxicity Testing Methods Manual, 2nd Edition*" (PUBL-WT-797; referenced in ss. NR 149.20 and NR 219.04 Table A, Wis. Adm. Code), which states required test procedures. The Methods Manual is referenced in WPDES permits and specifies test procedures that are required for use when determining compliance with permit requirements. The WET Guidance Document was created to supplement the Methods Manual, to assist staff when making decisions related to WET testing and WPDES permits, and to assist permittees and their labs when conducting WET tests in accordance with those permits.

Part One (Chapters 1.1-1.13) of this document was written as guidance to assist staff making permit-related decisions. It is important that staff be as consistent as possible when implementing WET requirements in permits, however, it is recognized that there may be circumstances when the general recommendations in this guidance are not appropriate for an individual situation. Decisions that don't follow the general recommendations in this guidance should be clearly documented in water quality based effluent limit (WQBEL) memos and/or permit fact sheets, so others can understand why decisions were made.

Part Two (Chapters 2.1-2.11) contains guidance and clarification of existing requirements for permittees and others, concerning issues of importance to the WET program. It is hoped that this guidance will provide permittees and their labs some assistance when making decisions regarding sampling and testing, and help them to better understand WET requirements.

The WET Guidance Document is expected to change as the Department gains experience with implementation of the WET program. This is due in part to the impact science and technology has on the WET program and the experience gained as we implement the Methods Manual and the guidance in this document. The maintenance of the guidance document is the responsibility of the Biomonitoring Coordinator, and it will be updated and improved with input from staff and other users, as needed. Staff, permittees, labs, and others should contact the Biomonitoring Coordinator (Kari.Fleming@wisconsin.gov) if they wish to suggest topics that may need to be addressed in future revisions or additions to the guidance.

WET Testing Regulations

Federal regulations:

- 40 CFR Part 122.21 (j) (5) (ii) requires permittees to submit WET tests on samples from each of their surface water outfalls during the last permit term on their permit application.
- 40 CFR Part 122.21 (j) (5) (ii) allows the permitting authority to determine the need for WET monitoring based on factors such as effluent variability, chemical-specific effluent data, wastewater treatment type, industrial contributions, available dilution, and receiving stream characteristics.
- 40 CFR Part 122.21 (j) (5) (v) requires that WET tests submitted with the permit application were conducted on multiple species (no less than two species; e.g., fish, invertebrate, plant), and that tests for chronic toxicity be done depending on the amount of receiving water dilution.
- 40 CFR Part 122.21 (j) (5) (viii) requires that WET testing be conducted using methods approved under 40 CFR part 136.
- 40 CFR Part 122.21 (j) (5) (x) requires that permittees provide information on the cause of toxicity and written details of any toxicity reduction evaluation conducted, if past WET tests revealed toxicity.
- 40 CFR Part 122.44 (d) (v) and 40 CFR Part 132, Appendix F, Procedure 6 (D) requires that WET limits be assigned when toxicity testing data, or other information, shows that a discharge causes, has the reasonable potential to cause, or contributes to an in-stream excursion above WET narrative criterion.
- 40 CFR Part 122.44 (d) (v) and 40 CFR Part 132, Appendix F, Procedure 6 (C) (1) (e) allows chemical-specific limits to be used in lieu of WET limits when sufficient to attain and maintain applicable numeric and narrative State water quality standards.
- 40 CFR Part 122.44 (i) (1) (iv) (B) requires that monitoring frequency be established on a case-by-case basis dependent on the nature of the discharge, but in no case less than once a year when a limit is given.
- 40 CFR Part 136.3 Table 1A identifies acute and chronic toxicity test methods required for use in permits and permit applications.

State statutes and regulations:

- Wis. Stat. § 283.11 State and federal standards.
- Wis. Stat. § 283.21 Toxic Effluent Limitations and Standards.

- Section NR 102.04 (1) (d), Wis. Adm. Code, states that substances in concentrations or combinations which are toxic or harmful to humans shall not be present in amounts found to be of public health significance, nor shall substances be present in amounts which are acutely harmful to animal, plant or aquatic life.
- Section NR 102.04 (4) (d), Wis. Adm. Code, states that unauthorized concentrations of substances are not permitted that alone or in combination with other materials present are toxic to fish or other aquatic life. Surface waters shall meet the acute and chronic criteria as set forth in or developed pursuant to ss. NR 105.05 and 105.06. Surface waters shall meet the criteria which correspond to the appropriate fish and aquatic life subcategory for the surface water, except as provided in s. NR 104.02 (3).
- Section NR 106.07(7), Wis. Adm. Code, states that the Department may establish a WET limitation according to s. NR 106.09, Wis. Adm. Code, as an alternative to a chemical-specific WQBEL based on a fish and aquatic life secondary acute or secondary chronic value determined according to ss.NR 105.05(4) and 105.06(6).
- Section NR 106.36, Wis. Adm. Code, allows effluent samples used in chronic fathead minnow tests to be modified to remove ammonia prior to testing when early life stage - absent ammonia criteria are in effect.
- Section NR 106.08, Wis. Adm. Code, describes the process for determining the need for WET testing requirements and limitations.
- Section NR 106.09, Wis. Adm. Code, describes the proper use of mixing zones in setting WET requirements and how WET limits are to be expressed in WPDES permits.
- Section NR 106.89, Wis. Adm. Code, allows chloride limits to be used in lieu of WET testing requirements and limitations until chloride source reduction actions are completed, under certain conditions.
- Section NR 149.20, Wis. Adm. Code, specifies requirements for laboratory certification for WET. The “State of Wisconsin Aquatic Life Toxicity Testing Methods Manual, 2nd Edition,” is incorporated by reference.
- Section NR 219.04, Table A, Wis. Adm. Code, specifies that WET compliance monitoring must be performed in accordance with the procedures in the “State of Wisconsin Aquatic Life Toxicity Testing Methods Manual, 2nd Edition,” Wisconsin Department of Natural Resources, 2004.

WET Methods Manual

The "*State of Wisconsin Aquatic Life Toxicity Testing Methods Manual, 2nd Edition*" (PUBL-WT-797; referenced in ss. NR 149.20 and NR 219.04 Table A, Wis. Adm. Code) provides laboratory procedures and technical guidance for permittees and laboratories performing WET testing for the WPDES permit program. The information given includes testing and sampling procedures, types of tests, definitions, quality control/quality assurance procedures, etc. The requirements contained in the Methods Manual apply to all permittees and labs doing WET testing for WPDES permit compliance and all WET tests conducted in accordance with a WPDES permit must be performed according to the Methods Manual. All site-specific conditions such as sampling type or instream waste concentration, which are different for each permittee, are specified in the WPDES permit. The Methods Manual can be found on the DNR website at: https://dnr.wi.gov/topic/wastewater/WET.html.

Laboratory Certification

Under Wis. Stat. § 299.11, the Department certifies laboratories to perform different types of environmental analysis. According to ch. NR 149, Wis. Adm. Code, in order for a laboratory to apply for certification or registration the laboratory must submit a completed application, a quality assurance plan, and appropriate fees to the laboratory certification program and pass an on-site evaluation. For additional information on laboratory certification and registration, contact the Laboratory Certification Program, 101 S. Webster St., Madison, WI 53707, (608) 267-7633, DNRLabCert@Wisconsin.gov or go to http://dnr.wi.gov/topic/wastewater/WETCertified.html.

CHAPTER 1.1 - Sampling For WET Testing

The purpose of this chapter is to provide staff with guidance regarding sample type and location that should 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.

Effluent Sampling Location

According to Section 2.2 of the "*State of Wisconsin Aquatic Life Toxicity Testing Methods Manual, 2nd Edition*" (s. NR 219.04, Wis. Adm. Code; http://dnr.wi.gov/topic/wastewater/WET.html) (Methods Manual), the effluent sampling location must be identified in the WPDES permit. The 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 (s. NR 219.04, Wis. Adm. Code) 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°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 (Methods Manual, Section 2.2.2, s. NR 219.04, Wis. Adm. Code). Intermittent or seasonal discharges may require deviations from this standard sampling schedule (see Chapter 1.6 for a discussion of intermittent discharges). 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 cases where sample security is paramount, where discharges are for short or intermittent periods, etc.). Sample type and date of collection must be noted on chain of custody forms and on WET test report forms (Methods Manual, Section 6.2.2, s. NR 219.04, Wis. Adm. Code).

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 (s. NR 219.04, Wis. Adm. Code) 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 of deficiency toxicity).

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

The following permit language is included in most individual WPDES permits where WET testing is required.

> *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.*

When this language is present in the permit, permittees are not allowed to shut down chlorination, chemical addition, or other wastewater treatment processes during WET test sampling. Instead, 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.

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) 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 may cause biological interference in WET tests or produce endotoxins that are toxic to the WET test organisms.

It is 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 each 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 (s. NR 219.04, Wis. Adm. Code):

"All containers that are reused shall be cleaned according to the following procedures, except where materials 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% HNO_3 (v:v) to remove scale, metals, and bases.* ***Caution****: HNO_3 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; s. NR 219.04, Wis. Adm. Code), 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 $\leq 10^{o}C$ 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 $10^{o}C$ 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 (s. NR 219.04, Wis. Adm. Code), 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 (s. NR 219.04, Wis. Adm. Code), 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 should 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 should 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 $\leq 10^{o}C$ 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 a 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, s. NR 219.04, Wis. Adm. Code), 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) 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, s. NR 219.04, Wis. Adm. Code). 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, s. NR 219.04, Wis. Adm. Code). 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 attachment contains advice for WET sample collection. Details are provided in the preceding chapter. Additional advice may be provided by the WET lab - permittees should discuss sampling details 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 for dilution).

2) Clean all sampling equipment (see preceding chapter), 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 the lab. NOTE: Courier services may guarantee delivery within certain time periods, with the purchase of shipping insurance. Shipping insurance may add costs per sample, but may prevent higher 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. ***Label coolers for "Saturday Delivery" <u>NOT</u> "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 $\leq 10^{o}C$ and there must be evidence that the sample was packed with ice during shipping and the amount of time from the end of the sampling period to the beginning of the test must not be > 36-hr., according to the Methods Manual (s. NR 219.04, Wis. Adm. Code). See the preceding chapter for more discussion.

CHAPTER 1.2 - Receiving Water Used to Make WET Determinations

The purpose of this chapter is to aid in the selection of the appropriate receiving water for use when determining dilution ratios, calculating the IWC, and as dilution waters.

Receiving water type, location, and dilution water are important factors to be considered when determining the appropriate WET requirements for a given situation. Since the magnitude of toxic effect usually increases as effluent concentration increases, one of the most important factors affecting toxicity potential is the dilution available in the receiving water into which the effluent is discharged. A very toxic effluent with extremely large dilution may cause less environmental damage than a less toxic effluent with very little dilution. Since dilution and mixing are important considerations, the relationship between receiving water flow and effluent flow should be evaluated when making decisions about WET permit requirements (see the WET Checklist process described in Chapter 1.3).

When making decisions related to WET monitoring and limits, it is important to use the appropriate effluent and receiving water flow information to calculate the receiving water to effluent flow ratio and instream waste concentration (IWC). Generally, cold water, warm water sport fish, and warm water forage fish waters support a more diverse aquatic life community and warrant a higher level of protection. Conversely, waters historically referred to as "variance" waters (limited forage fish, limited aquatic life) usually have less diverse fish populations or macroinvertebrate-only communities. In recognition of this, the following approach is recommended which provides protection from acute toxicity impacts to all waters, moderate protection from chronic impacts to variance waters, and full protection from chronic impacts to all waters classified as cold water, warm water sport fish, or warm water forage fish.

Since the aquatic communities that WET testing is designed to protect are always present in cold water (s. NR 102.04 (3) (a), Wis. Adm. Code), warm water sport fish (s. NR 102.04 (3) (b), Wis. Adm. Code), and warm water forage fish (s. NR 102.04 (3) (c), Wis. Adm. Code) communities, the receiving water used for making chronic WET determinations should be the first waterbody that the effluent encounters which supports any of these communities. If Department staff have information showing that the immediate waterbody supports a cold water, warm water sport fish, or warm water forage fish community, chronic WET requirements such as the IWC should be determined using that receiving water.

Staff should use their best professional judgment to select the appropriate receiving water flow to be used when making chronic WET determinations that will be protective of the appropriate aquatic life communities. Some example scenarios are illustrated at the end of this chapter. Once staff have chosen the appropriate waterbody to be used, the same waterbody should be used for stream flow to effluent flow ratios and IWC determinations.

Discharges to Limited Forage Fish and Limited Aquatic Life Streams

In situations where the effluent is discharged into a flowing waterbody that supports a limited forage fish (s. NR 102.04 (3) (d), Wis. Adm. Code) or limited aquatic life (s. NR 102.04 (3) (e), Wis. Adm. Code) community, the distance to the first waterbody that the effluent encounters which supports a cold water, warm water sport fish, or warm water forage fish community (or the distance between the discharge and where the direct receiving water supports any of these communities) should be determined. When this distance is less than 4 miles, it is recommended that the receiving water flow to be used for chronic WET determinations should be that of the first downstream cold water, warm water sport fish, or warm water forage fish waterbody, minus the effluent flow contributed by the discharger. If the distance to that waterbody is $\geq$ 4 miles, chronic WET monitoring is not usually

recommended, unless information is available that shows a potential exists for impacts due to chronic toxicity (for example, if there have been chronic WET failures).

Discharges to Lakes and Ponds

As specified in s. NR 106.06 (3) (b) 2, Wis. Adm. Code, when an effluent is discharged to a waterbody without unidirectional flow (e.g., lakes, bays, impoundments), the default stream flow to effluent flow ratio is 10:1 and the IWC is 9%. These default values should also be used whenever a “variance” stream empties into a waterbody without unidirectional flow (i.e., when the first downstream cold water, warm water sport fish, or warm water forage fish waterbody is a lake or pond). See Attachment 1 of this chapter for some examples of appropriate receiving water use in different discharge situations.

Using Receiving Water As Diluent in WET Tests

According to Section 4.4 of the Methods Manual (s. NR 219.04, Wis. Adm. Code), receiving water must be used in all chronic tests and in acute tests where an acute mixing zone - or zone of initial dilution (ZID) - has been approved. In acute tests that measure toxicity at the end of pipe (i.e., where no acute mixing zone has been approved), the Methods Manual allows the use of either receiving water or a standard laboratory water.

Correct identification of an appropriate receiving water sample location is important in cases where permittees are required to use receiving water as the diluent, because the objective of the WET test in those cases is to estimate the effect on the receiving water after the effluent has been mixed in and the extent to which the chemicals are biologically available to aquatic organisms. The Methods Manual (s. NR 219.04, Wis. Adm. Code) requires the use of receiving water as diluent because this increases the environmental relevance of WET testing by more directly representing real-world effluent and receiving water interactions in the test. Use of receiving water for dilution improves the ability of WET tests to predict in-stream effects.

Section 2.3 of the Methods Manual (s. NR 219.04, Wis. Adm. Code) requires at least one representative grab sample of receiving water be collected for use in each WET test. In stream and river situations, the receiving water sample should be collected upstream of the discharge, with every attempt made to avoid contact with the permittee's and any other discharges' mixing zones. In situations where a lake, impoundment, bay, or other waterbody without unidirectional flow has been identified as the receiving water to be used for WET tests, a sampling location should be chosen that is outside of the influence of all known discharges.

In situations where the water that is discharged into becomes a cold water, warm water sport fish, or warm water forage fish waterbody within 4 miles, receiving water samples should be collected upstream of the outfall, as described above. In situations where a separate downstream waterbody was used for WET determinations, the receiving water sample should be collected from a site upstream of the confluence of the waterbody that is discharged into and the waterbody that was used for WET determinations.

What if Samples Cannot Be Collected in the Receiving Water?

It may not be possible to collect receiving water samples under some conditions. For example, a river may lack sufficient flow during dry periods or it may be unsafe to collect samples in icy conditions during the winter months.

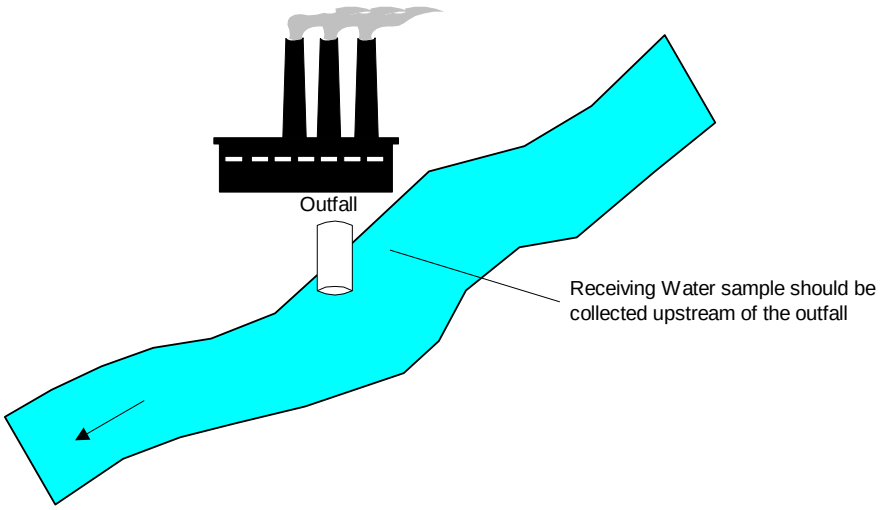
In limited cases where the Department has determined that the receiving water is regularly unavailable or inappropriate for use as a dilution water, an alternate dilution water may be specified in the permit. If specified in the permit, this alternate dilution water would apply to all tests conducted during the term of the permit. If the permit specifies the use of lab water for dilution, section 4.4.7 of the Methods Manual (s. NR 219.04, Wis. Adm.

Code) requires that the lab water have a hardness similar to that of the receiving water. If an alternate dilution water is specified in the permit, staff should document the reason for doing so in the water quality based effluent limit (WQBEL) memo and/or permit fact sheet, so others can understand why decisions were made.

If hazardous conditions occur during the permit term (such as flooding or icy conditions) that make it unsafe to collect receiving water samples, an alternate dilution water may be approved by the Department prior to conducting individual WET tests. In these situations, the permittee or their lab should contact the Biomonitoring Coordinator (Kari Fleming: kari.fleming@wisconsin.gov) to receive permission to use an alternate dilution water for the test in question.

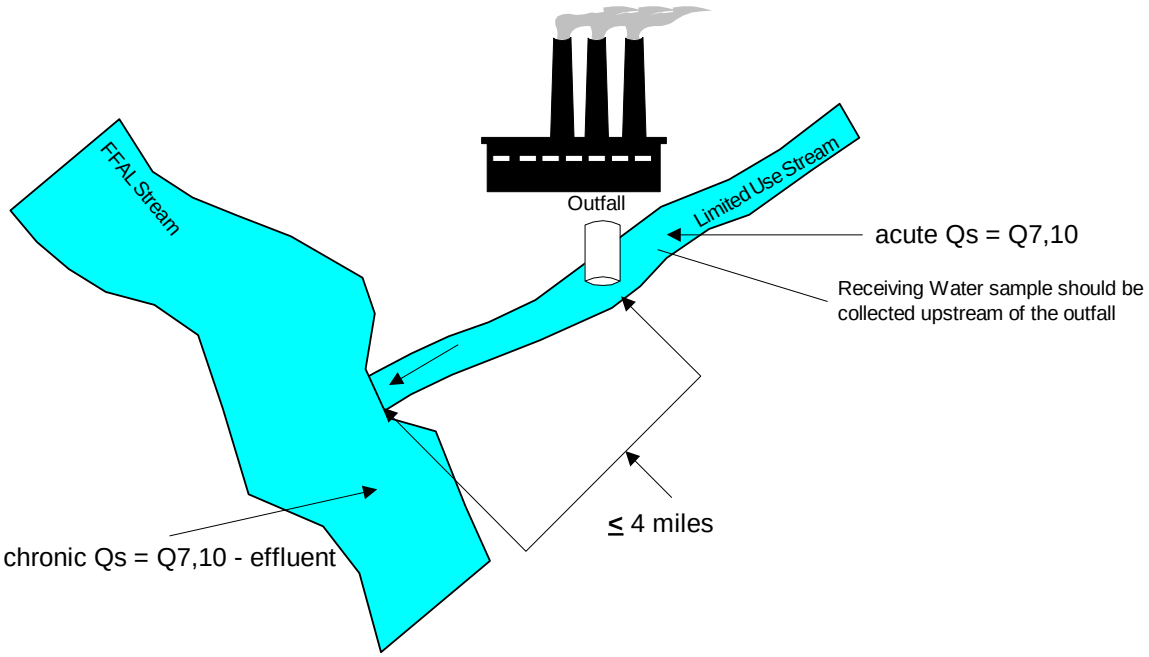
An alternate dilution water could be another surface water or a standard lab water having approximately the same characteristics as the receiving water. The next best option to the direct receiving water would be another surface water with similar characteristics (i.e., in the same watershed, similar water chemistry and physical characteristics). If no appropriate alternate surface water is identified, then a synthetic laboratory water may be used for dilution. However, using lab water for dilution should be a last resort. Naturally occurring materials that complex with and potentially detoxify some compounds are absent from lab waters, therefore tests using lab water may overstate effluent toxicity effects on the receiving water environment.

Example #1: Fish and Aquatic Life (FFAL) Stream



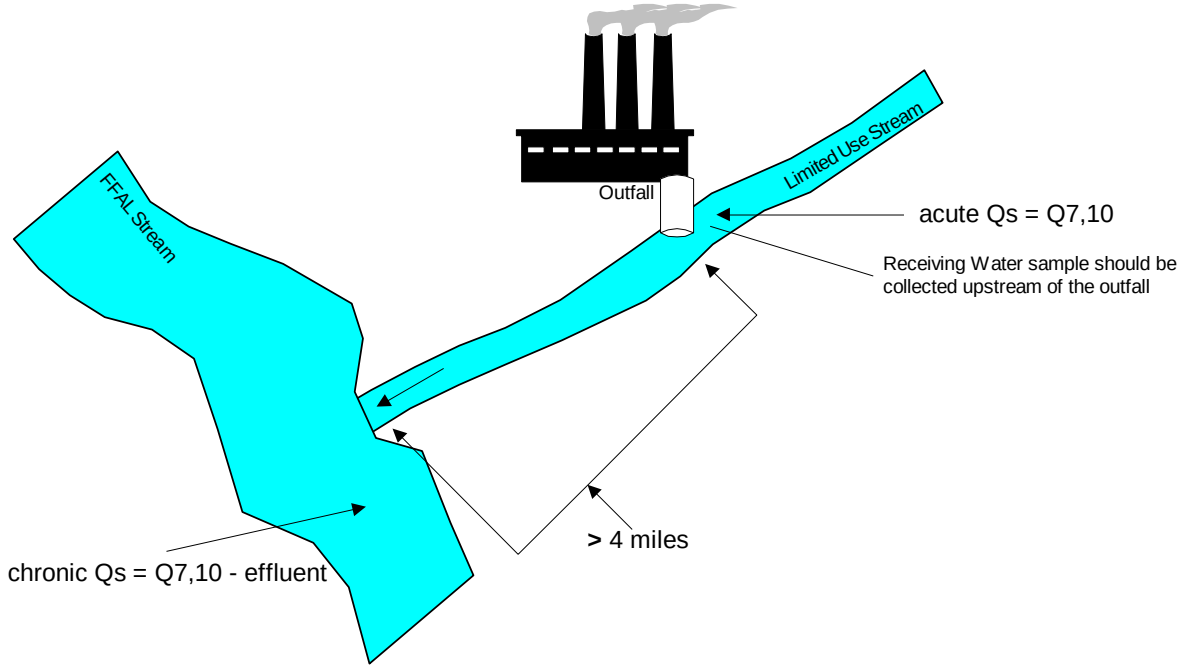
acute & chronic Qs = Q7,10 of receiving water
Need for acute and chronic testing evaluated using the WET Checklist:
Q7,10:Qe > 1,000:1 No WET usually recommended
Q7,10:Qe ≤ 1,000:1 & >100:1, Acute WET only usually recommended
Q7,10 ≤ 100:1 Acute & Chronic testing recommended

Example #2: Limited Use Stream w/Upstream Flow



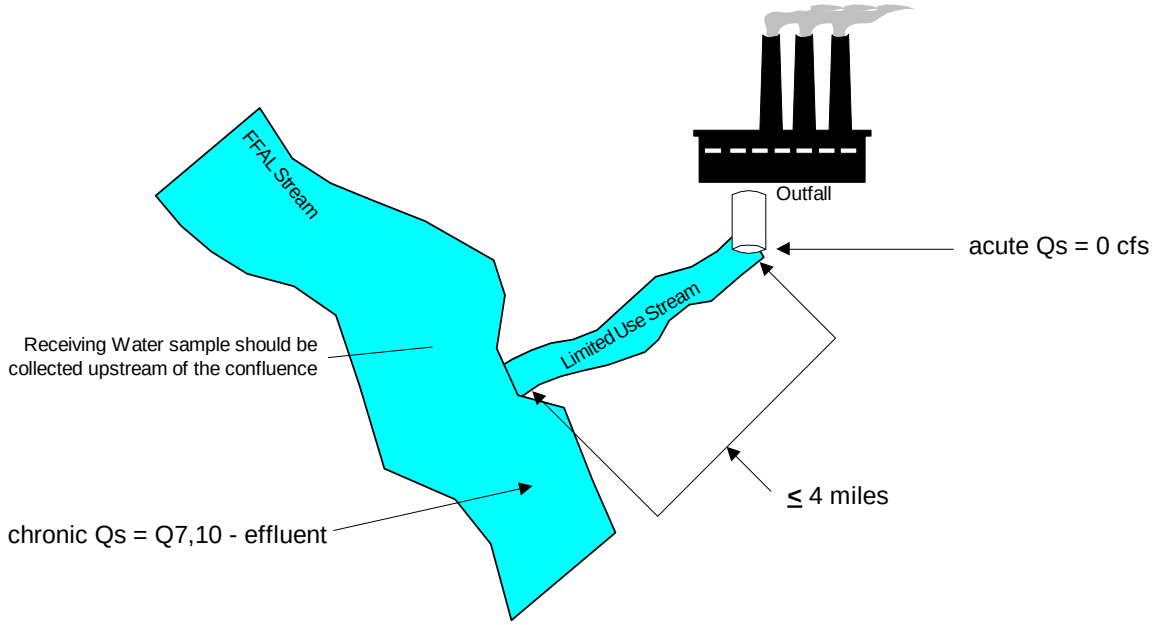
chronic Qs = Q7,10 of 1st downstream FFAL or higher
Need for acute and chronic testing evaluated using the WET Checklist:
Q7,10:Qe > 1,000:1 No WET usually recommended
Q7,10:Qe ≤ 1,000:1 & >100:1, Acute WET only usually recommended
Q7,10 ≤ 100:1 Acute & Chronic testing recommended

Example #3: Limited Use Stream w/Upstream Flow



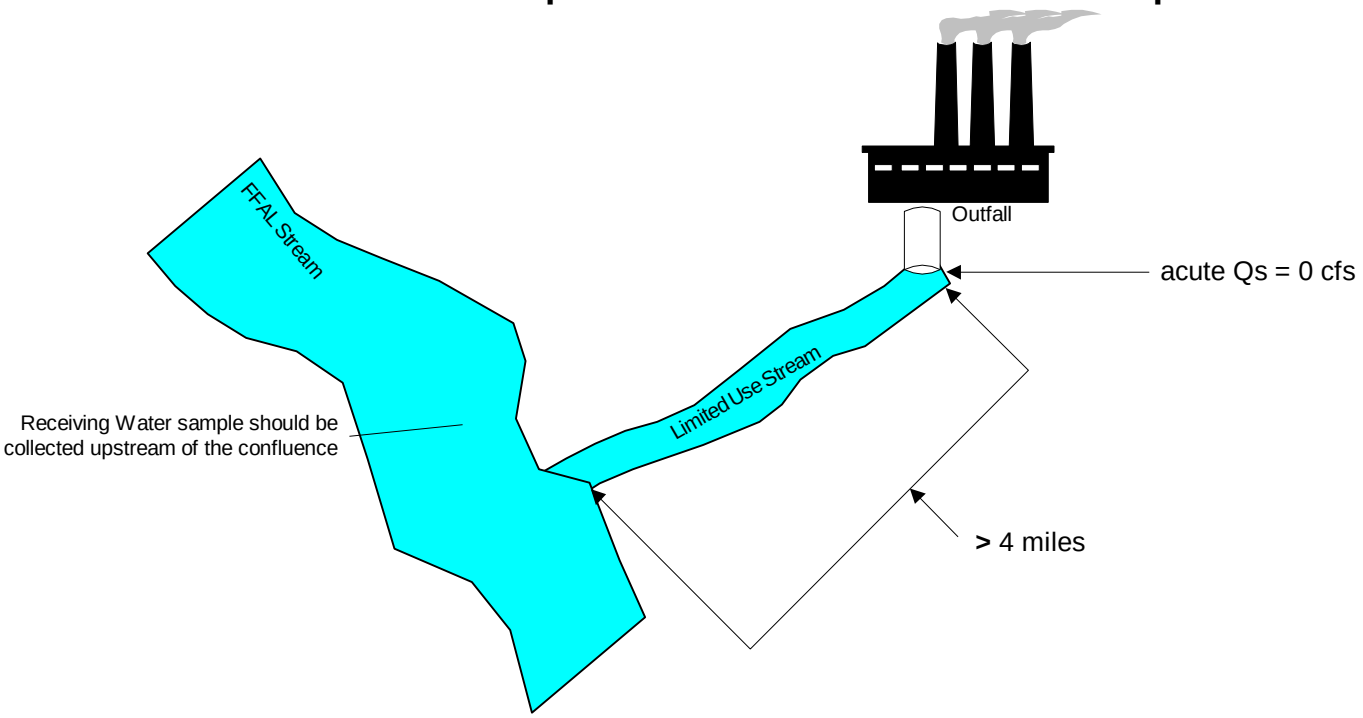
chronic Qs = Q7,10 of 1st downstream FFAL or higher
Need for acute and chronic testing evaluated using the WET Checklist:
Q7,10:Qe > 1,000:1 No WET usually recommended
Q7,10:Qe ≤ 1,000:1 Acute WET only usually recommended
No chronic recommended, unless historical toxicity problems are known.

Example #4: Limited Use Stream w/No Upstream Flow



chronic Qs = Q7,10 of 1st downstream FFAL or higher
Need for acute and chronic testing evaluated using the WET Checklist:
Q7,10:Qe >100:1, Acute WET only usually recommended
Q7,10 ≤ 100:1 Acute & Chronic testing recommended

Example #5: Limited Use Stream w/No Upstream Flow



Need for acute and chronic testing evaluated using the WET Checklist:
Qs = 0 cfs Acute WET usually recommended
No Chronic testing recommended unless historical toxicity problems are known

CHAPTER 1.3 - Representative Data, Reasonable Potential, Monitoring and Limits

This chapter was written to provide guidance for staff use when choosing representative data and considering facility-specific information in order to make decisions regarding WET monitoring and limitations, including instructions for use of the electronic WET Checklist.

The guidance in this chapter includes instructions for staff to follow a stepwise process (called the WET Checklist) that considers site-specific information in order to make WET monitoring recommendations. Also included in this chapter is guidance related to determining WET reasonable potential and the need for WET limits, according to methods required in 40 CFR Part 132, Appendix F, Procedure 6 (D), and s. NR 106.08, Wis. Adm. Code.

40 CFR Part 122.21 (j) (5) (ii) (C) requires that the need for WET monitoring be determined based on factors including:

- effluent variability (based on chemical-specific information, type of treatment plant, and types of industrial contributors);
- the ratio of effluent flow to receiving water flow;
- receiving water characteristics, including possible or known water quality impairments, and whether the discharge is to a Great Lake or a water designated as an outstanding natural resource water;
- other considerations including, but not limited to, the history of toxicity and compliance problems that could cause or contribute to adverse water quality impacts.

The WET Checklist and the guidance in this chapter are intended to help staff thoroughly evaluate these site-specific factors and to make appropriate WET monitoring recommendations. The WET Checklist asks staff about factors that affect effluent toxicity and adds points based on responses and the relative importance of each factor. The questions asked and points given in the WET Checklist are not arbitrary, rather they were chosen based on the best professional judgment and years of WET experience of the Checklist's creators (three DNR toxicologists, a State Lab of Hygiene toxicologist, a WET lab auditor, and two wastewater compliance engineers). However, in the end the monitoring recommendations made by the WET Checklist should be carefully considered and the final monitoring frequency should be based on the best professional judgment of staff that are knowledgeable about the discharge. Staff should use their own judgment and their knowledge of the facility to decide if the WET Checklist recommendations are appropriate for the discharge being evaluated.

The staff responsible for making WET determinations needs to be familiar with receiving water and effluent conditions, water quality-based effluent limit (WQBEL) recommendations, and other site-specific information, or be able to easily obtain it (have access to field staff with this knowledge). In order to make the best informed decisions possible, WQBEL and WET determinations should be made via a collaborative effort with permit drafter, compliance engineer, and WQBEL staff. In most cases WQBEL staff should make WET monitoring and limit recommendations concurrently with WQBEL recommendations, with compliance staff input related to facility-specific information. Once complete, WET recommendations should be given to permit drafters for incorporation into permits.

Making WET Determinations Using Representative Data

It is important that decisions about monitoring and limits be made using data that is representative of the discharge being evaluated, as specified in s. NR 106.08, Wis. Adm. Code (see Figure 1), and discussed further in the guidance below.

Figure 1. NR 106.08 Representative Data

NR 106.08 Determination of the necessity for whole effluent toxicity testing requirements and limitations.

(3) REPRESENTATIVE DATA. Toxicity test data available to the department shall be considered representative when those data meet the following conditions:

(a) Data are representative of normal discharge conditions and current effluent quality;
(b) Data were produced by a lab certified or registered under ch. NR 149;
(c) Data were produced from toxicity test procedures specified in the WPDES permit; and
(d) Data were produced from toxicity tests that met all applicable QA/QC requirements specified in the WPDES permit.

(4) NO REPRESENTATIVE DATA. If no representative discharge data are available for an effluent being discharged from a point source, whole effluent toxicity testing requirements are necessary if, in the judgment of the department, water quality standards may be exceeded. In such cases, all of the following factors shall be considered:

(a) Any relevant information which is available that indicates a potential for an effluent to impact the receiving water aquatic life community.
(b) Available dilution in the receiving water.
(c) Discharge category and predicted effluent quality.
(d) Proximity to other point source dischargers.

(7) DATA EXCLUSIONS. The Department may exclude data from a WET reasonable potential determination when those data meet any of the following conditions:
(a) Data are not representative pursuant to sub. (3);
(b) Positive WET results are caused by deficiency toxicity only; or
(c) Positive WET results are caused by groundwater or surface water remediation needed to correct or prevent an existing surface or groundwater contamination situation or a public health problem.

Steps 1 and 2 below include a description of criteria that staff may consider as they review WET data prior to making decisions about the need for monitoring and limits. Step 3 includes completing the WET Checklist and using best professional judgment to determine if limits are necessary and make WET monitoring frequency recommendations.

Step 1: Collect and summarize all WET data and other related information,

Step 2: Select WET data which is representative of the discharge being evaluated, and

Step 3: Complete the WET Checklist, determine the need for WET limits, and recommend a monitoring frequency.

The guidance in this chapter is intended to apply in most situations, but there may be situations where the general assumptions it is based on may not apply. Decisions that don't follow the general recommendations in this guidance should be shared with the Biomonitoring Coordinator (Kari.Fleming@wisconsin.gov) and clearly documented in WQBEL memos, so others can understand why decisions were made.

Step 1 - Data Collection and Summarization

As staff are preparing to make WET determinations, available WET test data and related information for each outfall being evaluated should be collected and summarized. As a first step, staff should review data in the System for Wastewater Applications, Monitoring, and Permits (SWAMP) WET database and print summary reports. Staff can find instructions for creating summary reports in the SWAMP user manual (http://intranet.dnr.state.wi.us/water/wq/ww/SWAMP.html).

Staff should compare tests listed in the summary report to requirements in the previous permit, to verify that all required tests have been submitted to the Department. Staff should contact field staff and/or the permittee to locate missing WET

tests. Also, there may be a delay between report submittal and data entry, so staff should contact the Biomonitoring Coordinator (Kari.Fleming@wisconsin.gov) if it appears that tests have been completed recently but have not yet made it into the database.

SWAMP summary reports allow for visual review of results and comparison to other effluent data. Since these reports include only summaries, staff should also refer to files saved in the Permit Documents tab in SWAMP. WET report forms, TRE plans and reports, and other related correspondence should be stored there. Staff should review these files, especially if there is a history of toxicity or if there are questions about the validity of data. A call or email to the Biomonitoring Coordinator can also be helpful when questions arise.

The Department uses its WET laboratory certification program, regular communication with labs and permittees, and staff and external customer training strategies to insure WET data quality. Data quality can be a complex issue and is determined for each test by the Biomonitoring Coordinator during the report review process (see Chapter 1.5 for more on data review). If data quality is questionable, a note is placed on the test report form and in the WET Database. Tests with poor data quality should be identified on reports from the WET Database, however, it may be necessary to check hard copies of report forms or talk with the Biomonitoring Coordinator.

Only WET tests with clear documentation of data quality problems should be eliminated from the data set at this stage in the process. WET Database reports should include comments noting when problems have occurred or if results are questionable (see discussion of "Qualified Data" below in the next section). A quick call or email to the Biomonitoring Coordinator to verify the appropriateness of using such data could make things go more smoothly later on. If available, it may be useful to consult other effluent data when making decisions regarding the representativeness of WET data. For instance, flow data and results of conventional pollutant testing may be an indicator of abnormal treatment plant operations. It may also be helpful to compare WET results with other effluent data to look for similar trends.

Staff should clearly indicate in the WQBEL memo which WET data was used in RP decisions (and which were not) and discuss why any changes were made to data sets, so that others can understand why decisions were made.

Step 2 - Selecting Representative Data

Decisions about the need for WET limits and appropriate monitoring frequencies should be based on data that is representative of the discharge being evaluated. Once all valid WET data have been compiled (Step 1), additional screening of the data may be necessary. At this point in the process, only data that are clearly flawed have been screened out. Now, additional decisions can be made based on the complete body of data and other factors. Following is a list of considerations when selecting representative data. For many of these, staff may have to rely on the permittee to know whether there is a potential problem. For others, staff may have to dig a little deeper if things don't seem right.

1. **Qualified Data**. The Biomonitoring Coordinator reviews all WET Test Report Forms and notes should be added to the WET database when there are QA concerns or unusual circumstances at the time of sampling that might render data unrepresentative. Reasons for disqualifying data may include lab error, poor organism health, interferences in the test that confounded test data, or other factors. In many cases when test acceptability concerns are noted, it may be appropriate to exclude the test from the data set. If staff have any questions about the quality of WET data, they should discuss them with the Biomonitoring Coordinator.

   Tests completed during upset conditions may be excluded if it is determined that conditions were not representative of normal effluent conditions. However, recurrent plant upsets should not be excused. Staff should judge whether the

problem regularly occurs or is due to poor operation. If regular upsets or poor operation represent normal conditions, the data should be used in making WET monitoring and limits decisions.

Occasionally, tests must be repeated due to poor QA. When this happens, only the unacceptable portions are repeated. Tests done under these conditions shouldn't be double-counted. For example, suppose toxicity tests were performed using the *Ceriodaphnia dubia* and fathead minnow. The fathead minnow portion was unacceptable, so that had to be repeated. The original acceptable *C. dubia* results and the repeated fathead minnow results should be counted together as one complete test. (Only 1 value, that of the most sensitive species, should be used in reasonable potential decisions).

2. **Laboratory capabilities and sample integrity.** Lab performance, results of recent audits, and sample quality may need to be considered when deciding whether to include WET data in reasonable potential decisions. All certified WET labs are audited regularly (on a ~3 year cycle) and audit reports are available. Any evidence of improper sample collection, preservation, or holding times should be considered (test results with these problems may have to be discarded). Tests done by labs not certified or registered according to ch. NR 149, Wis. Adm. Code, at the time the tests were done, are not acceptable for determining permit compliance. A list of currently certified WET labs can be found at: http://dnr.wi.gov/topic/wastewater/WETCertified.html

   The Department has reason to believe that tests completed by S-F Analytical Labs from July 2008 through March 2011 were not performed using proper test methods. WET data from this lab during this period has been disqualified and flagged as "not reliable" in the WET Database. These tests should not be used in reasonable potential decisions.

3. **"Inconclusive" tests.** Tests may be labeled "inconclusive" during the test report review process, when confounding factors have made the results difficult to interpret. For example, prior to changes made to WET test methods in 2004, inconclusive tests were often the result of the "pathogen effect" (a biological interference) in fathead minnow chronic tests (see Chapter 2.7 for a detailed discussion of this phenomenon). When the pathogen effect occurs, there is unusually high variability between replicates and an abnormal concentration-response (i.e., lower effluent concentrations have poorer performance than higher concentrations), which may make test results unreliable. In most cases, inconclusive tests cannot be used in reasonable potential decisions because confounding factors have made the results difficult to interpret and it is hard to tell whether the effluent would have "passed" or "failed" with the affected species. An exception to this would be when the unaffected species (i.e., the one that wasn't "inconclusive") showed toxicity. In that case, the portion of the test that failed should be used, even if the other half of the test was inconclusive. (Because even if the inconclusive half had passed, the failing half would be used in the RP analysis.)

4. **WET Method Changes and Older WET Data.** Significant changes were made to WET test methods in 2004 and these changes were assumed to be fully implemented by certified labs by no later than June 2005. It may be appropriate to exclude data collected before July 1, 2005, unless 1) it shows repeated toxicity that was never resolved or 2) older data is all that is available and no significant changes have occurred which obviously make it unrepresentative.

   Staff should use judgment when determining whether changes to wastewater treatment or industrial processes (or industrial contributors to a municipality) or other significant changes have occurred which would render data unrepresentative. Staff should evaluate test data to determine whether factors such as treatment plant upgrades, industrial process modifications, or other significant changes have caused WET data to no longer be representative of the discharge, and should not disqualify data simply because it was not generated during the last permit term.

5. **Split samples.** Care should be taken to count only tests conducted on unique effluent samples. Tests are occasionally conducted simultaneously at two different labs, as a check on laboratory performance and/or sampling procedures,

and should not be counted as separate tests in reasonable potential determinations. Information from these tests may point out problems, however, which may lead to data elimination (for example, if split samples indicate a contaminated sampler or lab error caused past toxicity problems).

6. **Contributing Sources.** It may be necessary to investigate source loadings to the WWTP, including industrial sources to a municipality. For example, abrupt changes in WET results may be explained by the shutdown of a local industry or the clampdown by a municipality on its industrial contributors. Wide fluctuations in data could represent slug loads from contributors that remain undetected for a time and then reoccur. Wide fluctuations in data caused by permanent industrial discharges or regularly discharged slug loads (for example, a high strength waste that is occasional, but expected) should not cause data to be thrown out. For industrial permittees, wide fluctuations in a data set could mean a change in manufacturing processes. Data gathered during a period when a particular process was used, that is no longer in use (and won't be used during the next permit term), are not likely to be representative of the present discharge and may be excluded. If significant changes have occurred to contributing sources within a treatment facility, WET data collected prior to these changes may no longer be representative.

7. **WWTP upgrades.** Consider whether treatment processes have been upgraded which could significantly affect toxicity removal through the plant. Remember that toxicity can be caused by many factors and an upgrade that only improves solids or BOD_5 removal may not affect effluent toxicity. Data collected prior to an upgrade should be thrown out, in most cases, only if data collected after the upgrade suggests a change in effluent toxicity.

8. **Toxicity Reduction Evaluations (TRE).** Data generated during toxicity reduction evaluations are not usually used in reasonable potential decisions, unless they were compliance-style tests done to demonstrate the successful completion of the TRE. Tests completed during a TRE often involve single-species, single dilutions, or modified samples, used in order to investigate toxicity and are not comparable to standard toxicity tests.

   Successful TREs usually identify the cause of toxicity, steps needed to eliminate toxicity, and results from WET tests conducted after implementation of changes showing that toxicity is gone (accounting for seasonal, process, source loading and other changes, when appropriate). Therefore, successfully completed TREs can significantly change a discharge's potential to exhibit toxicity. In most cases, successful completion of the TRE means that previously collected data (including that collected during the TRE) are no longer representative. When this is the case, only tests that were collected after the TRE that are representative of current discharge conditions should be used in reasonable potential decisions. If failures are present in the dataset, WQBEL staff should check permit files and talk to compliance staff and/or the Biomonitoring Coordinator to determine whether a TRE was conducted and completed successfully.

In order to demonstrate that previous WET data is no longer representative of the current discharge, information is needed that shows why it is no longer representative - for example, significant changes in wastewater treatment, contributing industries, or industrial processes. In most cases it will be necessary to provide WET data which shows a change in toxicity (e.g., data collected after changes were made). Depending on the seasonal nature of the discharge and other factors, 3-4 passing tests conducted under normal operating conditions (at least 30 days apart) are usually enough to demonstrate that changes have resulted in toxicity removal.

When making WET determinations as recommended by this guidance, staff should remember that data are not automatically representative of the discharge being evaluated. If it is determined that representative data are not available, staff should recommend WET monitoring and should not be bound to setting WET limits in the permit. When there is doubt regarding the representativeness of one or a few data points, additional WET data may clarify the representativeness of those data. When representativeness of existing data is questionable, more experienced permittees (or those helped along by supportive Department staff) will conduct additional tests when faced with results that could trigger a limit in the next permit term.

Other factors may cause data to be unrepresentative. Staff should use best professional judgment to determine when this is the case and talk to the Biomonitoring Coordinator (Kari.Fleming@wisconsin.gov) if questions arise.

It is essential that decisions be well documented in WQBEL memos and permit fact sheets. Decisions that don't follow the general recommendations in this guidance should be shared with the Biomonitoring Coordinator (Kari.Fleming@wisconsin.gov) and clearly documented in WQBEL memos, so others can understand why decisions were made.

Step 3 - Determination of Monitoring Frequency and Need for a Limit

Once it is determined which data are representative, it can be decided whether a limit is necessary and how much monitoring should be done. The need for WET limits is determined as specified in s. NR 106.08, Wis. Adm. Code. WET limits are given when data shows there is a reasonable potential for toxicity to be present.

Figure 2. NR 106.08(1) Determining the Need for WET Limits

NR 106.08 Determination of the necessity for whole effluent toxicity testing requirements and limitations.
(1) GENERAL. The department shall establish whole effluent toxicity testing requirements and limitations whenever necessary to meet applicable water quality standards as specified in chs. NR 102 to 105 as measured by exposure of aquatic organisms to an effluent and specified effluent dilutions. When considering the necessity for whole effluent toxicity testing requirements and limitations, the department shall consider in-stream biosurvey data and data from ambient toxicity analyses, whenever such data are available.

Should WET limits be carried over into the next permit? Whole effluent toxicity and other facility-specific data should be reassessed and the WET Checklist redone with each permit reissuance. In situations where a WET limit was previously given and a successful toxicity reduction evaluation (TRE) was completed (permanent changes were made to remove toxicity), a limit may no longer be required. However, if WET limit violations occurred during the previous permit term and changes were not made to fix previous toxicity problems, the WET limit should be carried over into the next permit term. If questions exist, staff should talk to the Biomonitoring Coordinator.

WET Checklist

In order to guide staff through the WET limit and monitoring decision-making process, an automated WET Checklist is provided in SWAMP. The Checklist is designed to assist staff when they are deciding whether WET limits are necessary and what amount of WET monitoring should be recommended for individual discharges, based on their potential to exhibit toxicity or exceed water quality standards. As the potential for toxicity increases, more points accumulate in the Checklist and more monitoring is recommended to insure that toxicity is not occurring. Step-by-step instructions and supporting guidance for use when completing the Checklist are provided below. Staff should use best professional judgment and their knowledge of the facility to decide if the WET Checklist monitoring recommendations are appropriate for the discharge being evaluated or if another monitoring frequency is appropriate.

The WET Checklist and this chapter are intended as guidance and do not contain mandatory requirements except where statute or administrative rules are referenced. The Checklist calculates WET reasonable potential and determines the need for WET limits according to the methods required in 40 CFR Part 132, Appendix F, Procedure 6 (D), and s. NR 106.08, Wis. Adm. Code.

