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

This chapter defines and discusses the Lethal Concentration (LC_{50}), Inhibition Concentration (IC_{25}), and Toxic Unit (TU_a or rTU_c) 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.

LC_{50} Value

Uses. A lethal concentration (LC) is the statistical analysis used in acute whole effluent toxicity (WET) tests to estimate the lethality of the effluent sample. Acute tests are used in Wisconsin to estimate "end of pipe" conditions (i.e., undiluted effluent, as it is discharged into the environment). The effluent concentration at which 50% of organisms die during the test (the LC_{50}) is used as the compliance endpoint for acute WET tests. In order to calculate an LC_{50}, at least one of the test concentrations must cause >50% mortality. The lower the LC_{50}, the more toxic the effluent. For example, an LC_{50} >100% means that full strength effluent did not kill more than half of the organisms. An LC_{50} = 50% means that half strength effluent killed 50% of the organisms.

Calculation. The LC_{50} is determined differently depending on the characteristics of the test data. The appropriate statistical tests used to calculate the LC_{50} are described in the "*State of Wisconsin Aquatic Life Toxicity Testing Methods Manual, 2nd Edition*" (Methods Manual), Section 5, Figure 5.2. 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* (EPA/600/4-90/027F). Copies of the statistical programs used to determine LC_{50} endpoints can be found at: http://cfpub.epa.gov/npdes/wqbasedpermitting/wet.cfm, or by sending a diskette with a written request to: USEPA, Ecological Monitoring Research Division, Environmental Monitoring Systems Laboratory, 26 West Martin Luther King Drive, Cincinnati, Ohio 45268.

IC_{25} Value

Uses. The inhibition concentration (IC) is the statistical analysis used in chronic WET tests to estimate the sublethal effects of the effluent sample. The IC_{25} is an estimate of the effluent concentration which causes a 25% reduction in growth or reproduction of test organisms. The IC_{25} 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_{25} is lower than the IWC (i.e., if effects are predicted at effluent concentrations expected to be present), the effluent may have the potential to cause sublethal effects to aquatic populations in the receiving water. (NOTE: the equation used to determine the IWC is given in Chapter 1.3, page 12.)

Calculation. The IC_{25} 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. Copies of the statistical program used to determine IC_{25} endpoints can be found at: http://cfpub.epa.gov/npdes/wqbasedpermitting/wet.cfm, or by sending a diskette with a written request to:

USEPA, Ecological Monitoring Research Division, Environmental Monitoring Systems Laboratory, 26 West Martin Luther King Drive, Cincinnati, Ohio 45268.

Confidence Intervals

The Methods Manual requires that test endpoints be reported as an LC_{50} (acute) or IC_{25} (chronic). The 95% confidence intervals associated with these endpoints should also be reported, as an estimate of the precision (uncertainty) around the LC_{50} or IC_{25} value. As the 95% confidence intervals of the point estimate increase, 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. 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 more detail in Chapter 1.5.

Toxic Units (TU)

Uses. The WDNR has developed a new computer system for the WPDES permit program, called the "**S**ystem for **W**astewater **A**pplications, **M**onitoring, and **P**ermits" (SWAMP). One of the functions of SWAMP is the tracking of WET monitoring and limits compliance. In order for SWAMP to "understand" WET results, test endpoints have to be converted to a number which appears like other permit limits (i.e., values that increase as toxicity increases). In the past, WET limits were expressed in a narrative form (e.g., "*It is a violation of this permit to discharge wastewater which exhibits toxicity…*") and WET results were reported only as an LC_{50} or IC_{25}. Now, WET results are also expressed in Toxic Units ("TU_a" or "rTU_c").

Calculation. Toxic Units are calculated as follows:

Acute Toxic Unit (TU_a) when Zone of Initial Dilution (ZID) is <u>not</u> allowed = $100/LC_{50}$
(NOTE: LC_{50} >100% = 1 TU_a)
An acute WET limit is expressed as 1.0 TU_a. See below for more discussion.

