

COMMENTS ON THE NATIONAL WHOLE EFFLUENT TOXICITY
IMPLEMENTATION GUIDANCE UNDER THE NPDES PROGRAM
FROM A WET TESTING LABORATORY'S PERSPECTIVE

Chadwick Ecological Consultants, Inc. (CEC) and Chadwick & Associates, Inc., and aquatic biological laboratory were requested by the Colorado Wastewater Utility Council (Utility Council) to review the Draft USEPA *National Whole Effluent Toxicity (WET) Implementation Guidance Under the NPDES Program*. The Colorado Wastewater Utility Council is a nonprofit corporation with more than 43 members comprised of municipal and special district wastewater treatment entities seeking to promote Clean Water Act goals through compliance with the regulations implementing those goals.

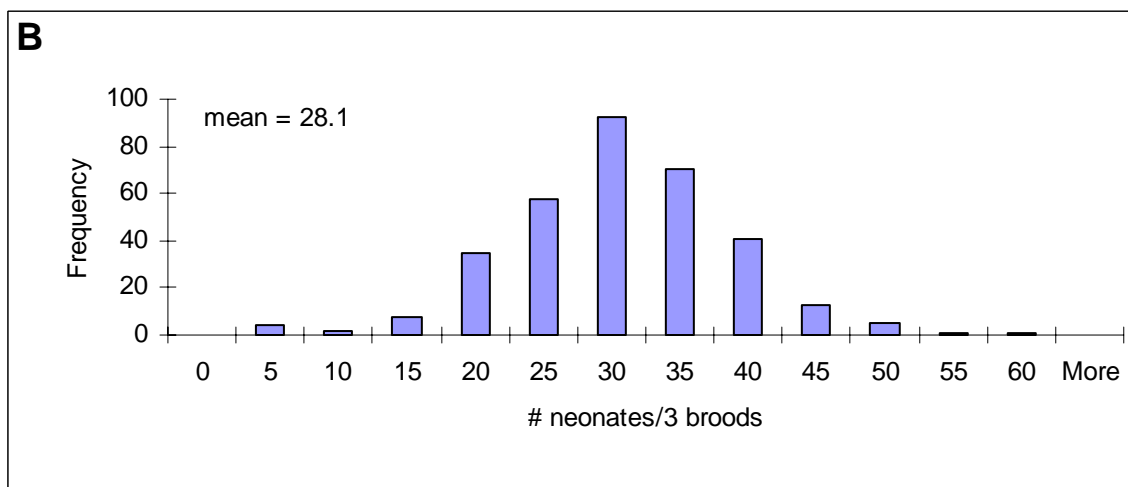
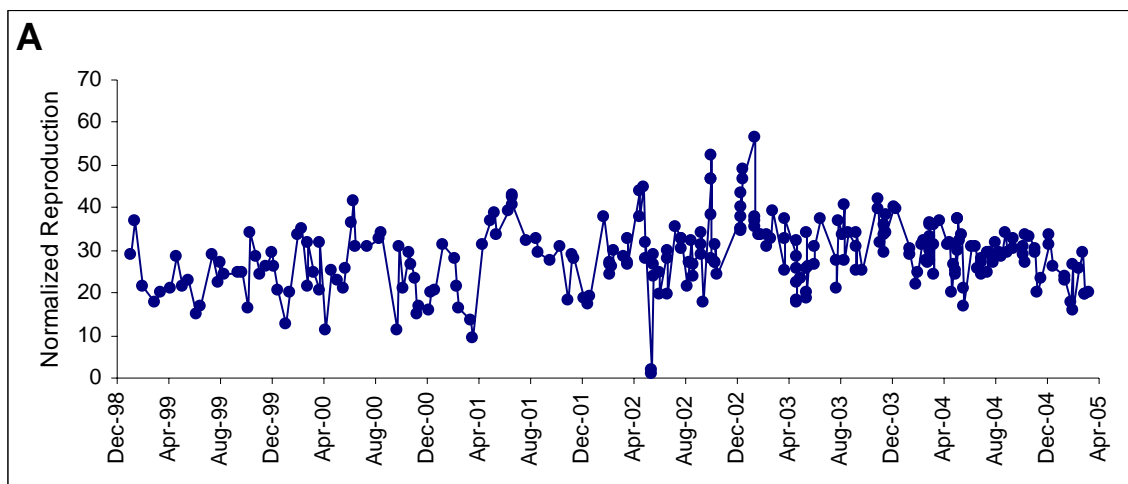
We are interested in NPDES implementation of WET testing from a number of different perspectives. However, a key issue is related to when our clients do “split” WET testing – i.e., sending the same effluent sample to two different laboratories – and get different results. A natural question is why this would happen? And this question is directly related to the real world implications of the variability in the precision of WET tests, which the court has said EPA must account for. CEC and their accompanying laboratory, Chadwick & Associates, Inc., have long-term data on *Ceriodaphnia dubia* neonate reproduction in control treatments (i.e., synthetic moderately-hard dilution water), as well as their sublethal response to reference toxicants and from other WET tests. We looked at the potential sources of WET test variability based on real world data from our own testing experience.