Points Assessed. The WET Checklist assigns points based on factors present that increase the chances for toxicity. Points are based on responses given and may be assessed towards acute, chronic, or both types of monitoring. Points given for

each question are shown below after each screen shot. The "Points Assessed" tables indicate whether points are added to acute, chronic or both. The completed Checklist recommends acute and chronic WET limits, when required according to 40 CFR Part 132, Appendix F, Procedure 6 (D), and s. NR 106.08, Wis. Adm. Code, and WET monitoring frequencies based on points accumulated during completion of the Checklist. Once the Checklist is completed, the user can generate a summary of points assessed and answers given, by clicking on the "Generate" button shown on the lower right corner of the screen.

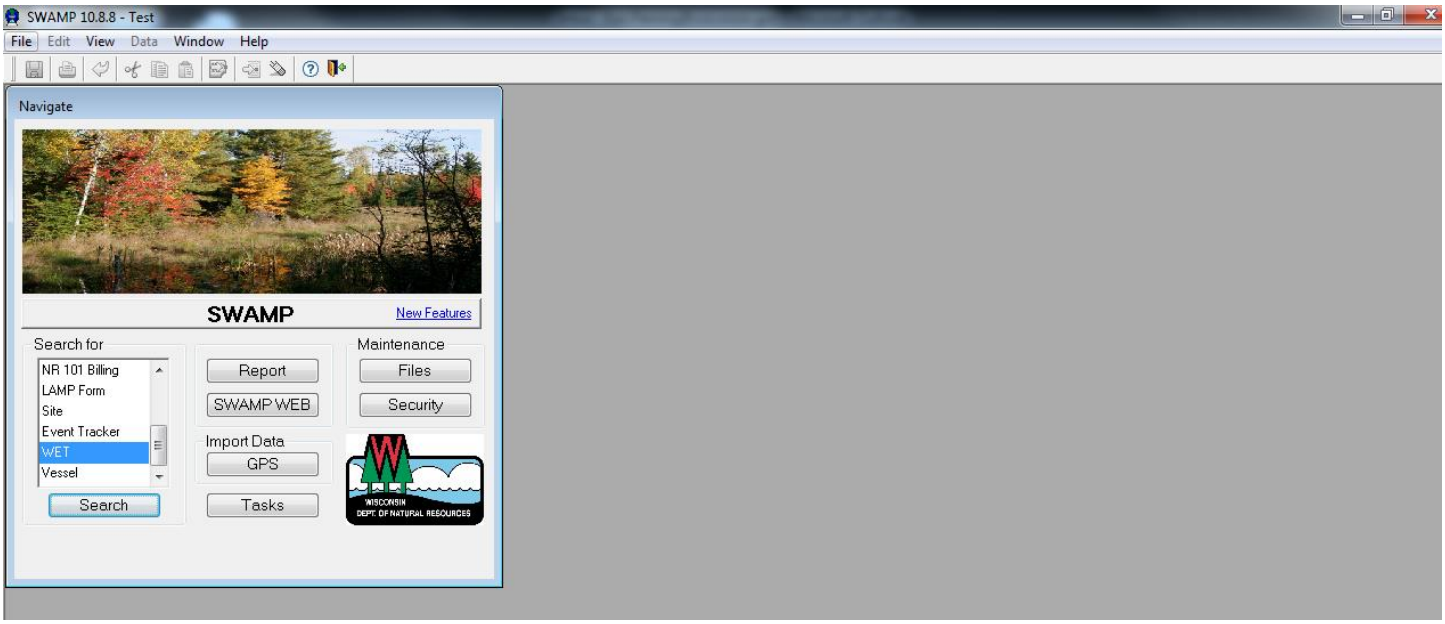
Minor Municipal Dischargers. If evaluating a minor municipal facility (< 1.0 MGD design flow) that receives only domestic wastewater, staff may consult the guidance in Chapter 1.11 to determine if further analysis using the WET Checklist is necessary. A pop-up screen in the WET Checklist will remind users of this additional guidance, when the user indicates that a municipal facility is being evaluated.

WET Checklist (Getting Started)

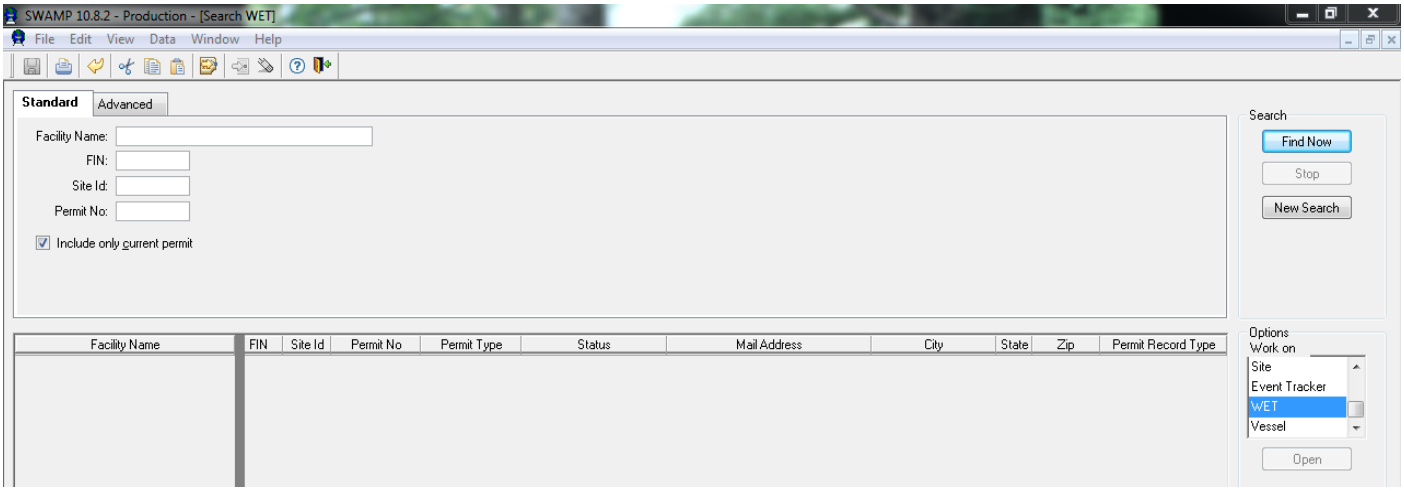
In order for the Checklist to work, information regarding effluent flow, percent effluent withdrawn from the receiving water, receiving water flow, receiving water classification, and acute mixing zone will need to be entered in the "Sample Point" tab in SWAMP. This information must be entered in order to successfully complete a new Checklist. (See attachment 3 at the end of this chapter for instructions on entering or changing data under the Sample Point tab in SWAMP).

Municipal effluent flows: Correct and up-to-date consultant engineered and DNR approved design flow information should be kept in the System tab area in SWAMP. Staff should confirm that the design flow they are entering into the Surface Water tab for use in WET determinations is not in conflict with the design flow information at the System tab in SWAMP.

Receiving water flows: The WET Checklist uses the $Q_{7,10}$ entered in the Sample Point tab in SWAMP. The Checklist is not programmed to use anything other than the $Q_{7,10}$ receiving water flow value. If another receiving water flow value is appropriate for the given discharge situation, staff will need to calculate stream flow to effluent flow ratios (Qs:Qe), acute mixing zones (AMZs), and instream waste concentrations (IWCs) outside of the checklist and note this in WQBEL memos.

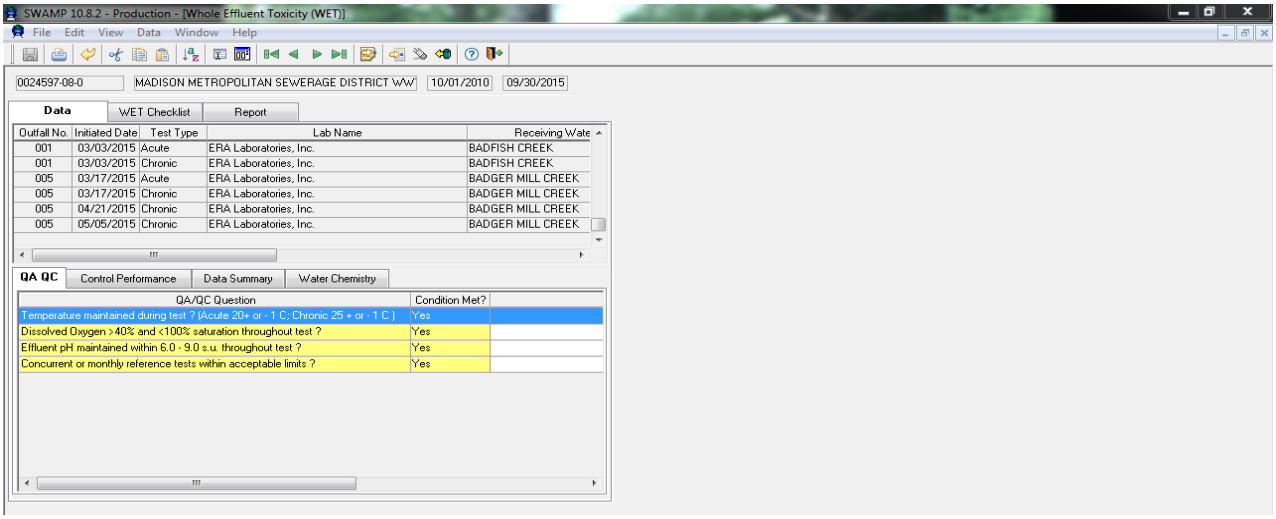


At the Navigate screen (shown above), click on "WET" in the "Search for:" box, then click on the "Search" button.



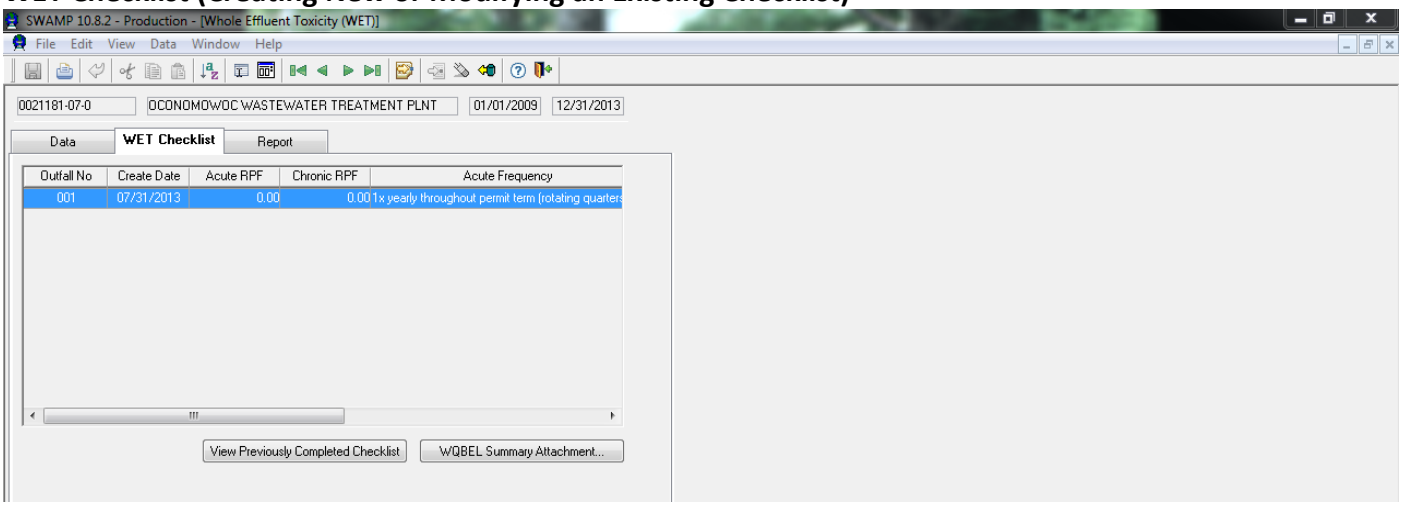
When the "Search WET" window appears (above), enter the permit number and click the "Find Now" button. From the list given, click on the facility you are interested in and then click on the "Open" button.

Previously created WET Checklists will appear in SWAMP attached to the permit that was in effect when the analysis was done. In other words, the WET Checklist for a permit marked "current" will be attached to the previous permit (now expired), since the previous permit was in effect when the Checklist was created.



The "Whole Effluent Toxicity (WET)" screen (above) appears with the Data tab displayed. To modify an existing Checklist or to create a new one, select the "WET Checklist" tab from the "Whole Effluent Toxicity (WET)" screen (shown above).

WET Checklist (Creating New or Modifying an Existing Checklist)

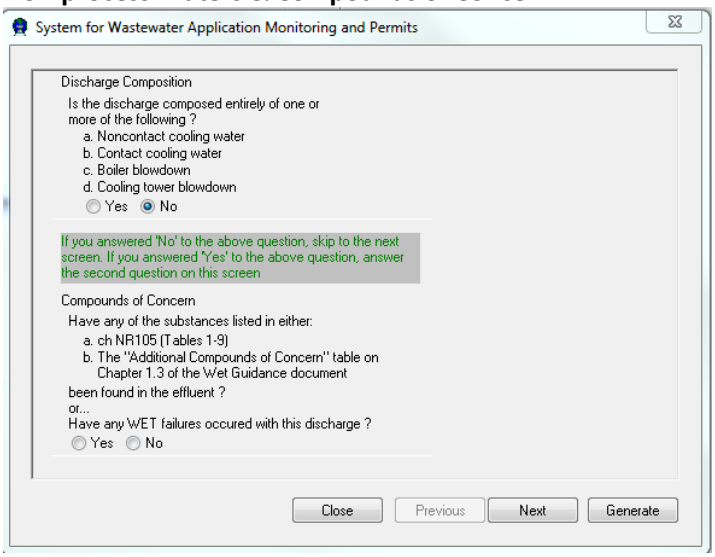


The WET Checklist screen (shown above) will show a list of previously completed checklists (if there are any) for the given facility, the date each was created, the person who completed the last update, and other pertinent information. The screen above also allows the user to create an abbreviated summary of a completed WET checklist to be attached to a WQBEL document (click the "WQBEL Summary Attachment" button), or a more complete summary showing answers given, point totals, and monitoring and limit recommendations (click on "View Previously Completed Checklist" button).

To modify an existing Checklist, select the appropriate row, then double-click. To create a new Checklist, right-click in the WET Checklist tab area, then choose "insert".

Completed WET Checklists will be marked "final" once the permit is reissued. Once marked final, users will no longer be able to modify the Checklist. Staff should contact the Biomonitoring Coordinator, if they need to modify a Checklist that has been marked as final.

Non-process Waters & Compounds of Concern

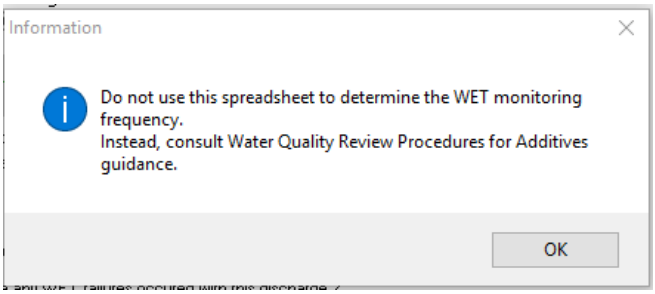


The questions shown in the screen above are designed to determine whether the user has chosen the correct tool for determining WET monitoring frequencies. If the discharge is <u>not</u> made up entirely of noncontact cooling water, contact

cooling water, cooling tower blowdown, or boiler blowdown (i.e., if the answer to the 1st question is "no"), the Checklist is appropriate and will continue to the facility information screen shown below (the answer to the 2nd question does not matter and the Checklist will not allow it to be answered). If it is solely a noncontact cooling water, contact cooling water, cooling tower blowdown, or boiler blowdown discharge (i.e., if the answer to the 1st question is "yes"), the Checklist only needs to be completed if WET failures have occurred or if "compounds of concern" have been detected (so the answer to the 2nd question will need to be given for the Checklist to continue).

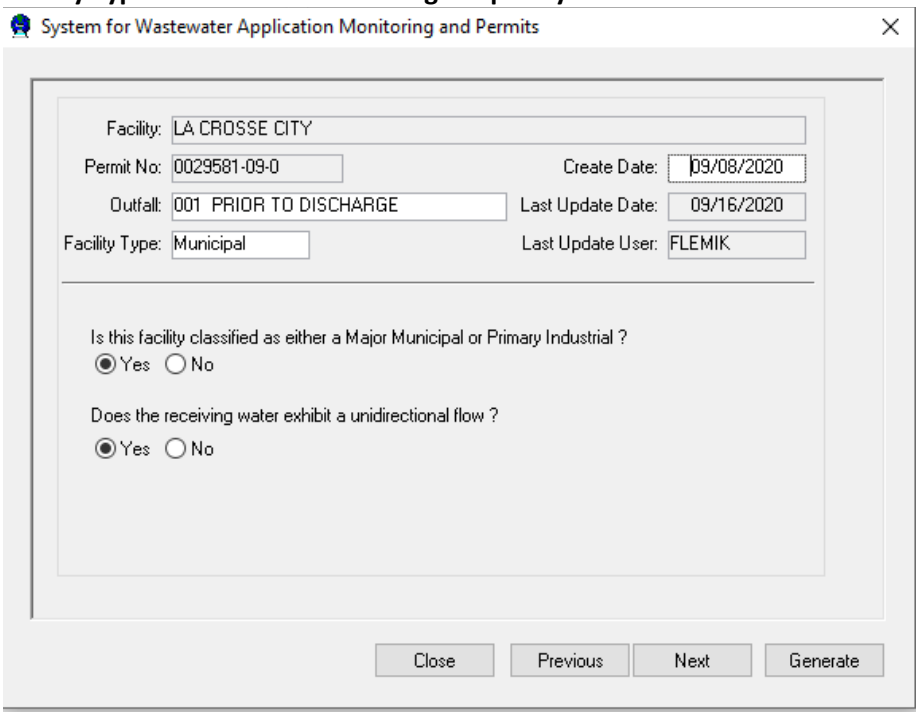
If the discharge is made up solely of one of the categories mentioned above and no compounds of concern have been detected in that discharge and no WET failures have occurred (in other words, a "yes" is given in the 1st question, and a "No" in the 2nd), an information box will appear instructing the user to consult the *Water Quality Review Procedures for Additives* guidance (see http://dnr.wi.gov/topic/wastewater/Guidance.html).

Additive Evaluations



If answers given in the Discharge Composition/Compounds of Concern screen indicate that the discharge is not composed entirely of noncontact cooling water, contact cooling water, boiler blowdown, or cooling tower blowdown, or if substances listed in ch. NR 105, Wis. Adm. Code, or the "Additional Compounds of Concern" table have been detected or WET failures have occurred, the Checklist will continue to the next screen.

Facility Type & Minimum Monitoring Frequency



Information entered in the screen above identifies the outfall being assessed, its discharge type, and other pertinent information. The WET Checklist can only evaluate one outfall at a time. Separate WET Checklists should be completed for each outfall at each facility, unless it has been decided that site-specific situations are better represented by conducting WET tests on combined outfalls (evaluations on combined outfalls will have to be done with the WET Checklist). The Checklist will automatically update the "last update date" and "last update user" each time the Checklist is revised.

Facility Type: The user chooses "municipal" or "industrial". (NOTE: all non-municipals are considered industrial for the purposes of this Checklist.) Indication of facility type is needed for the Checklist to continue, as future screens appear based on this designation.

Major Municipal or Primary Industrial: Federal regulations require that major municipal dischargers submit at least 4 acute and chronic WET tests with their permit reissuance application, as detailed in 40 CFR 122.21(j) (see Figure 3 below). It is recommended that these same minimums be applied to process wastewater discharges from primary industries. The federal regulations allow tests to be conducted within the previous term of the permit, so the Checklist is set up to recommend a minimum of 1x yearly acute and chronic monitoring (the need for chronic based on dilution) for major municipal and primary industrials, so that data can be available at the time of the next permit application. The Checklist does not assign points for this question, but instead evaluates whether the points given once the Checklist is complete are enough to satisfy this requirement. If less than 1x yearly testing would otherwise be recommended due to point totals, the Checklist recommends 1x yearly monitoring for these dischargers.

Figure 3. 40 CFR Part 122.21(j) Permit Application Requirements for Major Municipal Dischargers

§122.21 Application for a permit

(j) ***Application requirements for new and existing POTWs.***

(5) *Effluent monitoring for whole effluent toxicity.* (i) All applicants must provide an identification of any whole effluent toxicity tests conducted during the four and one-half years prior to the date of the application on any of the applicant's discharges or on any receiving water near the discharge.

(ii) As provided in paragraphs (j)(5)(iii)-(ix) of this section, the following applicants must submit to the Director the results of valid whole effluent toxicity tests for acute or chronic toxicity for samples taken from each outfall through which effluent is discharged to surface waters, except for combined sewer overflows:

(A) All POTWs with design flow rates greater than or equal to one million gallons per day;

….

(iv) Each applicant required to perform whole effluent toxicity testing pursuant to paragraph (j)(5)(ii) of this section must provide:

(A) Results of a minimum of four quarterly tests for a year, from the year preceding the permit application; or

(B) Results from four tests performed at least annually in the four and one half year period prior to the application, provided the results show no appreciable toxicity using a safety factor determined by the permitting authority.

(v) Applicants must conduct tests with multiple species (no less than two species; e.g., fish, invertebrate, plant), and test for acute or chronic toxicity, depending on the range of receiving water dilution…

(vi) Each applicant required to perform whole effluent toxicity testing pursuant to paragraph (j)(5)(ii) of this section must provide the number of chronic or acute whole effluent toxicity tests that have been conducted since the last permit reissuance.

(vii) Applicants must provide the results using the form provided by the Director, or test summaries if available and comprehensive, for each whole effluent toxicity test conducted pursuant to paragraph (j)(5)(ii) of this section for which such information has not been reported previously to the Director.

…

(ix) For whole effluent toxicity data submitted to the Director within four and one-half years prior to the date of the application, applicants must provide the dates on which the data were submitted and a summary of the results.

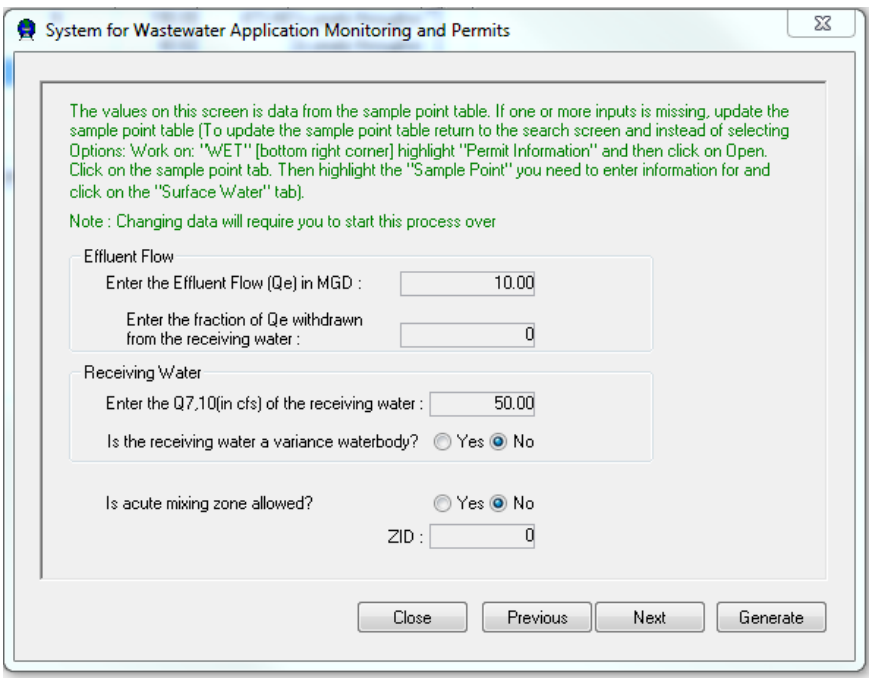
(x) Each POTW required to perform whole effluent toxicity testing pursuant to paragraph (j)(5)(ii) of this section must provide any information on the cause of toxicity and written details of any toxicity reduction evaluation conducted, if any whole effluent toxicity test conducted within the past four and one-half years revealed toxicity.

Available Dilution and Appropriate Mixing Zones

Because the magnitude of toxic effect increases as a toxicant's concentration increases, one of the most important factors affecting the potential for environmental impacts due to effluent toxicity is the dilution available in the receiving water. A very toxic effluent may cause less environmental damage if there is a lot of receiving water dilution available, than a less toxic effluent if there is very little available dilution. Since dilution and mixing are important factors to consider when determining the amount and types of WET testing that should be done, the Checklist evaluates this information.

The last question in the screen above asks whether the receiving water has a unidirectional flow in order to determine whether sufficient mixing is present at the point of discharge. If the receiving water is a flowing waterbody, the Checklist continues to the next screen. If the discharge is to a non-flowing waterbody (e.g., lake, pond, static wetland), a "No" should be given. Since adequate mixing does not occur in these situations, the Checklist assigns a default instream waste concentration (IWC) of 9% as required in s. NR 106.06 (4) (b) (2), Wis. Adm. Code, and skips to the "Calculate Reasonable Potential" screen.

Note: The Checklist is not programmed to use anything other than the default 10:1 ratio and IWC = 9% for non-flowing waterbodies. If a dilution factor other than 10:1 has been approved for use as allowed in s. NR 106.06 (4) (b) (2), Wis. Adm. Code, staff will have to calculate the AMZ and/or IWC outside of the checklist and note this in WQBEL memos.



If any values are missing from this screen, the Checklist will not continue. The user will need to update information in the Sample Point tab before continuing with the WET Checklist (see attachment 3 at the end of this chapter). In most cases, the appropriate effluent flow (Q_e) to be used in the WET Checklist should be the annual average design flow for municipals or the average annual actual flow for industrial dischargers. The withdrawal factor (f) should be entered as a decimal (for example, if ½ is withdrawn from the receiving water, enter 0.5). The Checklist uses this information to calculate the $Q_{7,10}$:Q_e ratio, AMZ, IWC, and chronic dilution series, as discussed below.

The waterbody type question in the screen above helps to determine the receiving water flow value that should be used to make WET determinations. This and the next 3 screens help choose that flow value as described in the guidance in Chapter 1.2. In most cases, the flow value used to determine the need for chronic testing, calculate the IWC, and choose the correct chronic dilution series should be the $Q_{7,10}$ of the first non-variance classified waterbody encountered by the discharge.

The type of low flow value used for WET determinations should usually be the same as that used to calculate other WQBEL limits based on chronic toxicity. In situations where a flow other than the $Q_{7,10}$ was used (for example, a $Q_{4,3}$), that value may be substituted for the $Q_{7,10}$ in this ratio.

Note: The Checklist is programmed to use $Q_s = 1/4\ Q_{7,10}$. If another low flow value or mixing ratio is more appropriate for the given situation, staff will have to calculate the AMZ and/or IWC outside of the checklist and note this in WQBEL memos.

Variance Waterbodies. If the receiving water is classified as a variance waterbody as discussed in Chapter 1.2, the Checklist continues to the next screen. If the receiving water is not a variance waterbody, the Checklist skips 3 screens and goes to the "Calculate Reasonable Potential" screen.

Acute mixing zone concentration (AMZ). Use of an AMZ is appropriate only in cases where a zone of initial dilution (ZID) has been approved as defined in s. NR 106.06 (3) (c), Wis. Adm. Code. See Figure 4 below for a discussion of how to use a ZID ratio to calculate the AMZ for each site-specific situation. Similar to the IWC for chronic testing, the AMZ is used as the compliance endpoint in acute tests (i.e., the LC_{50} must be $\geq$ AMZ% to pass). In cases where a ZID has not been approved for the given discharge, acute compliance is determined at the end of pipe (i.e., the LC_{50} must be $\geq$ 100% to pass). The Checklist only uses this information to calculate the AMZ concentration; no points are given based on the presence or absence of a ZID.

Figure 4. Acute Mixing Zone Concentration Calculation

When a zone of initial dilution, or acute mixing zone, has been granted for a discharge, it will typically be expressed as a ratio of the receiving water after it has mixed with the effluent compared to the effluent alone. For example, a ratio expressed as 19.5:1 means that 18.5 parts receiving water is mixing with 1 part effluent. The AMZ concentration to be used in WET tests for determining compliance is calculated as follows:

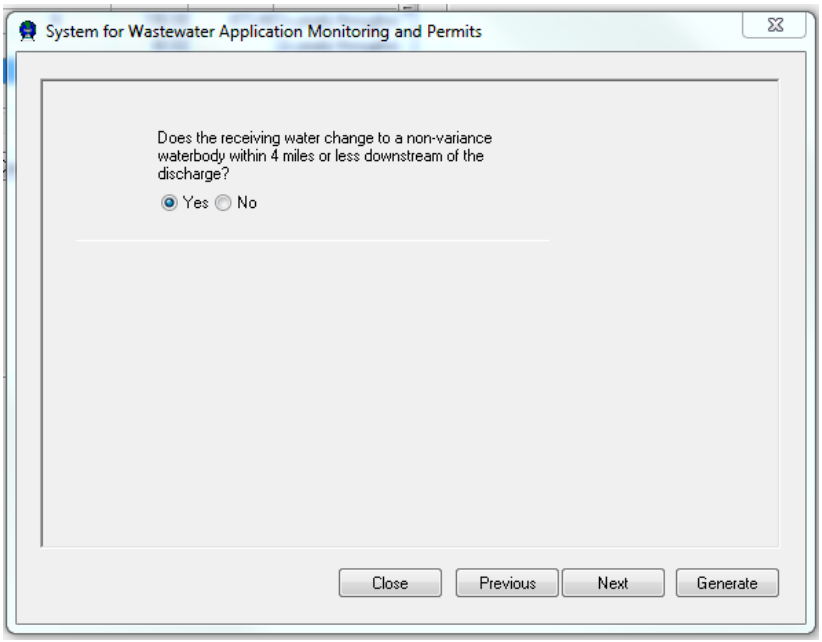
AMZ (as %) = (100 /receiving water + effluent ratio) x 3.3

For the example given above, where the dilution ratio is 19.5:1 (where 19.5 = 18.5 parts receiving water + 1 part effluent), the correct AMZ for acute tests would be:

(100 / 19.5) x 3.3 = 16.9

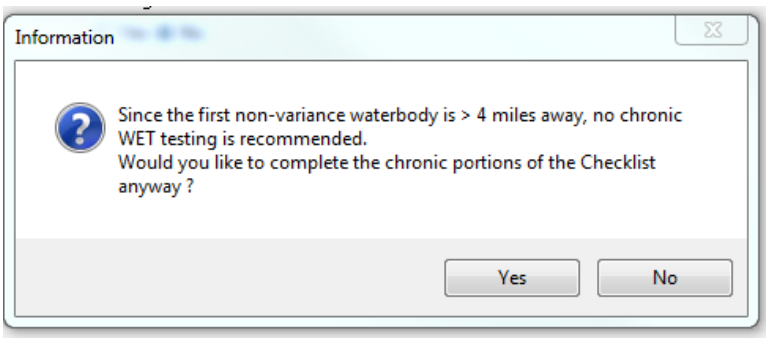
AMZ = 17%

Staff should be careful to note how the mixing zone dilution ratio was expressed in the first place - whether it includes the effluent or not. For example, if it's 19.5 parts receiving water only to 1 part effluent, then it should be expressed as 20.5 (total of effluent plus background) for the example above and the correct dilution in that case would be 16.1%. If it's 19.5 total to 1 (the 19.5 includes the effluent), then it's 16.9%.

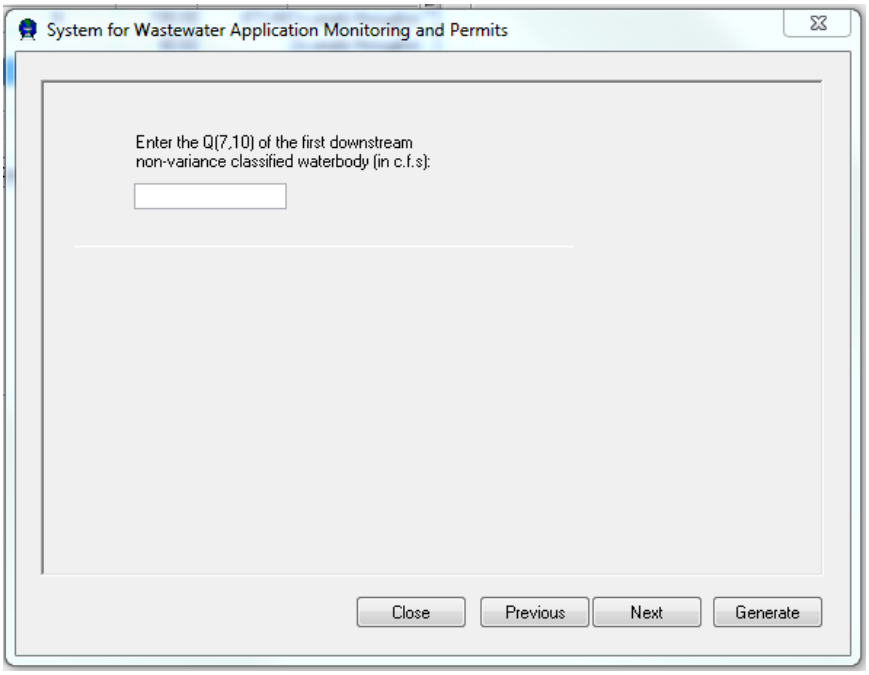


As described in Chapter 1.2, the Checklist uses the distance to the point where the receiving water becomes a non-variance waterbody to choose the low flow it will use in WET determinations. When this distance is ≤ 4 miles, the recommended flow used by the Checklist is that of the non-variance waterbody. If the user indicates that this distance is > 4 miles, chronic testing is usually not recommended unless data exists suggesting a potential for chronic impacts. The Checklist allows the user to choose whether or not chronic testing should be considered under these circumstances.

If chronic WET data exists for the outfall in question, and chronic WET failures have occurred, staff should consider whether additional chronic WET monitoring is necessary to insure that receiving water impacts are not occurring (see Chapter 1.2 for more discussion).



If the closest non-variance classified waterbody is > 4 miles away, the Checklist asks whether the user would like to evaluate chronic frequencies anyway, then continues to the reasonable potential screen. If ≤ 4 miles away, the Checklist goes to the next screen shown below, where the $Q_{7,10}$ of the 1st downstream non-variance waterbody is entered.



After the appropriate $Q_{7,10}$ and the Q_e have been entered in the screens above, the Checklist calculates the $Q_{7,10}$:Q_e ratio, the IWC, and the appropriate chronic dilution series, as described below.

$Q_{7,10}$:Q_e Ratio. The stream flow to effluent flow ratio ($Q_{7,10}$: Q_e) is calculated to determine whether WET testing should be recommended because of the amount of dilution that is present.

If the $Q_{7,10}$:Q_e > 1000:1, no WET testing is usually recommended, because dilution is high in the receiving water and therefore the potential for impacts due to effluent toxicity are extremely low. Staff may determine that testing is necessary despite high dilution and the Checklist allows the user to continue in these circumstances. The Checklist does not need to be completed if staff decide that no WET monitoring is necessary due to the large amount of available dilution, however this decision should be clearly documented in the WQBEL and permit file, so others can tell why decisions were made.

If the $Q_{7,10}$:Q_e $\leq$ 1000:1 and > 100:1, the need for acute monitoring should be evaluated, but chronic monitoring is usually not recommended, since dilution is high and the potential for impacts due to chronic toxicity are very low (the IWC is < 1%). Staff may determine that there is a need to evaluate chronic testing despite high dilution and the Checklist allows the user to continue in these cases.

If the $Q_{7,10}$:Q_e $\leq$ 100:1, the need for acute and chronic monitoring should be further evaluated. The Checklist will continue to the next screen and recommend acute and chronic monitoring frequencies based on final point totals.

Dilution Series. In the Methods Manual, Section 4.12 (s. NR 219.04, Wis. Adm. Code), the standard acute WET test dilution series is 6.25, 12.5, 25, 50, 100% and the standard chronic WET test dilution series is either 100, 30, 10, 3, 1% (if the IWC is $\leq$ 30%) or 100, 75, 50, 25, 12.5% (if the IWC is > 30%). These standard dilution series are appropriate for most situations and are recommended by the WET Checklist. The Methods Manual does allow for alternate dilution series, if requested by the permittee and specified in the permit. For more discussion of dilution series, see Chapter 2.11.

Instream waste concentration (IWC). The IWC is an estimate of the proportion of effluent (Q_e) to the total volume of water (Q_e +Q_s). Since toxicity typically increases as the concentration of a toxicant increases, one of the most important factors affecting the potential for impacts due to WET is dilution. A highly toxic effluent with a large amount of dilution available in the receiving water may cause less environmental impact than a slightly toxic effluent with very little available dilution. For

this reason, outfalls with higher IWCs are given more points by the WET Checklist. The IWC is calculated as required in s. NR 106.03(6), Wis. Adm. Code.

Figure 5. NR 106.03(6) Instream Waste Concentration Calculation

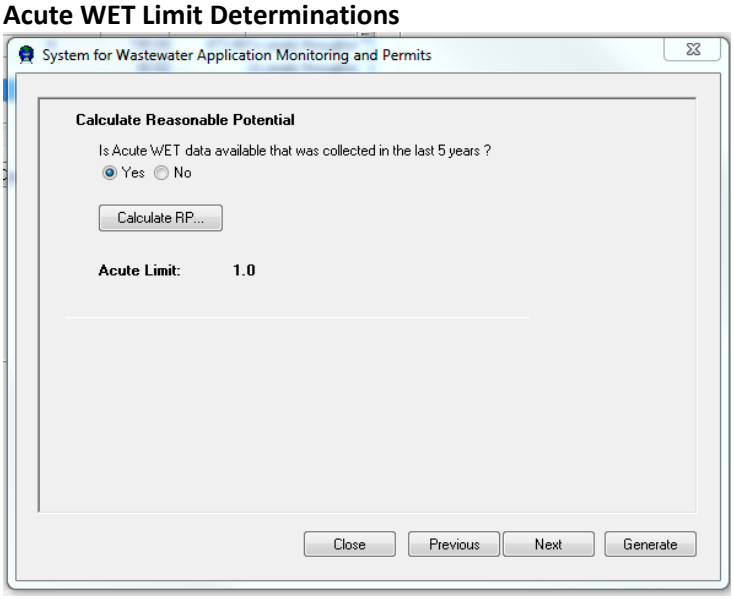
NR 106.03 Definitions. The following definitions are applicable to terms used in this chapter.
(6) "IWC" or "Instream waste concentration" means an estimate of the proportion of effluent to total volume of water (receiving water + effluent). The IWC is calculated according to the following equation:

$$\text{IWC (as \%)} = 100 \times \frac{Qe}{(1-f)Qe + Qs}$$

where:
Qe = effluent flow
f = fraction of the Qe withdrawn from the receiving water
Qs = receiving water flow (in most cases ¼ of a low flow value, such as the $Q_{7,10}$, is used in order to allow a free zone of passage for aquatic organisms).

POINTS ASSESSED (Chronic only)	
If the IWC is:	***Points given:***
≤ 35%	*0*
> 35 and ≤ 65%	*10*
> 65%	*15*

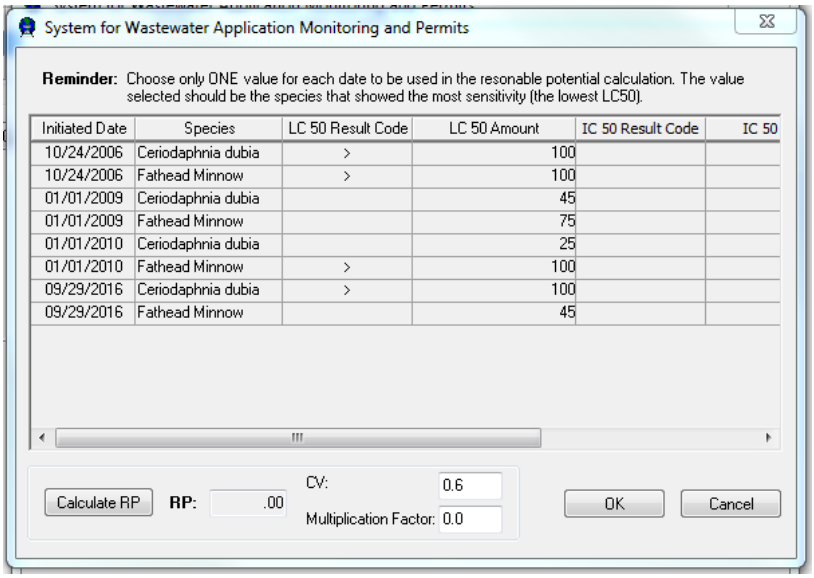
Acute WET Limit Determinations



In the screen above, the user indicates whether there is representative acute WET data available from the most recent 5 years for the discharge being evaluated. If no recent data is available, the Checklist assigns 5 points. This is done because more uncertainty exists in situations where testing has not been done in recent times than at those facilities that have produced recent data which shows toxicity problems are not a concern. The user clicks on "Calculate RP" if any representative data is available (even if ≥ 5 years old), in order to choose the data to be used to calculate the acute reasonable potential. The acute WET limit, calculated using the appropriate acute mixing zone (or no mixing zone) indicated for the facility, is also shown on this screen. (An acute WET limit = 100/AMZ, according to s. NR 106.09 (2) (e), Wis. Adm. Code.)

Points should be assigned (a "No" given) if no representative data is available from the last 5 years. This does not mean that older data cannot be used in reasonable potential decisions – it is recommended that all representative data be used in WET determinations. This is just a check to insure that WET monitoring has been done in the recent past.

Data Used to Calculate Reasonable Potential



Initiated Date	Species	LC 50 Result Code	LC 50 Amount	IC 50 Result Code	IC 50
10/24/2006	Ceriodaphnia dubia	>	100		
10/24/2006	Fathead Minnow	>	100		
01/01/2009	Ceriodaphnia dubia		45		
01/01/2009	Fathead Minnow		75		
01/01/2010	Ceriodaphnia dubia		25		
01/01/2010	Fathead Minnow	>	100		
09/29/2016	Ceriodaphnia dubia	>	100		
09/29/2016	Fathead Minnow		45		

The Checklist uses data selected in the screen above to calculate reasonable potential. WET limits are given whenever representative, facility-specific data shows the effluent may be discharged at a level that has the potential to cause or contribute to an excursion above a WET criterion, as specified in s. NR 106.08(6)(b). See Figure 6 below.

Decisions about reasonable potential, monitoring frequencies, and other WET determinations should be based on data that is representative of the discharge being evaluated, as discussed earlier in step 2. When the screen above opens, all valid WET data in the SWAMP WET Database for the selected outfall will appear. The user will have to select WET data to be used in the reasonable potential calculation, by highlighting <u>one species from each test date</u> to be used. Once the most sensitive species (see next paragraph) from each test has been highlighted, the user clicks "Calculate Reasonable Potential". The WET Checklist will calculate the resulting RP value.

Most sensitive species. The user should choose one value for each test date to be used in the RP calculation. The value selected should be the species that showed the most sensitivity (the lowest LC_{50} or $IC_{25/50}$) for each test. For example, if 2 tests were completed and the 1st resulted in an LC_{50} = 50% for *C. dubia* and an LC_{50} = 75% for the fathead minnow, and the 2nd resulted in an LC_{50} >100% for *C. dubia* and an LC_{50} = 25% for the fathead minnow, the user would select the *C. dubia* result (LC_{50} = 50%) from the 1st test and the fathead minnow result (LC_{50} = 25%) from the 2nd test.

Tests with one species. Permit-required WET tests require a battery of organisms, as specified in 40 CFR Part 122.21 (j) (5) (v) and s. NR 106.09, Wis. Adm. Code, in order to represent different trophic levels and taxonomic groups. Because species sensitivity can change as effluent quality and makeup changes, tests conducted with only one species should not be included in reasonable potential calculations, in most cases. An exception should be made for tests with only one species if the tested species failed – in this case, that species should be included in the RP analysis because even if both species had been included, the failed species would have been used in the RP analysis. Tests conducted in a manner consistent with permit-required test methods (specified in the Methods Manual, s. NR 219.04, Wis. Adm. Code), should be used; screening tests for TREs (100% effluent only) or other similar tests cannot be used because no LC or IC value can be generated from those tests.

Coefficient of Variation (CV). Section NR 106.08(6)(c), Wis. Adm. Code, requires that the multiplication factor chosen from Table 4 be based on a CV = 0.6 whenever there are less than 10 toxicity <u>detects</u> in the dataset. (See Figure 6 below.) Since this will be the case in almost all situations, the Checklist defaults to a CV = 0.6 in the screen shown above.

If there are more than 10 detects in the given dataset, the user will have to calculate the appropriate CV, then enter it and the corresponding multiplication factor from Table 4 into the spaces provided.

Table 4 Multiplication Factor. The multiplication factor from Table 4 in s. NR 106.08, Wis. Adm. Code, is used to convert the calculated effluent toxicity value to the estimated 95th percentile value. This multiplication factor must be chosen based on the number of detects (number of samples that resulted in an LC or IC < 100%) and the appropriate coefficient of variation. Staff should be careful to use the number of toxicity detects, not the total number of samples in the dataset, when selecting this multiplication factor.

When it has been determined that there is reasonable potential for a limit to be exceeded, that WET limit must be included in the permit, according to s. NR 106.08, Wis. Adm. Code. WET reasonable potential is determined by multiplying the highest toxicity value that has been measured in the effluent by the multiplication factor, in order to predict the likelihood (95% probability) of toxicity occurring in the effluent above the applicable WET limit. The factor used in the equation changes based on the number of toxicity detects in the dataset. The fewer detects present, the higher the factor, because there is more uncertainty surrounding the predicted value. **WET limits must be given, according to s. NR 106.08(6), Wis. Adm. Code, whenever the applicable RP equation (shown in Figure 6) results in a value greater than 1.0.**

Figure 6. NR 106.08(6) Reasonable Potential Calculations

NR 106.08 Determination of the necessity for whole effluent toxicity testing requirements and limitations.

(6) REASONABLE POTENTIAL TO RECEIVE AN ACUTE OR CHRONIC WHOLE EFFLUENT TOXICITY LIMIT.

(b) *Reasonable potential.*
1. If a zone of initial dilution has not been approved, the potential to exceed an acute criterion shall be calculated using the following equation:

(TUa effluent) (B) > 1.0

Where:

TUa effluent= maximum calculated TUa from the most sensitive species in the data set
B= Reasonable potential multiplication factor determined under par. (c)
1.0= Numeric acute WET limitation in acute toxic units (TUa) derived from narrative criterion in s. NR 102.04(1)(d)

2. If a zone of initial dilution has been approved, the potential to exceed an acute criterion shall be calculated using the following equation:

[(TUa effluent) (B) (AMZ)] > 1.0

Where:

TUa effluent= Maximum calculated TUa from the most sensitive species in the data set
B= Reasonable potential multiplication factor determined under par. (c)
AMZ= Acute mixing zone concentration based on presence of a zone of initial dilution as defined in s. NR 106.03(1) expressed as a decimal
1.0= Numeric acute WET limitation in acute toxic units (TUa) derived from narrative criterion in s. NR 102.04(1)(d)

3. The potential to exceed a chronic criterion shall be calculated using the following equation:

[(TUc effluent) (B) (IWC)]> 1.0

Where:

TUc effluent= Maximum calculated TUc from the most sensitive species in the data set
B= Reasonable potential multiplication factor determined under par. (c)
IWC= Instream waste concentration as defined in s. NR 106.03(6) expressed as a decimal
1.0= Numeric chronic WET limitation in chronic toxic units (TUc) derived from narrative criterion in s. NR 102.04(4)(d)

(c) *Reasonable potential multiplication factor*. The reasonable potential multiplication factor in par. (b) is used to convert the calculated effluent toxicity value to the estimated 95th percentile value. The department shall use the following methods to select a reasonable potential multiplication factor:

1. Where there are < 10 individual toxicity **detects**, the multiplication factor shall be taken from Table 4 and based on a coefficient of variation of 0.6.
2. Where there are ≥ 10 individual toxicity **detects**, the multiplication factor shall be taken from Table 4 and based on coefficient of variation calculated as the standard deviation of the WET test endpoints (IC25, IC50 or LC50) divided by the arithmetic mean of the WET tests.

