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***The effective date given is that of the most recent revision of each chapter. Since the WET Guidance Document is updated, as needed, users must insure use of the most recent version.**

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

Introduction

The Whole Effluent Toxicity (WET) Program Guidance Document is intended for use in conjunction with the "*State of Wisconsin Aquatic Life Toxicity Testing Methods Manual, 2nd Edition*" (Methods Manual), which states required test procedures. The Methods Manual is referenced in chs. NR 219 and NR 149, Wis. Adm. Code, and Wisconsin Pollution Discharge Elimination System (WPDES) permits, and is required for use when determining compliance with whole effluent toxicity (WET) requirements. The WET Guidance Document was created to supplement the Methods Manual and assist WDNR staff when determining permit requirements regarding WET testing and to assist permittees and their labs when conducting WET tests in accordance with these permits. 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.

Part I of the document was written as guidance for WDNR staff use when making permit decisions. It is imperative that WDNR staff be as consistent as possible when implementing WET requirements and Part I was created to help insure this. However, it is recognized that there will be situations when decisions contrary to the guidance may be necessary when the assumptions upon which it was based are not appropriate. Part II contains guidance and clarification of existing requirements for permittees and others, concerning issues of importance to the WET program.

The WET Guidance Document is expected to change as the WDNR gains experience with implementation of the WET program and it will be updated as program needs dictate. WDNR staff, permittees, labs, and others should contact the Biomonitoring Coordinator (Kari.Fleming@wisconsin.gov; 608-267-7663) if they wish to suggest topics that may need to be addressed in future revisions or additions to the guidance. Chapter effective dates are listed in the Table of Contents and on the bottom of every page in order to facilitate use of the most recent guidance.

As guidance was created or revised, the intended primary audience was specified in the table of contents in order to clarify the intent of the guidance. However, it is understood that each guidance chapter will be available to everyone. The identification of the primary audience is done only to provide an indication of the authors' focus when writing the guidance.

Why Require WET Testing?

The Clean Water Act (CWA, 1972) sets up the basic requirements for regulating toxic substances discharged to waters of the United States. The CWA established the National Pollutant Discharge Elimination System (NPDES) permits program. It is from this program that the WDNR has derived its WPDES permits program. When limits were first placed in permits, they were based on physical factors such as biological oxygen demand (BOD) and suspended solids. Water quality based effluent limits have also since been placed in permits for "priority pollutants". However water quality criteria (WQC), and therefore discharge limits, exist for only a few of the thousands of chemicals in use today. Another mechanism is needed to predict the effects of the thousands of chemicals which do not have criteria.

Federal policies recommend an integrated approach for controlling toxic pollutants that uses WET testing and chemical-specific analyses to protect aquatic life. The use of WET testing is necessary in addition to chemical-specific testing due to several factors, including: 1) the limitations of chemical analysis methods (for instance, limits of detection may not be low enough to show whether standards are being met), 2) inadequate toxicity data for some chemicals, and 3) the inability to predict the toxicity of chemicals when combined in an effluent (i.e., criteria for individual toxics provide protection against them individually, but do not account for the effects chemicals may have when combined).

In 1988, the WDNR began using WET testing, in addition to chemical-specific testing, to measure, predict, and control the discharge of materials that may be harmful to aquatic life. Recognizing that no single test or organism can be expected to satisfy a comprehensive approach to environmental conservation and protection, the WDNR requires a battery of aquatic toxicity tests which are broadly accepted and that measure different toxic effects using organisms representing different trophic levels and taxonomic groups.

What Are WET Tests?

In WET tests, aquatic organisms are exposed to effluent samples for a set time period. Test treatments consist of different effluent concentrations, usually diluted with the receiving water that it is discharged into. A control treatment (an exposure to dilution water with no effluent added) is used to measure the acceptability of the test by showing the quality of the organisms and the suitability of the dilution water, test conditions, and handling procedures. The organisms used in WET tests in Wisconsin are *Pimephales promelas* (fathead minnow), *Ceriodaphnia dubia* (zooplankton), and *Selenastrum capricornutum* (green algae).

There are typically two types of WET tests - acute and chronic. WPDES-required acute tests are conducted with *Ceriodaphnia dubia* and the fathead minnow; they last 48-hr and 96-hr, 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-required chronic tests are usually conducted using *Ceriodaphnia dubia* and the fathead minnow; both tested for 7 days. Wisconsin's WET Methods Manual also includes a chronic test with *Selenastrum capricornutum* (a 4 day test) but this species is usually only required in WPDES permits when there is thought to be potential for the effluent to impact 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.