Biological Variability

The biological variability associated with using live test organisms complicates the use of sublethal numerical or narrative WET limits in NPDES permits. Each WET test relies on the measured response of a new group of organisms to control water and a new effluent of unknown constituents. Even though experimental conditions are highly controlled, there is no way to control biological response.

Using data from 330 tests conducted from January 1999 through April 2005, it is clear that *C. dubia* neonate production in control treatments exhibits a wide range of values - even under control conditions (Fig. 1A and B). Normalized reproduction values for control treatments during a three brood

cycle for this dataset ranged from 1 to 57 neonates with a mean of 28.1 (Fig. 1A) and coefficient of variation (CV) of 29.5%. Not surprisingly, the *C. dubia* reproduction data from control water are normally distributed, with 95% of the data within the range of 12 to 45 neonates (Fig 1B). Of course, EPA protocols require control reproduction to be 15 neonates or higher, invalidating 4.2% of the data. More importantly, in the absence of data under 15 neonates, the underlying distribution for control organisms shifts from a normal to a log-normal distribution (Fig 1C) and shifts the mean up to 29 neonates. Combining natural, normally distributed reproduction data with the one-tailed control performance criteria of EPA protocols results in two different distributions for WET test treatments (Fig. 1B) and WET test controls (Fig. 1C) over the population of tests. This “built in” difference could be one source of the variability observed in WET test results.



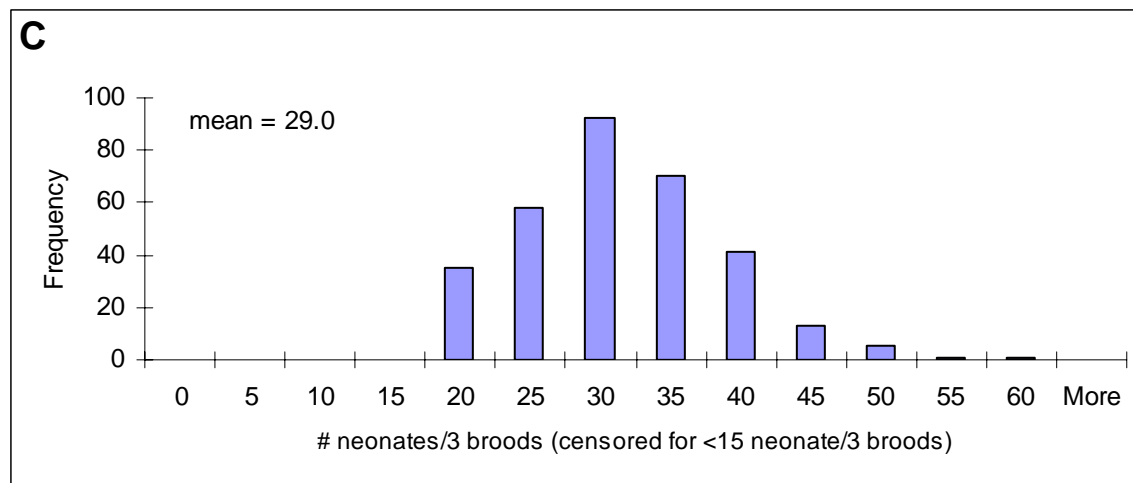


FIGURE 1: A) *Ceriodaphnia dubia* neonate production in reference toxicity and WET tests conducted by C&A since 1 January 1999. B) Frequency distribution of *C. dubia* neonate production during a three brood cycle using the same dataset. C) Frequency distribution of *C. dubia* neonate production during three brood cycle following imposition of EPA control performance criteria.