NR 106.08 (5) (c) Table 4 — Reasonable Potential Multiplication Factor.

	Coefficient of variation (CV)																			
Number of samples (n)	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0
1	-	-	-	-	-	6.2		-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	3.8		-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	3.0		-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	2.6		-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	2.3		-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	2.1		-	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	2.0		-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	1.9		-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	1.8		-	-	-	-	-	-	-	-	-	-	-	-	-
10	1.1	1.2	1.3	1.5	1.6	1.7	1.9	2.0	2.2	2.3	2.4	2.6	2.7	2.8	3.0	3.1	3.2	3.3	3.4	3.6
11	1.1	1.2	1.3	1.4	1.6	1.7	1.8	1.9	2.1	2.2	2.3	2.4	2.5	2.7	2.8	2.9	3.0	3.1	3.2	3.3
12	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9	3.0	3.0
13	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.5	2.6	2.7	2.8	2.9
14	1.1	1.2	1.3	1.4	1.4	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.3	2.4	2.5	2.6	2.6	2.7
15	1.1	1.2	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.8	1.9	2.0	2.1	2.2	2.2	2.3	2.4	2.4	2.5	2.5
16	1.1	1.1	1.2	1.3	1.4	1.5	1.6	1.6	1.7	1.8	1.9	1.9	2.0	2.1	2.1	2.2	2.3	2.3	2.4	2.4
17	1.1	1.1	1.2	1.3	1.4	1.4	1.5	1.6	1.7	1.7	1.8	1.9	1.9	2.0	2.0	2.1	2.2	2.2	2.3	2.3
18	1.1	1.1	1.2	1.3	1.3	1.4	1.5	1.6	1.6	1.7	1.7	1.8	1.9	1.9	2.0	2.0	2.1	2.1	2.2	2.2
19	1.1	1.1	1.2	1.3	1.3	1.4	1.5	1.5	1.6	1.6	1.7	1.8	1.8	1.9	1.9	2.0	2.0	2.0	2.1	2.1
20	1.1	1.1	1.2	1.2	1.3	1.4	1.4	1.5	1.5	1.6	1.6	1.7	1.7	1.8	1.8	1.9	1.9	2.0	2.0	2.0
21-100	(see table in NR 106)																			

NOTE: Red text is highlighted above to note that "number of samples" in column 1 is the number of detects in the dataset, not the total number of data.

(d) *Maximum toxicity values.* The Department shall set the TUc effluent and TUa effluent values in par. (b) equal to zero whenever toxicity is not-detected or the LC50, IC25, or IC50 equals or exceeds 100% effluent.

Reasonable Potential Could Be Present Even if a WET Failure Has Not Occurred

As a result of the WET reasonable potential procedures in s. NR 106.08 (6), Wis. Adm. Code (Figure 6), a limit will be required in almost all cases where a permittee has a WET failure in their dataset. Reasonable potential can also be indicated in situations where no WET failures have occurred, if toxicity was detected near enough to the applicable limit. The examples below demonstrate how this might occur.

NR 106.08(6)(b)
3. The potential to exceed a chronic criterion shall be calculated using the following equation:

[(TUc effluent) (B) (IWC)]> 1.0

Where:TUc effluent= Maximum calculated TUc from the most sensitive species in the data set
B= Reasonable potential multiplication factor determined under par. (c)
IWC= Instream waste concentration as defined in s. NR 106.03(6) expressed as a decimal
1.0= Numeric chronic WET criterion in chronic toxic units (TUc) derived from narrative criterion in s. NR 102.04(4)(d)

Example 1:

Date initiated	Chronic results		Maximum TUc (100/IC25)	Pass/ Fail
	C. dubia IC25	Fathead IC25		
12/31/2010	>100%	>100%	1.0	Pass
03/25/2011	>100%	>100%	1.0	Pass
06/10/2012	>100%	>100%	1.0	Pass
09/22/2013	85%	>100%	**1.18**	Pass
12/09/2014	>100%	>100%	1.0	Pass

- IWC = 10% (**0.10**)
- Most sensitive result: IC25 = 85%; Maximum TUc = 100/85 = **1.18** (test passed; toxicity detected well below the limit)
- 5 WET tests, 1 toxicity detect. Multiplication factor (B) from NR 106.08(5)(c), Table 4 = **6.2** (based on # of detects)

1.18 x 6.2 x 0.10 = 0.73; RP not shown, limit <u>not</u> required

Example 2:

Date initiated	Chronic results		Maximum TUc (100/IC25)	Pass/ Fail
	C. dubia IC25	Fathead IC25		
12/31/2010	>100%	>100%	1.0	Pass
03/25/2011	>100%	>100%	1.0	Pass
06/10/2012	>100%	>100%	1.0	Pass
09/22/2013	55%	>100%	**1.82**	Pass
12/09/2014	>100%	>100%	1.0	Pass

- IWC = 25% (**0.25**)
- Most sensitive result: IC25 = 55%; Maximum TUc = 100/55 = **1.82** (test passed; toxicity detected nearer to the limit)
- 5 WET tests, 1 toxicity detect. Multiplication factor (B) from NR 106.08(5)(c), Table 4 = **6.2** (based on # of detects)

1.82 x 6.2 x 0.25 = 2.82; RP is shown, limit is required

When Is a Compliance Schedule/Toxicity Reduction Evaluation Recommended?

Toxicity reduction evaluations (TRE) include investigations by the permittee and their WET lab to identify the chemical(s) causing toxicity. The **4th column** in Table 1 below shows when a TRE compliance schedule is recommended by the WET Checklist. (See Chapter 1.12 for more about what is included in a standard TRE compliance schedule.) In order to complete a successful TRE, toxicity needs to be present in the effluent often enough that sample manipulations can be done to characterize the toxicity and/or steps can be taken to trace the source of toxicity. Therefore, the WET Checklist recommends a TRE compliance schedule based on the percent failures that have occurred. In cases where data is limited or where toxicity has appeared infrequently, a TRE may not be recommended. In these cases, the WET Checklist often recommends more frequent monitoring instead, in order to determine whether toxicity reappears over time. Standard language typically included in WPDES permits requires the permittee to conduct a TRE if WET failures occur during the permit term, so repeated bouts of toxicity may still trigger the need for a TRE (see Chapter 1.14).

The Checklist recommendations shown in Table 1 should be appropriate in most situations. The TRE recommendations provided by the WET Checklist were chosen based on the best professional judgment of the Biomonitoring Coordinator. However, staff should carefully consider whether the WET Checklist recommendation is appropriate for the given situation based on their best professional judgment and their knowledge of the discharge. In order to make the best informed decisions possible, final decisions about whether or not to include a TRE compliance schedule in the permit should be made in many cases after discussions between WQBEL staff, compliance engineer, and the permit drafter.

Staff should use their best professional judgment to decide whether a TRE is needed in each case, especially where few failures have occurred or where other conditions might exist that make a compliance schedule inappropriate. A TRE should be required in most cases where valid, representative WET data indicates that reasonable potential is present and that the duration, magnitude, and frequency of WET failures is sufficient to be able to determine what is causing toxicity. A general rule of thumb may be that if more than 25% of completed tests have failed, and an explanation for toxicity has not been identified, then a TRE compliance schedule is probably appropriate. Staff should consult with the Biomonitoring Coordinator if they have questions or are uncertain whether a TRE is necessary.

Table 1. Compliance Schedules and Minimum Monitoring Frequencies

# data	# failures	Limit Required? (RP shown)	TRE schedule recommended?	Minimum monitoring frequency	Comments
1	0	No	No	NA	No limit or TRE recommended
1	0	Yes	No	1x annual	See "Reasonable potential may be present when a WET failure has not occurred".
1	1	Yes	No	1x annual	
2	0	No	No	NA	No limit or TRE recommended
2	0	Yes	No	NA	See "Reasonable potential may be present when a WET failure has not occurred".
2	1	Yes	No	1x annual	
2	2	Yes	No	1x annual	
3	0	No	No	NA	No limit or TRE recommended
3	0	Yes	No	1x annual	See "Reasonable potential may be present when a WET failure has not occurred".
3	1	Yes	No	1x annual	
3	2	Yes	**Yes**	2x annual	2x annual testing would be recommended only for the period after the compliance schedule ends and the WET limit has become effective.
3	3	Yes	**Yes**	Quarterly	Quarterly testing recommended after compliance schedule ends & the limit becomes effective.
4	0	No	No	NA	No limit or TRE recommended
4	0	Yes	No	1x annual	See "Reasonable potential may be present when a WET failure has not occurred".
4	1	Yes	No	1x annual	
4	2	Yes	**Yes**	2x annual	2x annual testing would be recommended only for the period after the compliance schedule ends and the WET limit has become effective.
4	3	Yes	**Yes**	Quarterly	Quarterly testing recommended after compliance schedule ends & the limit becomes effective.
4	4	Yes	**Yes**	Quarterly	Quarterly testing recommended after compliance schedule ends & the limit becomes effective.
≥ 5	0	No	No	NA	No limit or TRE recommended
≥ 5	0	Yes	No	1x annual	See "Reasonable potential may be present when a WET failure has not occurred".
≥ 5	< 25%	Yes	No	1x annual	
≥ 5	≥ 25% & ≤ 75%	Yes	**Yes**	Quarterly	Quarterly testing recommended after compliance schedule ends & the limit becomes effective.
≥ 5	> 75%	Yes	**Yes**	Quarterly	Quarterly testing recommended after compliance schedule ends & the limit becomes effective.

Minimum Monitoring Frequencies When Reasonable Potential is Indicated

The **5th column** in Table 1 shows the minimum WET monitoring frequencies recommended by the WET Checklist, based on the number of representative WET data available, the number of failures that have occurred, and whether or not reasonable potential has been indicated based on the procedure in s. NR 106.08, Wis. Adm. Code. The Checklist does not assign points based on this information, but instead evaluates whether the points given once the Checklist is complete are enough to satisfy this requirement. Other factors rated by the WET Checklist, such as the use of a large number of chemical additives or industrial contributors to a municipal treatment plant, may cause a higher monitoring frequency to be recommended. If less than the recommended monitoring frequency testing would otherwise be recommended due to point totals, the Checklist recommends the minimum monitoring frequency for these dischargers. According to federal regulations at 40 CFR Part 122.44(i)(2), monitoring must occur at least 1x yearly whenever a limit is present. **Therefore, in no case may staff recommend less than 1x annual monitoring when a WET limit is required.**

The recommendations shown in Table 1 reflect the minimums recommended by the WET Checklist and are intended to be appropriate in most situations. However, staff may decide that more or less monitoring is warranted in specific cases. Staff should use their best professional judgment to determine whether minimum monitoring frequencies given by the Checklist are appropriate. Staff should also use their best professional judgment to adjust monitoring frequencies if it has been decided that a TRE is not necessary. For example, if a TRE and quarterly monitoring for the period after the TRE was recommended by the WET Checklist, staff will need to decide if quarterly monitoring or some other frequency is appropriate for the entire term of the permit if it is decided that a TRE schedule will not be included in the permit.

WET Limit Trigger – Confirming Whether WET Data is Still Representative

WET limit triggers may be included in permits when staff are uncertain about the representativeness of WET data used in reasonable potential determinations. For example, say a permittee completed 4 WET tests between 2010-2014:

Date initiated	Chronic results		Pass/ Fail
	C. dubia IC25	Fathead IC25	
12/31/2010	25%	>100%	**Fail**
05/25/2011	>100%	>100%	Pass
06/10/2012	>100%	>100%	Pass
09/22/2013	>100%	>100%	Pass

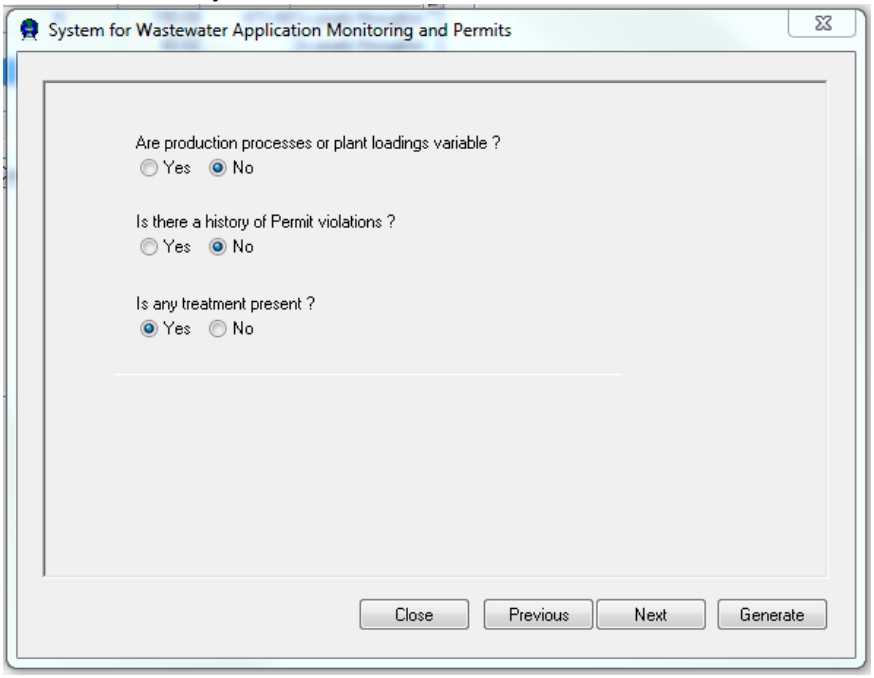
IWC = 55%

This hypothetical WPDES permit expired on 12/31/14 and is scheduled for reissuance in 2015. The permittee cannot provide definitive information, such as the results of a successful toxicity reduction evaluation, showing that the 2010 result is not representative of the current discharge. However, a local industry which contributes significantly to their influent changed their production process significantly in 2011 and it is likely that they are now sending a much less toxic wastewater to the POTW. Ideally, the permittee would have TRE investigations done before and after the changes were made at the industry which showed that toxicity was caused by that source and changes at the industry resulted in that toxicity being removed. Since the permittee in this example was not able to provide this type of information to the Department before their permit expired and the cause of the original toxicity is still uncertain, the 2010 result probably cannot be thrown out and should be included in the reasonable potential determination.

However, a limit trigger may be useful in an example like this to allow for more WET data to verify that toxicity was resolved by the change in industrial contribution (i.e., confirmation that the 2010 data in this example is no longer representative). A trigger usually allows for quarterly testing during the first 12 months of the permit to establish whether toxicity is present in the effluent or whether changes at the industry, in this example, truly resulted in the removal of toxicity. If no WET failures occur during that first 12 months, it is more likely that toxicity was removed and the older data is no longer representative, therefore, a limit would not be needed in this example situation. If a failure

did occur, then a limit would be necessary and the permittee could be required to complete a TRE compliance schedule designed to find and fix the source of toxicity. (See Chapter 1.12 for example trigger language.)

Effluent Variability and WWTP Performance

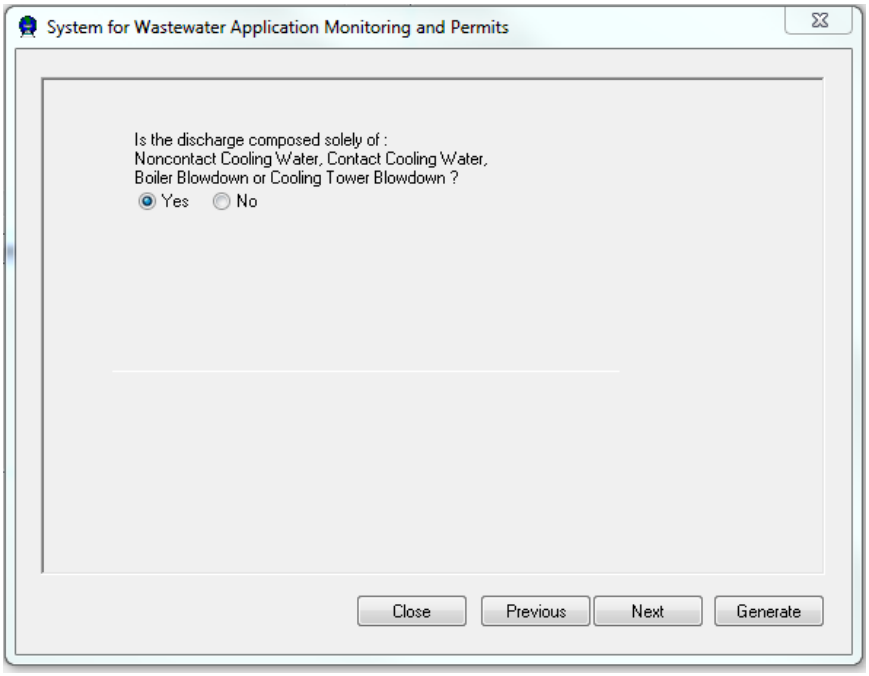


More variable effluents should be monitored more frequently because of the inconsistency of the effluent matrix. Less frequent monitoring may not represent effluent quality during different occurrences. Information requested in the screen above is used to assess whether the effluent is variable enough to warrant additional WET monitoring. Decisions here are subjective and should be based on the knowledge and best professional judgment of the staff most familiar with the permitted facility.

- **Question #1, Loading or Production Variability:** As effluent characteristics change (due to contributing industries, hauled waste, leachate, infiltration, process changes, spills, etc.), so may toxicity. Judgments should be made whether waste entering the system is resulting in a variable effluent, or if the system is handling incoming variability and effluent characteristics are relatively unchanged. The user should answer "Yes" if, in their judgment, industrial process changes, wastewater inputs, inconsistent treatment efficiency, or other changing conditions are resulting in a variable effluent.

- **Question #2, Compliance History:** A permittee's compliance history may be an indication of the quality and consistency of operations or the ability to handle incoming waste, which affects effluent variability. Staff should enter a "Yes" if the facility has had significant (for example, those warranting enforcement actions such as verbal or written NONs) or numerous violations. All effluent characteristics should be considered, not just toxics.

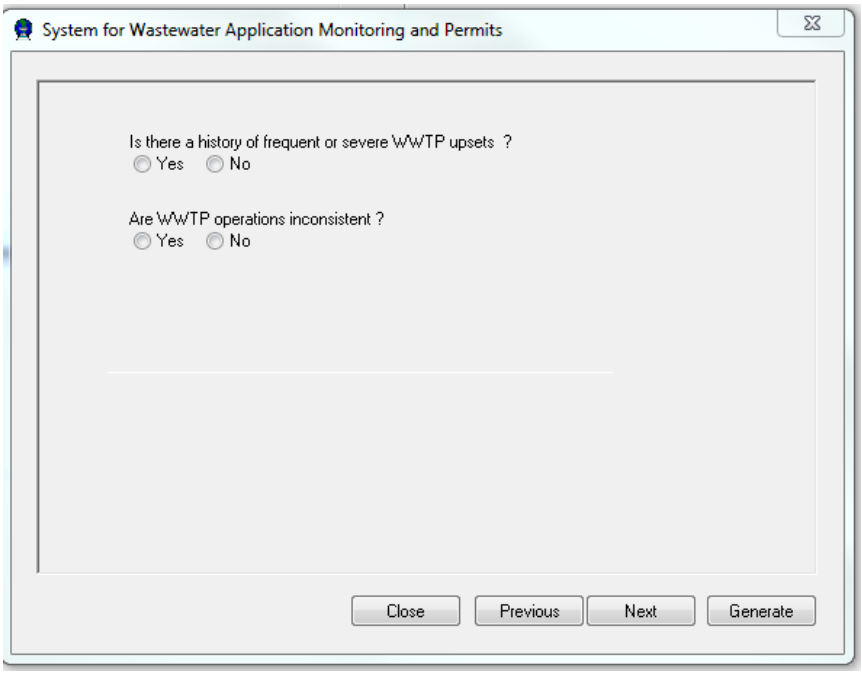
- **Question #3** is used to determine whether variability may be affected by inadequate treatment. A "No" should be given in question #3 for discharges that do not have wastewater treatment. If a no is given, the next screen will appear. If a yes is given to question #3, the next screen will be skipped and the subsequent screen will appear.



- **Question #4:** The screen above allows exemptions from points assessed for no treatment for four discharge categories. For all other discharge categories (including COW water discharges) a "No" should be given.

In situations where staff feel that points accumulated in this question would not be appropriate because the discharge doesn't otherwise warrant treatment, they should adjust final monitoring recommendations. Staff should NOT adjust points in the electronic Checklist by giving inaccurate answers, because this will confuse the record (i.e., others will not be able to tell why answers were given). Adjustments to final recommendations should be explained and well documented so that others can tell why decisions were made.

POINTS ASSESSED (Both Acute & Chronic)		
Question#	***Answer***	***Points***
1	*NO*	*0*
1	*YES*	*5*
2	*NO*	*0*
2	*YES*	*5*
3 & 4	*BOTH YES*	*0*
3 & 4	*BOTH NO*	*10*
3 & 4	*3 NO, 4 YES*	*0*
3 & 4	*3 YES, 4 NO*	*0*

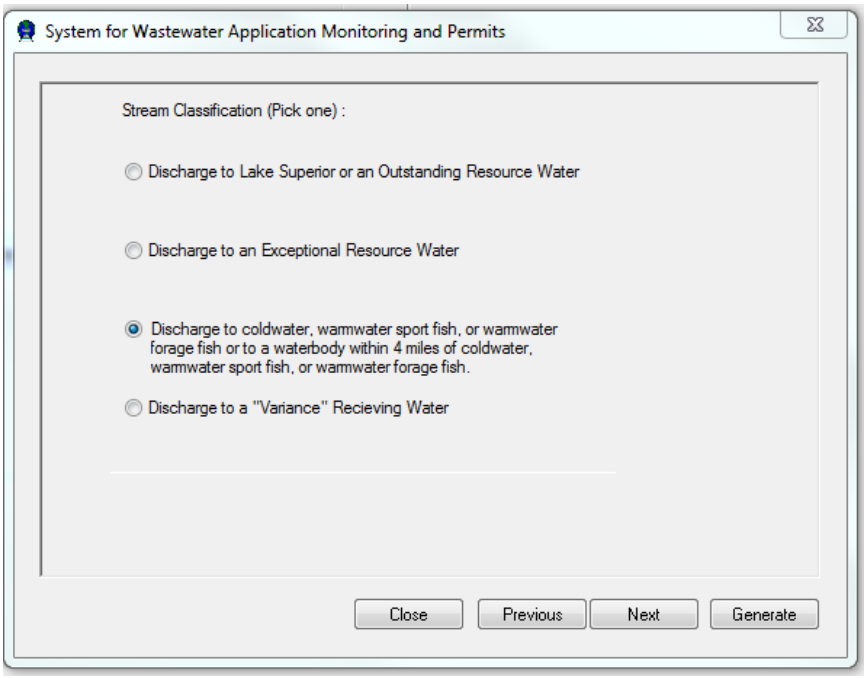


- **Question #1, Upsets:** Frequent or severe upsets may be an indication of poor operations, under design of a treatment plant, slug loads within the collection system, or other factors which raise the potential for toxicity. Staff should make judgments whether frequent upsets are unexplained or not handled properly, which may affect effluent variability.

- **Question #2, Operations:** The ability to maintain or restore quality treatment can impact effluent variability. If an operator is able to react quickly and effectively when treatment is upset, effluent characteristics are less likely to be altered for long periods. Conditions such as bulking and foaming, lost ability to nitrify, etc., may indicate poor treatment and impact toxicity. Concurrent cases of treatment problems and toxicity have been noted in many cases.

POINTS ASSESSED (Both Acute & Chronic)		
Question#	***Answer***	***Points***
1	*NO*	*0*
1	*YES*	*5*
2	*NO*	*0*
2	*YES*	*5*

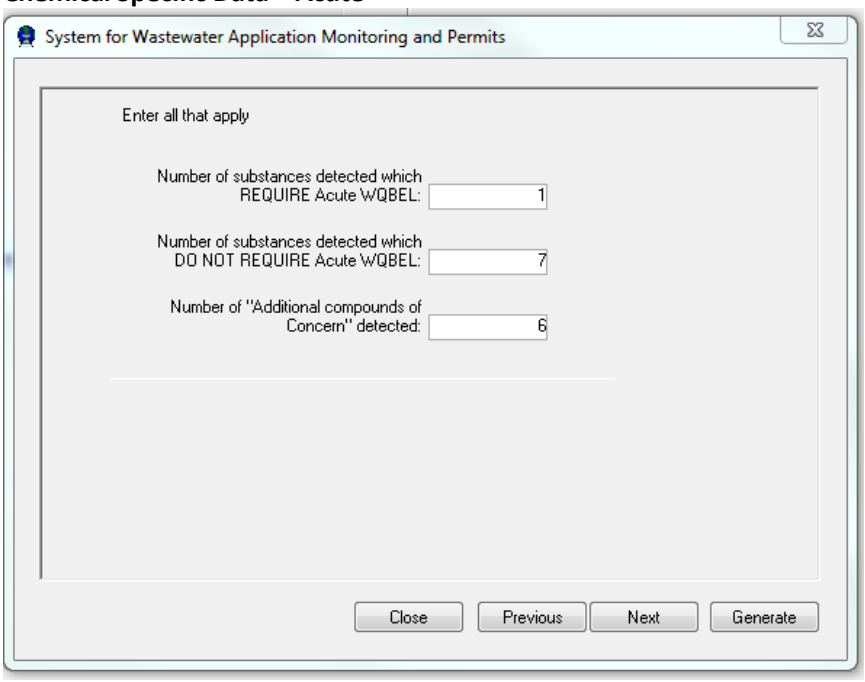
Stream Classification



WET tests use "indicator organisms" to mimic what happens in the environment when an effluent is introduced, to estimate the effluent concentration that may produce a harmful effect, and to predict concentrations that may interfere with the growth, development, and reproductive potential of aquatic organisms. Since "higher" classifications (e.g., exceptional/outstanding resource waters) designate waters where more sensitive populations or water quality exists, more monitoring should be done to insure protection of these waters and the Checklist assigns points accordingly.

POINTS ASSESSED (Both Acute & Chronic)	
Answer	***Points***
Lake Superior or Outstanding Resource Water	*15*
Exceptional Resource Water	*12*
Coldwater, Warmwater Sport, or Warmwater Forage Fish or < 4 mi from these waters	*5*
LFF/LAL	*0*

Chemical Specific Data – Acute

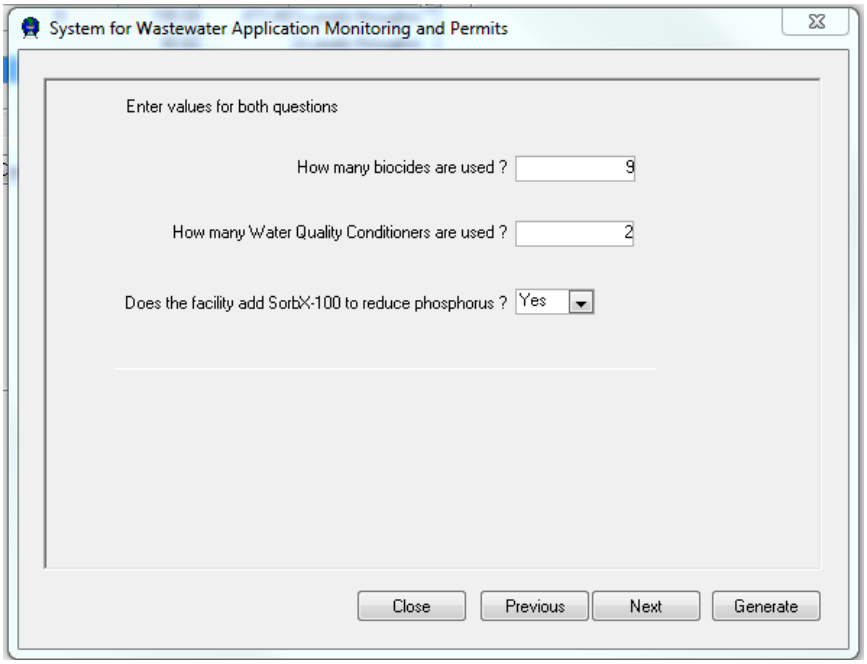


Water quality criteria are designed to be protective of aquatic life for the compounds that they limit. Chemical-specific limits alone can't account for additive or synergistic effects that occur when compounds are combined in an effluent. The more compounds present, the greater the potential is for additive or synergistic effects to occur. Staff should document which limits and/or detects were considered so it is clear to others why point totals were assigned. For a list of ch. NR 105, Tables 1 & 2, substances (i.e., those which may require acute WQBELs), and for a table of "Additional Compounds of Concern", see attachment 2 at the end of this chapter. Note: limits should not be counted if they are given only to satisfy limit expression requirements found in 40 CFR Part 122.45 (d) and s. NR 106.07, Wis. Adm. Code.

As mentioned in attachment 2, ammonia limits should usually be counted here only if representative effluent data demonstrates the need for a WQBEL (limits that are simply "carried over" from a previous permit term should not be counted). However, if ammonia has been detected in the effluent, it should be counted in that category.

POINTS ASSESSED (Acute only)	
Answer	***Points***
Acute WQBELs	*5 for 1st + 1 for ea. additional, not to exceed 10 pts.*
Detects w/out WQBELs	*1 for 1st + 1 for add. not to exceed 3*
Additional Cmpds of Concern	*2 (for ≥ 1 substance)*

Additives – Acute



Additives come in a wide variety of mixtures and forms and are used in a number of applications (e.g., biocides, corrosion inhibitors, boiler water treatments, scale control, pH control, clarifying agents, industrial process polymers, and other solids control products). Most additives have not undergone the level of toxicity testing needed to calculate water quality criteria, therefore secondary values should be derived according to s. NR 105.05, Wis. Adm. Code, and the *Water Quality Review Procedures For Additives* guidance (http://dnr.wi.gov/topic/wastewater/Guidance.html) followed, whenever an additive is discharged directly into a surface water without receiving treatment or an additive is used in the treatment process and is not expected to be removed before discharge.

The WET Checklist is used where more complex wastewaters are being evaluated, such as industrial process wastewaters and municipal POTW effluents. The more additives that are present in an effluent, the more complex that wastewater becomes and the greater the potential for toxic impacts due to the mixture. Additives should be counted in the WET Checklist analysis shown above if they are added during or after the wastewater treatment process (or if no treatment is present). If they are added prior to treatment (e.g., a production additive at an industrial facility) they should be included in the evaluation only when wastewater treatment is not expected to remove or significantly alter the toxicity of these chemicals (for example, if less than secondary treatment is present). Chemicals added at the WWTP or to the final effluent should be included in this evaluation, regardless of the treatment type.

Special attention should be paid in all cases where permittees are adding chemicals to remove phosphorus (FeCl, alum, SorbX-100, etc.), especially those that are optimizing treatment to meet more stringent or interim phosphorus limits. Overuse of these chemicals may cause effluent toxicity – care should be taken to use only the amount of chemical that is necessary and no more. Field staff should provide assistance, where needed, to help permittees determine the proper amount of chemical to be used.

SorbX-100 and Other New P Treatment Chemicals. Since about 2015, many permittees in Wisconsin have piloted the use of new phosphorus treatment chemicals. One example, SorbX-100 (a cerium chloride + lanthanum chloride mixture) was piloted by many in 2015-16, to see if this product could help them to meet lower phosphorus limits. Due to concerns raised by permittees, consultants, and staff about the potential risks of adding significant amounts of a new,

unknown product into the wastestream, the DNR requested toxicity information from the maker of the product. Using the information submitted by the manufacturer, staff were able to calculate secondary values, which can be used in place of water quality criteria when calculating WQBELs. Those values for SorbX-100 are as follows:

· Secondary Acute Value = 3.88 mg/L
· Secondary Chronic Value = 0.216 mg/L

If used correctly (not over-applied), this product should remain with the solids and not reach the surface water. However, for the purposes of determining whether or not a WQBEL may be warranted, the Department assumes that all that is added could reach the final effluent. Since the effective levels being added during these pilots were all reportedly well below these secondary value concentrations (and therefore likely discharged at even lower levels), no WQBELs appear to be required in most situations (i.e., if there is no reasonable potential to exceed the limit, no limit is needed). However, in cases where a permittee is at risk of discharging the product at levels above the secondary value, a WQBEL should be included in the permit. When WQBELs are determined not to be necessary, staff should share the secondary value numbers with permittees in order to inform them about potentially toxic levels to be avoided at all costs.

In addition to calculating secondary values, the Department asked the State Lab of Hygiene to conduct WET tests at facilities where this product was being piloted. All acute and chronic WET tests conducted before this guidance was written had passed. Though promising, this data was limited to only a few facilities.

Given that this product is not expected to be discharged into surface waters at significant amounts and information collected before the date of this guidance does not indicate a risk to aquatic life at expected usage levels, the Department concluded that its use in WWTPs should be handled as other phosphorus treatment products (FeCl, alum) have been handled to date. That is, the product is not an additive as defined in the *Water Quality Review Procedures for Additives* guidance (see http://dnr.wi.gov/topic/wastewater/Guidance.html) and no effluent limits will be necessary in most places where it is used. However, the following should be done in the near term to insure that problems do not occur:

1. Compliance staff should ask permittees to conduct WET tests during treatment pilots using SorbX-100 or other new treatment chemicals.
2. Compliance staff should share secondary values with permittees and advise them to keep usage rates/effluent concentrations below these levels.
3. The WET Checklist will add points for using SorbX-100, to collect additional effluent data and to further protect against toxicity under different use/discharge situations.

If other new treatment chemicals come onto the market in response to the need for meeting lower phosphorus limits, a similar process should be used to evaluate whether significant toxicity concerns exist. If staff have questions related to the use of these types of products or other treatment chemicals and their potential for toxicity, they should contact the Biomonitoring Coordinator.

Toxicity potential from treatment chemicals. While chemicals are often chosen as alternatives in wastewater treatment (i.e., chlorine to disinfect, chemicals to remove phosphorus, polymers to improve settling, etc.), they are not required and there may be alternatives which are less likely to cause toxicity. It is important to realize that the use of treatment chemicals increases the potential for toxicity in wastewater.

Examples:

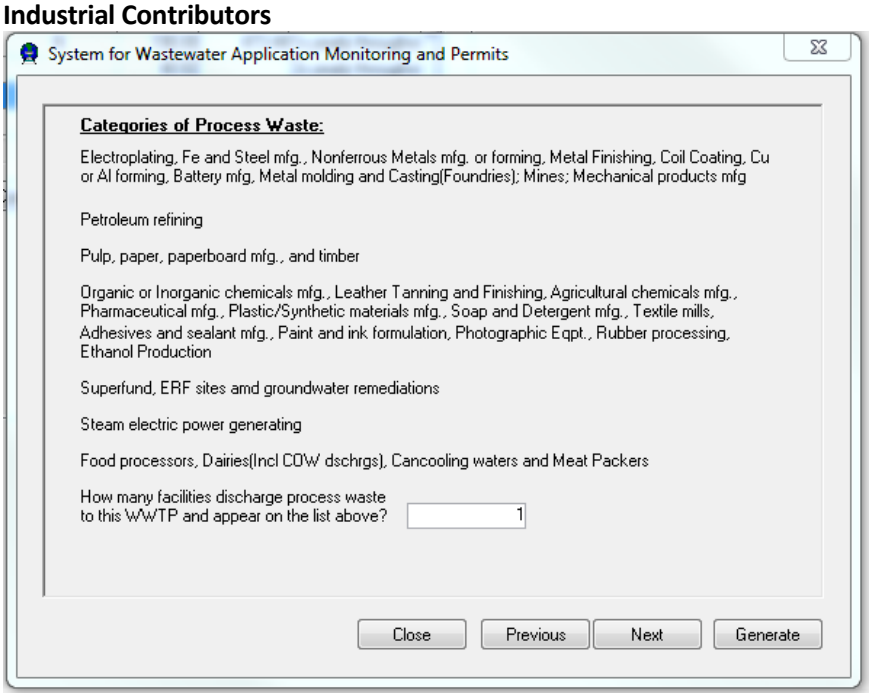
- ***Biocides*** - chlorine & other halogens, fungicides, herbicides, algaecides, bactericides, etc.

- ***Water Quality Conditioners*** - dechlorination chemicals, alum, ferric chloride, polymers, dyes, anti-scale, corrosion-inhibitors, pH adjustment chemicals, conditioning agents, etc. *Note: SorbX-100 is counted separately (see above) and should not also be counted as a water quality conditioner.*

If chlorination and dechlorination chemicals are added, staff should count both when asked for a count of additives in the Checklist (i.e., chlorine as a biocide, dechlorination chemicals as WQC). These products should also be included when accounting for WQBELs and chemical detects in the Checklist.

POINTS ASSESSED (Acute only)	
Type	***Points***
Biocides	*3 pts. each (not to exceed 20 pts when combined w/WQC)*
Water Quality Conditioners	*1 pt. each (not to exceed 20 pts when combined w/biocides)*
New P treatment chemicals	*15 points*

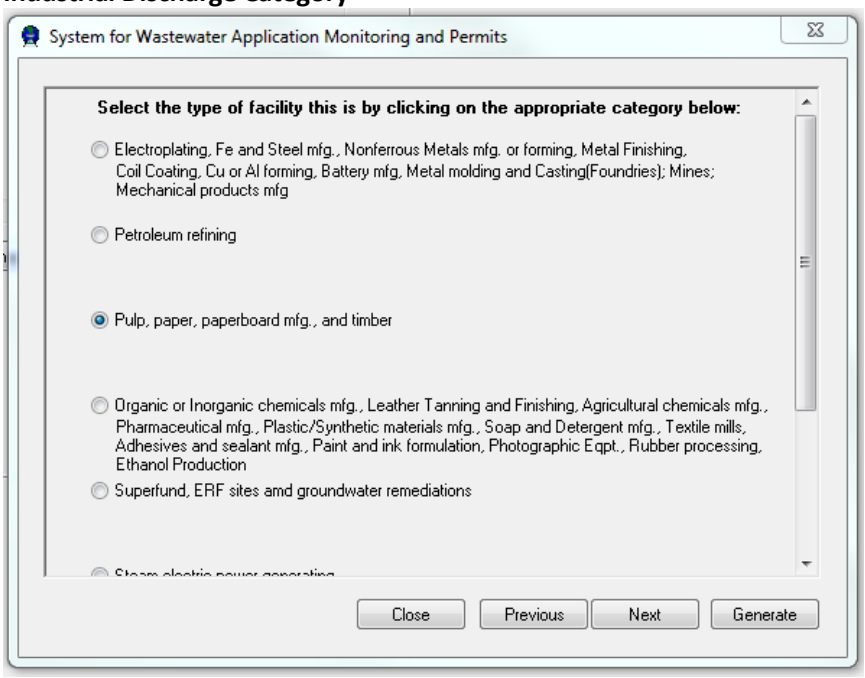
Industrial Contributors



Some industrial categories have more potential for causing effluent toxicity and industrial contributors to municipal treatment plants can increase their potential for toxicity, therefore, the Checklist asks for information related to industrial type and amounts of wastewater present. If the facility being evaluated is a municipality, the screen above will appear, asking for the number of industrial contributors. If the facility is industrial, the next screen will appear asking for the type of industrial discharger. In the screen below, points should be assessed only if the discharge contains **PROCESS** wastewater. Users should not include points for outfalls that contain only sanitary or other non-process wastewater.

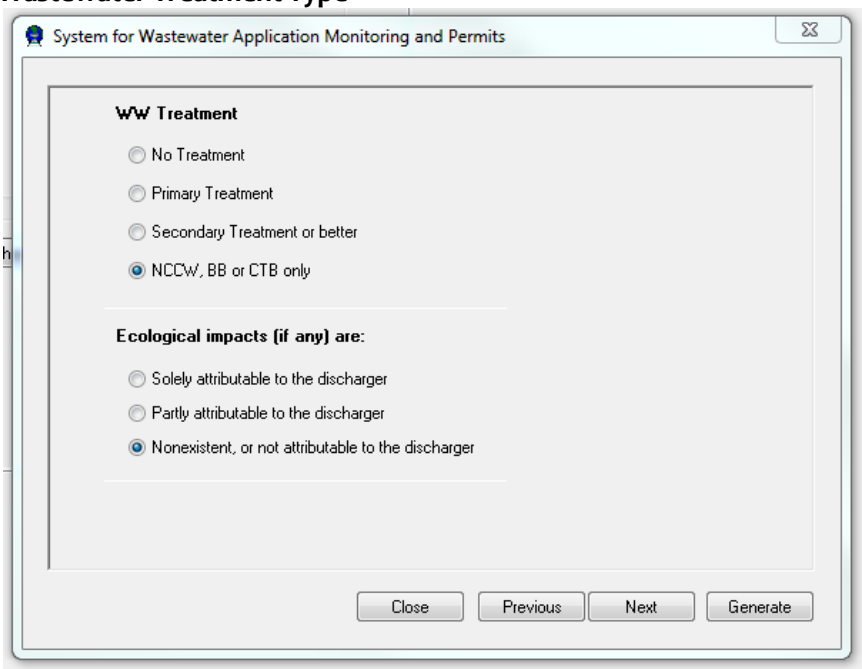
Staff should use their best professional judgment to include dischargers that do not fall strictly into one of the above categories. If staff feel that a discharger warrants the same points as one of the categories above, based on toxicity potential related to discharge type, they should assign points accordingly.

Industrial Discharge Category



POINTS ASSESSED (Acute & Chronic)	
Type	***Points***
Municipalities	*5 pts for 1st + 1 for ea. additional (not to exceed 15)*
Groups in bullets 1-3	*15*
Groups in 4th bullet	*10*
Groups in 5th bullet	*8*
Groups in 6th and 7th bullets	*5*
Groups in last bullet	*0*

Wastewater Treatment Type



Untreated wastewater has a higher potential for toxicity and therefore is assigned more points using information from the screen above. (No points are assigned for category 4.) If combined outfalls are being addressed and treatment differs, the most conservative points should be given (for example, if one is treated & the other isn't, 10 points should be given due to the presence of untreated wastewater).

Staff should assign points here for all discharges that do not have wastewater treatment (including those with cancooling waters, condensate of whey, etc.). In those situations where staff feel that points accumulated here are not entirely appropriate because the discharge would not otherwise warrant wastewater treatment, they should adjust final monitoring recommendations instead of putting incorrect information into the Checklist. Adjustments to final recommendations should be explained and well documented so that others can tell why decisions were made.

POINTS ASSESSED (Acute & Chronic)	
Type	***Points***
No Treatment	*10*
Primary Treatment Only	*8*
Secondary or Better	*0*
NCCW, Boiler or Cooling Tower Blowdown	*0*

Ecological Impacts

In situations where aquatic populations are under stress due to poor ecological conditions, toxicity from an effluent has a greater potential of causing environmental harm. Stressed individuals and populations may be less able to adapt or adjust to a toxic effluent. Since impacted areas could be more susceptible to toxicity, more severe impacts may occur to populations that are already stressed due to existing conditions, and past discharge problems may cause populations to be more sensitive to toxicity, it is appropriate to assign more monitoring to discharges that occur in areas where these concerns exist.

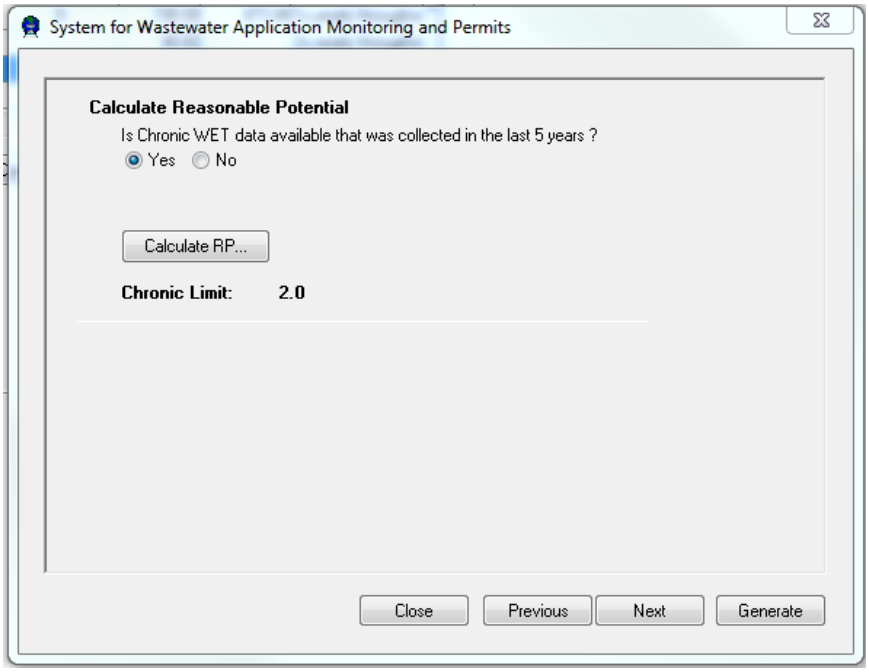
The second question in the screen above is designed to account for situations where data shows that a facility has contributed to problems in the receiving water (for example, fish kills or other impacts to benthic, macrophytic or aquatic

organisms). More points are given to discharges that are thought to be the sole source causing an ecological impact; less are given to those who may be a partial contributor. Water quality impacts caused by compounds typically characterized as toxics may be the easiest to determine points for in this category, however, staff should also consider situations where impacts may be present that are not necessarily caused by toxics. For example, low dissolved oxygen levels or impacts due to excessive nutrient levels may also cause concern in these situations. Staff should determine whether past receiving water problems have been addressed and assign points accordingly.

POINTS ASSESSED (Acute & Chronic)	
Type	***Points***
Impacts solely due to discharger	*20*
Impacts contributed to by discharger	*5*
No impacts known	*0*

If the user indicated earlier that it isn't necessary to evaluate the need for chronic WET monitoring because available dilution is high, the Checklist stops here.

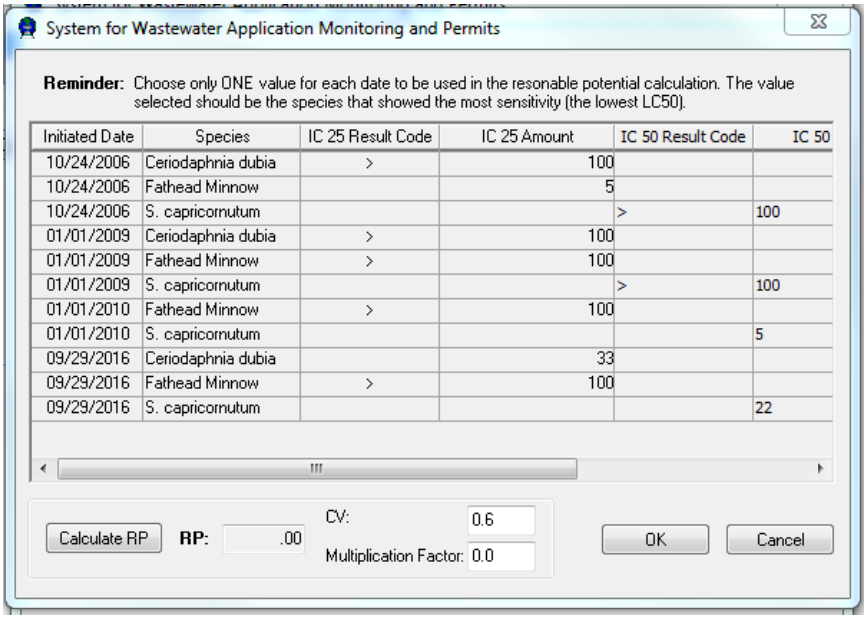
Chronic WET Limit Determinations



In the screen above, the user states whether there is representative chronic WET data available from the most recent 5 years for the discharge being evaluated. If no recent data is available, the Checklist assigns 5 points. This is done because more uncertainty exists in situations where testing has not been done in recent times than at those facilities that have produced recent data which shows toxicity problems are not a concern.

The chronic WET limit, calculated using the appropriate flow information indicated for the facility, is shown on this screen. (A chronic WET limit = 100/IWC, according to s. NR 106.09, Wis. Adm. Code.)

The user then clicks on “Calculate RP” if any representative data is available (even if $\geq$ 5 years old), in order to choose the data that is to be used to calculate the chronic reasonable potential.



