CHAPTER 2.11 – Dilution Series

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

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Does The Methods Manual Require A Certain Dilution Series?

The "*State of Wisconsin Aquatic Life Toxicity Testing Methods Manual, 2nd Edition*", (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 (e.g., ZID, if allowed, for acute or IWC for chronic) 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.

Tables 4.2-4.6 of the Methods Manual list standard dilution series for each test type. For acute tests, it says that *"6.25%, 12.5%, 25%, 50%, and 100% effluent plus any selected by permittee; alternate series may be selected at permit reissuance"*. 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 ZID is allowed), alternate dilution series would have little or no effect on compliance determination (i.e., since any $LC_{50} < 100\%$ is a "failure"). 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. Alternate dilution series for those situations can be handled in the same manner as chronic tests, as discussed below.

For chronic tests, the Methods Manual states that if the IWC is between 1-30%, then the default dilution series would be 100, 30, 10, 3, 1%. If the IWC is between 31-100%, then it would be 100, 75, 50, 25, 12.5%. The Methods Manual also allows *"any additional* [dilutions] *selected by the permittee or alternate dilutions specified in the WPDES permit"*. Like those for acute tests, these standard dilution series are intended as a default when little information is known about the effluent and 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.

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 (and no permit modification is planned), additional concentrations can be added to the permit-required series, but the permit-required series must be included (i.e., you can add dilutions, but you cannot subtract any).

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 (e.g., no mortality in 50% effluent, but 100% mortality in 100% effluent - See 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 (increased dilution factor) or the addition of intermediate effluent concentrations (see Figure 2.12.2).

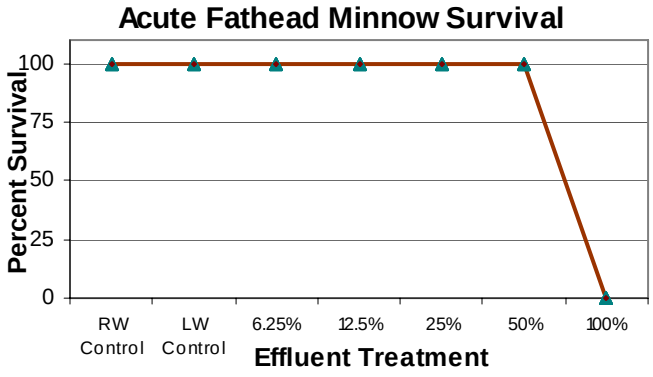

Figure 2.12.1: Standard Dilution Series
100% mortality in 100% effluent, no mortality in 50% effluent
LC_{50} = 75%

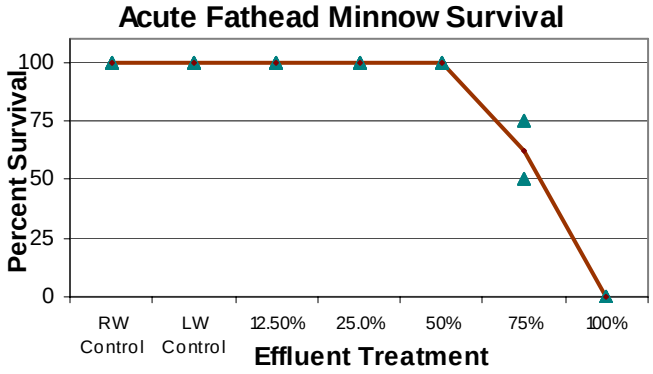

Figure 2.12.2: Alternate Dilution Series.
Replaced 6.25% with concentration closer to expected LC_{50}.
Partial response in 75% effluent. **LC_{50} = 79%.**

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 (e.g., if the concentration on 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, however, so they will not be revisited here. 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 will 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 can 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 wise 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 (i.e., 100, 75, 50, 25, 12.5%). 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 (and no permit modification is planned), additional concentrations can 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 will allow the most precise determination of point estimates around the IWC and will 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 can 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.