Within-test Variability

Another measure of variability in *C. dubia* performance is the percent minimum significant difference (PMSD) that quantifies the within-test variability. The PMSD is a measure of test precision based on the control performance and is calculated using the minimum significant difference (MSD) derived from the error mean square of ANOVA, and the critical value of Dunnett's test statistic (USEPA 2000). The measure indicates the minimum detectable percent difference that is statistically significant when comparing a series of treatment means to a control mean, and provides insight into the sensitivity of the analytical method.

Over the past eight years, we have over 260 WET tests which show that PMSD data also considerably variable (Fig. 2). The 10th and 90th percentile PMSD values from these 260 data points are 16% and 44%, respectively - approximately eight percentage points higher than the values presented by EPA in their method variability study (Table 3-6 of USEPA 2000). The PMSD data are log-normally distributed (Fig. 2B). Even though the C&A database includes values derived during both reference toxicant and NPDES WET tests, the range is indicative of the variability associated with sublethal *C. dubia* WET tests. The variability underlying experimental WET test controls, whether methodological or

biological, indicates the low precision expected with the sublethal *C. dubia* WET methodology endpoints. Results of the EPA method variability study using *C. dubia* (USEPA 2000) indicated that 18 of 33 laboratories (54%) had at least one sublethal reference toxicant test exceed the 90th percentile PMSD of 37%. Similarly, approximately 23% of the tests performed by C&A over the past 5+ years produced PMSDs above the upper bound recommended by the EPA. These data indicate that variability within *C. dubia* sublethal WET tests is not limited to a few laboratories or studies.