Initiated Date	Species	IC 25 Result Code	IC 25 Amount	IC 50 Result Code	IC 50
10/24/2006	Ceriodaphnia dubia	>	100		
10/24/2006	Fathead Minnow		5		
10/24/2006	S. capricornutum			>	100
01/01/2009	Ceriodaphnia dubia	>	100		
01/01/2009	Fathead Minnow	>	100		
01/01/2009	S. capricornutum			>	100
01/01/2010	Fathead Minnow	>	100		
01/01/2010	S. capricornutum				5
09/29/2016	Ceriodaphnia dubia		33		
09/29/2016	Fathead Minnow	>	100		
09/29/2016	S. capricornutum				22

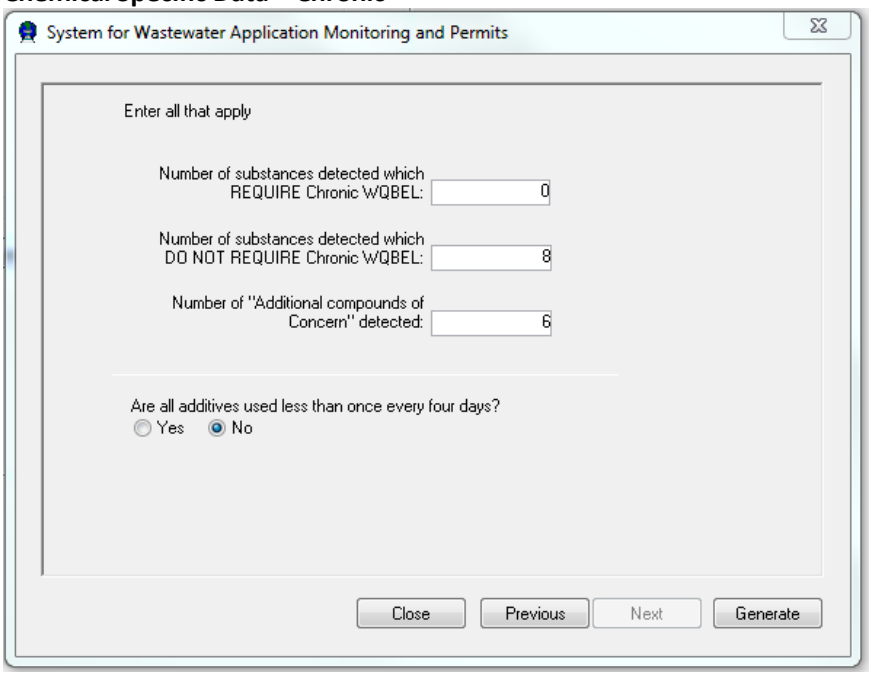
The Checklist uses data selected in the screen above to determine reasonable potential. Reasonable potential is defined as where an effluent "*is projected or calculated to cause an excursion above a water quality standard*" according to s. NR 106.08(6)(b), Wis. Adm. Code. WET limits should be given whenever representative, facility-specific data shows the effluent may be discharged at a level that has the potential to cause or contribute to an excursion above the WET criterion. Example reasonable potential decisions are shown in Attachment 1, at the end of this chapter.

Which data to use. When first opened, the WET data that appears in the screen above will include all valid data for that outfall in the WET Database. IC_{25}s are shown in the IC_{25} column for *Ceriodaphnia dubia* and fathead minnow tests; IC_{50}s are shown in the IC_{50} column for *Selenastrum* (green algae) tests. The user selects representative WET data that should be used in the reasonable potential determination, by highlighting one species from each test date that is to be used (the most sensitive species, or lowest IC value, from each test should be selected). Once the most sensitive species from each representative test has been highlighted, the user clicks the "calculate RP" button to determine the appropriate value.

Selenastrum capricornutum tests are often not included in the standard permit-required WET test battery. However, this species is included in chronic tests conducted by the UW-Madison State Lab of Hygiene and other labs. If *S. capricornutum* data has been collected for the discharge being evaluated, and it is believed to be representative of the discharge, it should be included in the RP analysis as described above (i.e., that species' test value should be selected, if it is the most sensitive endpoint). If this species is consistently the most sensitive in WET test failures, it may be appropriate to add it to the permit-required test battery. If staff have questions, they should contact the Biomonitoring Coordinator.

The same principles apply to the selection of chronic data, CV, and multiplication factors as that described in the section on acute data. Minimum monitoring frequencies, compliance schedules, and/or WET limit triggers are also recommended in similar situations for chronic as for acute.

Chemical Specific Data – Chronic



Water quality criteria are designed to be protective of aquatic life for the compounds that they regulate. However, chemical-specific limits are not designed to account for additive or synergistic effects that chemicals may have when they are combined in an effluent. WET testing can be used to determine whether additive or synergistic effects are occurring. The more compounds that are present in an effluent, the greater the potential may be for that effluent to exhibit these effects. Staff should document which limits and/or detects were considered (if any) so it is clear to others why point totals were assigned. Substances present at levels that cause chronic concerns (even if chronic limits are not given because acute limits are more restrictive) should be counted. For lists of substances found in ch. NR 105, Wis. Adm. Code, Tables 3 & 4 (i.e., substances which may require chronic WQBELs), and for a table of "Additional Compounds of Concern", see Attachment 2 at the end of this chapter. Note: limits should not be counted if they are given only to satisfy limit expression requirements found in 40 CFR Part 122.45 (d) and s. NR 106.07, Wis. Adm. Code.

As noted in Attachment 2, limits for ammonia should be counted as such in the WET Checklist only if effluent data shows the need for a WQBEL. Limits that are carried over from a previous permit, even though data suggests a limit is not needed, should not be counted as a limit in this evaluation. If ammonia has been detected in the effluent, it should be counted in that category.

POINTS ASSESSED (Chronic only)	
Answer	***Points***
Chronic WQBELs	*5 for 1st + 1 for ea. additional, not to exceed 10 pts.*
Detects w/out WQBELs	*1 for 1st + 1 for add. not to exceed 3*
Additional Compounds of Concern	*2 (for ≥ 1 substance)*

Additives – Chronic
Are all additives used less than once every four days? Additives used less than 1 in 4 days are not given points towards chronic testing because less potential for chronic impacts is believed present. If a "No" is given here, points are given based on the answers given previously in the biocides/water quality conditioners screen.

Final WET Checklist Summary

Once answers are entered into the last screen, the Checklist is complete. The user clicks on the "Generate" button to complete the Checklist and to preview a summary of answers, points assessed, and WET recommendations. The summary shows recommendations for limits and monitoring based on Checklist answers, the calculated AMZ and IWC and acute and chronic limits (as appropriate).

As noted at the beginning of this chapter, the WET Checklist and this chapter are intended to help staff evaluate site-specific factors and to make appropriate WET monitoring recommendations. The WET Checklist asks staff about factors that affect effluent toxicity and adds points based on responses and the relative importance of each factor. The questions asked and points given were chosen based on the best professional judgment of its creators. In the end, recommendations made by the WET Checklist should be carefully considered and the final monitoring frequency made by WQBEL staff should be based on their best professional judgment and knowledge of the discharge.

The guidance in this chapter is intended to apply in most situations, but there may be situations where the general assumptions it is based on may not apply. Decisions that don't follow the general recommendations in this guidance should be shared with the Biomonitoring Coordinator (Kari.Fleming@wisconsin.gov) and clearly documented in WQBEL memos, so others can understand why decisions were made.

WET Checklist Monitoring Recommendations

The monitoring recommendations that are given by the WET Checklist are shown in Figure 7 below.

Figure 7. Checklist Point Totals and Associated Monitoring Frequencies

Point Totals	Checklist Monitoring Recommendation	Comments
≤ 14 (ACUTE) **≤ 19 (CHRONIC)**	**No WET Tests Recommended**	WET testing is not usually recommended, since the potential for effluent toxicity is low.
15 - 24 (ACUTE) **20 - 24 (CHRONIC)**	**2 tests, per 5 year term**	Two tests each 5 years are recommended, since a few factors are present which cause concern. In order to insure that testing continues until permit reissuance, permit language should require that testing be done every other year, starting in year 2, until the permit is reissued (i.e., year 2, year 4, year 6, etc.). Tests should be required in different seasons, where possible.
25 - 34	**3 tests, per 5 year term**	3 tests each 5 years are recommended, due to a modest level of concern about toxicity. In order to insure that testing continues until permit reissuance, permit language should require that testing be done every other year, starting in year 1, until the permit is reissued (i.e., year 1, year 3, year 5, year 7, etc.). Tests should be required in different seasons, where possible.
35 - 44	**1x yearly**	One test is recommended each year during the permit term, due to a moderate level of concern about toxicity. Tests should be performed once each year, in successive quarters.
45 - 64	**2x yearly**	Two tests are recommended for each year during the permit term, due to a medium level of concern about toxicity. Tests should be performed during the 1st & 3rd quarters in odd numbered permit years (i.e., year 1, year 3, etc.) and the 2nd & 4th quarters in even numbered years (i.e., year 2, year 4, etc.).

65 - 84	**Quarterly**	Quarterly testing is recommended, due to a significant level of concern about effluent toxicity. Facilities that fall into this category usually have data that shows toxicity has been present.
≥ 85	**Bimonthly**	Testing every other month is recommended during the permit term, due to a substantial level of concern about toxicity. Facilities that fall into this category have historical data that shows toxicity to be present, and possibly data which shows an environmental impact has occurred due to the discharge. Tests should be performed at least 30 days apart, where possible.

Regardless of point totals, the following are true

- If the discharge is from a major municipal or primary industry, **a minimum** of annual acute & chronic (based on dilution) is recommended.
- If a limit is given, **a minimum** of annual acute & chronic (based on dilution) is recommended.

Wisconsin's State Lab

The UW-Madison's State Laboratory of Hygiene (SLH) Environmental Toxicology Section serves as a source of technical expertise and testing for staff. In situations where the Checklist recommends little or no monitoring, but staff determine during the permit term that more data is needed, the Environmental Toxicology Section can be asked to do testing in addition to that required in the permit. WET tests conducted at the SLH may be used to generate additional data for use in WET determinations, but cannot be used as a replacement or credit towards permit-required testing. You may contact the Biomonitoring Coordinator (Kari.Fleming@wisconsin.gov) to request a WET test.

Calculation and Expression of WET Limits

The following shows how acute and chronic WET limits are calculated, according to 40 CFR Part 132, Appendix F, Procedure 6 (D) and s. NR 106.09, Wis. Adm. Code.

In cases where no acute mixing zone has been approved, the acute WET limit will equal 1.0 Acute Toxic Units (TU_a). In cases where mixing zone studies or other information has been submitted and a zone of initial dilution (ZID) has been approved for the outfall, the acute WET limit is set at the edge of the approved acute mixing zone, as specified in s. NR 106.09 (2) (e), Wis. Adm Code, and described below. Acute WET limits must be expressed as a daily maximum, as specified in s. NR 106.09(2)(f), Wis. Adm. Code.

- **Acute WET Limit = 1.0 Toxic Unit (TU_a)** if no approved acute mixing zone
- **Acute WET Limit = 100/AMZ TU_a**, if a zone of initial dilution is allowed pursuant to s. NR 106.06 (3) (c), Wis. Adm. Code.

  AMZ = acute mixing zone concentration (see s. NR 106.09(2)(e), Wis. Adm. Code)

Chronic WET limits are set at the edge of the chronic mixing zone, using the applicable instream waste concentration, according to s. NR 106.09 (3) (c), Wis. Adm. Code. Chronic WET limits must be expressed as a monthly average, as specified in NR 106.09(3)(d), Wis. Adm. Code.

- **Chronic WET Limit = 100/IWC Toxic Units (TU_c)**

  IWC = instream waste concentration

Reasons For Adjusting WET Monitoring Recommendations

As noted above, the WET Checklist is intended to help staff evaluate site-specific factors and to make appropriate WET monitoring recommendations, final monitoring recommendations should be made by WQBEL staff based on their best professional judgment and knowledge of site-specific factors related to the discharge. Some example reasons why staff might adjust WET Checklist recommendations are given below.

Previous permit's tests. Tests that were required but not completed during the last permit term (e.g., postponed during a toxicity reduction evaluation, retests not completed, etc.) should be added to the next permit term's recommendations.

Delay at permit reissuance. A period of 1-3 months may be allowed between reissuance and the first permit-required WET test, in order to provide time for laboratory scheduling. Other reasons may exist, such as an upgrade or significant modification, which may warrant a delay between reissuance and WET testing. Staff should use their judgment to determine when adjustments are needed and document their reasons in the WQBEL memo or fact sheet, so that others can tell why decisions were made.

Seasonal discharges. If a discharge is noncontinuous or seasonal, staff should adjust recommendations so that tests would be done when the factors of concern that are driving the need for WET testing are present. For example, if the use of additives is a factor that contributed to WET monitoring recommendations, then testing should be done during periods of additive use. Or if the discharge will not occur long enough in a given year for the recommended monitoring frequency to be completed, the amount of testing should be adjusted accordingly. Additional guidance regarding monitoring frequencies and sampling schedules for seasonal or intermittent dischargers is given in Chapter 1.6.

Distance from a non-variance waterbody or higher amounts of available dilution. As discussed earlier in this chapter and in Chapter 1.2, chronic WET monitoring may not be recommended if the discharge is located greater than 4 miles from the nearest downstream non-variance classified waterbody or if available dilution is high (> 100 : 1 stream flow to effluent flow ratio). However, it is important to realize that this may not be appropriate in all situations. If data exists which suggests a higher potential for chronic toxicity (for example, if previously performed chronic WET tests have failed), it may be necessary to require chronic monitoring to insure that receiving water impacts are not occurring. If staff feel there are reasons to require chronic monitoring in these situations, they should make appropriate adjustments to the monitoring recommendations and document their reasons for doing so.

Groundwater remediation and other remediation type discharges. Where it has been determined that there is a need for WET testing of a discharge of this type, testing should begin as soon as possible after the discharge commences (first test usually within 90 days). These discharges are often of a short duration and any delays in testing may make testing difficult.

WWTP upgrades or other modifications. If a permit is to include a compliance schedule for a WWTP upgrade or another similar action that is expected to significantly change toxicity, WET monitoring may be postponed until that action is completed. The WET Checklist should be completed using the WET data and other factors that exists at the time of permit issuance, since it is necessary for the permittee to demonstrate that the upgrade has reduced their potential, unless data is present which shows that the WWTP improvements or other actions are certain to remove toxicity.

Compliance Schedules/Toxicity Reduction Evaluations. WET monitoring may be delayed during a WET limit compliance schedule and/or when a toxicity reduction evaluation (TRE) is being conducted. Chapter 1.12 provides guidance related to compliance schedules and Chapter 2.2 discusses TREs.

Permit terms of less than 5 years. The WET Checklist was designed for assessing discharges at the time of permit reissuance and its recommended monitoring frequencies are based on the standard 5 year permit term. Staff may use the Checklist during permit modifications or when permits are to be reissued for shorter than 5 year terms to assess a

discharge's toxicity potential, however, judgment should be used to adjust recommended frequencies to fit into the term of the reissued or modified permit. For example, if a modification is occurring with only 1 year left in the permit term and 3 tests are recommended, staff should determine whether 3 tests should be done during that year or if less testing would be sufficient to characterize the toxicity of the discharge.

Water quality variances. It may be appropriate to modify monitoring frequencies, test methods, or other WET requirements when a permittee has been granted a variance for a toxic compound that has the potential to cause (or may have already caused) WET failures. Changes to WET requirements should only be allowed if the permittee can demonstrate to the Department that the substance for which they were granted a variance is the only source of toxicity (i.e., the permittee would not be exempt from other toxicity sources) and should only be granted for the period for which the variance has been granted. All proposed WET monitoring, limit, or method changes due to variances should be discussed with the Biomonitoring Coordinator and documented in the permit file so that others can tell why changes were made. See Chapter 2.10 for a discussion related to WET monitoring and limits when a variance has been granted for chloride.

WET Limits as an Alternative to Secondary Values. Section NR 106.07(7), Wis. Adm. Code, states that the Department may establish a WET limitation according to s. NR 106.09, Wis. Adm. Code, as an alternative to a chemical-specific WQBEL based on a fish and aquatic life secondary acute or secondary chronic value determined according to ss.NR 105.05(4) and 105.06(6). The alternative WET limit has to meet all the following conditions:

1. The fathead minnow (*Pimephales promelas*) or the cladoceran *Ceriodaphnia dubia* were represented in the toxicological database used to generate the secondary value;
2. The permittee has requested the alternative WET limitation; and
3. WET testing shall be conducted at least once every three months during the entire term of the permit.

Deficiency Toxicity. Deficiency toxicity is defined as a condition where organisms are unable to survive because the surrounding water is lacking the necessary ions (e.g., sodium, calcium, magnesium, potassium, etc.) that must be available for them to survive. It is the opinion of DNR toxicologists that deficiency toxicity presented in a WET test will not have deleterious effects on receiving water organisms, as long as the necessary ions are introduced as soon as the effluent contacts receiving water, soils, or sediments. If it can be demonstrated that positive WET results are due to deficiency toxicity only, it is reasonable to allow WET monitoring frequencies to be reduced. The following guidance is provided for those who wish to make such a demonstration:

In order to show that toxicity is caused by deficiency toxicity, the following may be demonstrated:

1. Hardness (as $CaCO_3$) in the unaltered sample (i.e., the wastewater as it is discharged) is < 45 mg/l;
2. Mortality in the *Ceriodaphnia dubia* test, in unaltered sample, is > 50%; and
3. The permittee has WET data, involving *C. dubia*, from at least 2 tests that includes the following:
   a) parallel tests with unadjusted vs. adjusted (to 45 mg/l hardness) sample, using reagents that have been added proportionally according to Figure 8:

Figure 8. Deficiency Toxicity Hardness Adjustment

RECIPE FOR EFFLUENT SAMPLE HARDNESS ADJUSTMENT

REAGENT ADDED (mg/l)			
$NaHCO_3$	$CaSO_4H_2O$	$MgSO_4$	KCl
48.0	30.0	30.0	2.0

   b) Tests should include 4 replicates of at least 5 organisms in each; and
   c) The observed mortality in the altered sample is $\leq$ 10%.

If staff believe that deficiency toxicity exists, language may be placed in the permit allowing for a study similar to that above and for the dropping of WET monitoring after a successful demonstration. This demonstration should be made for each reissuance (exemptions from WET testing should only apply to one permit term).

Deficiency toxicity in condensate of whey (COW) discharges. Historically, it was believed that deficiency toxicity was responsible for results from WET tests at COW water discharges. Since the wastewater in these situations was thought to be only made up of condensate, the necessary ions were believed absent. In 1994-95, a study was conducted at the State Laboratory of Hygiene (SLH), in cooperation with the DNR and 17 dairies, in an attempt to identify the cause of COW water toxicity. Study results showed: 1 not acutely toxic, 4 acutely toxic due to deficiency toxicity, 4 acutely toxic due to deficiency toxicity <u>and</u> ammonia, and 8 acutely toxic for unknown reasons (i.e., it was not deficiency or ammonia toxicity). Based on these results, it is obvious that it is necessary to continue to evaluate the potential for WET in COW water, however, we may allow COW water dischargers to demonstrate that WET results are impacted by deficiency toxicity, as described above. The following language may be used to allow demonstrations in COW discharge permits (this language is available as a standard requirement choice in SWAMP):

> *"If discharges consisting of condensate of whey (COW) wastewater only or non-contact cooling water mixed with COW waters (NCCW/COW) pass the first two acute toxicity tests and the first two chronic toxicity tests (if chronic toxicity testing is required) then the permittee is not required to perform additional toxicity testing during this permit term. If positive toxicity is experienced in any of the first two acute or chronic toxicity tests, the permittee may attempt to demonstrate that toxicity is due to ion deficiency. If it can be demonstrated that ion deficiency is the sole cause of toxicity in at least two consecutive positive tests, and the Department agrees in writing, the permittee will not be required to perform additional toxicity testing during this permit term. If it cannot be demonstrated that ion deficiency is the sole cause of toxicity in these tests, the permittee must complete the remaining toxicity tests."*

Attachment 1: Example Reasonable Potential Calculations

Example 1: Acute WET Reasonable Potential Evaluation (no approved ZID, WET failure present)

Date initiated	Acute WET Results		Maximum TUc (100/LC50)	Pass/ Fail
	C. dubia LC50	Fathead LC50		
12/31/2010	>100%	>100%	1.0	Pass
03/25/2011	>100%	>100%	1.0	Pass
06/10/2012	>100%	>100%	1.0	Pass
09/22/2013	85%	>100%	1.18	Fail
12/09/2014	>100%	>100%	1.0	Pass

According to NR 106.08(6)(b), if a zone of initial dilution (ZID) has not been approved, reasonable potential to exceed the acute WET criterion is present when: (TUa effluent) (B) > 1.0, where TUa effluent is the most sensitive TUa in the data set and B is the multiplication factor from NR 106.08(5)(c), Table 4.

- Most sensitive result: LC50 = 85%; Maximum TUc = 100/85 = 1.18
- 5 WET tests in dataset, 1 toxicity detect. Multiplication factor (B) from NR 106.08(5)(c), Table 4 = 6.2 (based on # of detects)

1.18 x 6.2 = 7.32

7.32 > 1.0, RP shown, limit is required (limit = 1.0 TUa)

Example 2: Acute WET Reasonable Potential Evaluation (no approved ZID, no WET failure present)

Date initiated	Acute WET Results		Maximum TUc (100/LC50)	Pass/ Fail
	C. dubia LC50	Fathead LC50		
12/31/2010	>100%	>100%	1.0	Pass
06/10/2012	>100%	>100%	1.0	Pass
09/22/2013	>100%	>100%	1.0	Pass
12/09/2014	>100%	>100%	1.0	Pass

According to NR 106.08(6)(d), TUc and TUa effluent values are equal to zero whenever toxicity is not detected (i.e., when the LC50, IC25, or IC50 > 100%).

0 < 1.0, RP not shown, no limit is required

Example 3: Acute WET Reasonable Potential Evaluation (with an approved ZID)

Date initiated	Chronic results		Maximum TUc (100/LC50)	Pass/ Fail
	C. dubia LC50	Fathead LC50		
12/31/2010	>100%	>100%	1.0	Pass
03/25/2011	>100%	>100%	1.0	Pass
06/10/2012	65%	>100%	1.54	Pass
09/22/2013	35%	>100%	2.85	Fail
12/09/2014	>100%	>100%	1.0	Pass

According to NR 106.08(6)(b), if a zone of initial dilution has been approved, reasonable potential to exceed the acute WET criterion is present when: [(TUa effluent) (B) (AMZ)] > 1.0, where TUa effluent is the most sensitive TUa

in the data set and B is the multiplication factor from NR 106.08(5)(c), Table 4, and AMZ is the acute mixing zone concentration based on the zone of initial dilution approved according to NR 106.06 (3)(c).

- AMZ = 57% (0.57)
- Most sensitive result: LC50 = 35%; Maximum TUc = 100/35 = 2.85
- 5 WET tests in dataset, 2 toxicity detects. Multiplication factor (B) from NR 106.08(5)(c), Table 4 = 3.8 (based on # of detects)

2.85 x 3.8 x 0.57 = 6.17

6.17 > 1.0, RP shown, limit is required (limit = 100/AMZ = 1.75 TUa)

Example 4: Chronic WET Reasonable Potential Evaluation (toxicity detected well below the limit, no WET failures)

Date initiated	Chronic results		Maximum TUc (100/IC25)	Pass/ Fail
	C. dubia IC25	Fathead IC25		
12/31/2010	>100%	>100%	1.0	Pass
03/25/2011	>100%	>100%	1.0	Pass
06/10/2012	>100%	>100%	1.0	Pass
09/22/2013	85%	90%	1.18	Pass
12/09/2014	>100%	>100%	1.0	Pass

According to NR 106.08(6)(b), reasonable potential to exceed the chronic WET criterion is present when: [(TUc effluent) (B) (IWC)]> 1.0, where TUc effluent is the most sensitive TUc in the data set, B is the multiplication factor from NR 106.08(5)(c), Table 4, and IWC is the instream waste concentration calculated according to NR 106.03(6), Wis. Adm. Code.

- IWC = 10% (0.10)
- Most sensitive result: IC25 = 85%; Maximum TUc = 100/85 = 1.18 (test passed; toxicity detected well above the IWC)
- 5 WET tests in dataset, 1 toxicity detect. Multiplication factor (B) from NR 106.08(5)(c), Table 4 = 6.2 (based on # of detects)

1.18 x 6.2 x 0.10 = 0.73

RP <u>not</u> shown, limit is not required

Example 5: Chronic WET Reasonable Potential Evaluation (toxicity detected near to the limit, but no WET failures)

Date initiated	Chronic results		Maximum TUc (100/IC25)	Pass/ Fail
	C. dubia IC25	Fathead IC25		
12/31/2010	>100%	>100%	1.0	Pass
03/25/2011	>100%	>100%	1.0	Pass
06/10/2012	>100%	>100%	1.0	Pass
09/22/2013	55%	90%	1.82	Pass
12/09/2014	>100%	>100%	1.0	Pass

- IWC = 25% (0.25)
- Most sensitive result: IC25 = 55%; Maximum TUc = 100/55 = 1.82 (test passed; toxicity detected nearer to the IWC)
- 5 WET tests in dataset, 1 toxicity detect. Multiplication factor (B) from NR 106.08(5)(c), Table 4 = 6.2 (based on # of detects)

1.82 x 6.2 x 0.25 = 2.82

RP shown, limit is required (limit = 100/IWC = 4.0 TUc)

Attachment 2: NR 105 and Additional Compounds of Concern

CATEGORY	SUBSTANCES	
	ACUTE	CHRONIC
WQBEL required 5 pts for 1st + 1 for each additional, not to exceed 15 pts.	Ammonia[1], Arsenic, Cadmium, Chloride, Chlorine, Chlorpyrifos, Chromium, Copper, Cyanide, Dieldrin, Endrin, Gamma-BHC, Lead, Mercury, Nickel, Parathion, Pentachlorophenol, Toxaphene, Zinc	Ammonia[1,2], Arsenic, Cadmium, Chloride, Chlorine, Chromium, Copper, Cyanide, Dieldrin, Endrin, Lead, Mercury, Nickel, Parathion, Pentachlorophenol, Zinc
Substance detected, but no WQBEL needed 1 point each, not to exceed 3 pts	Substances above detected in the effluent (including those given chronic WQBEL), but not given acute WQBEL	Substances above detected in the effluent (including those given acute WQBEL), but not given chronic WQBEL
"Additional Compounds of Concern" detected 2 points given if any detected	Any substances in "Additional Compounds of Concern" table below detected in the effluent	Any substances in "Additional Compounds of Concern" table below detected in the effluent

[1] Ammonia limits should be counted only if representative effluent data demonstrates the need for a WQBEL (limits that are simply "carried over" from a previous permit term, even though effluent data suggests they are no longer needed, should not be counted as WQBEL limits). If ammonia has been detected in the effluent, it should be counted as described in the second row of the table above.

[2] Ammonia WQBELs based on 4-day chronic toxicity criteria and expressed in permits as weekly average limitations should be counted. WQBELs based on 30-day criteria and expressed as monthly averages are not indicative of conditions in chronic WET tests (since chronic tests last 7 days) and should not be counted. If ammonia has been detected in the effluent, it should be counted as described in the table above.

Information given above is from Tables 1 & 2 (acute) and Tables 3 & 4 (chronic), in ch. NR 105, Wis. Adm. Code, March 2004. Users should check recent versions of the code to make sure that they are using the most up-to-date lists.

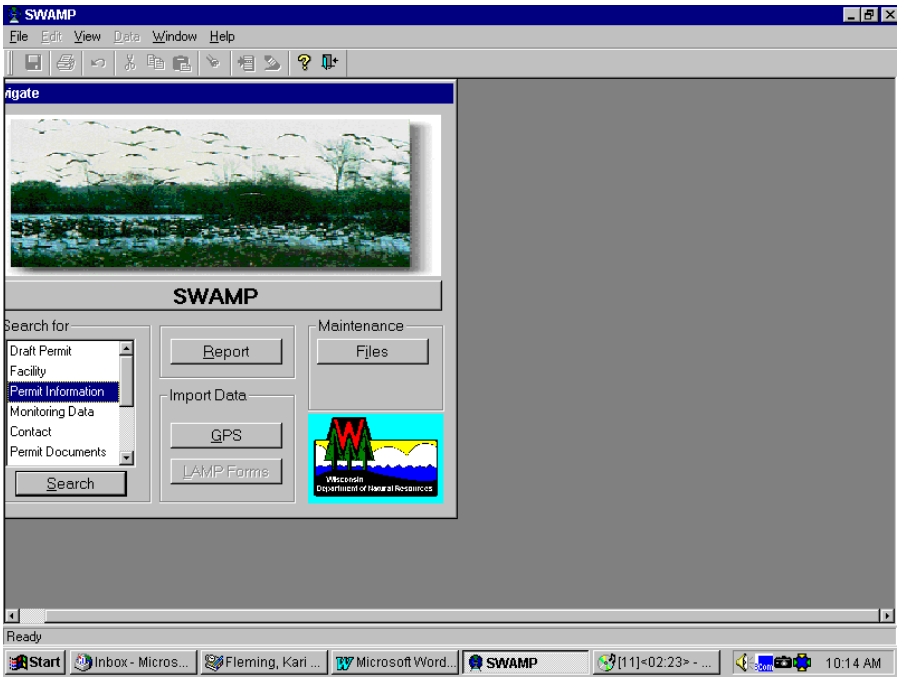
ADDITIONAL COMPOUNDS OF CONCERN (ACC)

Metals:	**Acid-Extractable Compounds:**	2,6-Dinitrotoluene	2,4-Dichlorophenoxyacetic acid
Antimony	P-Chloro-M-Cresol	Di-n-octyl Phthalate	Endosulfan
Beryllium	2-Chlorophenol	1,2-Diphenylhydrazine	Endosulfan Sulfate
Selenium	2,4-Dichlorophenol	Fluoranthene	Endrin Aldehyde
Silver	2,4-Dimethylphenol	Fluorene	Guthion
Thallium	4,6-Dinitro-O-Cresol	Hexachlorobenzene	Heptachlor
	2,4-Dinitrophenol	Hexachlorobutadiene	Heptachlor Epoxide
Volatile Organic Compounds:	2-Nitrophenol	Hexachlorocyclopentadiene	Malathion
Acrolein	4-Nitrophenol	Hexachloroethane	Methoxychlor
Acrylonitrile	Phenol	Indeno(1,2,3-cd)pyrene	PCBs
Benzene	2,4,6-Trichlorophenol	Isophorone	
Bromoform		Naphthalene	**Dioxin:**
Carbon Tetrachloride	**Base-Neutral Compounds:**	Nitrobenzene	2,3,7,8-TCDD (dioxin)
Chlorobenzene	Acenaphthene	N-Nitrosodimethylamine	
Chlorodibromomethane	Acenaphthylene	N-Nitrosodiphenylamine	**Other Non-Priority Pollutants:**
Chloroethane	Anthracene	N-Nitrosodipropylamine	Aluminum
2-Chloroethyl vinyl ether	Benzidine	N-Nitrosodiethylamine	Asbestos
Chloroform	Benzo(a)anthracene	N-Nitrosodi-n-butylamine	BHC-tech. grade
1,2-Cisdichloroethylene	Benzo(a)pyrene	N-Nitrosopyrrolidine	Bis(2-chloromethyl)ether
Dichlorobromomethane	3,4-Benzofluoranthene	Octachlorostyrene	3-Chlorophenol
1,1-Dichloroethane	Benzo(ghi)perylene	Pentachlorobenzene	4-Chlorophenol
1,2-Dichloroethane	Benzo(k)fluoranthene	Phenanthrene	Dichlorodifluoromethane
1,1-Dichloroethylene (vinylidene	Bis(2-chloroethoxy)methane	Pyrene	2,3-Dichlorophenol
chloride)	Bis(2-chloroethyl)ether	1,2,3,4-Tetrachlorobenzene	2,5-Dichlorophenol
1,2-Transdichloroethylene	Bis(2-chlorisopropyl)ether	1,2,4,5-Tetrachlorobenzene	2,6-Dichlorophenol

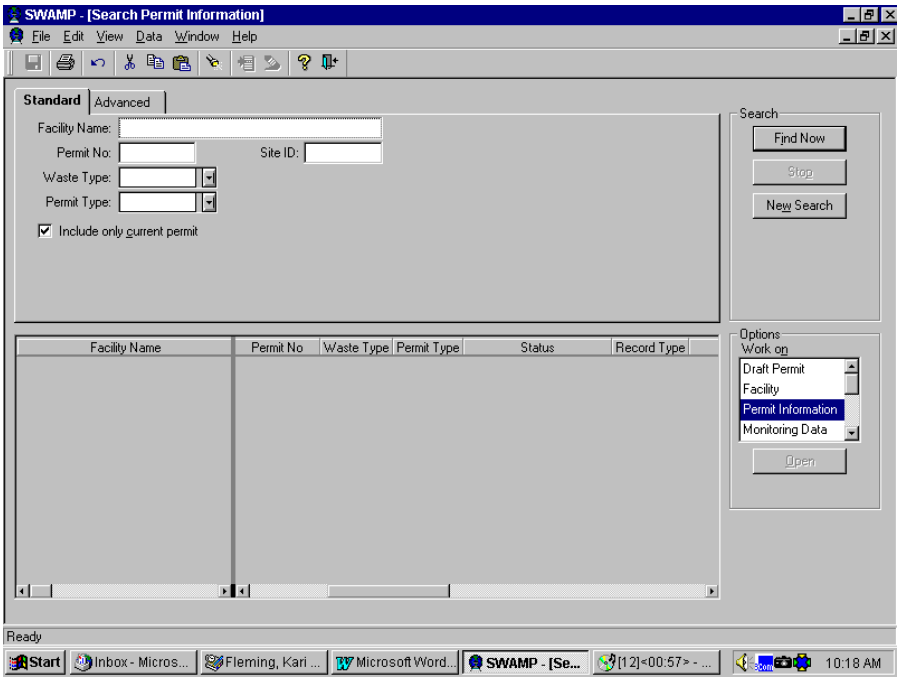
1,2-Dichloropropane	Di(2-ethylhexyl)phthalate (DEHP)	1,2,4-Trichlorobenzene	3,4-Dichlorophenol
1,1-Dichloropropene	4-Bromophenyl Phenyl Ether		1,3-Dichloropropane
2,3-Dichloropropene	Butyl benzyl phthalate	**Pesticides:**	2,3-Dinitrophenol
1,3-Dichloropropene	2-Chloronaphthalene	Aldrin	Fluoride
Ethylbenzene	4-Chlorophenyl Phenyl Ether	Alpha-BHC	Formalin
Methyl Bromide	Chrysene	Beta-BHC	Iron
Methyl Chloride	Dibenzo(a,h)anthracene	Delta-BHC	2-Methyl-4-Chlorophenol
Methylene Chloride	1,2-Dichlorobenzene	Chlordane	3-Methyl-6-Chlorophenol
1,1,2,2-Tetrachloroethane	1,3-Dichlorobenzene	Chlorpyrifos	Mirex
Tetrachloroethylene	1,4-Dichlorobenzene	4,4'-DDD	Photomirex
Toluene	3,3'-Dichlorobenzidine	4,4'-DDE	2,3,4,6-Tetrachlorophenol
1,1,1-Trichloroethane	Diethyl Phthalate	4,4'-DDT	Trichlorofluoromethane
1,1,2-Trichloroethane	Dimethyl Phthalate	Diazinon	2,4,5-Trichlorophenol
Trichloroethylene	Di-n-butyl Phthalate		
Vinyl Chloride	2,4-Dinitrotoluene		

Attachment 3: Changing Data in the Sample Point Table of SWAMP

In order for the WET Checklist to make decisions regarding the instream waste concentration (IWC), stream flow to effluent flow ratios, and other WET determinations, information regarding effluent flow (Qe), the fraction of Qe withdrawn from the receiving water (RW), RW flow ($Q_{7,10}$), and RW classification must be entered in the "Sample Point" table. This information must be entered before creating a new Checklist or revising an existing Checklist. This attachment includes instructions on how to enter this data into the Sample Point table.

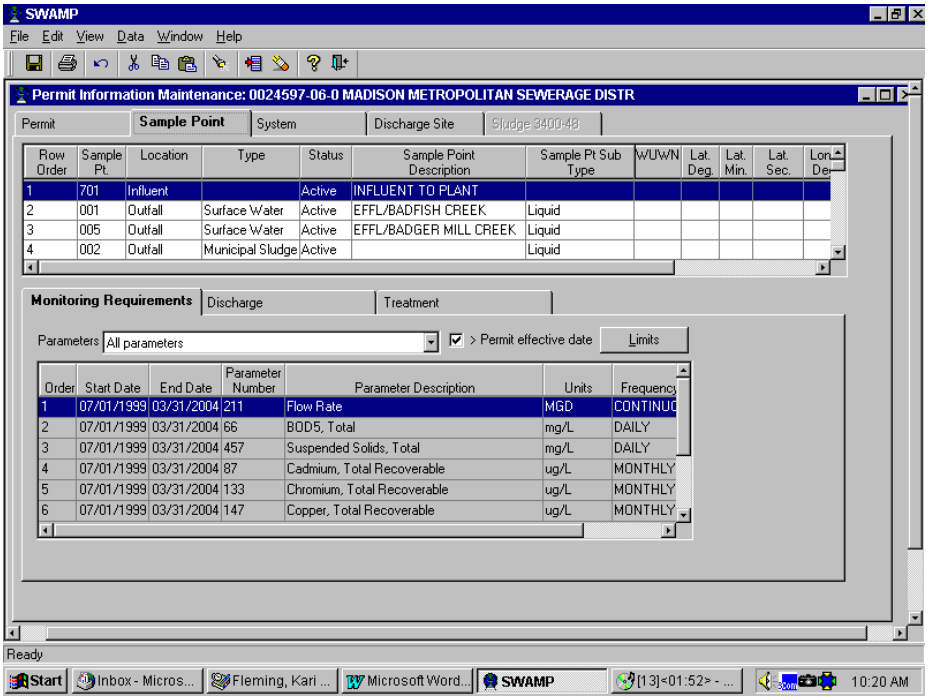


At the Navigate screen, click on "Permit Information" in the "Search for:" box, then click on the "Search" button.

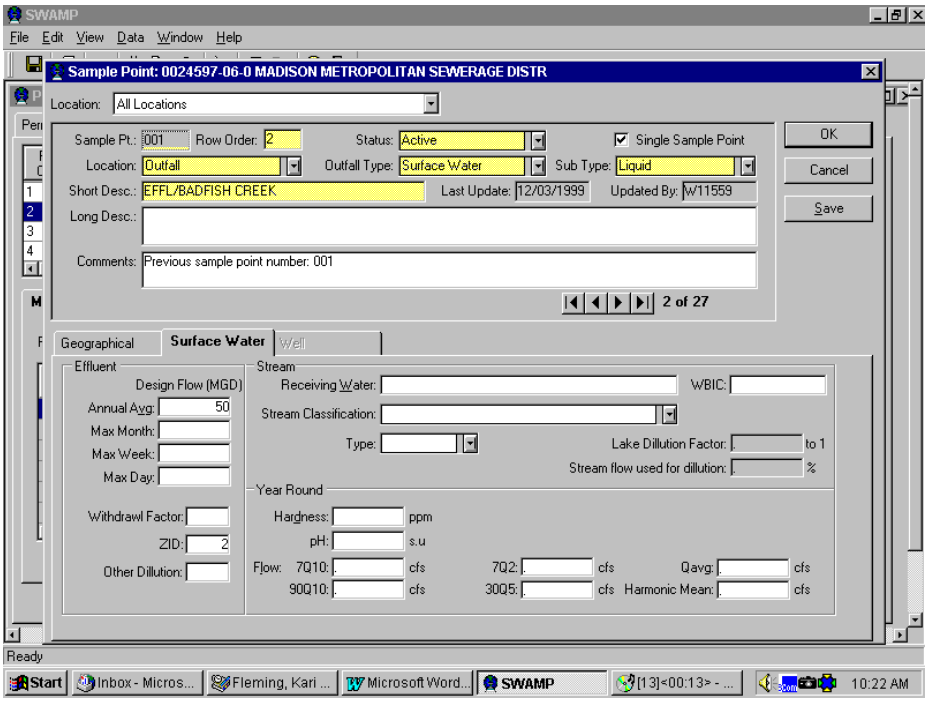


When this “Search Permit Information” screen appears, enter the facility name, FIN, Site ID, or permit number and then click on the “Find Now” button. The facility name and permit number will appear in the “Facility Name” box on the bottom half of the screen. Click on the name or permit number for the facility you are interested in and then click on the “Open” button.

In the “Permit Information Maintenance” screen (below), click on the “Sample Point” tab.



Double-click on the surface water outfall that you are interested in, then click on the “surface water” tab.



The effluent flow (Q_e) used in the WET Checklist is usually the annual average design flow for municipals or average annual actual flow for industrial dischargers. The Checklist will use this Q_e to determine the appropriate $Q_{7,10}$:Q_e ratio, IWC, and chronic dilution series. The withdrawal factor (f) should be entered as a decimal (for example, if the facility withdraws and uses 1/2 of the receiving water flow, enter 0.5). This value will be used as "f" in the IWC calculation. The $Q_{7,10}$ entered here is also used to determine the $Q_{7,10}$:Q_e ratio (used to determine need for acute and/or chronic testing), IWC, and to choose the chronic dilution series. Once the Q_e, $Q_{7,10}$, and f values are entered in the sample point table, return to the previously discussed screen in the WET Checklist.

CHAPTER 1.5 - WET Data Reporting, Review, and Interpretation

This chapter describes the WET test review process generally followed by the Biomonitoring Coordinator and provides guidance on the evaluation of dose-response information, as suggested in section 5.3 of the Methods Manual (s. NR 219.04, Wis. Adm. Code).

WET Test Report Forms

According to the Methods Manual (s. NR 219.04, Wis. Adm. Code), WET Test Report Forms must be submitted for demonstrating test completion and compliance with a WPDES permit. Instructions for completing the WET Test Report Form are found in Section 6 of the Methods Manual. According to that section, report forms (and any attachments) must contain all of the information needed to determine compliance with permit-required WET testing. That means that the test form must be filled out in its entirety, including a description of any abnormal test or sampling procedures, effluent conditions, or sample manipulations that may have been present during the test. The permittee and lab should provide any attachments or additional information which they believe to be relevant to the test. All other test documentation (e.g. bench sheets, record books, etc.) needed to fulfill Methods Manual requirements must be maintained at the lab for laboratory certification purposes (see Section 3.16, of the Methods Manual).

WET Test Report Forms should include observations made by the permittee or lab that may influence test results or data interpretation, such as: 1) unusual conditions (e.g., plant upsets, slug loads, weather conditions, etc.) during sampling periods, 2) deviations from test specifications or any sample manipulation (aeration, filtration, addition of chemicals, etc.) that is determined to be necessary for successful completion of a test, and/or 3) unusual behavior or appearance of test organisms (e.g., young developed in the brood pouch of the adults, but not released during the exposure period; partially or fully developed young released, but all dead at the end of the 24-h period; lethargy, hyperactivity, spots or filaments, discoloration, excessive ventilation, etc.).

WET Data Review

The Methods Manual (s. NR 219.04, Wis. Adm. Code) requires that the original, complete, signed version of the WET Test Report Form be sent to the Biomonitoring Coordinator (Bureau of Water Quality, 101 South Webster St., P.O. Box 7921, Madison, WI 53707-7921) by the date specified in the WPDES permit.

The Biomonitoring Coordinator (Kari.Fleming@wisconsin.gov) is primarily responsible for the review and interpretation of WET test results submitted for WPDES compliance, including the application of best professional judgment in the recognition and investigation of unusual test results. Once received, the Coordinator reviews results to determine if tests were acceptable and whether the effluent passed or failed. The Coordinator also provides technical advice and support to Department staff, permittees, and laboratories who make decisions about WET-related conditions. The Coordinator often works closely with permittees and their laboratories to evaluate and interpret WET test and toxicity reduction evaluation (TRE) information, especially in cases where effluent toxicity has been encountered. The following chapter describes some of the information that is considered during the WET data review process.

Test conditions. The Methods Manual (s. NR 219.04, Wis. Adm. Code) requires certain conditions (e.g., temperature, number of replicates, chamber size and volume, lighting, etc.) to be followed in all tests submitted for WPDES compliance. Before submitting results to the Department, the results of each test should be reviewed by permittees and lab staff to ensure that conditions were met as required by the Methods Manual. Any deviations from Methods Manual requirements should be clearly reported on the report form. The Biomonitoring Coordinator will check and attempt to verify this information while reviewing WET test data.

Reference toxicant testing. Reference toxicant tests (RTT) are tests that are conducted under the same conditions as effluent tests, substituting a known toxicant for effluent samples (Methods Manual Section 3.15, s. NR 219.04, Wis. Adm. Code). Reference toxicant testing is an important quality control practice that is required in order to 1) determine the sensitivity of the test organisms over time, and 2) assess the quality and comparability of within- and between-laboratory test results. RTT results can be used to help identify potential sources of variability, such as test organism health, difference among batches of organisms, changes in laboratory water or food quality, and performance by laboratory technicians. By standardizing RTT, test results can be compared within the same laboratory and between different laboratories.

Section 3.15 of the Methods Manual (s. NR 219.04, Wis. Adm. Code) requires RTT to be done monthly for each test method routinely conducted in a laboratory. A control chart must be maintained for each combination of species and test condition. A control chart is a running plot of the 20 most recent test endpoints. These charts can be used to evaluate the cumulative trend of these endpoints, which are examined to insure that they are within prescribed limits.

The Biomonitoring Coordinator regularly evaluates the test results and control charts submitted by each laboratory. Data are reviewed to look for outliers (values falling outside the upper and lower control limits) and trends of increasing or decreasing sensitivity. If it is determined that a series of reference toxicant tests are out of an acceptable range, effluent testing conducted during the same period may be rejected (Section 3.15 Methods Manual, s. NR 219.04, Wis. Adm. Code). Lab performance is expected to improve with experience and control limits generated by experienced, quality labs should gradually narrow over time.

Water chemistry data. Receiving water and effluent data for hardness, alkalinity, pH, total ammonia, and total residual chlorine are reported on WET Test Report Forms so that general sample characteristics can be assessed to determine their potential impact on test results. Values reported for hardness, alkalinity, and ammonia are from measurements taken upon arrival at the lab. Values reported for chlorine and pH are from measurements taken after samples have been warmed to test temperatures and just prior to use in tests. (Section 6 of the Methods Manual, s. NR 219.04, Wis. Adm. Code.)

Test acceptability criteria. The Methods Manual (s. NR 219.04, Wis. Adm. Code) provides criteria for each test type that must be met in order for WET tests to be acceptable. For example, each test method sets a minimum survival amount for control organisms. Test acceptability criteria are set in order to insure that any effects noted during WET tests are due to the effluent being tested, and not due to dilution water, lab error, or other factors. Tests not meeting test acceptability criteria may be considered invalid and repeat tests required (see Section 3.8 and 3.9 of the Methods Manual for details).

Organism response data. WET test results must be presented tabularly and graphically on WET Test Report Forms (Methods Manual Section 6, s. NR 219.04, Wis. Adm. Code). Replicate and mean survival, growth, and/or reproduction data are plotted against test concentrations in graphs for each species tested. Graphs give a visual picture of the dose-response, variability of the data, and highlight suspicious data and potential outliers.

Interpretation of Dose-Response Information

According to section 4.6 of the Methods Manual (s. NR 219.04, Wis. Adm. Code), the tests required for use in determining WPDES permit compliance are multi-concentration tests which provide a point estimate of effluent toxicity in terms of a Lethal Concentration (LC) or an Inhibition Concentration (IC). The dose-response is a fundamental concept of toxicology and the basis for the determination of point estimates in WET testing. This concept assumes that there is a relationship between the dose (or concentration) of a toxicant and the measured response. Typically, more severe responses are expected at higher concentrations and less severe responses at lower concentrations, as demonstrated in Figure 1.5.1 below. A biological response (mortality, growth, or reproduction) is measured at a range of effluent concentrations to develop a dose-response curve. From the resulting curve, the LC or IC point estimate effect concentration is calculated (see Figure 1.5.2). The effect concentration is an estimate of the concentration of effluent that will produce a specific level of response (e.g., 50% mortality, 25% inhibition).