Why These Organisms?

The species used in WET tests are known to be sensitive to toxic substances and are representative of aquatic populations found in Wisconsin waters. These so called "indicator organisms" are used to predict what may be happening in the environment as the effluent is introduced. All of the species mentioned above have been used in toxicity tests with effluents and chemicals for several decades.

Ceriodaphnia dubia belongs to a group of freshwater micro-crustaceans, referred to as water fleas, belonging to the Order Cladocera. These invertebrates are a major component of the freshwater zooplankton and are the dominant planktivorous (plankton-eating) herbivores in lakes. They are abundant in ponds, quiescent sections of streams and rivers, and lakes throughout North America. The selection of *C. dubia* for routine use in toxicity testing is appropriate for a number of reasons, including: 1) they are broadly distributed and present throughout a wide range of habitats, 2) they are an important link in aquatic food chains and a significant source of food for small fish, 3) they have a short life cycle and are easy to culture in the laboratory, 4) they are sensitive to a broad range of contaminants, and 5) their small size requires smaller volumes of test water, leading to easier sampling and transportation of wastewater samples.

Fathead minnows belong to the family Cyprinidae (carps and minnows), which is the dominant freshwater family in terms of number of species. The fathead minnow is native to much of North America and thrives in ponds, lakes, ditches, and streams. Fathead minnows are good laboratory fish, taking readily to that life and adapting well to the dry commercial fish food and brine shrimp that is necessary for laboratory culturing.

Selenastrum capricornutum is a freshwater green algae and is representative of higher order vascular plants. (It is NOT a blue-green algae, like those associated with nuisance and noxious conditions.) Like the other species used in WET tests, *S. capricornutum* was chosen because of its importance in the food chain and its ability to represent other species in its trophic level. It's use in toxicity testing is appropriate for a number of reasons, including: 1) They are broadly distributed and present throughout a wide range of habitats, 2) they are an important link in aquatic food chains and are a significant source of food for higher organisms, 3) they have a short life cycle and are easy to culture in the laboratory, 4) they are sensitive to a broad range of contaminants, and 5) their small size requires smaller volumes of test water.

Why Does My Facility Have To Do WET Tests?

WET testing has been a major part of the USEPA, and WDNR's, water pollution control program since the 1980s. In Wisconsin, all surface water discharges are evaluated to determine if WET testing should be conducted. Factors that are considered in determining whether WET monitoring should be required include, but are not limited to: 1) site-specific concerns, 2) designated uses of the receiving water, 3) dilution considerations, 4) facility type, 5) effluent variability, 6) chemical-specific data, 7) interactions between measured and unmeasured chemical constituents in the effluent, and 8) historical WET data. WET monitoring is used to determine whether a discharge has the potential to impact the aquatic life in its receiving waters. WET limits are considered when the WDNR determines through previous WET monitoring that there is evidence that the discharge may be harmful to the receiving water's aquatic life. (See Chapter 1.3 for more discussion of how WET monitoring and limits decisions are made: http://dnr.wi.gov/topic/wastewater/WETguidance.html.)

WET Methods Manual

The "*State of Wisconsin Aquatic Life Toxicity Testing Methods Manual, 2nd Edition*" (PUBL-WT-797; referenced in ss. NR 149.22 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.

Laboratory Certification

Under state statutes, the WDNR 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 for acute and chronic WET testing, 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 Bureau of Integrated Science Services' Laboratory Certification Program, 101 S. Webster St., Madison, WI 53707, (608) 267-7633, or go to: http://dnr.wi.gov/topic/wastewater/WETCertified.html.

Guidance Document Revisions

This document, while comprehensive, is meant to be dynamic - regularly being updated. This is due in part to the impact science and technology has on the WET program and the experience the WDNR will gain as we implement the Methods Manual and the guidance in this document. The maintenance of the guidance document will be the responsibility of the Biomonitoring Coordinator, and it will be updated and improved with input from WDNR staff and other users as program needs dictate. It is the responsibility of WDNR staff, permittees, labs, and others to contact the Biomonitoring Coordinator (Kari.Fleming@wisconsin.gov; 608-267-7663) if they wish to suggest issues or revisions that may need to be addressed.

Listed in the Table of Contents and at the bottom of every page is a "Chapter Effective Date", which indicates the date of the last revisions made to the guidance addressed in the chapter. This allows staff, permittees, and others to be aware of the most up-to-date guidance for the program. All guidance pertaining to the WET program is in this guidance document. Any guidance written prior to the dated chapters in this document are no longer appropriate for use in the WET program.