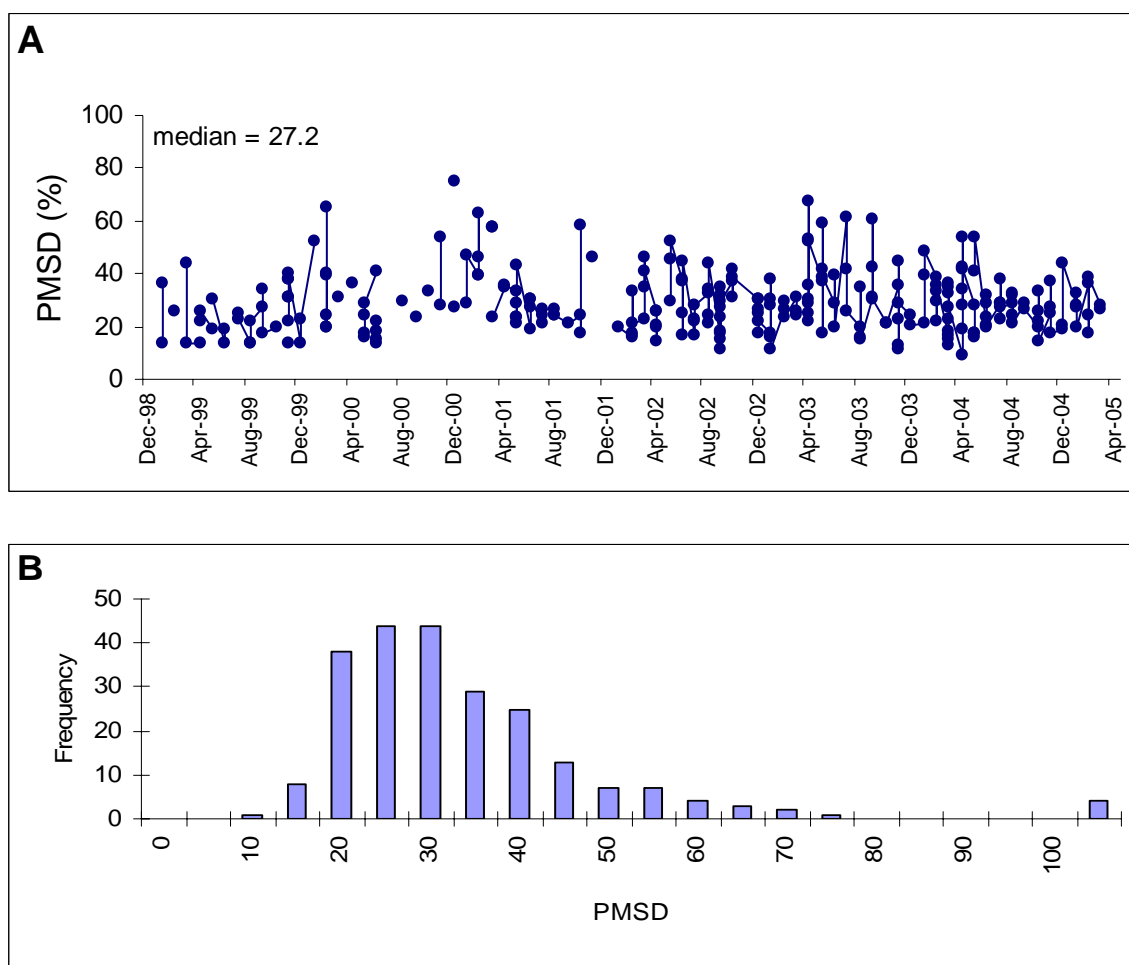


FIGURE 2: A) Percent minimum significant difference (PMSD) for *C. dubia* neonate production in reference toxicant and WET tests conducted by C&A since 1 January 1999. B) Frequency distribution of PMSD data from reference toxicant and WET tests.

Variable Response to Known Toxicant

Given the high degree of variability associated with *C. dubia* reproduction in laboratory control experiments (i.e., synthetic dilution water), it is reasonable to expect that this same variability will be expressed in regular WET testing statistics used for compliance testing (i.e., IC25). Since 2000, C&A reference toxicant tests (NaCl) with *C. dubia* have used a standardized dilution ratio from zero to 1200 mg/l NaCl, with 50 tests during this period. Despite use of a standardized dilution ratio and standardized test conditions required by USEPA methods, *C. dubia* IC25 values have exhibited a high degree of variability (Fig. 3) over time.

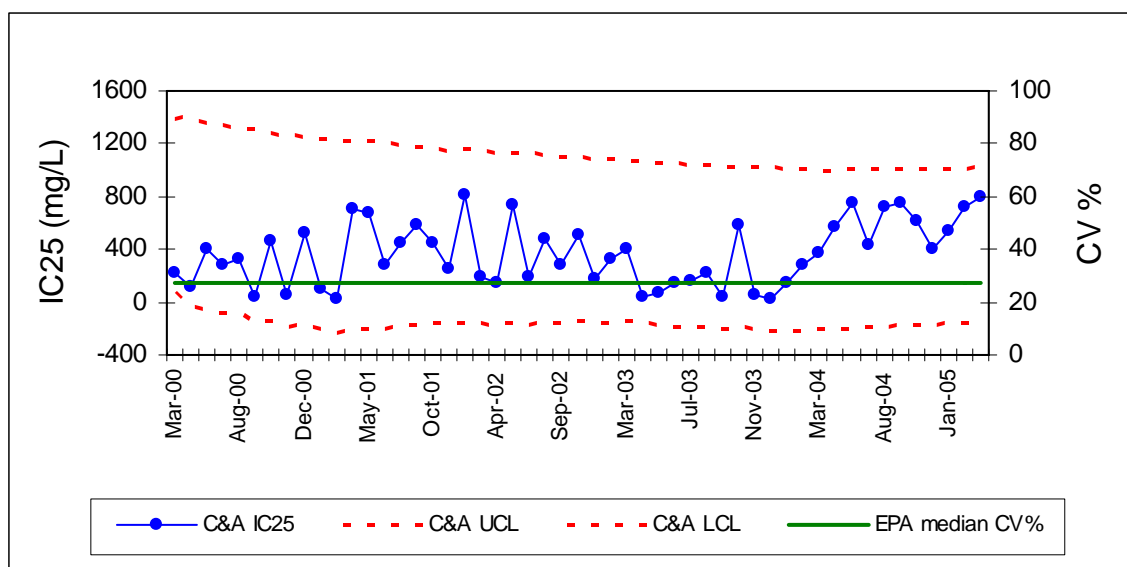


FIGURE 3: A) C&A reference toxicant (NaCl) inhibition concentrations and running laboratory coefficient of variation since June 2002, and EPA median CV% for *C. dubia* during WET method variability study

The median C&A within-laboratory coefficient of variation of 66% (Fig. 3A) has been considerably higher than the median within-laboratory CV of 27% presented by the EPA in their method variability study (USEPA 2000). Of greater interest is the fact that these data indicate there is no predictable response by *C. dubia* to a known toxicant. This has considerable implications to real world applications. If *C. dubia* reproduction endpoints exhibit such random responses to a known toxicant, why would we expect a predictable response to effluent testing?