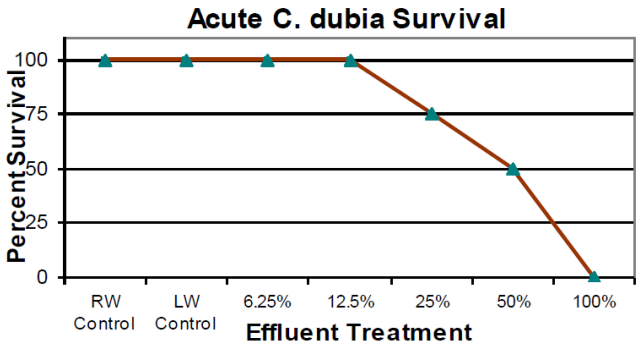


Figure 1.5.1. Example "normal" dose-response when toxicity is present.

Every toxicant should exhibit a dose-response relationship, assuming the appropriate response is measured and the concentration range evaluated is appropriate. Evaluation of the dose-response can be helpful in determining whether an effluent possesses toxicity and in identifying anomalous test results. Tests that exhibit unexpected dose-response relationships may indicate a need for further testing. As noted above, if a given effluent consistently produces an unusual dose-response, there is likely a physical, chemical or biological cause which should be further investigated.

Section 5.3 of the Methods Manual (s. NR 219.04, Wis. Adm. Code) requires dose-response relationships be reviewed to ensure that results are interpreted correctly. Section 5.3 states that based on the review of the concentration-response curve several determinations may be made, including: 1) the results are reliable and should be used for determining compliance, 2) the results are anomalous and should be explained, or 3) the results are inconclusive and tests should be repeated. Examples are given below of different dose-responses that may occur in WET data. Included are common patterns seen in toxicity test data, possible causes for unexpected patterns, and some discussion of when data may be suspect based on a certain dose-response (or lack thereof).

It should be noted that the determination of a valid dose-response is not always clear cut. Permittees and labs should review dose-response information, highlight potential problems on WET Test Report Forms, and discuss abnormalities with the Biomonitoring Coordinator. Tests that exhibit unexpected dose-response relationships may indicate a need for further investigation and possible retesting. Final decisions regarding the acceptability of tests based on dose-response information are made by the Biomonitoring Coordinator, as required by Section 5.3.3 of the Methods Manual (s. NR 219.04, Wis. Adm. Code). In general, when unexpected or apparently anomalous dose-response relationships are encountered, it is recommended that an effort be made to determine the cause. Review of specific test information or other (non-WET) effluent data may assist in determining a cause for an unexpected dose-response. Unexpected responses could be valid patterns or anomalies resulting from lab or sampling error, high test variability, or other causes. If a given effluent consistently produces an "unexpected" dose-response, there is likely a physical, chemical or biological cause.

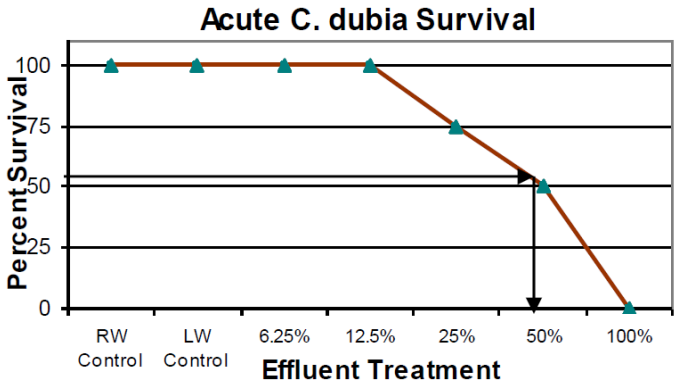


Figure 1.5.2. From the dose-response curve, a "point estimate effect

concentration" (LC_{50}) can be calculated. In this example, LC_{50} = 50% effluent.

Common Dose-Response Relationships

Several dose-response patterns are described below using hypothetical test data. Each section provides guidance for interpreting each dose-response pattern. The focus is on determining a cause for unexpected patterns by recommending a step-by-step review process. After thorough review it may be determined that it is appropriate to accept the calculated endpoint (LC_{50} or IC_{25}) as valid and reliable, the calculated results are anomalous, or that retesting is needed. Test results should be reported for all tests conducted, even if retesting is recommended.

This guidance on dose-response relationships is for informational purposes only and it is not intended to be used to recommend frequent disqualification or repetition of WET tests. Several conditions should be considered when using the guidance in this chapter. First, unexpected dose-response relationships should not occur with any regular frequency. Second, it is not recommended to reject only those tests in which toxicity is found at or below the concentration of concern. If screening is to be done for unexpected dose-response relationships, all tests should be screened in a similar manner. Third, all results must be reported to and reviewed by the Department, including those disqualified and repeated (Methods Manual Section 6.1.5, s. NR 219.04, Wis. Adm. Code). The Biomonitoring Coordinator will make final decisions about whether test results are to be rejected based on unusual dose-response patterns (Methods Manual, Section 5.3.3, s. NR 219.04, Wis. Adm. Code).

1) **Ideal dose-response relationship.** This response pattern (see Figure 1.5.1) shows a clear dose-response relationship, with multiple effluent concentrations identified as significantly different from the control. There is a monotonic decrease in response, meaning that the response steadily decreases for each higher effluent concentration. This pattern is indicative of a well-designed test with appropriately chosen concentrations that bracket the effluent's range of toxicity. Under these circumstances, point estimation techniques (LC_{50}, IC_{25}) recommended in the Methods Manual should provide reliable results.

2) **All or nothing response/significant effects only at highest concentration.** The "all or nothing" response pattern is very common in WET tests. This pattern (Figure 1.5.3) is characterized by a transition from no significant effect at one concentration to a complete effect at the next higher concentration. This response pattern is most often characterized by only the highest test concentration producing a significantly different response from the control. This pattern represents a valid dose-response relationship and point estimation techniques recommended in the Methods Manual should provide reliable results.

   When this pattern of response is shown, the concentrations used for testing should be re-evaluated for future tests, especially when the highest concentration is at or near the concentration of concern. The precision of future point estimates may be improved by closer spacing of effluent concentrations or the addition of intermediate concentrations. This approach should be used only if historical testing indicates that the effluent consistently shows this type of response and the effect concentration is not likely to fall below the adjusted test concentration series.

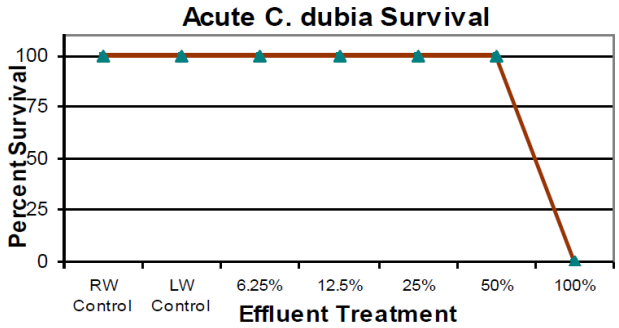


Figure 1.5.3 An example dose-response, showing an "all or nothing" response.

3) **Stimulatory response at low concentrations and detrimental effects at higher concentrations.** It is not uncommon for the lowest concentration in a toxicity test to demonstrate an effect that is greater than the control. The apparent stimulation of growth/reproduction by low toxicant doses is well known in pharmacology and toxicology and is referred to as hormesis. Hormesis can happen when an organisms biological system "overreacts" to a toxicant present at low levels. This is a well-known biological reaction and does not usually indicate an environmentally adverse effect. This dose-response pattern, while non-monotonic, is still a valid dose-response relationship and point estimation techniques required by the Methods Manual (s. NR 219.04, Wis. Adm. Code) should provide reliable results in most situations.

   A stimulatory response is characterized by an increase in response at low concentrations. This stimulation at low concentrations can be followed by a detrimental effect at higher concentrations (see Figure 1.5.4) or by no effect at higher concentrations (see 4.). The stimulatory pattern characterized in Figure 1.5.4 is more often found with chronic, sublethal endpoints such as reproduction and growth.

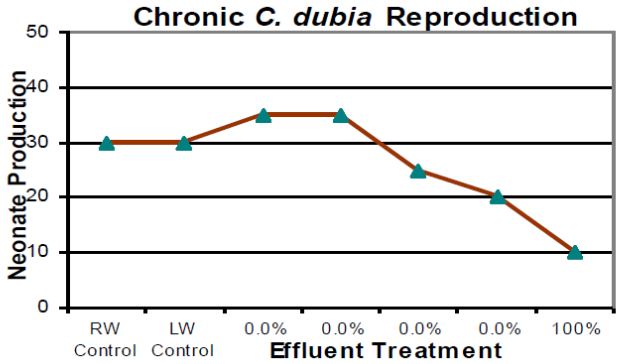


Figure 1.5.4 An example dose-response, showing stimulation at low concentrations and adverse effects at higher concentrations

4) **Stimulation at low concentrations but no significant effect at higher concentrations.** This dose-response relationship is similar to the previous example in that stimulation is observed at lower concentrations, but in this case, higher concentrations do not produce significant effects (see Figure 1.5.5). Results from point estimation techniques should be interpreted carefully when this response pattern is encountered, because statistical packages may result in endpoints that indicate toxicity at concentrations where the response is comparable to the control.

   For example, the inhibition concentration (ICp) procedure used for chronic tests assumes that responses are monotonically non-increasing, meaning that the mean response for each higher concentration is less than or equal to the mean response for the previous concentration. If this is not the case, the ICp uses a "smoothing" technique that averages means (including that of the control) with those of the next highest test concentration until responses

are monotonically non-increasing. In cases where the responses at the low effluent concentrations are much higher than the control, this smoothing process may result in a large upward adjustment in the control mean. This can lead to an ICp result that is less than the highest test concentration, even though this concentration was not significantly different from the control. If the response pattern shown in Figure 1.5.5 is encountered, the following should be done in addition to standard test review procedures:

a) **Evaluate the concentration range** - If the highest concentration was < 100% effluent, future tests should include higher concentrations to establish if a valid dose-response exists. This may not be necessary if the IWC is much < 100% and test results indicate no toxicity at that level and above.

b) **Evaluate control response** - It is possible that the response pattern shown in Figure 1.5.5 could result from poor control performance rather than stimulation at lower effluent concentrations. Poor control performance could cause a toxic effect at higher effluent concentrations to go undetected. To evaluate this possibility, compare the control response to the normal control performance for the laboratory. If 1) the test exhibits a response pattern similar to that shown in Figure 1.5.5 and 2) the control response is well below the laboratory's normal range of control performance, then retesting is recommended even if the minimum test acceptability criteria have been met. For example, if a laboratory usually achieves a control mean of 25-30 neonates in the *C. dubia* chronic test, a control mean of 15-18 neonates (in conjunction with a non-ideal dose-response curve) would warrant retesting. In this situation, suppressed control performance could be considered as the cause for this response pattern rather than stimulation. A review of control performance should also investigate the possibility of poor performance in a single replicate substantially reducing the mean control response. In this case, retesting is also recommended.

c) **Evaluate the ICp calculation** - If a test exhibits the pattern shown in Figure 1.5.5 and it has been determined that this is not due to poor control performance, then discrepancies may be due to bias from the ICp smoothing technique. To determine if this is the case, calculate the % difference between the response at the IWC and the control ([*mean response at IWC/mean control response] x 100*). If the observed percent difference between the response at the IWC and the control is < 25% and the response at the IWC is not statistically significantly different from the control response, then a calculated IC_{25} of less than the IWC should be noted as anomalous and the effluent determined to be non-toxic at the IWC. If the observed difference is $\geq$ 25%, then the calculated IC_{25} should be considered valid in most cases.

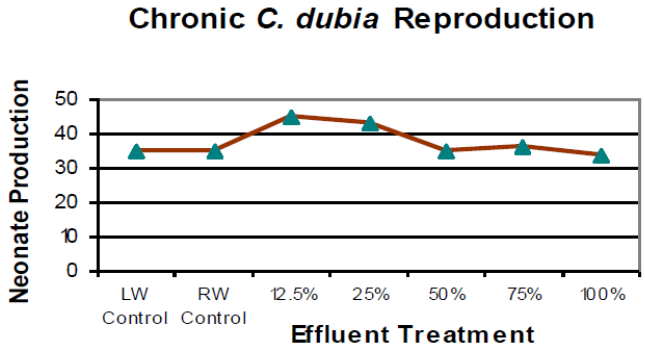


Figure 1.5.5 An example dose-response, showing a stimulatory response at low concentrations but no significant effect at higher concentrations

5) **Interrupted dose-response: significant effect bracketed by non-significant effects.** This response pattern is characterized by a single test concentration showing a significant difference from the control while adjacent higher and lower test concentrations do not differ significantly from the control (Figure 1.5.6). When this response pattern is encountered, point estimation techniques generally will yield reliable results.

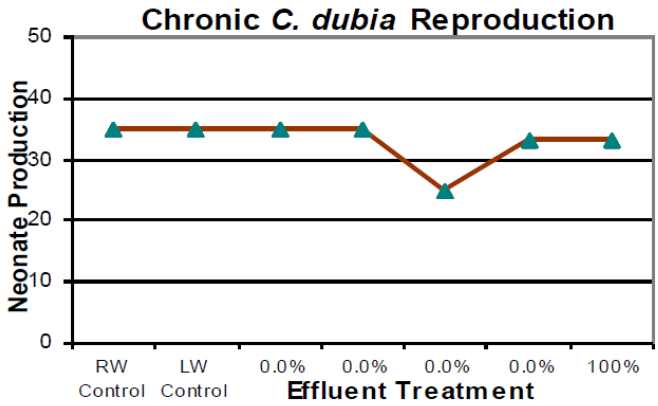


Figure 1.5.6 An example dose-response, showing an Interrupted dose-response: non-significant effects bracketed by significant effects

6) **Interrupted dose-response: non-significant effects bracketed by significant effects.** This response pattern is similar to the previous pattern in that the dose-response curve is interrupted, however, this pattern is characterized by two or more concentrations showing a significant difference from the control while an intermediate test concentration does not differ significantly from the control (Figure 1.5.7). When this response pattern is encountered, point estimation techniques will generally yield reliable results.

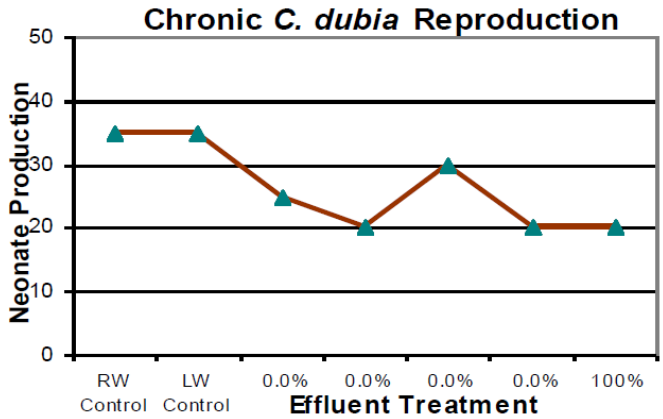


Figure 1.5.7 An example dose-response, showing an Interrupted dose-response: non-significant effects bracketed by significant effects

7) **Significant effects at all concentrations but flat dose-response.** This pattern is demonstrated in Figure 1.5.8. All test concentrations produce a response that is significantly different from the control response, but a clear dose-response relationship cannot be determined.

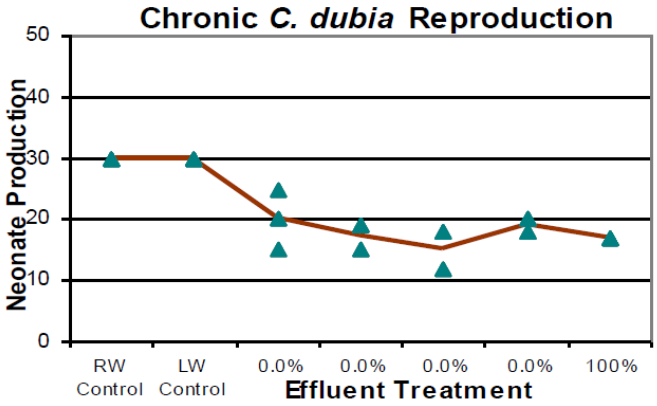


Figure 1.5.8 An example dose-response, showing significant effects at all test concentrations but flat dose-response curve

This response pattern could be due to: 1) low variability in the control, 2) an unusually high control response, 3) inappropriate dilution water or improper use of controls, (4) inappropriate test dilution series, (5) pathogen effects in the effluent, or (6) an unusual effluent-dilution water interaction. The following should be done to determine a cause for this dose-response and to determine the validity of calculated results:

a) **Evaluate the control response** - The pattern shown in Figure 1.5.8 could result from an unusually high control response. Labs are encouraged to track control performance over time. When a pattern like that in Figure 1.5.8 is exhibited, the control response should be compared to historic control performance in the lab. If the mean control response is above the normal range for that lab, a repeat test may be needed.

b) **Evaluate dilution water** - The improper use of dilution waters and controls could cause the dose-response pattern shown in Figure 1.5.8. It should be confirmed that test concentrations were compared to the dilution water control.

c) **Consider pathogen effect** - The pattern shown in Figure 1.5.8 could also be due to the presence of pathogens in the effluent. The most common identifier of pathogen effects are sporadic mortalities and high variability between replicates. The pathogen effect is more common in tests using fish species than in invertebrate testing and in chronic tests than acute tests. If within-treatment CVs for survival are >40% for effluent concentrations and relatively small for control replicates in standard synthetic water, a pathogen effect should be considered. If pathogen effects are suspected, the sample should be retested.

d) **Continued testing** - If all of the above scenarios have been investigated and have not revealed the cause of the response pattern, the results should be considered valid. However, further testing should be done in order to identify the cause of the response pattern. If an effluent consistently exhibits this response pattern, additional investigations could include chemical analysis or toxicity identification procedures.

8) **Significant effects at all concentrations with a sloped dose-response curve.** This pattern is similar to that identified in 7 above, except a dose-response can be identified at the higher concentrations (Figure 1.5.9). This is considered to be a valid dose-response relationship, and point estimates will generally yield reliable results.

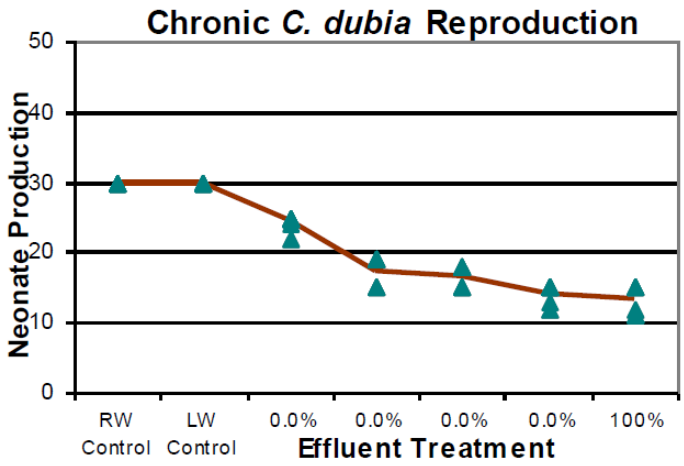


Figure 1.5.9 An example dose-response, showing significant effects at all test concentrations with a sloped dose-response curve

9) **Inverse dose-response relationship.** This pattern is characterized by a relationship in which adverse effects decrease with increasing effluent concentration (Figure 1.5.10). This is most often encountered in algal growth tests and sometimes in *C. dubia* chronic tests, and is typically caused by excess nutrients in the effluent. An inverse response pattern could also be due to the presence of pathogens in the effluent. While a valid dose-response relationship is demonstrated in this circumstance, the effluent may be nontoxic since the direction of the dose-response relationship indicates decreasing adverse effects. It should be noted that while the effluent may be non-toxic, the presence of excess nutrients still may pose a potential risk to the environment due to eutrophication and oxygen depletion.

   An inverse dose-response pattern may also occur in tests when the dilution water used is a receiving water. In such situations, the inverse pattern can result from toxicity or a lack of necessary ions/nutrients in the dilution water. Under such circumstances, the objective of the toxicity test should be evaluated. If the objective of the test is to determine the toxicity of the effluent in the natural receiving water, then the results indicate no toxicity in the sample. If the objective of the toxicity test is to determine the absolute presence of toxicity in the effluent, the sample should be retested using a standard synthetic dilution water.

   An inverse dose-response may also occur due to toxicity dependent characteristics of the effluent and dilution water. For example, if a parameter such as hardness changes with effluent concentration, and toxicity is hardness-dependent, an inverse dose-response could result (e.g., if hardness decreases with less effluent/more receiving water, and toxicity increases with decreasing hardness, an inverse dose-response like that shown in Figure 1.5.10 would be the expected result). Since a valid dose-response is demonstrated in this circumstance, the effluent would be considered toxic and retests may be necessary.

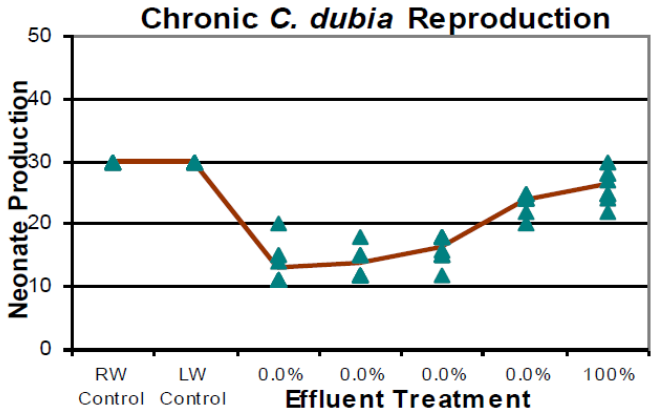


Figure 1.5.10 An example dose-response, showing an inverse dose-response relationship

Confidence Intervals

The Methods Manual (s. NR 219.04, Wis. Adm. Code) requires that test endpoints be reported as an LC_{50} (acute) or $IC_{25/50}$ (chronic). The 95% confidence intervals associated with these endpoints must also be reported, as an estimate of the precision (uncertainty) around the LC_{50} or IC_{25} value (Methods Manual Section 5.6, s. NR 219.04, Wis. Adm. Code). The Methods Manual requires that the proper statistical method be performed using EPA or commercially available software, which generally produce a point estimate with the associated 95% confidence intervals. It is important to note that under certain circumstances confidence intervals are not produced by the software or are unreliable. This can happen if test data do not meet specific assumptions required by the statistical methods, if point estimates are outside of the test concentration range, or if specific limitations of statistical software are encountered. Confidence intervals are not used when determining compliance, but must be reported (when available) and may be used as supplemental information when interpreting test results (Methods Manual section 5.5, s. NR 219.04, Wis. Adm. Code).

The 95% confidence intervals are a measure of the uncertainty of the endpoint calculated by the statistical package. As the 95% confidence intervals of the point estimate increases (i.e., get wider), the uncertainty in that estimate of the statistical endpoint increases. Conversely, the smaller the width of the confidence intervals, the more certain one can be that the endpoint determined by the statistical program is accurate. In WET testing, confidence intervals can be a measure of intratest variability. The confidence intervals for chronic endpoints are directly influenced by the variability between replicates in each treatment and the model used to interpolate the point estimate. The confidence intervals for acute test results using a point estimate approach, however, are not influenced by variability between replicates but by the characteristics of the dose-response relationship. As discussed in Chapter 2.12 of this guidance document, the certainty in point estimates is also a function of the dilutions tested and their proximity to the actual statistical endpoint being calculated. One will get a better estimate of the LC_{50} (tighter confidence intervals) if dilutions are tested near the concentration which actually results in 50% mortality.

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CHAPTER 1.6 - Intermittent Discharges

The purpose of this chapter is to address sample collection and retesting issues for intermittent dischargers (including "fill & draw" types) for staff use when determining permit requirements and for permittee use when conducting WET tests.

NOTE: Department approval is required (Methods Manual, Section 2, s. NR 219.04, Wis. Adm. Code) whenever deviating from WET test protocols (changes to sample type, number, holding time, etc.). Users should contact the Biomonitoring Coordinator to discuss specific situations, before changing test protocols.

Evaluating the Need for WET Testing of Non-Continuous Discharges

Discharges that occur continually for 1 hour or more in any 24 hour period should be evaluated to determine if acute WET testing is necessary. Acute tests aren't usually required for discharges less than 1 hour in duration, since the risk for adverse environmental impacts due to acute toxicity are typically low in these situations.

Discharges that occur continually for 96 hours or more in a 7 day (168 hour) period should be evaluated to determine if chronic testing is necessary. Chronic tests usually aren't required for discharges that are less than 96 hours in duration, since the risk for adverse environmental impacts due to chronic toxicity are typically low in these situations.

Monitoring Frequencies

The amount and frequency of WET testing for individual discharges should be determined by Department staff using the guidance in Chapter 1.3. Once monitoring frequencies are determined, special considerations may be needed when outlining WET requirements in permits for non-continuous discharges. For example, if the effluent is discharged seasonally it may not be possible for dischargers to conduct testing on a quarterly basis. It also may not be possible for tests to occur during different seasons, if the discharge in question occurs at the same time each year.

In situations where monitoring has been recommended and the discharge is non-continuous, it is important to insure that WET tests are done when the factors of concern that drove the need for testing are present. For example, if the use of a large amount of chemical additives raises concerns regarding the potential for effluent toxicity, then WET tests should be scheduled when those additives are in use.

When drafting permits, efforts should be made to require all of the recommended tests during expected periods of discharge, with tests completed at least 60 days apart. As an example, if quarterly testing is recommended but the discharge occurs only 2 months of the year, it may only be possible to conduct 1 test per year (since there are only 60 discharge days). If the same monitoring frequency was recommended for a discharge which occurred for more than 8 months out of the year, all four tests should be required.

Fill & Draw Discharges

Some permittees have fill & draw type discharges which might make scheduling and sampling for WET tests more difficult. In these situations, it may be necessary to alter standard sampling and retesting procedures. According to the Methods Manual (Section 2.2.2, s. NR 219.04, Wis. Adm. Code), two samples collected over a three day period are required to complete an acute test and three samples collected over a six day period are required to complete a chronic test. However, the Department does recognize that some situations may not fit this standard sampling schedule. Test methods allow for deviations from standard schedules in these situations, but these should be specified in the permit whenever possible.

Where fill & draw type discharge events last more than 6 days, WET sample collection should not be a problem. In most of these situations effluent quality is not expected to vary significantly over a 24-hour period, so grab samples are acceptable which makes WET sampling easier to coordinate with discharge schedules. However, in situations where the discharge will occur for less than 6 days it may be difficult to collect the 2 or 3 samples normally required to complete a WET test. Table 1.6 suggests some possible alternative sampling schedules that may be used in these situations.

Intermittent Or Seasonal Discharges

Some discharges, often times those from food industry, have intermittent or seasonal discharges, which might make scheduling and sampling for WET tests difficult. In some instances, it may be necessary to alter standard WET test sampling procedures when discharges are intermittent or seasonal. Test methods allow for deviations from standard sampling schedules in these situations, but these should be specified in the permit whenever possible (Section 2.2.2, s. NR 219.04, Wis. Adm. Code).

In situations where effluent quality is not expected to vary significantly over a 24 hour period, grab samples are acceptable and may be easier to coordinate with production and discharge schedules (e.g., a single grab sample may be acceptable for lagoons where detention time is longer). Normal WET sampling schedules are especially difficult to adhere to when discharge periods do not last for 3 days (72 hours) or more at a time. Table 1.6 contains possible adjustments to sample schedules, to accommodate shorter discharge periods.

Table 1.6 Adjusted Sampling Schedules for Intermittent Discharges

Discharge Duration	Maximum # of 24-h comp. samples to be collected	Comments
≥ 6 days (144 h)	3	Sampling for acute & chronic tests should not be a problem with proper planning
3-5 days (72-120 h)	2	Sampling for acute tests should not be a problem with proper planning Chronic tests should be conducted with 2 samples, with prior approval from the Department*; (Chronic tests usually not required if discharge < 4 days)
1-2 days (24-48 h)	1	Acute tests may be conducted with 1 sample, with prior approval from the Department*; (Chronic tests usually not required if discharge < 4 days)

** Test methods allow for deviations from standard sampling schedules, but these should be specified in the permit whenever possible. Written approval from the Department is required whenever sample type, number of samples, holding times or other changes are needed which deviate from WPDES permit requirements. (Section 2.2.2, s. NR 219.04, Wis. Adm. Code).*

Permittees with seasonal or intermittent discharges will need to make a special effort to be ready to collect WET samples as soon as discharge periods begin. Deviations from permit requirements must be noted on the WET report form required by Section 6 of the Methods Manual (s. NR 219.04, Wis. Adm. Code).

Completing Retests

WPDES permits typically require that 2 retests be completed within 90 days after a WET test failure. When discharges are intermittent, scheduling retests may become difficult if the discharge period is not expected to extend at least 90 days beyond the original test date. In order to avoid this problem whenever possible, every effort should be made to schedule the original tests so that retests may occur during the same discharge period (e.g., perform compliance tests in the 1st month of discharge so that retests can be done in the 2nd and 3rd months, if needed). If a discharge period does not last long enough to perform needed retests, retests may need to be postponed until the next discharge period. Permittees should contact the Biomonitoring Coordinator or Regional staff to discuss these types of situations and determine the best course of action.

CHAPTER 1.10 - Ammonia & Associated WET Requirements

The Department promulgated water quality standards for ammonia in s. NR 106.36, Wis. Adm. Code, on March 1, 2004, which addresses WET requirements in certain situations. The following guidance is given in two parts:

Part One: The first part of this chapter provides guidance for Department staff, WET labs and permittees, regarding requirements in s. NR 106.36, Wis. Adm. Code, which allows effluent samples used in chronic fathead minnow tests to be modified to remove ammonia prior to testing when early life stage (ELS) - absent ammonia criteria are in effect.

Part Two: The second part of this chapter gives guidance for staff to use when making adjustments to WET requirements when a permittee has been granted a water quality standards variance for ammonia.

Part One: WET Sample Modification When ELS-Absent Criteria Are In Effect

USEPA's *1999 Update of Ambient WQC for Ammonia* (https://nepis.epa.gov/Exe/ZyPDF.cgi/20003O3L.PDF?Dockey=20003O3L.PDF) contains a provision to adjust (relax) chronic water quality criteria (WQC) for ammonia when water temperatures are colder (< 15º C) and early life stages (ELS) are absent, in order to account for reduced sensitivity to ammonia by juvenile and adult fish at lower temperatures. In Wisconsin, this translates into higher (less stringent) limits for ammonia during winter months. Fathead minnow ELS were used, with other data, to develop the adjustment between ELS present and absent criteria, and the fathead minnow is known to be very sensitive to ammonia. Of the data used to develop WQC for ammonia, only *Hyalella* (an invertebrate), *Musculium* (a mussel), and *Lepomis* (blue gill) ELS were more sensitive to ammonia than the fathead minnow ELS. Like fathead minnow ELS, *Lepomis* ELS would not be expected in receiving waters during the ELS-absent period.

It has been pointed out that a conflict may exist between allowing this less stringent ammonia criteria during ELS-absent periods and requiring WET tests during those same periods, since chronic WET tests are conducted using fathead minnow ELS. Under this scenario, a situation could arise where the permittee is in compliance with its effluent limit for ammonia during an ELS-absent time period, but have a positive (failing) chronic fish WET test due to ammonia during that same period. Because of this, s. NR 106.36, Wis. Adm. Code, allows samples used in chronic fathead minnow WET tests to be modified to remove ammonia prior to testing when certain conditions are met (See Figure 1).

Permit Language When ELS-Absent Criteria Are Applied

The following standard language may be added to WPDES permits when WQBELs based on ELS-absent criteria are given:

"Effluent samples used in chronic fathead minnow tests may be modified to remove ammonia prior to testing, according to s. NR 106.36(2), Wis. Adm. Code, during periods when ammonia limits based on early life stage-absent criteria are in effect."

Figure 1. NR 106.36 Alternative whole effluent toxicity monitoring for certain discharges of ammonia.

(1) In addition to water quality based effluent limitations for ammonia, the department may establish whole effluent toxicity testing requirements and limitations pursuant to ss. NR 106.08 and 106.09.

(2) Chronic fathead minnow whole effluent toxicity test samples may be modified to remove ammonia prior to testing when all of the following conditions are met:
(a) The whole effluent toxicity test is being conducted during a period when ammonia effluent limitations based on early life stage absent criteria are in effect.
(b) The permittee has demonstrated compliance with applicable acute and chronic water quality based effluent limitations for ammonia during the testing period.
(c) Total ammonia measured in whole effluent toxicity test effluent samples is less than the applicable chronic water quality based effluent limitation contained in the WPDES permit, but greater than the "ammonia threshold number", determined as follows:
1. Measure the pH of the whole effluent toxicity test effluent sample after the sample has been warmed to the test temperature.
2. Using the pH value of the sample as determined in subd.1., determine the value of the ammonia multiplier in Table 1 for the pH range corresponding to the effluent pH.
3. Divide 100 by the appropriate in−stream waste concentration, as a percentage, contained in the WPDES permit; then multiply the resulting value by the ammonia multiplier determined in subd. 2. to obtain the ammonia threshold number.

(3) If all of the criteria in sub. (2) have been met, ammonia may be removed from the test sample.

Table 1.

Effluent pH (s.u., after warming)	Ammonia multiplier (mg/l total ammonia)
6.0 – 6.5	30
6.6 – 7.0	25
7.1 – 7.5	15
7.6 – 8.0	5
8.1 – 9.0	1

Modification of WET Samples During ELS-Absent Periods

As outlined above, it may be appropriate to conduct the fathead minnow portion of the chronic WET test on effluent samples that have been treated to remove ammonia prior to testing. When those conditions are met, effluent samples may be treated with zeolite resin prior to testing. Samples should be treated daily, before use in WET tests, rather than batch treated for multiple day usage. Ammonia, pH, hardness, and alkalinity should be measured prior to and after zeolite treatment. A blank (an extra negative control) should also be run through zeolite, to account for toxic artifacts due to the zeolite treatment. Samples used for fathead minnow chronic tests should not be modified in any way other than ammonia removal with the zeolite resin. Samples used in concurrent acute tests (fathead minnow and *Ceriodaphnia dubia*) and chronic tests with *C. dubia* cannot be modified prior to testing, since they are not included in the s. NR 106.36 (2) exemption for fathead minnow ELS.

Decisions regarding WET monitoring frequencies and scheduling should be made using guidance in Chapter 1.3 and acute and chronic WET tests should be required during ELS-absent periods, if they otherwise would be required there. Continued WET testing during winter months is important, when possible, because wastewater treatment effectiveness (and, therefore, effluent toxicity) can be different during colder weather. Chronic fathead minnow WET tests conducted during periods when ELS-absent ammonia criteria are in effect will still be used to assess whether toxicity is present due to other compounds. Toxicity will also be assessed with acute WET tests and the *C. dubia* chronic test conducted during these periods.

Can This Be Applied To Other Pollutants?

It is important to note that this is not just a matter of meeting the WQBEL for a chemical and failing a WET test that identifies that chemical as the toxicant. This is a special case only for ammonia because the provision to adjust the chronic WQC during periods of the year when water temperatures are colder and fish ELS are absent is unique to ammonia. Ammonia is the only chemical for which data showing a difference in sensitivity between ELS and adult fish has been used to allow for an adjustment in the WQC. This approach is believed to be appropriate for ammonia because the fathead minnow ELS (and other ELS that this species is used as a surrogate for) are not found in receiving waters during ELS-absent periods. Therefore, it is believed that a positive chronic fathead minnow WET test result caused by ammonia toxicity would not be indicative of negative effects in the receiving water because the life stages that experience those toxic effects would not be present. Chronic toxicity due to ammonia that may be harmful to other non-fish species present in the receiving water during colder periods should be indicated by *C. dubia* chronic tests.

Will Zeolite Remove More Than Ammonia?

Zeolite is composed of natural or synthetically created crystalline, hydrated alkali-aluminum silicates. When zeolite is exposed to an aqueous solution (such as an effluent), the positively charged resin removes cations from the solution. Since it is an effective ion exchange resin, zeolite is often used in toxicity identification work, specifically to remove the ammonium ion (NH_4^+) from effluent samples (*Methods for Aquatic Toxicity Identification: Phase II TIE Procedures*, EPA/600/R-92/080; https://www3.epa.gov/npdes/wettraining/module8/story_content/external_files/TIE%20Phase%202%201993.pdf).

However, because of its ion exchange properties it may also remove other cations such as heavy metals. In addition, although the primary action of zeolite is chemical (ion exchange), the physical manipulation of filtration also occurs during the process. Removal of compounds via filtration through zeolite may include surfactants and polymers. Changes in the ionic balance of the sample caused by the zeolite treatment may also cause chemicals that would not have caused toxic effects before zeolite treatment to be rendered biologically available.

While it is true that modification of samples with zeolite can remove substances other than ammonia and may modify the sample in other ways, the potential for this method to reduce the ability of the WET test to detect toxicity due to other substances should be small in most cases. Substances other than ammonia that may be removed by zeolite, especially heavy metals, are typically more toxic to *C. dubia* than the fathead minnow. Since samples used in the *C. dubia* test are not being modified, that toxicity should still be detected. Additionally, since the presence of ammonia in a sample may mask toxicity caused by other substances, the removal of ammonia could allow for the detection of substances that may have been missed had ammonia been present.

Historical WET Data From Previous ELS-Absent Periods

In some cases, a permittee may have collected WET data during previous years when ELS-absent ammonia criteria would have been applicable. The question arises then of how to consider WET tests conducted under these conditions when making permit-related decisions. The results of a fathead minnow chronic test completed during ELS-absent periods should not be used when assessing reasonable potential or choosing the WET monitoring frequency (i.e., in the WET Checklist process) if all of the following are true: 1) the test appears to have failed due to ammonia toxicity, 2) concurrent acute tests and chronic *C. dubia* tests did not show toxicity, and 3) the conditions listed in s. NR 106.36, Wis. Adm. Code (shown in Figure 1 above), are met.

It is important to keep in mind that this exclusion applies only to ammonia because it is the only WQC that has an adjustment for the presence or absence of fish ELS. Any WET tests which showed toxicity in an acute test or a *C. dubia* chronic test, or in a fathead minnow chronic test due to any toxicant other than ammonia should not be excluded from WET determinations. Results generated by acute tests and *C. dubia* chronic tests conducted during periods when ELS-

absent ammonia criteria are in effect are still applicable for assessing effluent toxicity from all toxicants, including ammonia. If staff have questions regarding WET data collected during ELS-absent periods, they should contact the Biomonitoring Coordinator (Kari.Fleming@wisconsin.gov).

Part Two: WET Monitoring When an Ammonia Variance Has Been Granted

WET testing has been conducted on municipal and industrial effluents since the 1980s. Ammonia has been shown to be a common toxicant found in wastewater effluents and has been addressed accordingly in the WET program. WET test failures caused by ammonia are treated the same way as those caused by other toxicants. Ammonia-related WET failures occur most often during the winter months, especially in cases where stabilization ponds and aerated lagoons are used, because wastewater treatment is less effective (or ineffective) in colder temperatures. In most cases, higher ammonia levels will occur in wastewater during the months of December through May.

Permittees may be granted a variance to the ammonia water quality standard (WQS) as allowed in s. 283.15, Wis. Stats., due to socio-economic impacts or other factors. (See http://dnr.wi.gov/topic/wastewater/variances.html for more information on WQS variances.) As a condition of the variance, permittees may be given higher (less stringent) ammonia limits during the term of the variance. In these cases, it may be necessary to also adjust WET monitoring schedules to account for periods when levels of ammonia in the effluent are allowed to be present above WQBEL that were applicable before the variance was granted.

Depending on the level of ammonia present in the effluent, toxicity may occur in acute and chronic tests and to one or all of the tested species. It is generally accepted that the fathead minnow will experience acute toxicity if ammonia is present at levels above 30 mg/l (at pH $\leq$ 7.5) and chronic toxicity if ammonia > 15 mg/l (at pH $\leq$ 7.5 and 100% IWC). *C. dubia*, on the other hand, would not be expected to show acute toxicity until ammonia is above 60 mg/l (at pH $\leq$ 7.5) and chronic *C. dubia* toxicity wouldn't be expected until levels are > 50 mg/l (at pH $\leq$ 7.5 and 100% IWC). Due to the change in ammonia toxicity at different pH levels, staff should consult with the Biomonitoring Coordinator when deciding whether or not toxicity would be expected in WET tests at site-specific pH and ammonia levels.

Depending on the level of ammonia expected to be present in the effluent, staff may determine that it is appropriate to not require WET testing at certain times of the year or to require that only *C. dubia* be tested. For example, if the maximum daily amount of ammonia present in the effluent is expected to stay above 15 mg/l, but below 50 mg/l in December through May, the permit may require that chronic WET tests be conducted at other times of the year (June – November). If it is desirable to collect WET data during the months of December – May in this same example, staff could require that testing be done using *C. dubia* only.

If it has been determined that the permittee should be granted (or has previously been granted) a variance for ammonia, staff should make decisions on whether or not to use previously collected WET data using the same logic as described above. If WET tests were collected under conditions that are being excluded for testing in the future, then it may be appropriate to remove those data from WET limit and monitoring determinations. WET test results that indicate toxicity is due to substances other than ammonia should still be used when making WET determinations.

Ammonia Toxicity and pH Drift

Natural processes which act to regulate the pH of natural waters also occur in mixtures of natural surface waters and effluents. With the exception of lagoon systems, effluent pH values are often lower than those in receiving waters, due to the presence of excess carbon dioxide resulting from the artificially high rates of respiration of microorganisms in wastewater treatment plants. When the effluent is discharged to surface waters (or mixed with receiving waters in laboratory settings), respiration rates fall to more natural levels and excess carbon dioxide is stripped, causing a pH rise or drift upwards. Significant pH drift can sometimes occur in WET tests, due to the static conditions present in the test.

This can impact how much toxicity is expressed due to ammonia in these WET tests, since a higher pH results in more ammonia toxicity. Extra care should be taken in tests where ammonia is at or near toxic levels. Chapter 2.8 discusses the use of CO2 entrapment methods to control pH drift and when it is required in permit-required WET tests.

Questions regarding WET test design, data interpretation, or the applicability of historical data should be directed to the Biomonitoring Coordinator (Kari.Fleming@wisconsin.gov).

CHAPTER 1.11 – WET Testing of Minor Municipal (< 1.0 MGD) Discharges

This chapter provides a streamlined procedure for determining whether the WET Checklist should be completed for a minor municipal discharge.

Prior to 1996, dischargers of municipal wastewater that had effluent design flows less than 0.25 million gallons per day (MGD) were not required to perform WET tests for WPDES compliance. This practice was set in the early stages of the WET program when little toxics and WET data was available on different types of discharges. It was decided that the minor municipal dischargers would be tested first during a "trial period" to determine whether WET was a concern at minor municipal facilities. The decision to limit the number of municipalities to be tested was based solely on workload considerations and not on scientific information, WET data, or any type of judgment that these effluents would not be toxic.

When the WET Guidance Document was created, Department toxicologists reviewed available WET data for all municipal facilities, including those dischargers just above and below the 0.25 MGD cutoff. Table 1.11 below summarizes WET data collected from 1992-2001 from municipal dischargers. This and other data showed that effluent flow volume does not influence a facility's potential for toxicity. Instead, factors such as industrial contribution, available dilution, treatment efficiency, additive use, etc. was found to play a much more important role in a facility's toxicity potential.

Table 1.11 WET Data from Municipal Dischargers (data from 01/01/1992 - 10/31/2001)

Design Flow (MGD)	Acute			Chronic		
	# Tests done	# failed	**% failed**	# Tests done	# failed	**% failed**
≤ 0.25	136	33*	**24.3%**	75	21*	**28.0%**
> 0.25 & ≤ 0.50	227	23	10.0%	125	24	19.2%
> 0.50 & ≤ 1.00	221	14	6.3%	184	54	29.3%
> 1.00 & ≤ 5.00	483	44	9.0%	370	78	21.1%
> 5.00	246	38	15.0%	156	17	10.9%
TOTAL	**1,313**	**152**	**11.6%**	**910**	**194**	**21.3%**

Total tests conducted by municipals: 2,223 (poor QA, TIEs, and inconclusive tests not included)

* Ammonia may be the cause of toxicity in 16/33 of acute and 11/21 of chronic tests done by minor municipal dischargers < 0.25 MGD. This is based on a very generalized assumption about this data - the fathead minnow was the most sensitive species in these tests, which usually suggests that ammonia is at fault because fish are more sensitive to ammonia than invertebrates. When these failures are removed from the above dataset, dischargers < 0.250 MGD failed 19/119 (13.4%) of acute tests and 10/64 (15.6%) of chronic tests.

A Quick Check to Determine if the WET Checklist is Necessary

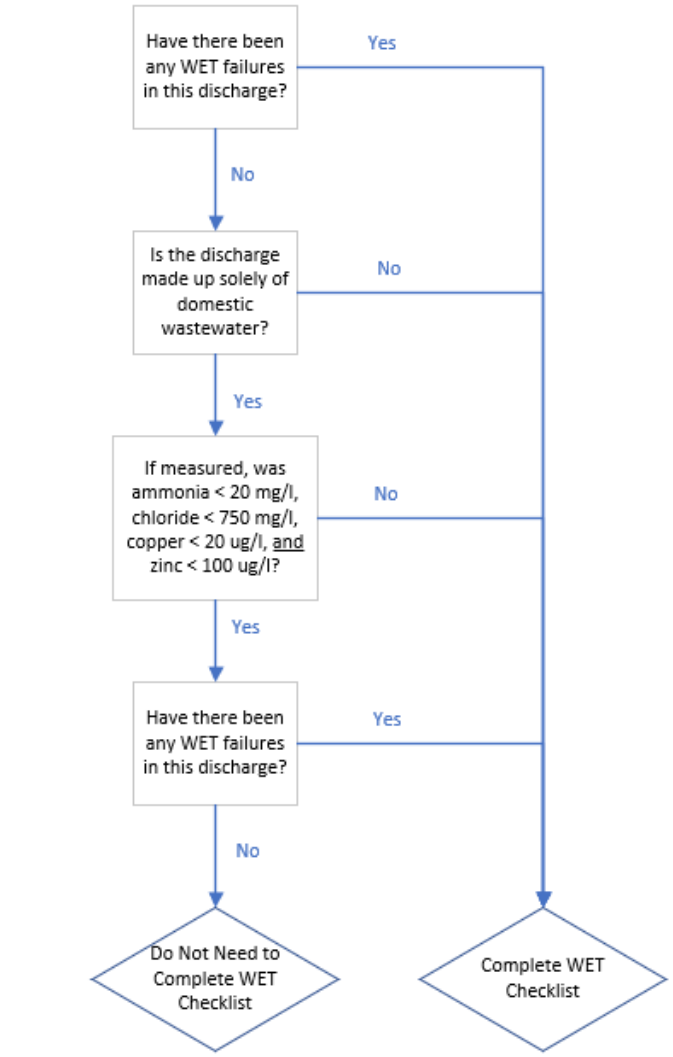
The WET Checklist was created along with the WET Guidance Document in 1996 to help staff make WET limit and monitoring decisions by considering site specific factors such as industrial contribution, available dilution, treatment efficiency, additive use, and others to assess a facility's toxicity potential. Effluent design flow is not considered (except as a factor in determining available dilution), since WET data shows that facility size alone does not play a role in a facility's toxicity potential. The Checklist was designed to assist staff that must decide how much WET monitoring to assign to individual discharges, based on their potential to exhibit toxicity. As the potential for toxicity increases, more points accumulate and more monitoring is suggested to insure that toxicity is not occurring (see Chapter 1.3).

The WET Checklist provides a more data-driven, scientific, and defensible process for staff to use when establishing WET monitoring requirements. However, a complicated analysis may not be necessary in cases where the municipal discharger is very small and is lacking the factors included in the Checklist to determine toxicity potential. This chapter includes a quick check process which may be used to determine whether a more in-depth analysis via the WET Checklist is

necessary to determine the need for WET testing at a minor municipal facility. By answering a few easy, Yes/No type questions, staff should be able to determine quickly whether further evaluation via the WET Checklist is needed. The flowchart in Figure 1.11 contains questions about information that is normally evaluated as part of the WET Checklist process and which usually play the biggest part in determining a municipal facility's WET potential. (NOTE: If staff wish to skip this "quick check" and go directly to the WET Checklist, they may do so.)