Relative Acute Toxic Unit (rTU_a) when ZID <u>is</u> allowed = 3.3 x ZID%/LC_{50}
(NOTE: LC_{50} > 3.3 x ZID% = 1 rTU_a)
An acute WET limit is expressed as 1.0 rTU_a. See below for more discussion.

Relative Chronic Toxic Unit (rTU_c) = IWC/IC_{25} (NOTE: IC_{25} >100% = 1 rTU_c)
A chronic WET limit is expressed as 1.0 rTU_c. See below for more discussion.

WPDES Permit Language - Determination of Positive Results

Standard acute tests with no mixing zone. Normally, in situations where an acute ZID has not been granted, the acute Toxic Unit (TU_a) = $100/LC_{50}$. A permittee must have a $TU_a \leq 1.0$ (i.e., an $LC_{50} \geq 100\%$ effluent) in order to pass the WET test. Standard WET language in SWAMP looks like this:

> ***Determination of Positive Results:*** *An acute toxicity test shall be considered positive if the Toxic Unit - Acute (TU_a) is > 1.0 for either species. The TU_a shall be calculated as follows: $TU_a = 100/LC_{50}$. An $LC_{50} \geq 100$*

equals a TU_a of 1.0. A chronic toxicity test shall be considered positive if the Relative Toxic Unit - Chronic (rTU_c) is > 1.0 for either species. The rTU_c shall be calculated as follows: $rTU_c = IWC/IC_{25}$. An $IC_{25} \geq IWC$ equals an rTU_c of 1.0.

Acute tests when a zone of initial dilution (ZID) has been approved. When a ZID has been granted, compliance in the acute WET test will need to be determined a little differently. The acute Toxic Unit will be called a "relative" Toxic Unit (or "rTU_a") and will be defined as (3.3 x ZID%) / LC_{50}. The permittee will still have to have an **r**$TU_a \leq 1.0$ [i.e., an $LC_{50} \geq$ (3.3 x ZID%)] in order to pass a WET test. When a ZID is given, it is recommended that the permit drafter replace the standard WET language shown above with the following:

Determination of Positive Results: *An acute test shall be considered positive if the Relative Toxic Unit - Acute (rTU_a) is > 1.0 for either species. Since a ZID has been granted for this outfall, the rTU_a shall be calculated as follows: rTU_a = [put (3.3 x ZID%) number here] / LC_{50}. A chronic toxicity test shall be considered positive if the Relative Toxic Unit - Chronic (rTU_c) is > 1.0 for either species. The rTU_c shall be calculated as follows: $rTU_c = IWC/IC_{25}$. An $IC_{25} \geq IWC$ equals an rTU_c of 1.0.*

In this situation, the permit drafter will need to know the ZID% in order to determine the appropriate value to put into the rTUa equation in the above paragraph. This should be determined as follows:

rTU_a = # / LC_{50}

where ZID% = 100/(dilution ratio + 1) (the dilution ratio should be given in the mixing zone approval from DNR modelers)

and # = ZID% x 3.3

For example, the following recommendation may appear in a water quality based effluent limits memo:

"*Relative toxic units used to determine compliance with the acute test = (3.3 X ZID%)/LC_{50}. ZID% = 100 / (20+1) = 4.76%, so rTUa = 15.7%/LC_{50}. An acute toxicity test shall be considered positive if the rTU_a is greater than 1.0.*"

In this situation, the permit language would look like this:

Determination of Positive Results: *An acute toxicity test shall be considered positive if the Relative Toxic Unit - Acute (rTU_a) is > 1.0 for either species. Since a ZID has been granted for this outfall, the rTU_a shall be calculated as follows: rTU_a =* ***15.7%****/LC_{50}. A chronic toxicity test shall be considered positive if the Relative Toxic Unit - Chronic (rTU_c) is > 1.0 for either species. The rTU_c shall be calculated as follows: $rTU_c = IWC/IC_{25}$. An $IC_{25} \geq$ IWC equals an rTU_c of 1.0.*