Laboratory Performance

We have evaluated the variation observed in these reference toxicant tests and can only conclude that the majority of the variation is primarily due to the physiological variability associated with using live organisms – not laboratory performance. Chadwick & Associates, Inc. (C&A) is an accredited WET testing facility with 15 years of experience performing acute and chronic toxicity tests for a variety of municipalities, mining, and industrial facilities in the western United States. Since 1992, C&A has received annual WET test certification by the States of Washington and California, and has successfully passed WET testing audits by the USEPA in recent years. Furthermore, C&A participates annually in the acute and chronic portions of the EPA DMR-QA studies, meeting the test acceptability criteria annually. C&A performs both acute and chronic toxicity tests using *Ceriodaphnia dubia*, *Daphnia magna*, *Pimephales promelas*, *Selenastrum capricornutum*, and *Oncorhynchus mykiss*. In fact, C&A’s DMR-QA results, using the same methodology as in the reference toxicant tests, have consistently been within USEPA’s accepted range of values (Fig. 4).

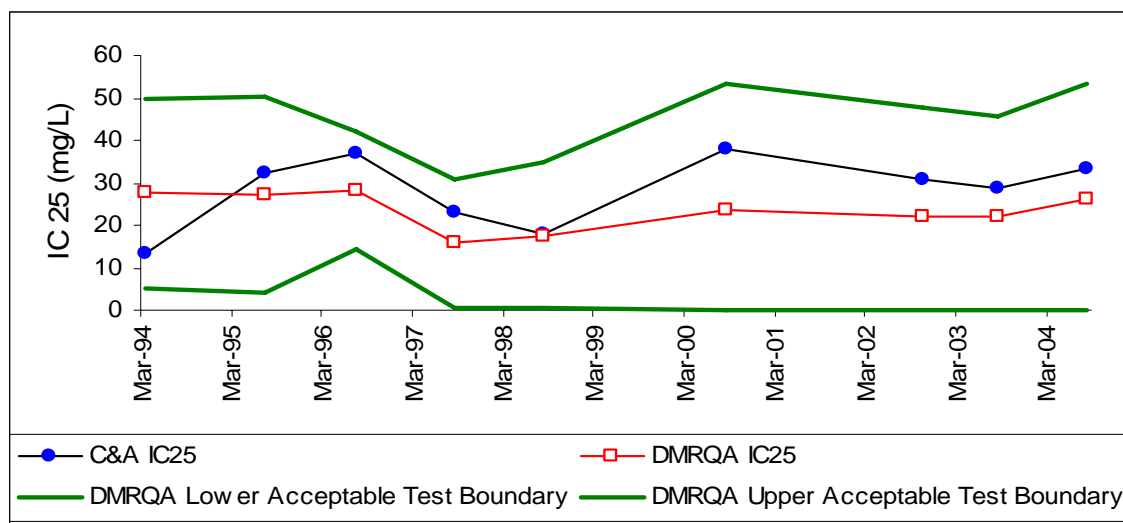


FIGURE 4: C&A performance in the DMR-QA program using *C. dubia* in chronic toxicity tests.

Recommendations

We are concerned that the variation observed in sublethal WET test endpoints using *C. dubia* is so great, that the results of many of the statistical tests is largely meaningless - and it would be difficult to

attribute these differences to effects of water quality or inherent biological variability. Of course, we recognize the importance of the use of WET tests in maintaining surface water quality, but we believe the use of *C. dubia* sublethal endpoints in the development of numerical or narrative triggers would be inappropriate. However, CEC believes their use would be more appropriate in the use of Reasonable Potential determination. If chronic toxicity results indicated that an effluent would likely cause or contribute to an excursion above water quality standards then the establishment of WET triggers should be based on acute or chronic-lethality toxicity limits.

As such, we believe that the NPDES implementation guidance should focus on acute and/or chronic lethal endpoints and allow individual States and tribes a greater flexibility in an approach for using sublethal endpoints. Specifically, we would recommend:

TUse only lethal endpoints (e.g., LC50, IC50) to establish WET triggers in NPDES permits

TUse sublethal endpoints only as a guide for establishing Reasonable Potential determination

References

USEPA. 2000. *Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination System Program*. Eds. Denton, D.L., Fox, J., Fulk, F.A., Greenwald, K., Narvaez, M., Norberg-King, T.J., Phillips, L. EPA/833/R-00-003. Office of Water, Washington, DC.