In effect, the flowchart shown in Figure 1.11 leads the user through a shortened, cursory review of the information that is entered into the WET Checklist. The information needed to answer the questions in the flowchart is basic knowledge about the discharge which permit staff will already need to know in order to complete a WQBEL recommendation. In most cases, if the answers to the questions in the flowchart end at the "*Do Not Need to Complete the WET Checklist*" box, a more complete review including the WET Checklist would not have recommended WET monitoring for that facility. If a minor municipal facility has no WET failures, no industrial contributors, no detects of chemicals other than ammonia, chloride, copper, and zinc, and levels of these compounds are below that which would be expected to cause WET problems, then the potential for WET is low and WET monitoring is not recommended.

Figure 1.11 Flowchart to Determine Use of WET Checklist



WET Monitoring Probably Not Needed
Staff do not need to complete the WET Checklist, unless they have other information suggesting that WET monitoring may be needed

The flowchart above may be used by WQBEL staff to decide whether a minor municipal discharge should be further evaluated via the WET Checklist. This flowchart may be applied to any minor municipal discharge < 1.0 MGD. However, because of minimum monitoring requirements and the complexity of their effluents, this quick check process cannot be used for major municipal or industrial discharges. Major municipal and industrial discharges should be evaluated using the WET Checklist (see Chapter 1.3).

NOTE: The WET Checklist (described in Chapter 1.3) and the "quick check" described in this chapter are both based on certain assumptions. One of these assumptions is that each discharge has been monitored for chemical-specific parameters, especially those substances with water quality criteria for the protection of fish and aquatic life in Tables 1 & 2 (acute) and Tables 3 & 4 (chronic), in ch. NR 105, Wis. Adm. Code. When data regarding the presence or absence of those parameters have not been collected for the effluent being evaluated, neither the WET Checklist nor this quick check can adequately determine the discharge's potential for toxicity. Users should be aware of these potential shortcomings in WET determinations, if data for these parameters are not available.

WET Reviews and WQBEL Recommendations

Staff should document in their WQBEL memo that based on this preliminary review, the WET Checklist was not completed and WET testing is not recommended. For example, the following explanation would suffice:

> "*This is a minor municipal facility (< 1.0 MGD) which has no historical WET failures, no known industrial contributors, no detects of chemicals other than ammonia, chloride copper, and zinc, and these compounds are below that which would be expected to cause WET problems. Therefore, no further WET evaluations were deemed necessary and WET testing is not recommended at this time.*"

If staff believe that there are other factors which may contribute to the potential for WET problems (for example, known ecological impacts, other special environmental conditions in the area of the discharge, or other information), the WET Checklist should be completed and WET monitoring and/or limits given based on staff's best professional judgment. If any questions or problems arise, staff should contact the Biomonitoring Coordinator.

CHAPTER 1.12 – WET Limit Compliance Schedules

This chapter is intended to help staff make decisions regarding appropriate time frames and requirements for WET limit compliance schedules in WPDES permits.

Standard WET Limit Compliance Schedule

WET limits are required in a permit according to s. NR 106.08, Wis. Adm. Code, whenever representative, facility-specific WET data demonstrate that the effluent is or may be discharged at a level that will cause, have the potential to cause, or contribute to an excursion of a water quality standard. The Department evaluates all surface water dischargers to determine the need for WET limits and monitoring at the time of permit reissuance. See Chapter 1.3 for more discussion of WET reasonable potential and how staff determine whether a WET limit is needed in each situation.

When a new WET limit is required for the first time in a WPDES permit, it may be necessary to include a compliance schedule in order to allow the permittee time to come into compliance with the new limit. Standard WET limit compliance schedules allow a Toxicity Reduction Evaluation (TRE) to be completed, which may be necessary to determine what is causing toxicity and what actions are needed to remove it. (See Chapter 2.2 for a more complete discussion of TREs.)

A TRE compliance schedule should be given in most cases where representative WET data suggests that repeated toxicity is present in the effluent. A TRE compliance schedule may not be appropriate, however, in cases where limited WET data is available or where toxicity has not occurred for some time. Chapter 1.3 (Table 1) shows when the WET Checklist recommends that a TRE accompany a new WET limit, based on the percent of WET tests that have failed. In cases where data is limited or toxicity has appeared infrequently, more frequent monitoring is recommended instead of a TRE, in order to determine whether toxicity reappears over time. Standard language in WPDES permits typically requires that a TRE be completed if WET failures occur during the permit term (see Chapter 1.14).

In cases where a TRE compliance schedule is required, regular WET monitoring is usually established after the compliance schedule is completed and the limit has become effective. Toxicity screening is usually done during the TRE, so regular permit-required, compliance-type WET tests are not necessary in addition to that screening during the compliance schedule period. Only the monitoring which accompanies the limit type should be postponed. (For example, if a chronic WET limit is given, chronic monitoring starts at the end of the compliance schedule, but acute WET monitoring begins at reissuance.) Monitoring should be required after the compliance schedule is completed and for the remainder of the permit term in order to demonstrate compliance with the WET limit.

The following is an example of typical TRE compliance schedule language and suggested dates for completion of each step. A schedule such as this is usually given when a permittee needs time to complete a full toxicity reduction evaluation. This includes time to investigate the source(s) of toxicity and to choose the best method for removing toxicity after the source has been identified. This schedule allows about 3 years from permit issuance to complete a TRE and meet the limit. Required steps or dates due should be adjusted if the permittee has already completed some of the work described.

Standard Compliance Schedule Dates

Step	Required Action	Date Due
1	Submit **part one** of a Toxicity Reduction Evaluation (TRE) plan describing procedures to be used to identify the source(s) responsible for the effluent toxicity.	*3 months from permit issuance*
2	Implement part one of the TRE plan, make a reasonable attempt to identify the source(s) of the toxicity, and submit a report to the Department presenting the results of the evaluation.	*18 months from permit issuance*
3	Submit **part two** of the TRE Plan describing actions to be taken to reduce or eliminate the toxicity identified in part one of the TRE and the dates by which those actions will be implemented.	*19 months from permit issuance*
4	Submit a progress report identifying the actions taken to date to implement part two of the TRE plan.	*28 months from permit issuance*
5	Complete all actions identified in the TRE plan and achieve compliance with the effluent toxicity limitation.	*36 months from permit issuance*

Possible dates are listed in the schedule shown above, but permit drafters should consider each individual situation to determine whether these time periods are appropriate for the given situation. This version of the compliance schedule (without suggested dates) is available in the SWAMP picklist when drafting a permit. When including a TRE compliance schedule for a WET limit in a permit, staff should give specific dates for each step in the schedule rather than using a narrative such as "3 months from permit issuance." Chapter 2.2 includes some guidance for permittees and their labs regarding how they might complete each step of a TRE compliance schedule.

Reasons for Deviating From the Standard Compliance Schedule

As suggested above, TRE compliance schedules typically last about 3 years since past experience shows that this is a reasonable timeframe in which to find and fix toxicity problems. Most successful TREs have included fixes such as the removal, reduction, substitution, or pretreatment of the source(s) and can usually be completed during a 3 year schedule. According to s. NR 106.117, Wis. Adm. Code, a WPDES permit limit compliance schedule cannot exceed 5 years in length, except when performing a study to alter a secondary value, therefore a WET limit compliance schedule cannot extend beyond 5 years.

Although most TREs can be completed in 3 years, it may be necessary to deviate from the standard schedule in some circumstances. Construction of a whole new treatment system or another major action are possible justifications for lengthening the time allowed by the compliance schedule. Conversely, a permittee may not need the full 3 years if they have already completed some parts of the TRE prior to permit reissuance. For example, if the permittee has already identified the source of toxicity and only needs time to remove it, a shorter schedule would be appropriate. Other circumstances may exist which call for a longer or shorter schedule, but it is important to insure that there is enough time (ideally, at least 1 year) between the end of the TRE and the end of the permit, to allow time for WET monitoring. It is necessary to conduct WET monitoring after the compliance schedule is complete in order to demonstrate compliance with the WET limit before the permit is reissued.

The following are some examples of reasons why staff may want to modify the standard 3 year TRE compliance schedule. There may be other reasons which are not discussed here. Reasons for giving a compliance schedule and the length of time allowed should be explained in fact sheets, so that others can tell why decisions were made. If staff have questions or would like to discuss timelines that may be appropriate for a given situation, they should contact the Biomonitoring Coordinator.

Major Modification or Construction of a New WWTP. Construction of a whole new treatment system or some other major action are possible justifications for lengthening the time allowed by the compliance schedule. In some cases, especially when staff have good reason to believe that past toxicity will be resolved by new treatment processes, it may not be necessary to include a separate TRE compliance schedule if another schedule (e.g., one for new facility construction) is

expected to account for all of the steps needed to achieve compliance with the WET limit. If a TRE compliance schedule is not given for these reasons, the WET limit should be made effective and monitoring should start as soon as the other compliance schedule is complete.
Staff should remember that toxicity can be caused by many factors and an upgrade that only removes more solids or BOD_5 may not necessarily improve the treatment or removal of the toxic substance(s) causing WET failures. If it is unclear whether an upgrade will resolve toxicity problems, it may be wise to require TRE studies prior to the upgrade to more clearly understand the cause. It is usually easier to judge whether treatment upgrades will better treat effluent toxicity when the cause of toxicity is known. If staff suspect that the upgrade will resolve past toxicity problems, but do not know this for sure, another option may be a WET limit and/or TRE "trigger" (see discussion below).

Chloride Source Reduction and WET Compliance Schedules. Special allowances may be given in situations where chloride is shown to be the sole cause of whole effluent toxicity. In some situations, it may be necessary to allow time to make this demonstration. More discussion and example schedules for these situations are given in Chapter 2.10.

Intermittent Discharges. The standard 3 year compliance schedule may not be appropriate if a discharge is intermittent or seasonal. In these cases, time allowed for each step may need to be adjusted to account for shorter discharge periods. Since these discharges occur for fewer days in a given year, more time may be needed between compliance schedule steps, in order to allow the permittee time to conduct toxicity investigations. For example, if an intermittent discharge only occurs for < 6 months every year, it may be necessary to allow up to 2 calendar years for the completion of the first step, so that the permittee has enough time to collect samples, perform toxicity identification work, and confirm any findings.

WET Triggers. In some cases, staff may suspect that past failures which are driving WET limit recommendations are no longer representative of the current discharge, but there may not be enough conclusive data to leave those failures out of the reasonable potential calculation. In these cases, staff have a couple of options to consider. One is to extend the TRE compliance schedule beyond the standard 3 years, in order to allow more time to determine whether toxicity recurs in the effluent. Another option is to place a "trigger" in the permit. See Chapter 1.3 for discussion of when a WET limit trigger may be appropriate.

A WET limit trigger may be most appropriate for situations where available toxicity data is dated, questionable, or limited, and staff feel that it is necessary to gather more WET data prior to the imposition of a WET limit or TRE compliance schedule. If a trigger is to be used in the permit, quarterly WET monitoring should be required, at a minimum, for the first twelve months of the permit and WET footnote and TRE compliance schedule language may be modified as shown below.

> **Trigger Language** (to be placed into the WET footnote directly below the "WET Testing Frequency" section):
>
> **WET Limit Applicability:** If any *(acute/chronic)* WET test completed during the first twelve months of this permit shows positive results, the remaining tests will be waived and the Whole Effluent Toxicity Compliance Schedule *(see p. X)* will be initiated. After the compliance schedule is completed, the *(acute/chronic)* WET limit will become effective and quarterly *(acute/chronic)* monitoring will be required for the remainder of the permit term. If no *(acute/chronic)* tests conducted in the first twelve months of this permit show positive results, the compliance schedule will be waived, the limit will not become effective, and the *(acute/chronic)* monitoring shown above will be required.

WET compliance schedule language when using a trigger

Step	Required Action	Date Due
1	Submit **part one** of a Toxicity Reduction Evaluation (TRE) plan describing procedures to be used to identify the source(s) responsible for the effluent toxicity.	*3 months after WET failure*
2	Implement part one of the TRE plan, make a reasonable attempt to identify the source(s) of the toxicity, and submit a report to the Department presenting the results of the evaluation.	*24 months after permit issuance*
3	Submit **part two** of the TRE Plan describing actions to be taken to reduce or eliminate the toxicity identified in part one of the TRE and the dates by which those actions will be implemented.	*25 months after permit issuance*
4	Submit a progress report identifying the actions taken to date to implement part two of the TRE plan.	*32 months after permit issuance*
5	Complete all actions identified in the TRE plan and achieve compliance with the effluent toxicity limitation.	*40 months after permit issuance*

The permit will need to include WET monitoring to be required if the WET limit is not triggered. In most cases, monitoring frequencies can be reduced from quarterly if no failures occur during the first twelve months.

Reduction in Monitoring After the Successful Completion of a TRE

Permit language may be written to allow a reduction in monitoring, in certain circumstances, after a TRE has been successfully completed. For example, if frequent monitoring (bimonthly or quarterly) is to be included in the permit, permit language may be added which allows monitoring to be reduced after the permittee has submitted at least 12 months of WET data (if no toxicity occurs). It is important to note that when WET limits are given, the minimum monitoring frequency allowed by federal regulations at 40 CFR 122.44 (i) (2), is 1x annually. The reduced monitoring frequency should be determined at the time of reissuance and placed in the permit. Staff may choose to use the WET Checklist to help them decide on appropriate monitoring frequency, by completing the WET Checklist under the assumption that toxicity is no longer present. WET limits cannot drop out and must remain effective until the permit is modified or reissued.

CHAPTER 1.13 – Spills Toxicity Testing: Guidance for Compliance Staff, 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.

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

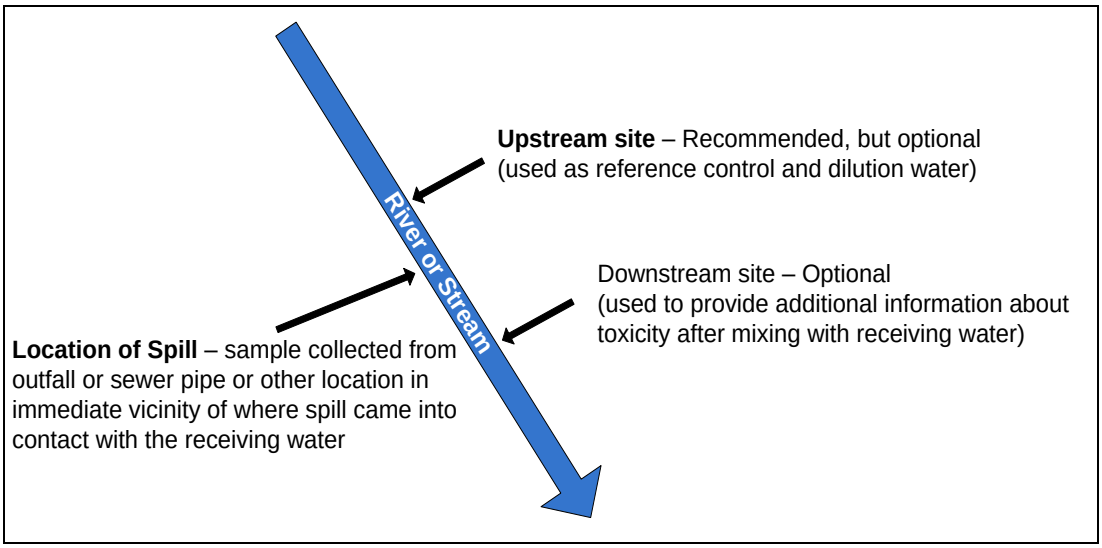
If you believe a spill has caused, or has the potential to cause, lethal or sublethal affects to fish or aquatic life of the waterbody in question, toxicity testing should be conducted. A visual inspection of the site may help determine if toxicity testing is warranted, but keep in mind that toxics can 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.

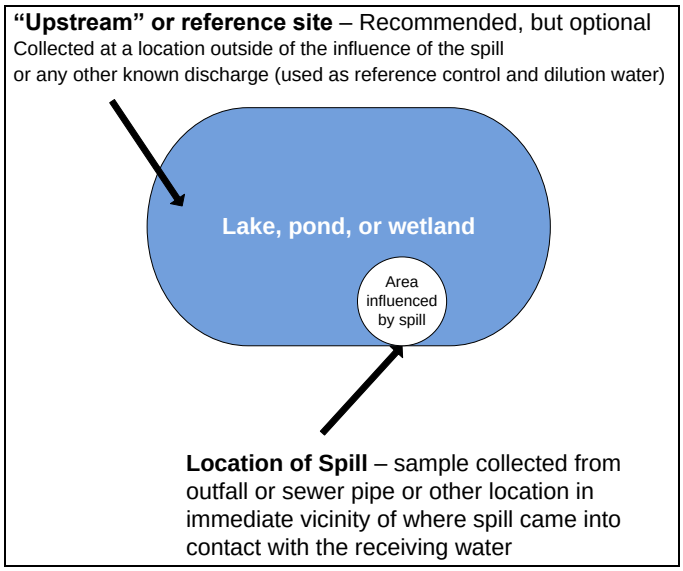
Please note: When responding to spills and illicit discharges, staff should not be serving as a first responder. Remember, safety is the first priority in any spill response. Staff must "stop, look and listen" before attempting to collect field samples to make sure the site is safe and they will not 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)

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 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, Fleet Farm, Wal-Mart, Menards, etc.). If Cubitainers® are not available, other containers can be used, as long as they are new and/or clean (see guidance on cleaning methods in Chapter 1.1). For guidance on which types of TIEs may be appropriate for a situation, contact Kari Fleming in the Bureau of Water Quality (contact information 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 WET testing, is available in Chapter 1.1 of this document.

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 Department 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 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 the Department’s Liaison to the State Lab (Zana Sijan, 608-264-8589 or Zana.Sijan@wisconsin.gov) 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 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.

Kari Fleming
Biomonitoring Coordinator
Bureau of Water Quality
(608) 400-2851
Kari.Fleming@wisconsin.gov

Wisconsin State Laboratory of Hygiene
Environmental Toxicology Lab
2601 Agriculture Drive
Madison, WI 53718
(608) 224-6230
biomonitoring@mail.slh.wisc.edu
(Note: this phone & email reaches all staff in the ET Lab)

CHAPTER 1.14 – Standard WET Permit Language

The purpose of this chapter is to provide staff with guidance regarding how WET requirements should be specified in WPDES permits.

This chapter is intended to help staff use the proper language in WPDES permits to implement state and federal WET regulations, and the requirements of the "*State of Wisconsin Aquatic Life Toxicity Testing Methods Manual, 2nd Edition*". Shown below is standard template language that appears in SWAMP. The callout boxes shown below provide some advice to permit drafters about how to modify this standard language, as needed to adapt it for individual discharge situations. Permit drafters may need to make other modifications to permit language to fit site-specific situations. They should contact the Biomonitoring Coordinator (Kari.Fleming@wisconsin.gov) with questions about alternate language. See Chapter 1.12 for advice about WET compliance schedule language.

WET Footnote

(appears beneath the effluent limits table for each outfall where WET testing is required)

1.1.1 Whole Effluent Toxicity (WET) Testing

Primary Control Water:

> Lab water is allowed in acute tests where a ZID is not allowed (compliance is determined in 100% effluent). Receiving water is required in chronic and acute with a ZID.
> See Chapter 1.2 for more information.

Acute Mixing Zone Concentration (AMZ):

> Add this line if a zone of initial dilution (ZID) is approved for the discharge.

Instream Waste Concentration (IWC):

> This line is applicable only if chronic WET is required. Remove this line if there is no chronic monitoring.

Dilution series: At least five effluent concentrations and dual controls must be included in each test.

- **Acute:** 100, 50, 25, 12.5, 6.25%, and any additional selected by the permittee.

> Pick the applicable dilution series, based on the permittee's IWC. Delete the series that is not applicable.

- **Chronic:** *100, 30, 10, 3, 1% (if the IWC ≤30%)* ***or*** *100, 75, 50, 25, 12.5% (if the IWC >30%)* and any additional selected by the permittee.

WET Testing Frequency:

Pick one.

- **Acute** tests shall be conducted *(once every other year, once each year, twice each year, quarterly, or bimonthly)* in rotating quarters, in order to collect seasonal information about the discharge. For example, tests are required during the following quarters:

  Enter each applicable calendar quarter

  Specify the quarter.

  Acute WET testing shall continue after the permit expiration date (until the permit is reissued) in accordance with the WET requirements specified for the last full calendar year of this permit. For example, the next test would be required in *(list appropriate quarter)*.

Pick one.

- **Chronic** tests shall be conducted *(once every other year, once each year, twice each year, quarterly, or bimonthly)* in rotating quarters, in order to collect seasonal information about the discharge. For example, tests are required during the following quarters:

  Enter each applicable calendar quarter

  Specify the quarter.

  Chronic WET testing shall continue after the permit expiration date (until the permit is reissued) in accordance with the WET requirements specified for the last full calendar year of this permit. For example, the next test would be required in *(list appropriate quarter)*.

Testing: 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.

Reporting: The permittee shall report test results on the Discharge Monitoring Report form, and also complete the "Whole Effluent Toxicity Test Report Form" (Section 6, "State of Wisconsin Aquatic Life Toxicity Testing Methods Manual, 2nd Edition"), for each test. The original, complete, signed version of the Whole Effluent Toxicity Test Report Form shall be sent to the Biomonitoring Coordinator, Bureau of Water Quality, 101 S. Webster St., PO Box 7921, Madison, WI 53707-7921, within 45 days of test completion. The Discharge Monitoring Report (DMR) form shall be submitted electronically by the required deadline.

Remove acute sentence if no acute is required.
Remove chronic sentence if no chronic is required.

Determination of Positive Results: An acute toxicity test shall be considered positive if the Toxic Unit - Acute (TU_a) is greater than [X] for either species. The TU_a shall be calculated as follows: $TU_a = 100 \div LC_{50}$. A chronic toxicity test shall be considered positive if the Toxic Unit - Chronic (TU_c) is greater than [Y] for either species. The TU_c shall be calculated as follows: $TU_c = 100 \div IC_{25}$.

Enter the correct values for X and Y:
X = 1.0, if a ZID is not allowed, or 100 ÷ AMZ, if a ZID is allowed
Y = 100 ÷ IWC
See Ch. 1.3 for more information.
AMZ and IWC are entered as whole numbers (e.g. IWC = 55% means 100/55)
WET Limits should be expressed using two significant digits (100/55 = 1.8 TUc)

Additional Testing Requirements: Within 90 days of a test which showed positive results, the permittee shall submit the results of at least 2 retests to the Biomonitoring Coordinator, on "Whole Effluent Toxicity Test Report Forms". The 90 day reporting period shall begin the day after the test which showed a positive result. The retests shall be completed using the same species and test methods specified for the original test (see the Standard Requirements section herein).

WET Standard Requirements (appears near the end of the permit, in the "Standard Requirements" section)

Placed in all permits with WET monitoring:

2.2.2 Whole Effluent Toxicity (WET) Monitoring Requirements
In order to determine the potential impact of the discharge on aquatic organisms, static-renewal toxicity tests shall be performed on the effluent in accordance with the procedures specified in the *"State of Wisconsin Aquatic Life Toxicity Testing Methods Manual, 2nd Edition" (PUB-WT-797*, November 2004) as required by NR 219.04, Table A, Wis. Adm. Code). All of the WET tests required in this permit, including any required retests, shall be conducted on the *Ceriodaphnia dubia* and fathead minnow species. Receiving water samples shall not be collected from any point in contact with the permittee's mixing zone and every attempt shall be made to avoid contact with any other discharge's mixing zone.

Change the species in this paragraph, if species other than C. dubia & fathead minnow are to be used in testing (rarely occurs).

Placed in all permits with WET monitoring:

2.2.3 Whole Effluent Toxicity (WET) Identification and Reduction
Within 60 days after the completion of a retest which showed positive results, the permittee shall submit a written report to the Biomonitoring Coordinator, Bureau of Water Quality, 101 S. Webster St., PO Box 7921, Madison, WI 53707-7921, which details the following:

- A description of actions the permittee has taken or will take to remove toxicity and to prevent the recurrence of toxicity;
- A description of toxicity reduction evaluation (TRE) investigations that have been or will be done to identify potential sources of toxicity, including some or all of the following actions:
  (a) Evaluate the performance of the treatment system to identify deficiencies contributing to effluent toxicity (e.g., operational problems, chemical additives, incomplete treatment)
  (b) Identify the compound(s) causing toxicity
  (c) Trace the compound(s) causing toxicity to their sources (e.g., industrial, commercial, domestic)
  (d) Evaluate, select, and implement methods or technologies to control effluent toxicity (e.g., in-plant or pretreatment controls, source reduction or removal)
- Where corrective actions including a TRE have not been completed, an expeditious schedule under which corrective actions will be implemented;
- If no actions have been taken, the reason for not taking action.

The permittee may also request approval from the Department to postpone additional retests in order to investigate the source(s) of toxicity. Postponed retests must be completed after toxicity is believed to have been removed.

Placed in permits with WET monitoring & a chloride variance:

2.2.4 Whole Effluent Toxicity and Chloride Source Reduction Measures

Section NR 106.89, Wis. Adm. Code, states that chloride limitations can be used in the permit in lieu of whole effluent toxicity testing requirements and limitations until chloride source reduction actions are completed, under the following conditions.

When an acute chloride limitation is included in the permit, acute whole effluent toxicity testing and limitations may be discontinued until chloride source reduction actions are completed, according to s. NR 106.89, Wis. Adm. Code, if either:

- The permittee can demonstrate to the satisfaction of the department that the effluent concentration of chloride exceeds 2,500 mg/l, or

- The permittee can demonstrate to the satisfaction of the department that the effluent concentration of chloride is less than 2,500 mg/l, but in excess of the calculated acute water quality-based effluent limitation, and additional data are submitted which demonstrate that chloride is the sole source of acute toxicity.

When a chronic chloride limitation is included in the permit, chronic whole effluent toxicity testing and limitations may be discontinued until chloride source reduction actions are completed, according to s. NR 106.89, Wis. Adm. Code, if either:

- The permittee can demonstrate to the satisfaction of the department that the effluent concentration of chloride exceeds 2 times the calculated chronic water quality-based effluent limitation, or

- The permittee can demonstrate to the satisfaction of the department that the effluent concentration of chloride is less than 2 times the calculated chronic water quality-based effluent limitation, but in excess of the calculated chronic water quality-based effluent limitation, and additional data are submitted which demonstrate that chloride is the sole source of chronic toxicity.

Following the completion of chloride source reduction activities, the department shall evaluate the need for whole effluent toxicity monitoring and limitation.

Placed in permits with WET monitoring & a condensate of whey (COW) discharge:

2.2.5 COW/NCCW or COW Discharges - WET Testing Requirements

If discharges consisting of condensate of whey (COW) wastewater only or non-contact cooling water mixed with COW waters (NCCW/COW) pass the first two acute toxicity tests and the first two chronic toxicity tests (if chronic toxicity testing is required) then the permittee is not required to perform additional toxicity testing during this permit term. If positive toxicity is experienced in any of the first two acute or chronic toxicity tests, the permittee may attempt to demonstrate that toxicity is due to ion deficiency. If it can be demonstrated that ion deficiency is the sole cause of toxicity in at least two consecutive positive tests, and the Department agrees in writing, the permittee will not be required to perform additional toxicity testing during this permit term. If it cannot be demonstrated that ion deficiency is the sole cause of toxicity in these tests, the permittee must complete the remaining toxicity tests

Placed in permits with WET monitoring, when ammonia limits based on ELS-absent criteria:

2.2.6 Whole Effluent Toxicity and ELS-absent Criteria for Ammonia

Effluent samples used in chronic fathead minnow tests may be modified to remove ammonia prior to testing, according to s. NR 106.36(2), Wis. Adm. Code, during periods when ammonia limits based on early life stage-absent criteria are in effect.

CHAPTER 2.1 - Selecting a WET Laboratory

The purpose of this chapter is to provide some guidance and answers to frequently asked questions for permittees who are selecting a new WET lab.

The first thing that should be done when evaluating a lab for its ability to perform WET tests is to verify that it is certified in Wisconsin to perform WET tests (s. NR 149.20, Wis. Adm. Code). You should ask the lab to provide you with a copy of their certificate or you may contact the Lab Certification Program to obtain this information (the Bureau of Integrated Science Services, 101 S. Webster St., Madison, WI 53707). A list of certified laboratories is also provided at: http://dnr.wi.gov/topic/wastewater/WETCertified.html.

You should ask the lab if they've had an on-site evaluation conducted by the DNR or other accrediting authority. If there has been an on-site evaluation, you may want to see a copy of the audit report and the lab's response to any deficiencies cited in the report. You may also contact the Lab Certification Program and ask to speak to the lab's auditor or go to the Bureau of Integrated Science Services in Madison and review the lab's file.

Lab Selection Based on Quality, Not Cost

Because lab selection can be such an important factor in test results, it is important that the experience of the analysts be carefully considered. The educational qualifications and experience of the lab individuals who will actually perform the tests, as well as the qualifications of the supervisory staff, should be reviewed prior to lab selection. The toxicity testing lab should demonstrate a serious commitment to a QA/QC program that extends beyond analyst experience. Considerations such as an ongoing reference toxicant program, a two-tiered review process for all toxicity test data and summary reports, a good sample custody tracking system that is always used, proper equipment maintenance, dilution water quality monitoring, facility maintenance, and attention to test organism health are all characteristics of a lab that is committed to generating quality data.

The costs associated with more experienced and better qualified laboratories can be higher than those of the less qualified laboratories, and many entities are constrained by existing procurement regulations that require the selection of the least expensive (and potentially least qualified) bidder. Perhaps one way to improve this situation is to convince the individuals responsible for making procurement decisions that WET testing is a professional service (much like engineering and chemical analyses services), which may give more latitude in selecting better qualified laboratories, rather than simply those that charge the least. It is advisable to define lab acceptance criteria in the specifications released for bid. Only those labs which can meet the specifications would be considered responsive, and only the lowest bid among those responsive would be considered for contract.

Probably the best lab-selecting tool is obtaining recommendations from others who have the expertise to critique lab performance. Since WET testing is required for many reasons, one can always find several individuals or firms who have been required to perform compliance toxicity tests, and it is very easy and straightforward to obtain information from them on how well (or poorly) their WET lab met their needs. The regulated community has every reason to be honest in their assessments (in fact, there is a real incentive not to be dishonest if they value their relationship with the person asking the question), and as a consequence, this is probably the best source of information currently available for making a decision regarding selection of a WET lab.

Organism Health And Performance

Lab visit. If visiting the lab, it may be wise to view the organisms used in testing. Aquariums, culturing chambers, and culturing areas should be clean and organized. Culture areas should be separate from testing areas so that cross-

contamination can't occur. The organisms should appear active and healthy. If fish appear sluggish, this may be an indication of health problems. *C. dubia* should be free-swimming, not floating or immobile.

Culture Records. The lab should be able to provide records documenting where their organisms came from originally. The Methods Manual (Section 3.6; s. NR 219.04, Wis. Adm. Code) requires that species confirmation occur annually and the lab should have records on file documenting that they use the correct species. If organisms from an outside supplier are used, ask who the supplier is and have the lab provide the documentation from the supplier regarding the health of the organisms. If the lab performed a reference toxicant test (see below) when the organisms first arrived in the lab, ask for a copy of those results.

Reference Toxicant Testing. Another way to compare laboratories is to look at the reference toxicant tests that have been generated. The Methods Manual (Section 3.15; s. NR 219.04, Wis. Adm. Code) requires that each lab demonstrate its ability to obtain consistent, precise results with reference toxicants before it performs tests with effluents for permit compliance purposes. Prior to lab certification or registration, each lab must establish an individual precision benchmark for each species and test type performed. This benchmark is defined as the coefficient of variation (CV) between replicates of the test endpoint (acute = LC_{50}; chronic = IC_{25}) calculated from $\geq$ 5 tests conducted with the reference toxicant sodium chloride (NaCl). These tests are done at the same concentrations, with the same test conditions, and same data analysis methods as effluent tests. Once established, ongoing lab performance must be continually compared to this benchmark. This ongoing performance is tracked in a "control chart" (see Figure 2.1.1).

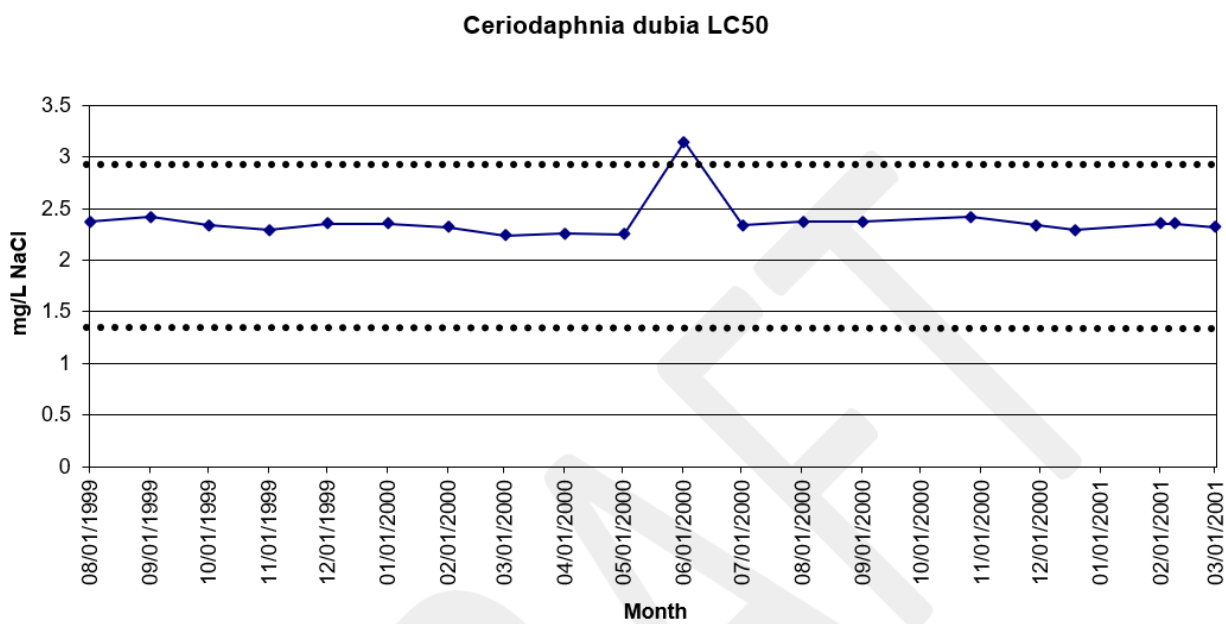


Figure 2.1.1 Example control chart showing mean LC_{50} and control limits (mean $\pm$ 2 standard deviations)

A control chart, like the example shown in Figure 2.1.1, is a graphical representation of the mean LC_{50} or IC_{25} and upper and lower control limits (mean $\pm$ 2 standard deviations) of each reference toxicant test using data from the previous 20 reference toxicant tests. The size of a control chart's upper and lower control limits is an indication of a lab's capability to reproduce the desired endpoints of a reference toxicity test. Laboratories with very wide control limits, and/or many points outside of the control limits, should be investigating problems related to the quality of the data being produced. A series of exceedances of either the upper or lower control limit after establishment of the control chart should prompt a review of the culture and test systems, as they may cause effluent tests completed during the month(s) of the exceedances to be questioned.

It is important to note that control chart limits are a function of the test species, test type and biological endpoint (survival, growth, etc.) being used. These factors must be considered before drawing conclusions regarding lab performance. Analyst performance should improve with experience, and the control limits for point estimates should gradually narrow. However, control limits of ± 2 S.D., by definition, will be exceeded 5% of the time, regardless of how well a lab performs. Highly proficient laboratories may develop very narrow control limits. A lab that has experienced a test which falls just outside the control limits, but has very narrow control limits, may not warrant the same level of concern as a similar test done at a lab with wider control limits. The width of the control limits should be considered in determining whether or not points outside of the normal range are a signal of poor lab performance.

The permittee should ask the lab to provide a copy of their reference toxicant results and control charts. Reference toxicant testing is required by the Methods Manual (Section 3.15; s. NR 219.04, Wis. Adm. Code) in order to determine the sensitivity of test organisms over time and to assess the lab's ability to obtain consistent test results using a known toxicant. Results can be used to identify potential sources of variability, such as test organism health, difference among batches of organisms, changes in lab water or food quality, and performance by lab technicians. Performance of reference toxicity tests as recorded by control charts is a criterion that may be used by a permittee when selecting which laboratory to use for WET tests. Charting the performance of a lab's controls relative to its reference toxicity test results can also be a good way to track the lab's performance and to identify when the lab's performance is not acceptable.

Lab and Individual Analyst Experience and Qualifications

A number of factors are important in reducing test variability and improving test quality. Some of the most important of these factors are careful adherence to test guidelines, adequate analyst expertise, and conscientious selection of contract laboratories. Analyst experience and organism health are probably the two most important aspects of any successful WET test. Wisconsin's WET laboratory certification program requires that each lab meet certain experience and qualification requirements for their staff (see Section 3.17 of the Methods Manual, s. NR 219.04, Wis. Adm. Code).

The ability to successfully complete toxicity tests is a direct function of the training and expertise that an analyst has accumulated. If laboratories have staff who are responsible for the toxicity testing program but have no training in the biological sciences or little practical experience in WET testing, this can be a major source of test variability. The DNR has written and codified the Methods Manual (s. NR 219.04, Wis. Adm. Code), in order to set Wisconsin-specific test methods and test acceptability criteria. The Methods Manual also contains specific requirements for lab analyst training and expertise. Section 3.17.2 of the Methods Manual says that "*These methods are restricted to use by or under the supervision of analysts experienced in the use or conduct of aquatic toxicity testing and the interpretation of data from aquatic toxicity testing. Each analyst must demonstrate the ability to generate acceptable test results with these methods using the procedures described in this methods manual*". One would not consider chemical analysis of water samples, including effluents, without adequate analyst expertise and proper quality assurance/control (QA/QC). Since WET test results are as important as chemical analyses in most regulatory decisions, it seems only appropriate to have the same high standards for these test methods.

Permittees should ask to see the training files of lab staff and insure that each lab technician has the necessary combination of experience and education to adequately demonstrate a specific knowledge of their particular function and a general knowledge of lab operations, analytical test methods, QA/QC procedures, and records management. Lab management should be able to certify that personnel with appropriate educational and/or technical background perform all tests for which the lab is accredited.

CHAPTER 2.2 - Toxicity Reduction Evaluations

This chapter provides guidance to aid dischargers and their consultants when conducting a TRE. Included is a description of the basic steps usually included in a TRE and the reports usually submitted to the Department.

Additional TRE/TIE Guidance

The following documents provide guidance on designing and conducting TREs and provide case studies illustrating approaches used successfully by others. They describe steps that may be considered when doing a TRE and provide more detail than given here. These documents are recommended supplemental reading for those doing a TRE on complex wastewaters.

Society of Environmental Toxicology and Chemistry (SETAC) Publications

- *Toxicity Reduction and Toxicity Identification Evaluations for Effluents, Ambient Waters, and Other Aqueous Media* (2005) Almost 25 years after the WET program was initiated, SETAC invited participants representing universities; government, research, and regulatory agencies; mining and chemical industries; and consulting services to a Pellston Workshop on TRE/TIE to update and advance the understanding of the TRE process and the science of TIEs. What resulted was this comprehensive guide to TRE/TIE, detailing procedures and including more than 30 case studies describing various aspects of the process. 978-1-880611-64-7 , 496 pp. (Order #: SB02-18) (available free of charge at: http://www.setac.org (store))

USEPA Guidance

- *Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures, 2nd Ed.* (1991) Methods to characterize the chemical/physical nature of constituents which cause toxicity.1 of 3. (EPA-600-R-91-003) https://nepis.epa.gov/Exe/ZyPDF.cgi/300011NY.PDF?Dockey=300011NY.PDF

- *Toxicity Identification Evaluation: Characterization of Chronically Toxic Effluents, Phase I* (1992) Methods to characterize the chemical/physical nature of the constituents which chronic toxicity. (EPA 600-6-91-005F). https://www3.epa.gov/npdes/pubs/owm0255.pdf

- *Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity* (1993) Guidance on how to identify the cause of toxicity. 2 of 3. (EPA-600-R-92-080) https://www3.epa.gov/npdes/wettraining/module1/story_content/external_files/TIE%20Phase%202%201993.pdf

- *Methods for Aquatic Toxicity Identification Evaluations: Phase III Toxicity Confirmation Procedures for Samples Exhibiting Acute and Chronic Toxicity* (1993) Guidance on how to confirm the cause of toxicity. 3 of 3. (EPA-600-R-92-081) https://www3.epa.gov/npdes/pubs/owm0341.pdf

- *Toxicity Reduction Evaluation Guidance for Municipal Wastewater Treatment Plants* (1999) Guidance document to permittees, permit writers and consultants on the general approach and procedures for conducting TREs at municipal WWTPs. Intended as a guide to approaches that have been successfully used in municipal TREs (EPA 833-B-99-022) https://nepis.epa.gov/Exe/ZyPDF.cgi/20004C2J.PDF?Dockey=20004C2J.PDF

- Generalized Methodology for Conducting Industrial Toxicity Reduction Evaluations (1989) A generalized methodology for the design and performance of a TRE at an industrial facility. (EPA 600-2-88-070) https://nepis.epa.gov/Exe/ZyPDF.cgi/30000H2L.PDF?Dockey=30000H2L.PDF

The terms “toxicity reduction evaluation” (TRE) and “toxicity identification evaluation” (TIE) may be used interchangeably, separately, or together (as in "TI/RE"). A TRE is the complete evaluation intended to determine the actions needed to remove toxicity. A TIE is one step in the TRE where effluent samples are taken into a lab and manipulated in various ways in order to identify the chemical(s) causing toxicity. A TRE usually includes steps to identify the source(s) of toxicity and steps designed to identify ways to reduce toxicity. It may identify a simple solution such as improved housekeeping procedures or require a more extensive investigation to identify cost-effective treatment or source reduction options.

At the beginning of a TRE, the review of effluent data and facility-specific information is important in order to define study objectives, identify what is known, and provide clues as to the cause of toxicity. Included should be a review of facility housekeeping practices, treatment plant operation, and the selection and use of process and treatment chemicals, in an attempt to identify/reduce potential sources of toxicity. If none of these practices is identified as the source of toxicity, a TIE is usually the next step. The objective of a TIE is to characterize and identify the chemical(s) causing toxicity so that they can be traced back to their source. Once the chemical compound and its source are identified, the TRE process usually goes in one of two directions. One approach is to evaluate options for treating the final effluent, the other is to remove/reduce the source of toxicity through upstream pretreatment, source reduction, or process modifications. A decision can be made to pursue both approaches, and then to select the most technically and economically attractive option.

Flexibility in the design of a TRE is important. Each successive step in a TRE can hinge on the results of previous steps. Since every discharge and toxicity situation is different, the approach needed to investigate potential sources is usually different, too. However, there are some investigative techniques and approaches that are common to many TREs. The guidance provided here is intended to describe general approaches which have been used successfully in the past by Wisconsin permittees and their consultants.

Communication and cooperation between the permittee, their consultant, and the Department is essential in TRE plan development, review, and implementation and will help ensure achievement of the TRE objective. No better way exists to establish this communication than face to face meetings between the permittee, the consultant hired to perform the TRE, and Department staff. Routine meetings and interim reports during the course of the TRE can be beneficial to assess progress and steer future activities. This type of communication insures that there are no surprises to any of the participants and maximizes the efficiency of TRE processes.

Finding A Qualified TRE Consultant. Probably the most important beginning step in any TRE is the identification of a qualified consultant that can help coordinate the toxicity investigations. Since most permittees do not have experience with these types of investigations, they must rely on their consultant to help steer them through the process. Permittees sometimes rely on the lab that conducted previous WET tests to conduct the TRE, which may not be appropriate in all cases. TRE work can be much different than normal WET testing, and not all labs and consultants have similar levels of experience doing successful TRE work.

The selection of a consultant requires careful review and evaluation. Potential consultants should be asked to provide references, with special emphasis given to the company’s knowledge, experience and successful resolution of TREs in general, and specifically with the same type of facility and testing requirements. Relevant TRE expertise far outweighs the selection of the lowest bidder because the latter often lack staff experience and do not have a track record of solving similar toxicity reduction challenges. When properly written, requests for bids that include specific capabilities and experience can avoid problems when awarding contracts.

When selecting a consultant to address toxicity problems, the chief criterion for that choice should be a history of success with TREs. A simple ability to conduct select TIE procedures alone does not constitute this experience. A consultant should demonstrate the capacity to put together a multifaceted team of individuals including toxicologists and chemists, and possibly engineers and other professions pertinent to the client’s situation so that the results of TIE and TRE activities can be effectively funneled from one expertise to the next.

It is crucial to the success of a TRE that the primary investigator has experience conducting successful TREs. The quality of the work being done may be entirely dependent on the experience and knowledge that the lab or consulting firm can bring to the project. A good TRE consultant, whether part of the WET lab's staff or a separate individual, should be a good "detective". Ideally, he/she should tour the facility, understand treatment plant operations and contributing wastestreams, review process and WET records, and become familiar with staff and operations at the facility. The best way to choose a consultant that is up to the challenge may be to talk to other permittees who have performed TREs, in order to determine which consultants have been the most successful at identifying toxicity sources. You should try to identify labs that have had success identifying the cause of toxicity during these studies, and separate those from investigations that were long and costly without identifying a cause.

TIE costs can vary greatly, depending on the amount and type of work needed. Permittees should not limit their search to local labs, since the lab work is usually much more expensive than shipping costs. The Department cannot officially endorse or recommend labs or consultants, but can identify those that have performed TREs successfully in the past (see http://dnr.wi.gov/topic/wastewater/WETlabTRE.html).

WET Requirements Driven by Limited Data or Intermittent Toxicity. Federal and state regulations sometimes require that a WET limit be given when a small number of WET failures have occurred, even if toxicity has not occurred in the effluent for some time or if toxicity is not always present in the effluent. (See Chapter 1.3 for a discussion of when limits are required.) Although past WET failures may suggest that there is reasonable potential for adverse environmental impacts caused by effluent toxicity, which requires the imposition of a WET limit (s. NR 106.08, Wis. Adm. Code), TREs are more difficult to perform when toxicity is not present consistently. In situations where toxicity has not recurred, a successful TRE may include monitoring that shows that toxicity in past tests is no longer present even if the reason for the disappearance is not fully known. If no toxicity occurs during monitoring performed during the TRE period, it may not be possible for past sources of toxicity to be identified. If monitoring done during the permit term (during the TRE or after) shows that toxicity is no longer present in the discharge, it may be possible to argue that older WET failures are no longer representative of the discharge.

When Is A TRE Necessary?

The Department strongly encourages permittees to begin a TRE as soon as their effluent has shown severe or repeated bouts of toxicity. The following is language that is typically included in the Standard Requirements sections of WPDES permits where WET monitoring is required.

> **WET Identification and Reduction**
>
> *Within 60 days of a retest which showed positive results, the permittee shall submit a written report to the Biomonitoring Coordinator, Bureau of Water Quality, 101 S. Webster St., PO Box 7921, Madison, WI 53707-7921, which details the following:*
>
> - *A description of actions the permittee has taken or will take to remove toxicity and to prevent the recurrence of toxicity;*
> - *A description of toxicity reduction evaluation (TRE) investigations that have been or will be done to identify potential sources of toxicity, including some or all of the following actions:*
>
> *(e) Evaluate the performance of the treatment system to identify deficiencies contributing to effluent toxicity (e.g., operational problems, chemical additives, incomplete treatment)*
> *(f) Identify the compound(s) causing toxicity*
> *(g) Trace the compound(s) causing toxicity to their sources (e.g., industrial, commercial, domestic)*
> *(h) Evaluate, select, and implement methods or technologies to control effluent toxicity (e.g., in-plant or pretreatment controls, source reduction or removal)*

- *Where corrective actions including a TRE have not been completed, an expeditious schedule under which corrective actions will be implemented;*

- *If no actions have been taken, the reason for not taking action.*

The permittee may also request approval from the Department to postpone additional retests in order to investigate the source(s) of toxicity. Postponed retests must be completed after toxicity is believed to have been removed.

When an effluent has shown a severe or persistent toxicity problem, the Department may modify or reissue the permit to include additional monitoring and WET limits, since the potential for exceedance of water quality standards exists. If there is evidence that severe or persistent toxicity exists at the time of permit reissuance, the Department will likely reissue that permit with a WET limit and a TRE compliance schedule that requires the permittee to find and fix the source(s) of that toxicity. However, if permittees can find and fix the problem on their own before the permit is modified or reissued, they may avoid WET limits, compliance schedules, and potential enforcement.

