

## CHAPTER 2.4 – Toxic Units, LC<sub>50</sub>, and IC<sub>25</sub> Values

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

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

### Lethal Concentration (LC) Value

Acute whole effluent toxicity (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<sub>50</sub>) within the prescribed period of time (48 or 96-hr). The lower the LC<sub>50</sub> value, the more toxic the effluent. For example, an LC<sub>50</sub> >100% means that full strength effluent did not kill half of the organisms. An LC<sub>50</sub> = 50% means that half strength effluent killed 50% of the organisms.

**Calculation.** The LC<sub>50</sub> is calculated differently depending on the characteristics of test data. The appropriate statistical tests used to calculate the LC<sub>50</sub> are described in the “*State of Wisconsin Aquatic Life Toxicity Testing Methods Manual, 2<sup>nd</sup> Edition*” (see <http://dnr.wi.gov/topic/wastewater/documents/WETMethodsManualEdition2.pdf>, Section 5). They are the graphical, probit, Spearman-Kärber, and trimmed Spearman-Kärber 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<sub>25</sub>” 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<sub>50</sub> 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 (<http://dnr.wi.gov/topic/wastewater/WETguidance.html>).

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

### Confidence Intervals

The Methods Manual requires that test endpoints be reported as an LC<sub>50</sub> for acute tests and an IC<sub>25</sub> (for *Ceriodaphnia dubia* and fathead minnow) or IC<sub>50</sub> (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  $TU_c$ ).

## WPDES Permit Language - Determination of Positive Results

An acute WET test will be considered a failure (or a “positive” result) if the acute Toxic Unit ( $TU_a$ ) is greater than **X** for any tested species.

In most cases, acute WET requirements are applied at “end of pipe” (no mixing is allowed) and **X** will equal 1.0  $TU_a$ .

### ◆ Acute WET Limit = 1.0 Toxic Unit ( $TU_a$ )

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, **X** would be set at the edge of the approved acute mixing zone, as described below.

### ◆ Acute WET Limit = 100/AMZ Toxic Units ( $TU_a$ )

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

Acute WET limits are expressed in the permit as a daily maximum, as specified in s. NR 106.09(2)(f), Wis. Adm. Code.

Chronic WET requirements are set at the edge of a site-specific chronic mixing zone, using the applicable instream waste concentration (IWC), as shown below. A chronic WET test will be considered a failure (or a “positive” result) if the chronic Toxic Unit ( $TU_c$ ) is greater than  $100/IWC$  for any tested species. The IWC will be specified in the permit.

### ◆ Chronic WET Limit = 100/IWC Toxic Units ( $TU_c$ )

IWC = instream waste concentration

Chronic WET limits are expressed in the permit as a monthly average, as specified in s. NR 106.09(3)(d), Wis. Adm. Code.