Whether voluntary or permit-required, TRE studies should be well thought out and study objectives and results well documented and communicated to the Department. This chapter was created in order to provide some guidance regarding the basic information that should be included in TRE plans and reports. The primary purpose of TRE plans and reports are to inform the Department about work that was done (or is to be done) to identify the source of toxicity. It is important to remember that although TRE plans and reports are intended primarily for the Biomonitoring Coordinator who is the Department's technical expert on WET, others (staff, local government, public, industry, environmental groups, etc.) may also want to read and understand them as well. For this reason, TRE plans and reports should be written so that the general public can understand them.

Toxicity Reduction Evaluation Plans and Reports

The submittal and completeness of TRE plans and reports required by WPDES permits is the responsibility of the permittee, even if parts or the whole are written by consultants. The following guidance describes the TRE plans and reports typically required by WPDES permits, but may also be used by permittees who are doing a voluntary TRE. In order for readers to understand the goals of the TRE, plans should include specific steps to be taken, specific dates when each step is to be completed, and a description of what each step is meant to accomplish. In order to help permittees and their consultants design plans and reports, some general outlines of information that may be included are given in the following pages.

In order for permit-required TRE plans and reports to be approved by the Department, they should generally include the information given below for each step. However, the Department does recognize that each TRE will be facility specific, some of the areas outlined may not apply in some situations, and that in some cases more or different information may be necessary. Communication and cooperation between the permittee and the Department is essential in TRE plan development, review, and implementation and will help ensure achievement of the TRE objective.

A TRE is usually divided in two parts: 1) identification and 2) removal of toxicity. Decisions on the most appropriate way to remove toxicity are usually dependent on the cause. The standard WET limit compliance schedule (described in Chapter 1.12) is usually set up so that sufficient time is allowed for both parts of the TRE to be completed. Descriptions of the steps included in that schedule, including descriptions of the TRE plans and reports that should be submitted, are given below.

Example Compliance Schedule

Step	Required Action	Date Due
1	**Submit part one of a Toxicity Reduction Evaluation (TRE) plan** describing procedures to be used to identify the source(s) responsible for the effluent toxicity.	*3 months from permit issuance*
2	Implement part one of the TRE plan, make a reasonable attempt to identify the source(s) of the toxicity, and submit a report to the Department presenting the results of the evaluation.	*18 months from permit issuance*
3	Submit part two of the TRE Plan describing actions to be taken to reduce or eliminate the toxicity identified in part one of the TRE and the dates by which those actions will be implemented.	*19 months from permit issuance*
4	Submit a progress report identifying the actions taken to date to implement part two of the TRE plan.	*28 months from permit issuance*
5	Complete all actions identified in the TRE plan and achieve compliance with the effluent toxicity limitation.	*36 months from permit issuance*

Step One: Submittal of Part One of the TRE Plan

The first step in the typical WET limit compliance schedule shown above usually involves the development of a plan describing actions to be taken to investigate sources of toxicity. Typically, this plan is required to be submitted to the Department within about 3 months of permit reissuance. This plan should be designed to provide the Department with a description of work to be done to identify toxicity, a schedule for conducting specific tasks and reporting results, relevant background information on the facility, and identification of the lab and/or consultants who will be involved in the investigations.

This TRE plan should include: a description of tasks to be done to identify the source of toxicity, a schedule for conducting these tasks, relevant background information, objectives of the study, and scheduled completion dates and milestones. The following is an outline of suggested information to be included in these plans.

1. Introduction. *(1-2 pages)*

A. Narrative description of past WET Tests, toxicity identification work done to date (if any)

B. Summary *(tables, graphs, etc.)* of specific WET test results, TIE work done (if any)

2. Outline or flowchart of study. *(1-2 pages)*

A. Timelines for when each phase of the work is expected to be done.

B. Discussion of data gathering & review steps, facility-specific investigations that will be done

1) Review of in-house data *(effluent data, operational records, treatment chemicals, etc.)*

2) Field data collection *(inventory of hauled wastes, user surveys, etc.)*

C. Discussion of Phase I, II, and II TIE steps planned *(usually more detail regarding Phase I, Phase II & II in general terms, since they're dependent on results of previous Phase)*.

3. Toxicity Identification Evaluation (TIE) & other laboratory investigation steps. *(4-5 pp)*

A. Species used, type *(acute or chronic, screening vs. dilution series, etc.)* & frequency of tests

B. Description of TIE manipulations - what's done, what results mean if a reduction in toxicity is noted *(1-2 paragraphs on each manipulation)*

1) Initial toxicity tests *(if applicable)*

2) Baseline tests *(if applicable)*

3) pH adjustment *(if applicable)*

4) filtration *(if applicable)*

5) aeration *(if applicable)*

6) C_{18}/SPE *(if applicable)*

7) sodium thiosulfate/oxidant reduction *(if applicable)*

8) EDTA chelation *(if applicable)*

9) any additional selected

4. Reporting timelines for the project *(1-2 pages)*

A. Description of when progress reports will be made to the Department

B. Dates when Phase I, II, & III reports will be due *(options include separate dates for each as they are completed or one final report when all work is finished)*

C. Date when report is expected *(on or before next date specified in compliance schedule)*

5. Summary, potential future work *(1-2 pages)*

Other steps can be taken to identify toxicity sources. The permittee and their lab/consultant may be the best source of ideas for these additional steps. The Biomonitoring Coordinator and other Department staff can assist permittees, where possible. Permittees should provide regular updates during the TRE, in order to insure that studies are proceeding as planned. Communication and cooperation between the permittee and the Department is essential in TRE plan development, review, and implementation and will help ensure achievement of the TRE objective.

Things To Avoid When Planning Or Conducting A TRE:

1. **Reliance on "gut feelings" & "trial and error" studies.** Since TIEs are expensive, some often narrow their search by limiting studies to look for sources they "feel" may be the cause. Substantial modification of the Phase I TIE methods or preconceived assumptions about the cause of source of toxicity should be avoided in most cases. Trial and error type studies often result in starting over from scratch when preconceived ideas are proven wrong. While toxicity sources may be identified through this type of approach, they often take longer and the cost of doing so may quickly surpass the cost of a more organized TRE.

   Guessing the cause or source of toxicity can increase the probability that toxicity resolution will be delayed, continued non-compliance will occur, additional cost will be incurred, and/or that public trust will be diminished. For these reasons, the TRE investigator should approach effluent characterization without a preconceived notion as to the cause of toxicity.

2. **Reliance on priority pollutant scans.** Toxicity may be caused by compounds which don't appear on chemical analysis lists or may be caused by a combination of compounds. Priority pollutant scans may provide helpful information to supplement TIE tests, but shouldn't be relied on to provide all of the answers.

3. **Confirmation steps not carried out for identification or treatability studies** Money can be wasted if conclusions are rushed and not confirmed before treatment options are considered.

4. **Quick solutions without modifying ongoing behavior.** In most cases, solutions to toxicity should become standard operating procedure. In order to avoid additional WET test failures and possible limit violations, TRE activities should not be once in time but continuing activities.

Example Compliance Schedule

Step	Required Action	Date Due
1	Submit part one of a Toxicity Reduction Evaluation (TRE) plan describing procedures to be used to identify the source(s) responsible for the effluent toxicity.	*3 months from permit issuance*
2	**Implement part one of the TRE plan**, make a reasonable attempt to identify the source(s) of the toxicity, and submit a report to the Department presenting the results of the evaluation.	*18 months from permit issuance*
3	Submit part two of the TRE Plan describing actions to be taken to reduce or eliminate the toxicity identified in part one of the TRE and the dates by which those actions will be implemented.	*19 months from permit issuance*
4	Submit a progress report identifying the actions taken to date to implement part two of the TRE plan.	*28 months from permit issuance*
5	Complete all actions identified in the TRE plan and achieve compliance with the effluent toxicity limitation.	*36 months from permit issuance*

Step Two: Implementation of Part One of the TRE Plan and Submittal of a Report

Following the plan submitted in step one, the permittee should begin investigating the sources of effluent toxicity as laid out in their plan. The first stage is usually the collection of data and facility-specific information. These activities are often used to define study objectives, identify what is known, and provide clues as to the cause(s) of toxicity. This information may also suggest immediate actions which may be useful in reducing effluent toxicity. After data collection, the next action usually involves optimization of facility operations in an attempt to reduce effluent toxicity. Three areas are usually investigated during this step: facility housekeeping, treatment plant operation, and the selection and use of process and treatment chemicals.

After it has been established that sampling protocols, facility housekeeping, treatment plant operations, and treatment chemicals are not causing toxicity, a TIE is often done. The objective of the TIE is to characterize and identify the compound(s) causing toxicity so that they can be traced back to their source. In a TIE, effluent samples are taken to the WET 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 effluent 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. Multiple samples are usually needed for a TIE and one objective of a TIE may be to determine if and how toxicity varies over time. Once a specific class or individual compound has been identified as a potential cause of toxicity, the investigation turns towards finding the source (e.g., contributing industrial process, commercial source, etc.) of that compound.

Specific tasks that may be done during Step Two of a TRE:

- **Check For Sampler Contamination.** Sampling locations and gear should be checked for possible toxic contributions and to see whether equipment was new and/or properly cleaned prior to testing (see Chapter 1.1 for guidance on sampler cleaning). Sampler contamination and sampling procedures can also be studied by conducting concurrent tests with different samplers or different WET labs.

- **Data Gathering.** TREs usually begin with information gathering and study of on-site situations. Data gathering steps may include: 1) checking effluent data to see if other limits were exceeded or operational upsets may have occurred at the same time as effluent toxicity; 2) identifying potential sources of toxicity within the process or wastewater treatment plant, such as cleaning or disinfection agents, process side-streams, or treatment chemicals (e.g. phosphorus removal chemicals, polymers, flocculants, etc.); 3) looking for significant levels of individual chemicals in the combined wastestream (using pretreatment data, Merck index, or other chemical references) and 4) inventorying what is entering the sewer system by industry, commercial, domestic, & batch loads accepted by the headworks or elsewhere.

A thorough inventory of all contributions to the waste stream is often helpful. Attachment 1 at the end of this chapter includes an example "User Survey", which has been used by POTWs successfully in the past to collect this type of information.

Industries should identify all possible contributions from their plant into the wastewater stream, including floor drains. Unwanted materials may be getting into the discharge without wastewater staff's knowledge. An understanding of all potential constituents and their relative presence (in time and concentration) may be beneficial to resolving toxicity problems. In addition to the obvious process waste streams, side streams such as cooling tower discharges, boiler blowdown, and other potential sources should be reviewed for the presence of toxic chemicals. In many cases, toxicity sources have been identified that are not process-related, but rather side streams where unintentional releases or unconsidered chemicals (e.g., shift-dependent equipment sterilization, highly ionic wastes, chlorinated tap water, etc.) contributed to toxicity problems.

- **Public and Employee Education.** Employee education regarding proper disposal and use of process and treatment chemicals has reduced toxicity in many instances. Public education has been important in toxicity reduction for some municipal dischargers. Commonly used household pesticides and cleaners have been implicated at POTWs as sources of toxicity. Educating the public and employees about the environmental and monetary costs of improper disposal of toxic substances has resulted in reductions in effluent toxicity in some instances. In a few cases, toxicity has disappeared from a municipal effluent after efforts were made to alert the public about toxicity problems.

- **Facility Housekeeping.** It is important to verify that process chemicals are not overused, housekeeping practices are not contributing wastes directly to the effluent, and other facility practices are not contributing to toxicity (see Attachment 2 "Housekeeping Logic Flow") and the selection and use of process and treatment chemicals. Chemical optimization is a process that can be performed in conjunction with the housekeeping parts of the TRE. The goal of this process is to identify simple solutions to toxicity problems by evaluating and possibly modifying chemical use at the facility.

  A general inspection of housekeeping practices by the TRE investigation team may indicate possible problem areas that may be contributing to toxicity. Treatment chemicals used within the facility should be reviewed to determine if any new chemicals (or different concentrations) are being discharged by industrial processes or used at the treatment plant that could potentially explain the effluent toxicity. Some common sources of toxicity that have been identified in past TREs are chemical additives (especially surfactants, polymers, and biocides), wastewater treatment chemicals (polymers and defoamers), and disinfection/cleaning chemicals.

- **Optimization Of Facility Operation.** An attempt should be made to see what operational adjustments could be made that might reduce toxicity, such as increasing aeration basin detention time, sludge age, etc. and develop a scheme for testing how such adjustments in process control may reduce toxicity in the effluent (allowing time at each adjusted setting to ensure several detention times occur). See Attachment 3 "Treatment Plant Optimization Logic Flow".

- **Additional Monitoring To Demonstrate Absence Of Toxicity.** In some cases, toxicity may be infrequent, disappear inexplicably, or have been removed by actions taken prior to initiation of the TRE. In these cases, permittees may wish to demonstrate that toxicity is no longer present by conducting additional WET tests. These tests should be performed using the procedures specified in the permit. The number of tests required to make this demonstration may depend on factors such as the seasonality, severity, and cause(s) of toxicity.

- **Toxicity Identification Evaluation (TIE).** TIE methods include bench-top treatment steps designed to indicate the general types of compounds that are causing effluent toxicity. Initial toxicity tests are performed to determine if samples are toxic, then manipulations for removal or alteration of effluent toxicity are performed and the resulting treated samples are tested for toxicity. The physical/chemical characteristics are indicated by the treatment steps that

reduce toxicity relative to a baseline test. It is recommended that the full suite of Phase I-III procedures be performed on effluent samples, in order to characterize, identify, and confirm the cause of toxicity. As information is obtained on the nature and variability of toxicity, additional tests may focus on the steps that are successful in affecting toxicity. (See the list of TIE methods given at the beginning of this chapter.)

- **Three Major Parts to a TIE**

  ⇒ **Phase I, Characterization:** Intended to characterize the physical/chemical properties of the compounds causing toxicity. Such characteristics as solubility, volatility, pH sensitivity, polarity, and filterability are determined without specifically identifying toxicants. Usually a 1st step in identifying toxicity but can also be used to develop treatment methods to remove toxicity without specific identification of toxicants.

  ⇒ **Phase II, Identification:** Specifically identifies toxicants if non-polar organics, ammonia, or metals.

  ⇒ **Phase III**, **Confirmation:** Confirmation of suspected toxicants.

  As stated previously, it is recommended that the investigator approach effluent characterization without a preconceived notion as to the cause of toxicity. The Phase I TIE should be completed in its entirety to minimize the chance of overlooking a toxicant. It is possible to overlook classes of compounds that contribute to toxicity if the Phase I process is substantially cut short. Abbreviated Phase I TIEs may result in the loss of valuable and necessary information on the characteristics of the substances responsible for toxicity, which may lead to inconclusive results or erroneous conclusions.

- **Test Frequency.** TIEs require that toxicity be present so that toxicants can be characterized and identified. Enough toxicity screening should be done to assure consistent presence of toxicants for characterization. Usually monthly or bimonthly testing is recommended. In order to reduce costs, it may be wise to do more frequent screening tests (100% effluent only) to determine if toxicity is present, rather than full dilution series less frequently.

- **Most Sensitive Species.** Tests done during a TIE may be limited to the species shown to be the most sensitive in previous WET tests. It is assumed that by removing toxicity to the most sensitive species, toxicity to others is removed as well.

- **Toxicity Source Evaluation.** Once the toxicants have been identified, steps can be taken to locate their source. This evaluation may include a review of existing pretreatment data or data from the collection and analysis of samples from industrial users. Information gathered from an "Industrial and Commercial User Survey" (see Attachment 1) can be invaluable at this point in the TRE.

Part One Report

The following is an outline of suggested information to be included in a part one TRE Report (required during step two of the example compliance schedule shown above). Plan submittals intended to comply with permit-required compliance schedules should include: a description of specific tasks done by the permittee and their consultant to identify the source of toxicity (e.g., industry, commercial, domestic) and any relevant background information.

1. Introduction. *(1-2 pages)*

A. Description of data gathering/analyses, education efforts, and facility-specific investigations done
B. Summary of specific toxicity test results, TIE work done
C. General discussion of Phase I, II, and II steps completed and conclusions reached

D. Any deviations from "normal" WET test procedures *(e.g., feeding schedules, temperatures, pH control, aeration, etc.)* should be highlighted

2. Results of Toxicity Identification Evaluation & other laboratory investigation steps. *(4-5 pp.)*

A. Discussion of test type used *(acute/ chronic, screening vs. dilution series, etc.)* and frequency of tests

B. Description of specific TIE manipulations - what was done, what results were and what they mean *(1-2 paragraphs on each manipulation - should contain tables showing survival and growth/reproduction results from each manipulation, when appropriate)*

3. Toxicity Source Evaluation.

A. review of existing pretreatment data or data from the collection and analysis (chemical-specific and/or toxicity) of additional samples from industrial users. Information gathered from an "Industrial and Commercial User Survey" should be reported. Conclusions reached as to the specific industrial, commercial, or other source(s) of effluent toxicity should be discussed.

4. Summary & Conclusions *(1-2 pages)*

A. Summary of work done to identify source(s) responsible for toxicity and what results indicate.

B. Identification of potential source(s) *(e.g., industrial, commercial, internal to WWTP)* of toxicity, based on data review and collection, frequency & duration of toxicity, TIE work.

Example Compliance Schedule

Step	Required Action	Date Due
1	Submit part one of a Toxicity Reduction Evaluation (TRE) plan describing procedures to be used to identify the source(s) responsible for the effluent toxicity.	*3 months from permit issuance*
2	Implement part one of the TRE plan, make a reasonable attempt to identify the source(s) of the toxicity, and submit a report to the Department presenting the results of the evaluation.	*18 months from permit issuance*
3	**Submit part two of the TRE Plan** describing actions to be taken to reduce or eliminate the toxicity identified in part one of the TRE and the dates by which those actions will be implemented.	*19 months from permit issuance*
4	Submit a progress report identifying the actions taken to date to implement part two of the TRE plan.	*28 months from permit issuance*
5	Complete all actions identified in the TRE plan and achieve compliance with the effluent toxicity limitation.	*36 months from permit issuance*

Step Three: Submittal of Part Two of the TRE Plan

The third step of the TRE compliance schedule usually requires the permittee to submit a plan describing actions to be taken to investigate ways to remove the sources of toxicity identified in previous steps. To meet the intent of this third step of the compliance schedule, part two of the TRE plan should include: a description of tasks to be done to identify alternatives for removing/reducing toxicity, a schedule for conducting these tasks, background information from part one of the TRE, identification of those conducting the evaluation (e.g., lab, consultants, etc.), objectives of the study, and scheduled completion dates and milestones. The following is an outline of suggested information to be included in these plans:

1. Introduction. *(1-2 pages)*

A. Narrative description of toxicity identification work done & results shown

B. Summary *(tables, graphs, etc.)* showing specific WET test results, TIE work done *(optional)*

2. Outline or flowchart of study. *(1-2 pages)*

A. General description of plan to investigate ways of reducing or eliminating toxicity, including an evaluation of options for treating the final effluent and an evaluation of upstream pretreatment options, source reduction, and/or process modifications.

B. Timelines for when each phase of the work is expected to be done.

3. Toxicity Reduction Evaluation (TRE) & other investigation steps. *(4-5 pages)*

A. Discussion of studies to be done to identify toxicity removal alternatives, which may include:

1) Source reduction alternatives

2) Assessment of the treatment options: trials of modified treatment procedures in existing works or the evaluation of different procedures or works through bench or pilot scale simulation

3) Cost/benefit analysis *(factors such as cost effectiveness and long term effects should be considered when choosing the best alternative)*

B. Tests to confirm toxicity removal

1) Tests should be done according to procedures specified in the permit.

2) Number of tests will depend on seasonality, severity, and cause of original toxicity *(usually 3 or 4 tests, performed at least 60 days apart, is sufficient)*.

4. Reporting timelines for the project *(1-2 pages)*

A. Description of when progress reports will be made to the permittee and the Department

B. Compliance schedule for installment of pretreatment *(when applicable)*

C. Date when final report is expected *(on or before date specified in last step of compliance schedule)*

5. Summary, potential future work *(1-2 pages)*

Example Compliance Schedule

Step	Required Action	Date Due
1	Submit part one of a Toxicity Reduction Evaluation (TRE) plan describing procedures to be used to identify the source(s) responsible for the effluent toxicity.	*3 months from permit issuance*
2	Implement part one of the TRE plan, make a reasonable attempt to identify the source(s) of the toxicity, and submit a report to the Department presenting the results of the evaluation.	*18 months from permit issuance*
3	Submit part two of the TRE Plan describing actions to be taken to reduce or eliminate the toxicity identified in part one of the TRE and the dates by which those actions will be implemented.	*19 months from permit issuance*
4	**Submit a progress report** identifying the actions taken to date to implement part two of the TRE plan.	*28 months from permit issuance*
5	**Complete all actions** identified in the TRE plan and achieve compliance with the effluent toxicity limitation.	*36 months from permit issuance*

Steps Four & Five: Submittal of Progress and Final Reports

Once the cause and source of toxicity have been identified, decisions on the most appropriate approach for removing toxicity should be made. To do this, the TRE process usually goes in one of two directions. One approach is to evaluate options for treating the final effluent; the other is to evaluate upstream pretreatment options, source reduction, or process modifications. A decision can also be made to pursue both approaches, and then to select the most technically and economically attractive option. This is the work that should have been planned during the third step of the compliance schedule and includes efforts to meet the WET limit which will become effective at the end of the compliance schedule. (If the TRE was permit-required.)

A progress report is usually required about ½ way through part two of the TRE, in order to allow for mid-course corrections, if needed. This report should contain what has been learned to date regarding treatability, reduction, or removal options. If an alternative has been selected, the report should include a detailed schedule for when implementation is expected. This report might also include information regarding whether the TRE is on track to meet final compliance schedule dates.

Specific tasks that may be done during part two of a TRE may include:

- **Investigating Alternatives For Removing Toxicity.** Once the source has been identified, it is possible to investigate ways of reducing or eliminating the toxicity. Source reduction is often the preferred method of toxicity reduction, but a concurrent or alternative approach may be the assessment of wastewater treatment options. Cost/benefit decisions often drive decisions between source reduction or treatment. Paths taken to address treatability most often take the form of either trials of modified treatment procedures in existing works or the evaluation of different procedures or works through bench or pilot scale simulation.

- **Choosing The Best Toxicity Control Alternative.** Criteria for the selection of preferred toxicity control options most often include: 1) compliance with WET limits, 2) compliance with other regulations (air, solid & hazardous waste, etc.), 3) capital, operational, and maintenance costs, 4) ease of implementation, 5) reliability, and 6) environmental impact (not necessarily in that order). Cost may often be the driving factor, however, the selected option should offer the best potential for consistent, reliable toxicity reduction with the least environmental impact.

- **Performing Tests To Confirm Toxicity Removal.** Once appropriate toxicity control options have been implemented, WET tests should be done to demonstrate that toxicity has been reduced to an acceptable level. WET tests should be performed using procedures specified in the permit. The number of tests required to make this demonstration will depend on factors such as seasonality, severity, and cause(s) of toxicity. Usually, four tests performed at least 60 days apart are recommended. WET tests required in the permit after the TRE compliance schedule end date may be used to demonstrate that a TRE was successful, however, the permittee risks permit violations if failures occur after a WET limit becomes effective.

Final Report & WET Limit Compliance

The following is an outline of suggested information to be included in the final TRE Report. In order to comply with permit-required compliance schedules, this final report should include: a description of work that was done to remove the source(s) of toxicity and any relevant background information.

1. Introduction. *(1-2 pages)*

A. Narrative description of toxicity identification & reduction work done
B. Summary of specific WET test results, TIE work done *(optional)*

2. Results of Toxicity Reduction Evaluation (TRE) & other investigation steps. *(4-5 pages)*

A. Discussion of studies done to find most cost effective toxicity removal alternative(s)
B. Results of WET Tests competed to confirm toxicity removal

3. Summary & Conclusions *(1-2 pages)*

A. Report of which toxicity removal alternatives were chosen, why they were chosen, and whether or not they were successful *(based on results of WET Tests competed to confirm toxicity removal).*

ATTACHMENT 1: Example Industrial/Commercial User Survey

(City/village/town) is performing a toxicity reduction evaluation, since toxicity test results have indicated the presence of substances in the effluent that are potentially harmful to aquatic life. In order to reduce or eliminate effluent toxicity, (city/village/town) must identify and locate its source. As a part of this investigation, (city/village/town) is conducting a survey of industrial and commercial dischargers that may contribute toxic substances to the wastewater treatment facility. This questionnaire has been prepared to assist (city/village/town) in gathering information for this purpose. Please complete the form by filling in answers to the following questions, and provide a copy to (city/village/town). Use additional sheets as necessary.

1. Name and Address of Business:

2. Who should be contacted at your facility for additional information?

Name: _____Telephone No.: _____

3. Product(s) manufactured or service(s) performed:

4. What is your average volume discharge to the sanitary sewer system in gallons per day?

5. Does your discharge to the sanitary sewer system include process or cleaning-sanitizing wastewater other than normal sanitary wastewater from rest rooms and employee facilities? If yes, please provide the average and peak daily volumes of process wastewater discharged to the wastewater treatment facility. Include any discharge other than restroom and employee facility wastewater. YES ( ) NO ( )

******IF NO, YOU MAY DISREGARD THE REMAINING QUESTIONS ON THIS FORM***

6. Attach copies of any wastewater analytical data from your facility which has been collected in the last five years. Identify whether such data is for your entire discharge or for samples from your process wastewater flow(s) only (specify which flows).

7. Does your facility store any raw materials, cleaners, etc., used in your production processes, which contain any of the pollutants listed on the attached toxic pollutant list? If yes, what specific compounds or formulations (indicate volumes stored)? YES ( ) NO ( )

8. Do you use any substances containing any of the substances on the attached sheet in production or cleanup activities (e.g., sanitizers, cleaning agents, cooling water additives, etc.) If yes, please provide a list of all substances used and a copy of the Material Safety Data Sheets (MSDS) for each substance. Also, please provide the following information for any compounds or formulations identified: name of pollutant and containing compound, process compound used in, and amount of compound used per month. YES ( ) NO ( )

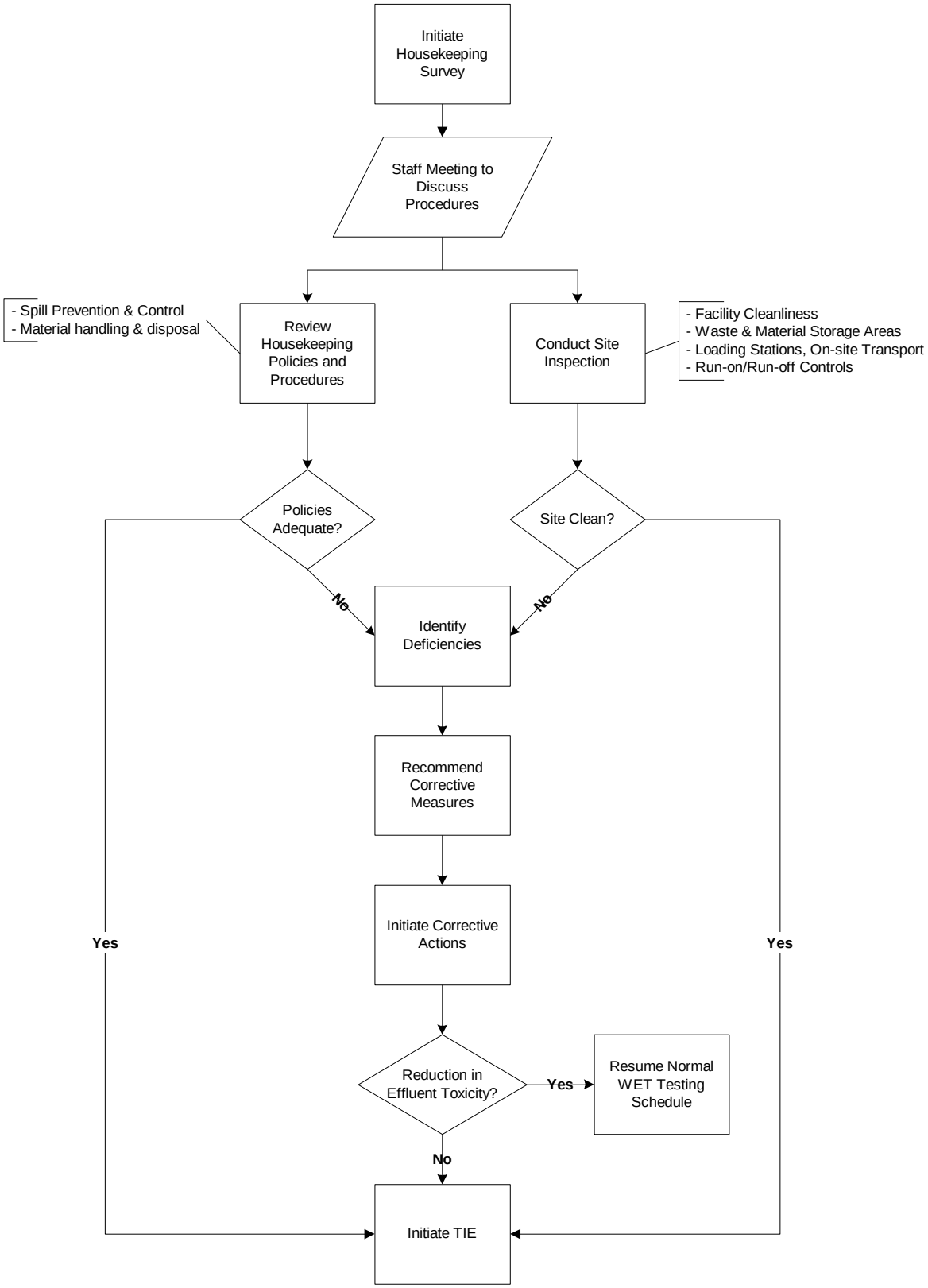
9. Are there particular days of the week and/or times of the day when potentially toxic discharges from your facility may be highest? If so, please describe by providing peak day to average ratios, peak hourly concentrations and flow rates, etc., to the extent this information may be available. YES ( ) NO ( )

Your assistance with this survey is appreciated. If you have questions, or need additional information, please call _____ at the (name and phone no. of facility).

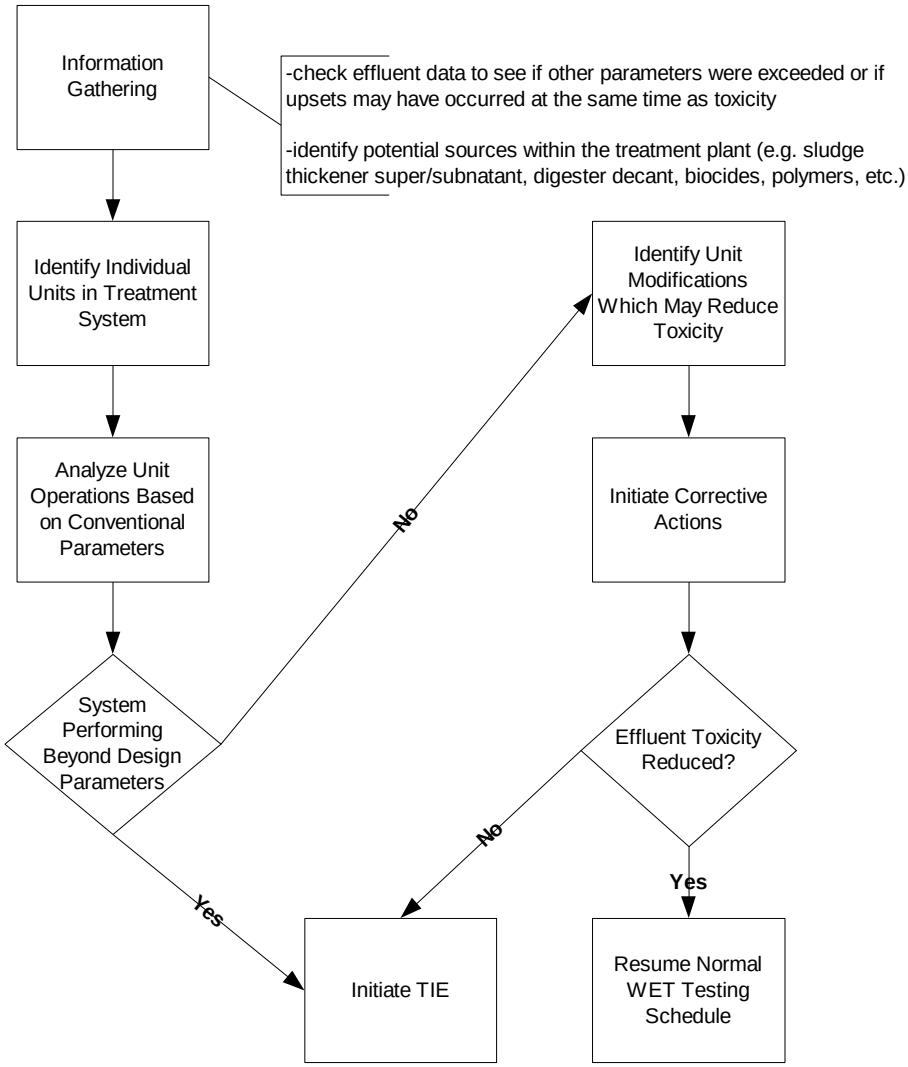
TOXIC SUBSTANCES LIST INDUSTRIAL/COMMERCIAL USER SURVEY
(sorted by priority pollutant category)

Metals:	Toluene	Dimethyl Phthalate	Diazinon
Antimony	Toxaphene	Di-n-butyl Phthalate	2,4-Dichlorophenoxyacetic acid
Arsenic	1,1,1-Trichloroethane	2,4-Dinitrotoluene	Endosulfan
Beryllium	1,1,2-Trichloroethane	2,6-Dinitrotoluene	Endosulfan Sulfate
Cadmium	Trichloroethylene	Di-n-octyl Phthalate	Endrin Aldehyde
Chromium	Vinyl Chloride	1,2-Diphenylhydrazine	Guthion
Copper		Fluoranthene	Heptachlor
Cyanide	**Acid-Extractable Compounds:**	Fluorene	Heptachlor Epoxide
Lead	P-Chloro-M-Cresol	Hexachlorobenzene	Malathion
Mercury	2-Chlorophenol	Hexachlorobutadiene	Methoxychlor
Nickel	2,4-Dichlorophenol	Hexachlorocyclopentadiene	PCBs
Selenium	2,4-Dimethylphenol	Hexachloroethane	
Silver	4,6-Dinitro-O-Cresol	Indeno(1,2,3-cd)pyrene	**Dioxin:**
Thallium	2,4-Dinitrophenol	Isophorone	2,3,7,8-TCDD (dioxin)
Zinc	2-Nitrophenol	Naphthalene	
	4-Nitrophenol	Nitrobenzene	**Other Pollutants:**
Volatile Organic Compounds:	Phenol	N-Nitrosodimethylamine	Aluminum
Acrolein	2,4,6-Trichlorophenol	N-Nitrosodiphenylamine	Ammonia
Acrylonitrile		N-Nitrosodipropylamine	Asbestos
Benzene	**Base-Neutral Compounds:**	N-Nitrosodiethylamine	BHC-tech. grade
Bromoform	Acenaphthene	N-Nitrosodi-n-butylamine	Bis(2-chloromethyl)ether
Carbon Tetrachloride	Acenaphthylene	N-Nitrosopyrrolidine	Chloride
Chlorobenzene	Anthracene	Octachlorostyrene	Chlorine
Chlorodibromomethane	Benzidine	Pentachlorobenzene	3-Chlorophenol
Chloroethane	Benzo(a)anthracene	Phenanthrene	4-Chlorophenol
2-Chloroethyl vinyl ether	Benzo(a)pyrene	Pyrene	Dichlorodifluoromethane
Chloroform	3,4-Benzofluoranthene	1,2,3,4-Tetrachlorobenzene	2,3-Dichlorophenol
1,2-Cisdichloroethylene	Benzo(ghi)perylene	1,2,4,5-Tetrachlorobenzene	2,5-Dichlorophenol
Dichlorobromomethane	Benzo(k)fluoranthene	1,2,4-Trichlorobenzene	2,6-Dichlorophenol
1,1-Dichloroethane	Bis(2-chloroethoxy)methane		3,4-Dichlorophenol
1,2-Dichloroethane	Bis(2-chloroethyl)ether	**Pesticides:**	1,3-Dichloropropane
1,1-Dichloroethylene	Bis(2-chlorisopropyl)ether	Aldrin	2,3-Dinitrophenol
(vinylidene chloride)	Di(2-ethylhexyl)phthalate (DEHP)	Alpha-BHC	Fluoride
1,2-Transdichloroethylene	4-Bromophenyl Phenyl Ether	Beta-BHC	Formalin
1,2-Dichloropropane	Butyl benzyl phthalate	Delta-BHC	Gamma-BHC
1,1-Dichloropropene	2-Chloronaphthalene	Chlordane	Iron
2,3-Dichloropropene	4-Chlorophenyl Phenyl Ether	Chlorpyrifos	2-Methyl-4-Chlorophenol
1,3-Dichloropropene	Chrysene	Dieldrin	3-Methyl-6-Chlorophenol
Ethylbenzene	Dibenzo(a,h)anthracene	4,4'-DDD	Mirex
Methyl Bromide	1,2-Dichlorobenzene	4,4'-DDE	Photomirex
Methyl Chloride	1,3-Dichlorobenzene	4,4'-DDT	2,3,4,6-Tetrachlorophenol
Methylene Chloride	1,4-Dichlorobenzene	Endrin	Trichlorofluoromethane
Pentachlorophenol	3,3'-Dichlorobenzidine	Parathion	2,4,5-Trichlorophenol
1,1,2,2-Tetrachloroethane	Diethyl Phthalate		
Tetrachloroethylene			

ATTACHMENT 2: Housekeeping Logic Flow



ATTACHMENT 3: Treatment Plant Optimization Logic Flow



CHAPTER 2.3 - WET Testing of Lagoon Systems

The purpose of this chapter is to explain why the Department may require WET testing at facilities that use lagoons for wastewater treatment, even if WET test species are already present in the systems.

The Department does not disqualify wastewater treatment systems from WET testing because they may have aquatic species in their lagoons. This is not appropriate, because individual organisms present in a lagoon system would not be good representatives of those that would normally be found in the wild. Organisms that are reared in lagoon systems are likely to be adapted to the effluent conditions that have been present throughout their life cycle and may have become acclimated to potential toxicants in the system. These organisms may be able to tolerate low levels of contaminants that would otherwise impact natural populations of the same organism. Additionally, WET testing is conducted under controlled conditions of temperature, feeding, light, pH, dissolved oxygen, and other variables. The test organisms are compared to control organisms to assess the potential impact of the discharge in the environment. WET test organisms are raised under controlled conditions to be genetically diverse and representative of native populations, not resistant to potential toxicants.

Fathead Minnows
Laboratory raised fathead minnow used in WET tests are intended to represent more sensitive fish that are normally found in Wisconsin receiving waters. The Methods Manual (s. NR 219.04, Wis. Adm. Code) requires that WET test minnows be between 4 - 14 days old in acute tests and < 24-hours old in chronic tests (Methods Manual Section 4, Tables 4.1 - 4.4). Test-specific age categories are necessary to evaluate the potential effects of toxicants on the most sensitive life stages of these fish. While it is possible that these life stages may inhabit a lagoon system, there are no means to determine a recruitment rate of the population - the percentage of fertilized embryos that reach sexual maturity. This is an important metric, because a low recruitment rate would be reflective of an imbalance in the receiving stream fish and aquatic life community.

***Selenastrum capricornutum* (green algae)**
Algae that appear in lagoons and other bodies of water during bloom conditions (rapid increases in algae, most noticeable during the summer months) are usually blue-green algae. These blue-greens are not true algae, but instead are cyanobacteria.

The green algae (*Selenastrum capricornutum*) in WET tests is used as a surrogate for natural aquatic plant populations. Green algae are more often the dominant algal forms found in lower nutrient and non-human impacted lakes. The presence of these types of algae is an indicator of a healthy aquatic environment. *S. capricornutum* is a freshwater green algae and is therefore more representative of higher order aquatic plants. It is not a blue-green species that would normally be associated with nuisance conditions. This species was chosen for use in WET tests because it's an important source of food for higher organisms, and because it is a good representative of other aquatic plants.

CHAPTER 2.4 – Toxic Units, LC_{50}, and IC_{25} Values

This chapter defines and discusses Lethal Concentration, Inhibition Concentration, and Toxic Unit toxicity test endpoints.

Lethal Concentration (LC) Value

Acute WET is measured using a multi-concentration test consisting of a control and five effluent concentrations. These tests are designed to provide dose-response information, expressed as the percent effluent concentration that is lethal to 50% of the test organisms (LC_{50}) within the prescribed period of time (48 or 96-hr). The lower the LC_{50} value, the more toxic the effluent. For example, an LC_{50} >100% means that full strength effluent did not kill half of the organisms. An LC_{50} = 50% means that half strength effluent killed 50% of the organisms.

Calculation. The LC_{50} is calculated differently depending on the characteristics of test data. The appropriate statistical tests used to calculate the LC_{50} are described in Section 5 of the Methods Manual (s. NR 219.04, Wis. Adm. Code). They are the graphical, probit, Spearman-Karber, and trimmed Spearman-Karber methods. An in-depth discussion on the appropriate use of each statistical package is given in *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms* (http://water.epa.gov/scitech/methods/cwa/wet/).

Inhibition Concentration (IC) Value

The inhibition concentration (IC) is the statistical analysis used in chronic WET tests to estimate the sublethal effects of an effluent sample. An "IC_{25}" is an estimate of the concentration of effluent that causes a 25% reduction in a nonlethal endpoint, such as reproduction or growth, in a given time period (usually 7 days). An IC_{50} is an estimate of the effluent concentration that would cause a 50% reduction. The IC is compared to the instream waste concentration (IWC) for the effluent to determine whether there is potential for the effluent to cause sublethal effects to aquatic populations, once it has mixed with the receiving water. If the IC value is lower than the IWC, the effluent has the potential to cause chronic impacts in the receiving water. Methods used to calculate the IWC are described in Chapter 1.3.

Calculation. The IC is calculated using a computer program developed by the USEPA, called the *IC_p* program. This program will generate a linear interpolation (e.g., IC_{25}), a bootstrap mean, and 95% confidence limits, when appropriate.

Confidence Intervals

The Methods Manual (s. NR 219.04, Wis. Adm. Code) requires that test endpoints be reported as an LC_{50} for acute tests and an IC_{25} (for Ceriodaphnia dubia and fathead minnow) or IC_{50} (for green algae) for chronic tests. The 95% confidence intervals associated with these endpoints should also be reported, as an estimate of the precision (uncertainty) around the LC or IC value. As the 95% confidence intervals of the point estimate increase, the uncertainty in that estimate of the statistical endpoint increases. The smaller the width of the confidence intervals, the more certain one can be that the endpoint determined by the statistical program is accurate. The certainty in point estimates is also a function of the dilutions tested and their proximity to the actual statistical endpoint being calculated. Confidence intervals and data interpretation are discussed in Chapter 1.5.

Toxic Units (TU)

LC and IC values may be somewhat counterintuitive, since the lower the value, the greater the toxicity. Because this feature of standard toxicity endpoints sometimes tends to confuse non-toxicologists, an alternative way of expressing toxicity data was developed, called a Toxic Unit.

An acute Toxic Unit (TU_a) = $100/LC_{50}$, which is the reciprocal of the effluent concentration that causes 50 percent of the organisms to die by the end of the acute exposure period. The chronic Toxic Unit (TU_c) = $100/IC_{25}$ or $100/IC_{50}$, which is the reciprocal of the effluent concentration that causes significant inhibition to the test organisms by the end of the chronic exposure period. This has the advantage that as toxicity increases, so does the TU value. WET limits are expressed in permits using Toxic Units (TU_a or TU_c).

The standard permit language typically used in WPDES permits for WET requirements in described in Chapter 1.14.

CHAPTER 2.5 - Relationship Between WET and Chemical-Specific Limits

The purpose of this chapter is to describe the differences between WET and chemical-specific applications and to discuss why these may be used separately or in lieu of one another.

The Department uses an integrated approach for controlling toxic pollutants that includes WET testing and chemical-specific analyses to protect aquatic life from toxics in toxic amounts (ss. NR 102.04 (1) (d) and 102.04 (4) (d), Wis. Adm. Code). The use of WET testing in addition to chemical-specific testing is necessary to adequately protect aquatic life from toxicity due to several factors, including: 1) the limitations of chemical analysis methods (inadequate limits of detection), 2) insufficient toxicity data for some chemicals, and 3) the inability of single chemical tests to predict the toxicity of chemicals when combined. Water quality criteria (WQC) for individual pollutants provide protection against these compounds individually, but do not account for the effects they may have when combined in an effluent. The Department is required to establish WQBELs for toxic substances according to ss. NR 106.05, 106.06, and 106.07, Wis. Adm. Code, and for WET according to ss. NR 106.08 and 106.09, Wis. Adm. Code.

WET In Addition to WQBELs for Toxics

The Department has been using WET since the 1980s to measure, predict, and control the discharge of materials that may be harmful to aquatic life. Since then, there have been many occasions where a positive WET test result was attributed to a compound that did not have promulgated WQC. The WET program has several advantages over its counterpart chemical-specific approach with regards to water quality protection. Among the most important of those advantages is the ability of the WET test to evaluate the collective impact of all chemical constituents of an effluent. The entire chemical matrix has an effect on whether or not the organisms exposed to the effluent react in an adverse fashion. Using WET test procedures, factors such as additivity (1+1=2), synergism (1+1=3), and antagonism (2+2=3) can be addressed without the need for expensive chemical analysis for a myriad of known and unknown chemical compounds.

Establishment of water quality criteria for chemical compounds requires controlled laboratory conditions, including the use of clean laboratory water to eliminate the risk of introducing bias. WET tests offer another advantage in that they can evaluate the potential for impact to a fish and aquatic life community by exposing the test organisms to a mix of effluent and the natural receiving water that the effluent is discharged into. Natural waters often contain ligands with binding sites for many compounds that help render them unavailable to sensitive aquatic life, thus potentially changing the measured toxicity. Further, compounds with toxicity related to water quality may be released or sequestered by naturally occurring conditions of the effluent/receiving water mix.

Chemical-specific Limits in Lieu of WET Limits

Federal regulations allow the use of a chemical-specific limit in lieu of a WET limit under certain circumstances. To do so, it must be shown that the chemical-specific limit(s) are sufficient to attain and maintain water quality standards without the WET limit. (40 CFR Part 122.44 (d) (v) and 40 CFR Part 132, Appendix F, Procedure 6 (C) (1) (e).) For instance, if the permittee can identify and confirm that a certain chemical is responsible for an effluent's toxicity, then a chemical-specific limit for that identified toxicant may be appropriate in lieu of a WET limit. The chemical in question would have to have an established WQC or secondary value, according to ch. NR 105, Wis. Adm. Code, so that an appropriate WQBEL could be established.

One example of this would be the Department's policy for addressing chloride toxicity in wastewaters. (See Chapter 2.10 for more details.) The ultimate goal of that policy is for dischargers to comply with their WQBEL for chloride, however, in

recognition of the impracticality of end-of-pipe treatment options for chloride, the rules allow permittees to implement a source reduction plan that works towards the WQBEL. When a permittee gets a source reduction based permit, s. NR 106.89, Wis. Adm. Code, allows permittees to demonstrate whether chloride is the sole source of effluent toxicity. If chloride is the sole cause of WET, the Department can include chloride limits in the WPDES permit in lieu of WET testing requirements until source reduction actions are completed.

CHAPTER 2.7 - Dilution Water and Test Controls

The purpose of this chapter is to address the use of receiving waters as diluent and controls in WET tests, discuss some problems that have been noted with receiving water controls in the past, and discuss how the Department may interpret a WET test when a dilution water has not met test acceptability criteria.

Dilution Waters Used in WET Tests

In Wisconsin, WET tests are intended to measure the aggregate effect of all toxic contaminants in an effluent and the extent to which the chemicals are biologically available to organisms living near to or downstream from the discharge. By requiring the use of receiving water as the test diluent in most tests, WET test protocols found in the Methods Manual (s. NR 219.04, Wis. Adm. Code) are intended to account for many site-specific factors (e.g., bioavailability, pH, hardness, alkalinity, etc.) that may influence whether toxicity is manifested in the environment. The Methods Manual requires that receiving water be used in all chronic tests and in acute tests where an acute mixing zone - or zone of initial dilution (ZID) - has been approved. In acute tests that measure toxicity at the end of pipe (i.e., where no acute mixing zone has been approved), the Methods Manual allows the use of either receiving water or a standard laboratory water. (See Section 4.4.)

Choosing the dilution water can be an important step in the WET testing process. Using laboratory water may decrease costs associated with the sampling and shipping of receiving water samples, however, WET tests which use lab water for dilution may overstate the effects of an effluent on the receiving water. This is because the bioavailability of a toxic substance is influenced by a number of factors. For example, natural waters may affect traditional toxicants such as metals by altering their chemistry and decreasing the toxicity that is elicited in the environment. The naturally occurring materials that tend to complex and detoxify certain materials are usually absent from lab waters. Using receiving water for dilution increases the environmental relevance of WET testing by simulating real world effluent/receiving water interactions in the test. Use of receiving water for dilution improves the ability of WET tests to predict in-stream effects.

What if the Receiving Water Control "Fails"?

Though use of natural receiving waters for dilution in WET tests increases their environmental relevance, it also increases the complexity of the test and may occasionally present other problems related to background effects. There are a few reasons why a receiving water may not meet test acceptability criteria in a WET test. Failure of a receiving water to meet test criteria does not necessarily mean that the receiving water is toxic. Toxicity in an effluent is defined as greater than 50% mortality in an acute test or greater than a 25% reduction in reproduction or growth in a chronic test. Receiving water control test acceptability criteria are lower than this, allowing only 10% mortality in an acute test and 20% in a chronic test.

The following factors may have the potential to introduce toxicity or sample contamination into a receiving water control, causing it to show less than perfect performance: 1) the sample was collected in the mixing zone of the permittee's or another discharge, 2) a storm event has washed pollution into the waterbody, 3) the sample was collected in a container that was not properly cleaned and conditioned, 4) natural bacteria in the receiving water may cause adverse effects to laboratory organisms (discussed in greater detail below), or 5) the presence of natural toxicants (e.g., cyanobacteria) in the receiving water.

Poor Receiving Water Performance in Past Tests

In WET tests, organisms are exposed to a series of effluent concentrations for a specific time period in order to estimate the effluent’s toxicity. A receiving water control (an exposure of test organisms to dilution water with no effluent added) is used in most tests to monitor the suitability of the dilution water. A laboratory control water is also included to

monitor the health of the organisms, test conditions, and handling procedures (Methods Manual, Section 4, s. NR 219.04, Wis. Adm. Code).

According to the Methods Manual (s. NR 219.04, Wis. Adm. Code), in order for an acute WET test to be acceptable, organism survival in receiving water and laboratory water controls must be ≥90%; for a chronic test to be acceptable, control survival must be ≥80% (see Methods Manual Sections 3.8 & 3.9). As shown in Table 1 below, receiving water controls in chronic tests completed between 1988-1998 showed a surprisingly high failure rate.

TABLE 2.7.1
Control Failure Rates in WET Tests 1988-1998

TEST TYPE	TOTAL TESTS	UNACCEPTABLE RECEIVING WATER CONTROLS	UNACCEPTABLE LAB WATER CONTROLS
ACUTE	2,308	78 (3.4%)	43 (1.9%)
CHRONIC	1,497	382 (26%)	44 (2.9%)

Close examination of those chronic tests where unacceptable receiving water controls were noted revealed the following common characteristics:

1. Lowered survival in receiving water controls in fathead minnow (*Pimephales promelas*) 7-day chronic tests but not in concurrent chronic tests with *C. dubia* or concurrent acute tests with either organism.

2. High variability in survival among replicates. It was not uncommon for mortality in the receiving water control and/or lower effluent concentrations to range from 0 to 100% among replicates.

3. Non-monotonic dose responses (mortality not always highest in the highest sample concentrations). In tests with unacceptable receiving water controls, receiving water controls and lower effluent concentrations often showed similar lowered survival and high replicate variability while higher effluent concentrations did not show effects.

4. Mortality often first noted in receiving water controls and lower effluent concentrations on day 4 of the chronic test, but not before (and not in the 4 day acute test). Once mortality is noted in an individual replicate, most, if not all of the fish in that replicate succumbed before the test was completed.

5. Presence of fungal growth on the gills of dead and/or dying fish. This fungal growth may be attributed to *Saprolegnia* sp. and may have been a secondary infection.

While some of these characteristics were not always observed in tests with unacceptable receiving water controls, items 1,2 and 5 together were the most common.

The characteristics listed above suggest that the lowered survival in receiving water controls was not due to chemical toxicity. In tests where chemical toxicity is shown, higher effects are expected in higher effluent concentrations, usually along with very low between-replicate variability. However, in the tests described above, the opposite was often true - lower effluent concentrations would show higher effects and high between-replicate variability would be present. These types of reverse dose-response patterns are most often associated with contaminated samples (e.g., samples collected with unclean equipment). Other evidence pointing away from chemical toxicity includes the fact that the *C. dubia* species was not affected while the fathead minnow was. *C. dubia* is known to be more sensitive than the fathead minnow to most chemicals (ammonia being the most well-known exception). The fact that the fish was affected and *C. dubia* was not eliminates many chemical toxicants as suspects for causing this phenomenon.

Additional information comes from other states who have noted similar problems. Some laboratories outside of Wisconsin have performed toxicity identification evaluations on receiving water samples which showed similar effects to be pathogenic (Downey, et al, 2000; Kszos, et al 1997; Grothe, et al, 1996). Attempts to associate lowered survival with

chemical toxicity were not successful in those TIE trials. Sample manipulations designed to remove metals, organics, and other chemical classes did not improve survival or lower between-replicate variability in receiving water samples with these symptoms. Further toxicity characterization of similar samples showed that only sterilization was effective in eliminating or reducing mortality. Autoclaving, pasteurization, addition of antibiotics, filtration and irradiation with ultraviolet light all improved survival and lowered between-replicate variability in affected receiving water samples. Another set of experiments showed that when living fish carrying fungus were removed from test beakers, the remaining fish were much more likely to survive (Downey, et al, 2000; Kszos, et al 1997).

In Texas, eight power plants observed similar effects as noted above in once-through cooling waters and receiving water controls. There, the effects occurred most often during late fall to early spring. Tennessee also reported this phenomenon in a number of streams. Data from New England indicate that shallow, slow running, highly urbanized streams were most likely to experience this phenomenon and a seasonal effect was also noted. Data from Massachusetts and Rhode Island show some rivers which consistently produce this phenomenon, others which show it periodically and some which do not show it at all (Downey, et al, 2000).

In Wisconsin, this phenomenon seemed to occur whenever surface waters were used as diluents in WET testing, throughout the year, and on a wide variety of streams and lakes (including headwaters, outstanding resource waters, & other locations where no point or non-point impacts were expected). As shown in Table 2.7.1 above, of a total 1,497 chronic *P. promelas* tests performed between 1988-98, 26% showed unacceptable survival in receiving water controls. Of a total of 124 receiving waters used in these tests, 91 (73.3%) showed these effects during one or more tests. These receiving waters range in size from large rivers to shallow, intermittent streams; and also occurred with waters taken from lakes, pools, and impoundments. Due to the numerous differences between the physical, chemical, anthropological, and climatic influences on all of these receiving waters, it is highly unlikely that the same chemical toxicant would be found in all of these locations.

Collectively, the factors listed above provide strong evidence which suggests that a microbiological interference, rather than chemical toxicity, was responsible for unacceptable receiving water controls.

A Solution to the Problem

Research was conducted at the University of Wisconsin-Madison's State Lab of Hygiene (SLH) from 1998-2000 to determine why receiving water controls were performing poorly (Geis, 2003). The SLH conducted tests on 18 receiving water sites. Initially, microbiological work was done to isolate pathogenic organisms from receiving waters, the fish and their food. This work showed that pathogens (e.g. *Flexibacter spp.*, *Aeromonas hydrophila*) could be found everywhere and that attempts to remove them from the lab (e.g. through decontamination of the fish and their food) were unsuccessful. This suggested that the problem was not the presence of bacterial organisms in certain samples, since these bacteria were always present, but rather conditions in the receiving water and WET test that caused these organisms to flourish (e.g., optimum nutrients, light, temperature, food, etc.).

Since this research suggested that the problem lay in the method itself, the SLH switched its focus from trying to identify the organisms at fault to how to change test methods to eliminate the phenomenon. The laboratory began testing different variations of the test set-up in order to try to eliminate this pathogenic effect. Analysis of their data showed that manipulations like filtering or irradiating the receiving water, using older (48-h) fish, using clean test cups each day of the test, and using smaller test cups with fewer fish per cup all significantly reduced the occurrence of the effect. This again reinforced the theory that these effects were pathogenic and not toxicological. The use of smaller test chambers (30 mL cups) with 2 fish per chamber was significantly better at reducing the effect than all of the other treatments. It was also the simplest modification, which did not have as much potential to alter the chemical nature of effluent samples.

The Department asked certified WET laboratories to pilot this method from about 1999-2004. In all tests where this new method was used, no receiving water controls were unable to meet test acceptability criteria. Because of the overwhelming success of this research and subsequent test pilot, the USEPA modified its WET methods in 2002 to

allowed for this test method modification (40 CFR Part 136.3, Table I A, *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*, EPA-821-R-02-013. 4th Edition, October 2002). The Department then modified it's chronic fathead minnow test methods in the Methods Manual (s. NR 219.04, Wis. Adm. Code) in November 2004 to require the use of the new smaller test chambers/2 fish per chamber method.

Action Following a Test With An Unacceptable RW Control

Since the new fathead minnow chronic test methods described above have been put in place, there have been no more incidences of the "pathogen effect". Therefore, if proper test methods are used and the receiving water control still does not meet test acceptability criteria, the permittee should evaluate the situation to see if there are other factors that may have contributed to the poor performance. The first recommended step would be for the permittee to verify that proper sampling protocols were used, including proper cleaning of all containers used for effluent and receiving water sample collection. Receiving water samples should be treated with the same care as effluent samples. All buckets, funnels, or other equipment used to collect receiving water samples should be new or cleaned according to the requirements discussed previously in Chapter 1.1, in order to avoid contamination.

Other actions the permittee can take is to check if the sample was taken near another discharge, a dam or other physical structure, or another potentially toxic source. When poor receiving water performance has been noted, it may be necessary to identify another location on the same receiving water or another, similar surface water within the same basin or watershed that can be used in future testing. Every attempt should be made to identify a waterbody with similar physical and chemical characteristics (e.g., pH, alkalinity, hardness) as the receiving water. The receiving water control results, subsequent sampling evaluation, and change in sampling location (if applicable) should be noted on WET Test Report Forms or an accompanying cover letter (Methods Manual Section 6, s. NR 219.04, Wis. Adm. Code).

In situations where no alternative receiving water can be identified, it may be necessary to substitute laboratory water as the primary control water and diluent. If the receiving water has shown poor performance repeatedly and no obvious cause or contributing factor can be found, and no alternative receiving water location can be identified, subsequent tests of the discharge may be completed using laboratory water as the primary control water and diluent after receiving written approval from the Department (Methods Manual Section 4.4, s. NR 219.04, Wis. Adm. Code). A receiving water control should be set in conjunction with the test, as the secondary control, so receiving water performance can be monitored. If after subsequent tests the receiving water performance appears to have improved, the Department may again ask that the receiving water be used as the primary control and diluent. Acceptable laboratory control water can be synthetic (reconstituted) or natural uncontaminated ground or surface waters collected from another source. When laboratory water is used as the primary test control or diluent water source, hardness (as $CaCO_3$) must be adjusted as required in the Methods Manual (Section 4.3; s. NR 219.04, Wis. Adm. Code).

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CHAPTER 2.8 - The CO_2 Entrapment Method

The purpose of this chapter is to discuss the CO_2 entrapment method required by the Methods Manual and how it controls pH and eliminates artifactual toxicity.

Methods Manual CO2 Requirements

The Methods Manual (s. NR 219.04, Wis. Adm. Code) requires acute tests be conducted in a 2.5% CO_2 atmosphere as follows:

4.15.7.5 All static-renewal acute tests must be conducted in a CO_2 atmosphere (a 2.5% mixture, or an equivalent mixture shown to work successfully in the lab) or under flow-through conditions that maintain the pH at a level no lower than the measured effluent pH at the time of discharge. Static and static-renewal chronic tests are not required to be conducted in a CO_2 atmosphere or under flow-through conditions, but if pH control measures are used, the pH shall be maintained at a level no lower than the receiving water pH…"

What is Artifactual Toxicity?

The Methods Manual requires acute tests be conducted in a 2.5% carbon dioxide (CO_2) atmosphere to maintain pH at a level that is more representative of discharge conditions, in order to avoid "artifactual" toxicity. Artifactual refers to something that is created due to an outside, unnatural (i.e., human-influenced) factor having been introduced. In other words, it is something that would not exist under normal or natural conditions. In this case, it is referring to the toxicity that occurs when a pH drifts unnaturally high in a toxicity test.

During the conduct of static or static-renewal WET tests, the pH in test containers can change from the initial pH value. This pH drift can be upwards or downwards, depending on test conditions and sample characteristics, but usually is an upward drift. For instance, the addition of food substances such as algae may cause a decrease in pH, while the loss of CO_2 from supersaturated effluent samples often causes an increase in pH.

Any degree of pH drift may interfere with test results if the sample contains a compound with toxicity that is pH-dependent at a concentration that is near the toxicity threshold. Compounds with pH-dependent toxicity are those with chemical characteristics that allow sufficient differences in dissociation, solubility, or speciation to occur within a certain pH range. Examples of pH-dependent toxicants include ammonia, metals, hydrogen sulfide, cyanide, and ionizable organics.

The most common example of artifactual toxicity occurs when ammonia is present in an effluent sample. Most municipal effluents have pH values in the 7.0 to 7.5 range when discharged, but the pH of highly organic effluents may drift as high as pH 8.5 under static test conditions without any type of pH control. The toxicity of ammonia can increase by an order of magnitude between pH 7 and pH 9. Concentrations of ammonia common to many municipal and industrial effluents may cause toxicity in standard effluent toxicity tests when effluent pH level rises, yet show no toxicity at lower pH levels. Ammonia toxicity caused by this abnormal pH drift during a WET test is what is often referred to as artifactual ammonia toxicity, because it would not be expected to occur under real-world discharge conditions.

As pH increases, the toxicity of ammonia also increases, so upward pH drift may increase sample toxicity. For metals, toxicity may increase or decrease with increasing pH. Lead and copper have been shown to be more toxic at a lower pH, while nickel and zinc are more toxic at a higher pH. A change in pH during testing means that an effluent sample might be tested for toxicity at a pH different than what is actually present at the point of discharge. Under certain

circumstances, this pH drift could influence sample toxicity and be considered a test interference. For these reasons, pH control measures are required in all acute tests and recommended in most chronic tests completed for WPDES permit compliance, as specified in the Methods Manual (Section 4.15.7.5; s. NR 219.04, Wis. Adm. Code).

The CO_2 Method and pH Control

Artifactual toxicity caused by a shift in pH during testing can be reduced or eliminated by exposing the test chambers to a CO_2 controlled atmosphere. Advantages of using CO_2 over other pH control measures include less alteration of normal test solution chemistry and use of a natural buffer system to achieve ongoing pH control. An alternate but much more costly approach would be to conduct onsite flow-through toxicity tests with a turnover rate in the test chamber which maintains the pH of the test solution to that of the effluent.

In most natural waters and in many effluents, pH is controlled by the carbonate buffer system. In this buffer system, carbon dioxide and water combine to form carbonic acid and carbonate salts. When a solution contains a weak acid (such as carbonic acid) and a salt of that acid (such as carbonate salt), the solution is referred to as a buffered solution. In a buffered solution, small additions of acid or base will produce very little change in pH. In pure water, pH is controlled by the partial pressure of carbon dioxide in the atmosphere. In many effluents, high partial pressures of CO_2 may be present, due to high biological activity, causing pHs to drift upwards when placed in static WET tests. Abnormal pH drift can be controlled in static toxicity tests by introducing more CO_2 into the atmosphere over the test chambers. Introducing more CO_2 will encourage the interaction of CO_2 and water to form the buffer system mentioned above.

Since this method is not thought to alter the effluent and more closely maintains the effluent's pH as it was discharged, the Methods Manual requires the CO_2 entrapment method be used for all acute tests and also recommends it's use during chronic tests. This method cannot be used to artificially produce a certain pH, but to maintain the pH of the effluent at a level which is comparable to the pH at the time the sample arrived at the laboratory. (Methods Manual section 4.15, s. NR 219.04, Wis. Adm. Code) By using the CO_2 entrapment method, the effluent can be tested for toxic effects without interference from the artifactual toxicity caused by pH drift.

CHAPTER 2.9 – WET Test Variability

The primary purposes of this chapter are to discuss potential sources of variability and to present ways for addressing and reducing that variability in WET testing.

WET test variability has been discussed by a variety of groups in many different forums in past years. This chapter is an attempt to generally discuss WET variability and ways to control it. WET variability issues were first formally discussed at a "Pellston Workshop", held September 16-21, 1995, at the University of Michigan Biological Station on Douglas Lake in Pellston, Michigan (https://www.setac.org/page/SETACWorkshopPubs). This workshop included participation of experts from academia, industry, and government who were selected because of their experience and knowledge of WET test methods. The workshop provided a structured environment for the exchange of ideas and debate such that consensus positions could be derived and documented for some of the issues surrounding the science of WET testing. The participants at the 1995 Pellston Workshop categorized the different types of WET variability and highlighted ways that WET variability could be reduced (Grothe, et al., 1996). This group categorized WET variability into 3 types:

- **Intratest (within-test) variability.** Sources of intratest variability include the number of replicates, the number of organisms per replicate, and the sensitivity differences between organisms.

- **Intralab (within-lab) variability.** Intralab variability is that which is measured when tests are conducted under reasonably constant conditions in the same lab. Sources of intralab variability include those sources described for intratest variability, plus differences in: 1) test conditions (e.g., seasonal differences in dilution water & environmental conditions), 2) organism condition/health, and 3) analyst performance.

- **Interlab (between-lab) variability.** Interlab variability reflects the degree of precision that is measured when a sample is analyzed by multiple labs using the same methods. Variability measured between labs is a consequence of variability associated with both intratest and intralab variability factors, plus differences allowed within the test methods, technician training programs, sample and organism culturing/shipping effects, testing protocols, and testing facilities.

Pellston Workshop participants determined that both the regulatory and regulated communities could significantly influence factors that affect WET variability. They found that WET variability could be limited by controlling the factors that have the most influence:

- **Strict adherence to clearly specified methods.** Improper utilization of WET methods can have a substantial impact on variability. Since United States Environmental Protection Agency (EPA) methods must be written to apply to a wide range of different situations (e.g., different regions, climates, environments), the methods contain optional steps which can be used to fit different situations. In order to control WET variability, it is necessary to limit "optional" test methods that can cause differences in how tests are conducted.

- **Increasing analyst & regulator experience.** The experience and qualifications of the analyst performing the test will dictate how well culture and test methods are followed and the extent to which good judgment is exercised when issues arise in the process of conducting the test, analyzing data, and interpreting results. The issue of experience is of concern not only in relation to test results, but also in relation to the development and implementation of WET requirements by regulators. Although regulator experience does not directly influence WET variability, it is a key factor that determines how WET is implemented in a regulatory context.

- **Selection of quality labs.** Along with organism health (which is linked to lab quality), lab quality was considered by Pellston workshop participants to be one of the most important factors affecting test variability. Quality WET labs should be able to demonstrate a serious commitment to a quality assurance/control program that extends beyond analyst experience. Considerations such as an ongoing reference toxicant program, a review process for all toxicity test data and reports, a good sample custody tracking system that is always used, proper equipment maintenance, dilution water quality monitoring, facility maintenance, and attention to test organism health are all characteristics of a lab that is committed to generating quality data.

In 1998, the USEPA further investigated WET test variability by conducting an inter-lab study to evaluate the precision of 3 freshwater chronic tests, 4 marine chronic tests, and 5 acute tests. As a result of these studies, EPA a final report discussing variability in acute and chronic WET tests (https://nepis.epa.gov/Exe/ZyPDF.cgi/P100IK48.PDF?Dockey=P100IK48.PDF).

In addition to this EPA report, earlier peer-reviewed data was published which discussed WET variability. The "*Technical Support Document for Water Quality-based Toxics Control*" (EPA/505/2-90-001, 3/91; http://www.epa.gov/npdes/pubs/owm0264.pdf) references published studies that show "*the precision of WET tests is similar to chemical-specific methods*" (pp. 11-12). In addition, inter-lab studies have been completed and published for the fathead minnow and *C. dubia* chronic tests (see references, below). These studies showed good reproducibility for these methods. Other researchers have also agreed that the precision of each of these methods is acceptable. Rue, et al, concluded that WET methods "*are comparable to accepted analytical methodologies*". Another study by Grothe, et al, concluded that "*when comparing CVs for select toxicity test methods and commonly accepted analytical methods...the precision of both techniques is similar*".

What Has Wisconsin Done To Control WET Variability?

Wisconsin has addressed many of the issues that have the potential to influence WET variability. The changes recommended at the Pellston workshop to improve WET variability have already been addressed in Wisconsin:

- **Strict adherence to clearly specified methods.** In addition to analyst experience and organism health, the following factors can affect WET variability: 1) sampling procedures (sample volume, type, storage conditions; frequency of composite sub-sampling), 2) sample holding time, 3) test duration, 4) deviations in feeding & environmental conditions (light, pH, temperature, DO, etc.), 5) dilution water, 6) number of concentrations and replicates tested, and 7) number of organisms per replicate. Each of these is addressed in EPA methods, but flexibility is allowed so states can make tests fit into their specific situations. The more flexibility allowed in test methods, the higher the chance that tests will be done differently between labs or between tests, resulting in increased WET variability.

  In order to control WET variability and improve the consistency of methods used by Wisconsin labs and permittees, the DNR created the Methods Manual in 1995 and updated it in 2004. The Methods Manual is incorporated by reference in ss. NR 149.20 and NR 219.04, Wis. Adm. Code, which require that labs be certified to conduct WET tests and that these procedures be used when completing WET tests for WPDES compliance. The Methods Manual contains Wisconsin-specific procedures for sampling and testing that labs must follow when performing WET tests for permit compliance.

- **Increasing analyst & regulator experience.** In order to insure that WET tests are conducted by qualified, experienced analysts, section 3.17 of the Methods Manual sets forth minimum qualifications and training requirements that each lab must follow in order to maintain their certification.

Additionally, Wisconsin has an advantage over many states due to the dedication of an experienced toxicologist that is responsible for the review and interpretation of WET tests and making decisions regarding unusual data or problems that have occurred during testing. In other states, there is no WET expert and decisions related to WET testing fall to permit drafters or compliance staff that may not have any toxicology experience.

- **Selection of quality labs.** In order to insure labs are of the highest quality and are able to demonstrate a serious commitment to a quality assurance/control program, the Department, under Wis. Stat. § 299.11, certifies labs to perform different types of environmental analysis. According to ch. NR 149, Wis. Adm. Code, in order for a laboratory to apply for certification or registration the laboratory must submit a completed application and a quality assurance plan to the laboratory certification program and pass an on-site evaluation. WET labs must have an ongoing reference toxicant program, a review process for all test data and reporting, a good sample custody system, proper equipment maintenance, dilution water quality monitoring, facility maintenance, and attention to test organism health, and make other demonstrations of good lab practices according to the Methods Manual in order to pass an audit.

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CHAPTER 2.10 - Chlorides and WET Testing

This chapter provides guidance for making demonstrations that chloride is causing effluent toxicity, as allowed by s. NR 106.89, Wis. Adm. Code.

In most cases, the Department doesn't make a distinction about what causes toxicity when making WET-related permit requirements, because it is the permittee's responsibility to achieve and maintain WET compliance, regardless of the cause. When toxicity failures occur, permittees typically have to take steps to identify the source of toxicity and fix it however they can (e.g., source reduction, pretreatment, in-plant modifications). Chloride is unique, however, since it behaves conservatively and wastewater treatment processes designed to remove it have high capital equipment costs, operating & maintenance costs, high energy requirements, and produce large volumes of solid waste which make them undesirable environmental alternatives. Therefore, in cases where all of these things are true, permittees may seek a variance to the water quality standard and work towards reducing chloride in their effluent via source reduction instead of wastewater treatment (s. NR 106.83 (2), Wis. Adm. Code).

WET-related Requirements in Wisconsin's Chloride Rule

Wisconsin chloride regulations are given in s. NR 106.80, Wis. Adm. Code, which spells out requirements for point sources that discharge wastewater containing chloride to surface waters of the state. The ultimate goal of that policy is for dischargers to comply with their WQBEL for chloride. However, in recognition of the impracticality of end-of-pipe treatment, the rules allow permittees to request a source reduction based permit with a schedule to work towards the WQBEL, rather than a traditional permit which immediately imposes the WQBEL. When a source reduction based requirement is established in the WPDES permit, s. NR 106.89, Wis. Adm. Code, allows permittees to demonstrate that chloride is also responsible for past WET failures. If chloride can be shown to be the sole cause of WET problems, chloride limits can be used in lieu of WET requirements until chloride source reduction measures are complete:

NR 106.89 Alternative whole effluent toxicity monitoring and limitations for dischargers of chloride.

(1) GENERAL. In addition to interim, target and calculated water quality-based effluent limitations and target values for chloride, the department may establish whole effluent toxicity testing requirements and limitations pursuant to ss. NR 106.08 and 106.09.

(2) FINDINGS. The department finds all of the following:

(a) Acute whole effluent toxicity limitations cannot be attained if the effluent concentration of chloride exceeds 2,500 mg/L;

(b) Chronic whole effluent toxicity limitations cannot be attained if the effluent concentration of chloride exceeds 2 times the calculated chronic water quality-based effluent limitation;

(c) Chloride limitations will be used in lieu of WET limitations to attain and maintain narrative criteria in ss. NR 102.04(1)(d) and NR 102.04(4)(d) in the cases where chloride is the sole source of acute or chronic whole effluent toxicity.

(3) CHLORIDE LIMITS IN LIEU OF ACUTE WET LIMITS. Chloride limitations shall be included in the WPDES permit in lieu of acute whole effluent toxicity testing requirements and acute whole effluent toxicity limitations until source reduction actions are completed if any of the following apply:

(a) The permittee can demonstrate to the satisfaction of the department that the effluent concentration of chloride exceeds 2,500 mg/L, or

(b) The permittee can demonstrate to the satisfaction of the department that the effluent concentration of chloride is less than 2,500 mg/L, but in excess of the calculated acute water quality-based effluent limitation, and additional data are submitted which demonstrate that chloride is the sole source of acute toxicity.

(4) CHLORIDE LIMITS IN LIEU OF CHRONIC WET LIMITS. Chloride limitations shall be included in the WPDES permit in lieu of chronic whole effluent toxicity testing requirements and chronic whole effluent toxicity limitations until source reduction actions are completed if either:

(a) The permittee can demonstrate to the satisfaction of the department that the effluent concentration of chloride exceeds 2 times the calculated chronic water quality-based effluent limitation, or

(b) The permittee can demonstrate to the satisfaction of the department that the effluent concentration of chloride is less than 2 times the calculated chronic water quality-based effluent limitation, but in excess of the calculated chronic water quality-based effluent limitation, and additional data are submitted which demonstrate that chloride is the sole source of chronic toxicity.

(5) DECISION DOCUMENTATION. The department shall specify the decision to include chloride limitations in lieu of whole effluent toxicity limitations in the permit fact sheet.

(6) RE-EVALUATION. The department shall re-evaluate the need for whole effluent toxicity and chloride monitoring or limitations upon permit reissuance.

Chemical-specific chloride limits can be used in place of WET monitoring and limits according to s. NR 106.89, Wis. Adm. Code, when chloride is the sole source of toxicity. Standard permit language is provided in SWAMP and discussed in Chapter 1.14. Reasons for excluding WET requirements should be spelled out in the permit fact sheet. Once chloride source reduction is complete and WQBELs are being met, the need for WET monitoring and limits should be reevaluated. Monitoring may be necessary to show that all toxicity has been removed with the reduction of chloride or to check for other toxicants in the effluent.

Allowing for Additional Data to be Collected

In some cases, there may be some question as to whether chloride is the sole source of toxicity when a permit is being reissued. If this happens, a schedule may be placed in the permit to allow time to make this demonstration. The WET limit and appropriate monitoring (based on the assumption that chloride is not the cause) should be placed in the permit, in the event that the permittee cannot successfully demonstrate that chloride is the sole source of toxicity. Below are some example schedules that may be used in these situations.

If monitoring only recommended:

Whole Effluent Toxicity Compliance Schedule

Required Action	**Date Due**
Submit a study plan describing procedures to be used to demonstrate chloride is the sole source of effluent toxicity.	*3 months from permit issuance*
Implement the study plan, make a reasonable attempt to identify the source of toxicity, and submit a report to the Department presenting the results of the evaluation. If the Department determines that chloride is the sole source of toxicity, the **(acute/chronic)** WET monitoring required in section **(X)** will not be required. If this demonstration is not successful, the permittee must complete the **(acute/chronic)** WET monitoring required in section **(X)**.	*18 months from permit issuance*

If monitoring and WET limit recommended:

Whole Effluent Toxicity Limit Compliance Schedule

Required Action	**Date Due**
Submit a study plan describing procedures to be used to determine the cause of effluent toxicity.	*3 months from permit issuance*
Implement the study plan, make a reasonable attempt to identify the source of toxicity, and submit a report to the Department presenting the results of the evaluation. If the Department determines that chloride is the sole source of toxicity, the remainder of this schedule, **(acute/chronic)** WET monitoring in section **(X)**, and the **(acute/chronic)** WET limit will not become effective. If this demonstration is not successful, the permittee must complete the remaining portions of this schedule and meet the WET limit in section **(X)**.	*18 months from permit issuance*
Submit **part two** of the TRE Plan describing actions to be taken to reduce or eliminate the toxicity identified in part one of the TRE and the dates by which those actions will be implemented.	*19 months from permit issuance*
Submit a progress report identifying the actions taken to date to implement part two of the TRE plan.	*28 months from permit issuance*
Complete all actions identified in the TRE plan and achieve compliance with the effluent toxicity limitation.	*36 months from permit issuance*

What Additional Data Is Needed To Show That Chloride Is Causing Toxicity?

Section NR 106.89, Wis. Adm. Code, says the Department can place chloride limitations in the WPDES permit in lieu of WET monitoring and limits if either chloride is present at a preset level (2,500 mg/l for acute or twice the WQBEL for chronic) or if effluent concentrations are above the WQBEL and additional data are submitted which demonstrate that chloride is the sole source of toxicity.

Normally, when an effluent has shown repeated toxicity, a permittee is required to perform a Toxicity Reduction Evaluation (TRE) to identify and fix the source(s) of toxicity. In toxicity identification steps, effluent samples may be manipulated to remove suspect chemicals (e.g., metals, organics) and then re-tested to see if toxicity remains. If a specific effluent manipulation removes toxicity, then the researcher has a clue about the source of toxicity. However, chloride is a unique substance that is not easily altered by chemical reactions, therefore traditional investigation methods do not work well for identifying chloride as the cause of toxicity. However, other information can be used to help determine if chloride is responsible for toxicity.

Most Sensitive Species. As shown in Table 1 (below), there is a significant difference in the sensitivities of WET test organisms to chloride, and that difference can provide useful information when determining whether chloride is the sole cause of toxicity. *Ceriodaphnia dubia* is the most sensitive to chloride (as NaCl), with an average acute critical concentration (LC_{50}) of about 2,500 mg/l and an average chronic critical concentration (IC_{25}) of about 720 mg/l. The algae species is less sensitive with an average IC_{50} of about 2,200 mg/l. The fathead minnow is the least sensitive of these 3 species, with an average LC_{50} more than twice as high as that for *C. dubia* and an IC_{25} more than four times as high. This relative sensitivity pattern is useful as a first step towards determining whether chloride is a significant source of toxicity. If the LC_{50} for the fathead minnow is lower than that for *C. dubia*, it is safe to rule out chloride as the primary toxicant. If the situation is reversed and chloride levels are near levels of concern, further data may be needed to verify whether chloride is the primary toxicant in the effluent.

TABLE 1. CHLORIDE TOXICITY VALUES (mg/l)		
	Acute	**Chronic**
Water Quality Criteria (according to ch. NR 105, Table 1 & Table 5)	757	395
Water Quality Based Effluent Limits (WQBEL) (according to ss. NR 106.06(3) and (4)).	1514	$\frac{(395)(Q_s+(1-f)Q_e) - (Q_s-fQ_e)(C_s)}{Q_e}$
Reference toxicant information (NaCl) (average of data from 5 labs, except algae data which represents SLH data only); (Acute = LC_{50}; chronic = IC_{25})	2500 (*C. dubia*)[1] 5830 (fhm)	720 (*C. dubia*)[2] 2220 (algae) 3080 (fhm)

1 *Range of last 20 LC_{50} values from reference toxicant tests using sodium chloride (NaCl) from all certified labs in 2014 was 1,710 – 3,540 mg/l.*
2 *Range of last 20 IC_{25} values from reference toxicant tests using NaCl from all certified labs in 2014 was 210 – 1,730 mg/l.*
· Q_s= receiving water flow (usu. $Q_{7,10}$/4); Q_e= effluent design flow (municipal) or average annual effluent flow (industrial); f = fraction of the effluent withdrawn from the receiving water; C_s= background concentration of the substance.
· fhm = fathead minnow (*Pimephales promelas*)

Effluent Chloride Concentration. Additional insight can be obtained by determining the chloride concentration in the effluent. As a general rule, if chloride levels are near or above the reference toxicant values shown in Table 1, the concentration may be high enough to adversely affect WET test species. If effluent levels are significantly lower than these values, chloride may not be the primary source of toxicity.

Phase I TIEs. If Phase I TIE manipulations on effluent with high chloride levels indicate that toxicity cannot be eliminated or significantly reduced by any of the treatment steps, the chloride concentration in the effluent may be responsible for toxicity and should be further evaluated. Since the toxicity of chloride may be masked or affected by associated ions (see below), it may be necessary to include a determination of specific ion concentrations in the effluent before and after each step of the Phase I TIE protocol. (See Chapter 2.2 for more discussion of Phase I TIEs.)

One can also assess the cause(s) of toxicity by evaluating the concentration of major ions that compose the effluent's total dissolved solids (TDS). Measured concentrations of ions can be compared to literature or to laboratory-derived effect concentrations to determine if ion concentrations are above effect concentrations. Chemical fractionation schemes can provide additional information on whether inorganic toxic constituents are contributing to toxicity. Chromatographic columns containing cation and anion exchange resins have also been successfully used by researchers to help determine if inorganic salts are playing a role in toxicity.

Ionic Composition vs. Toxicity. TDS, conductivity, and salinity are often used as measures for ions in effluents. However, the correlation between increasing TDS or conductivity and toxicity may vary with ionic composition and therefore may not be the best predictor of toxicity due to chloride. Because chloride is not usually present as individual constituents but rather in combination with other ions, the toxicity of chloride may be masked or affected by associated ions. Therefore, it may be necessary to understand the effects of the various ions alone and to consider those caused by the combination of ions in the effluent.

For example, in one study, the effects of more than 2,900 ion solutions on *C. dubia*, *D. magna*, and the fathead minnow (*P. promelas*) were studied (Mount, et al, 1997). The relative ion toxicity was found to be $K^+ > HCO_3 > Mg^{2+} > Cl > SO_4^2$. For all of the salts tested, *C. dubia* was found to be the most sensitive species, when compared to *D. magna* and *P. promelas.* For certain salts, such as $CaSO_4$, toxicity to the three species was found to be similar, whereas for others (i.e., NaCl), the difference was great. In addition, the toxicity of Na^+ and Ca^{2+} salts was primarily attributable to the corresponding anion. For *C. dubia* and *D. magna,* the toxicity of chloride was sometimes reduced in solutions that were enriched with more than one cation.

Synthetic Effluents. Synthetic or "mock" effluents, which mimic the major ions in the effluent under evaluation, have also proven useful for the assessment of TDS toxicity. In this procedure, aliquots of the effluent are mixed with various amounts of synthetic effluent (based on chemical evaluation of the effluent for the major ions) in an effort to determine if the concentration of the measured anions and cations cause toxicity to the test organism. The hypothesis of this procedure is that if the effluent is diluted with various amounts of synthetic effluent that contain only the salts found in the effluent, then any unknown toxicants potentially in the effluent will also be diluted, resulting in a lessened acute or chronic toxicity response of the test organism. However, if TDS is the toxicant of concern in the wastewater, the corresponding acute or chronic toxicity responses would be similar.

Chloride Toxicity May Be Indicated If:

- There is greater sensitivity by *Ceriodaphnia dubia* compared to *Selenastrum capricornutum* (algae) and the fathead minnow, together with high conductivity and/or chloride measurements.

- Phase I TIE manipulations show that: 1) pH adjustments don't remove toxicity and a precipitate is not visible in the pH adjustment test, pH adjustment and filtration test, or pH adjustment and aeration test; 2) there is no loss of toxicity in the post C18 SPE column tests, or a partial loss of toxicity, but no or little change in conductivity; 3) there is no change in toxicity with the EDTA addition test, sodium thiosulfate addition test, or the graduated pH test; 4) toxicity is not removed or reduced by passing the effluent over activated carbon; and 5) toxicity is removed or reduced by ion exchange resin.

- A mock effluent prepared with the same ions as the effluent exhibits similar toxicity as the effluent.

The above approaches may be used individually or together in a weight-of-evidence approach to demonstrate the part that chloride plays in effluent toxicity. Because of the differences between production and treatment processes and wastewater effluents, flexibility in the design of these studies is important and approaches used may be facility specific. The guidance provided here is intended to describe general approaches which may be used to identify chloride as the primary cause of toxicity. When required by their permit the permittee, with help from their WET lab or consultant, will need to develop a study plan and determine what is necessary to determine the cause of toxicity.

REFERENCES:

Goodfellow, et al. "Major Ion Toxicity In Effluents: A Review With Permitting Recommendations"; *Environmental Toxicology and Chemistry:* Vol. 19, No. 1, pp. 175–182.

Mount, et al. "Statistical Models to Predict Toxicity of Major Ions to *C. dubia*, *D. magna*, and fathead minnows"; *Environmental Toxicology and Chemistry:* Vol. 16, pp. 2009-2019.

CHAPTER 2.11 – Dilution Series

This chapter provides guidance regarding alternate dilution series for WET tests.

The Methods Manual provides standard dilution series for acute and chronic tests, but allows some latitude if permittees wish to choose an alternate series. The dilution series given for each test is intended as a default when little information is known about the effluent and when existing data suggests the concentration of interest is within the range of that dilution series. In some situations, a more appropriate dilution series may be necessary based on experience from past testing. The appropriate selection of a dilution series can be important for accurately identifying dose-response relationships and increasing the precision of point estimates derived from those relationships. An alternate series may be used if approval is obtained from the Department prior to use. (See Methods Manual section 4.12, s. NR 219.04, Wis. Adm. Code.)

Tables 4.2-4.6 of the Methods Manual list standard dilution series for each test type. As mentioned above, sometimes alternate dilution series or additional effluent concentrations in the standard dilution series can help better define the point estimate around the concentration of concern. However, since the effluent concentration of concern is usually 100% in acute tests (when no mixing zone or ZID is allowed), alternate dilution series would have little or no effect on compliance determination. Therefore selection of an alternate dilution series is rarely necessary for acute tests. However, if a ZID has been approved for the discharge, an alternate dilution series may be appropriate in some cases.

In the case of chronic tests, the Methods Manual lists standard dilution series based on the IWC. If between 1-30%, then the default series is 100, 30, 10, 3, 1%; if between 31-100%, then 100, 75, 50, 25, 12.5%. Like those for acute tests, these dilution series are intended as a default when the IWC is expected to be within the range of concentrations in the standard series. In some situations, a more appropriate dilution series may be deemed necessary based on experience gained during historical WET testing of the effluent.

Section 4.12 of the Methods Manual requires that dilution series be specified in the WPDES permit, so alternate dilution series must be chosen prior to permit reissuance. If an alternate dilution series is needed during the permit term, additional concentrations can be added to the permit-required series, but the all of the dilutions from the permit-required series must be included (i.e., dilutions can be added, but cannot be subtracted).

When Is Selecting A Different Dilution Series Important?

A specialized, site-specific dilution series for WET tests may be important in producing more precise results in some cases. The appropriate selection of a dilution series can be important for better definition of dose-response relationships and increasing the precision of point estimates determined from those relationships. For example, toxicants or effluents with steep dose-response curves, often produce “all or nothing” results when using a standard dilution series. An all or nothing response means that one effluent concentration produces no effect and the next highest concentration produces a complete effect (see the example in Figure 2.12.1). Under these circumstances, the point estimate is graphically determined between the no effect and complete effect concentrations. This all or nothing response is very common in WET tests and is not a cause for concern. However, the point estimate (LC_{50}, IC_{25}) derived in this situation is less precise than when multiple concentrations with partial effects occur, which can be important if the point estimate is at or near the concentration of concern. Under these circumstances, the precision of the point estimate can be improved by closer spacing of effluent concentrations or the addition of intermediate effluent concentrations (see Figure 2.12.2).

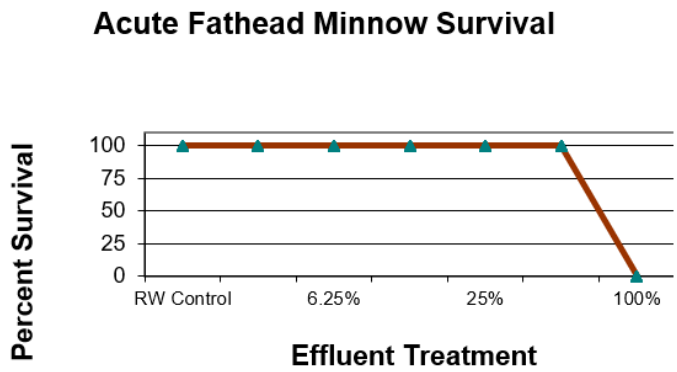

Figure 2.12.1: Standard Dilution Series
100% mortality in 100% effluent, no mortality in 50% effluent
LC_{50} = 75%

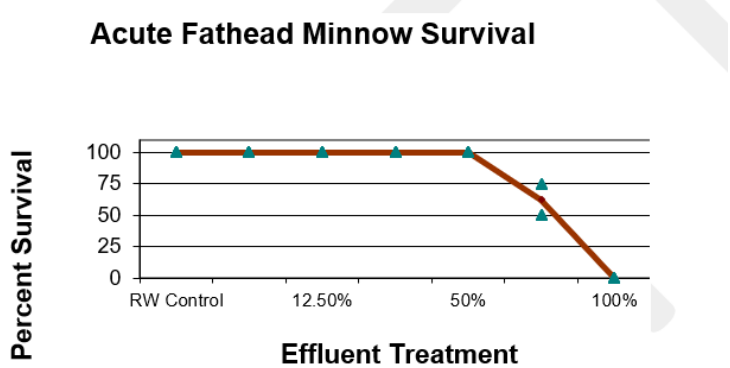

Figure 2.12.2: Alternate Dilution Series.
Replaced 6.25% with concentration closer to expected LC_{50}.
Partial response in 75% effluent. **LC_{50} = 79%.**

The proper selection and spacing of effluent concentrations used in the test can increase the chances of obtaining a dose-response relationship that exhibits smooth transitions from no effect to partial effect to complete effect. Figures 2.12.1 and 2.12.2 illustrate a simplistic example of how additional or alternate concentrations may affect test outcomes. The difference in the statistical outcome between the two tests is small (LC_{50} = 75% vs. 79%), but could be important if the effluent concentration of concern falls between these two endpoints – for example, if the compliance concentration of concern was 77%, Figure 2.12.1 would be a "failure", Figure 2.12.2 a "pass".

Choosing Alternate Dilutions

Chapter 1.5 discusses different dose-response patterns typically observed in WET tests and suggests in some cases that the dilution series be re-evaluated to see if additional or alternate effluent concentrations may help better define dose-response relationships. Those circumstances discussed in Chapter 1.5 are less likely to occur than the one discussed above. In general, situations where the LC_{50} or IC_{25} is at or near the concentration of concern will generate the most need for alternate dilution series. Whatever the reason for an alternate concentration or dilution series to be considered, however, the following should be taken into account:

- **Consider historic WET testing information for the given effluent** - Appropriate dilution series selection should be based on knowledge of the effluent from historical testing and permit information rather than simply on standard laboratory practice. Historic testing information on a given effluent may provide a typical range of effects that can characterize the consistency of the effluent's toxicity. This information is valuable and should not be overlooked. If historical testing shows toxicity consistently within a specified range of concentrations, the test dilution series for future tests could be selected to focus on that range.

  For example, if the IC_{25} for a given effluent is consistently estimated to be about 75% effluent, it may be needless to continue testing concentrations as low as 6.25% effluent. A larger dilution factor, such as 0.75 could be used to provide a dilution series of 100%, 75%, 56%, 42%, and 32% (each concentration is determined by multiplying the previous concentration by 0.75). The analyst should be cautious not to narrow the range of concentrations too much, to avoid causing the point estimate to fall outside the test concentration range when an unusually toxic sample is encountered.

- **Use the effluent concentration of concern (IWC or ZID concentration) as a test concentration** - In some cases, it may be helpful to include the concentration of concern, for example the IWC in chronic tests, as one of the concentrations in the dilution series. For example, if the IWC is 60% and previous WET tests have shown IC_{25}s near 60%, a dilution series equal to 100, 75, **60**, 25, and 12.5% could be recommended, instead of the standard dilution series. As mentioned above, alternate concentrations or dilution series must be specified in the WPDES permit.

  If an alternate dilution series is needed during the permit term, additional concentrations may be added to the permit-required series. For example, in the case given above where the IWC is 60%, the permittee can accomplish this by adding the 60% concentration to the standard dilution series (i.e., making it 100, 75, **60**, 50, 25, and 12.5%).

- **Bracket the IWC with test concentrations** - In some cases, test concentrations selected should not only include the IWC, but also should bracket the IWC (unless the IWC is 100%). This would allow the most precise determination of point estimates around the IWC and could aid in the determination of a valid dose-response relationship. This can be accomplished by setting the dilution factor equal to the IWC value.

  For example, if the IWC for a given effluent is 60% effluent, the dilution factor of 0.60 could be used to provide a dilution series of 100%, 60%, 36%, 22%, and 13% (each concentration is determined by multiplying the previous concentration by 0.60). The analyst should be cautious, however, not to narrow the range of concentrations too much, to avoid causing the point estimate to fall outside the test concentration range when an unusually toxic sample is encountered.

- **Consider adding test concentrations within a given range of interest** - Although the Methods Manual recommends a standard dilution series, the permittee can always collect additional information (e.g., add effluent concentrations to the dilution series) that may provide more precise results. For better test resolution and more precise point estimates, additional test concentrations may be added within a given range of interest. This may be most beneficial when testing an effluent or toxicant that possesses a steep dose-response relationship. Additional test concentrations placed between concentrations of no effect and complete effect may allow for partial effects to be measured and improve the precision of calculated point estimates.

  For example, if no effect was observed at 100% effluent concentration and a complete effect was observed at 50% effluent concentration, an additional test concentration of 75% could be added to improve the precision of calculated point estimates (this was previously discussed - see Figures 2.12.1 and 2.12.2). The addition of test concentrations also may be beneficial when very shallow dose-response relationships are encountered. In this case, additional test concentrations can be added to extend the concentration range tested (e.g., 3.125%, 6.25%, 12.5%, 25%, 50%, and 100%).

- **Consider increasing the dilution factor used to space effluent concentrations** - Increasing the dilution factor for a test (i.e., reducing the space between concentrations) is encouraged if historic testing of the given effluent indicates relative consistency, and the given point estimate is not expected to lie outside of the concentration range. Similar to adding test concentrations, increasing the dilution factor has the effect of narrowing the test focus on a concentration range of interest. This effect is accomplished while maintaining a logarithmic spacing of test concentrations, which is standard practice in toxicity testing.