Toxicity Work Group recommendation for evaluating toxicity data from tests conducted at a non-standard test temperature

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BACKGROUND

Toxicity test guidance documents produced by the U.S. EPA recommend the temperature at which the test is to be conducted, and require that the temperature remain within a prescribed range during the course of the test. Sediment toxicity tests with the amphipod *Hyalella azteca* are typically conducted at 23°C, with a required range of ± 3°C (U.S. EPA, 2000). It has long been recognized that some pyrethroid pesticides are more toxic at colder temperatures (Coats et al., 1989), and this characteristic has been used as a toxicity identification evaluation (TIE) tool to diagnose pyrethroid-associated toxicity (Anderson et al., 2008). A similar response to cold temperature was observed with DDT, but to a lesser extent (Weston et al., 2009). In a SWAMP statewide study of urban creek toxicity, Holmes et al. (2008) used this attribute to help identify pyrethroids as the likely cause of toxicity to *H. azteca*. Increasing toxicity with decreasing temperature has been demonstrated specifically with *H. azteca* in more recent studies (Weston et al., 2009), and also with the larval black fly *Chironomus dilutus* (Harwood et al., 2009). Harwood et al. (2009) showed this is due to slower metabolic breakdown of pyrethroids at lower temperatures and increased nerve sensitivity.

The Stream Pollution Trends Program (SPoT) conducts baseline sediment toxicity testing with *Hyalella azteca* on samples from all of its monitoring stations, and conducts reduced temperature tests on a subset of samples (Phillips et al., 2014). The baseline tests are conducted at 23°C and the cold temperature tests are

conducted at 15°C. Tests of samples from the SPoT stations demonstrate that a significantly greater percentage of samples were toxic when tested at 15°C, and the magnitude of toxicity was much greater at the lower test temperature. Samples were approximately 2-3 times more likely to be toxic when tested at 15°C. These results suggest that pyrethroid pesticides likely played a role in the increased incidence of toxicity in these samples. Although DDT can cause a similar response at colder temperatures, the concentrations of DDT in the sediments were well below toxicity thresholds for *H. azteca*.

The average statewide surface water temperature was calculated for water samples collected in SPoT hydrologic units as part of various SWAMP surveys. Samples represented daytime water temperatures measured at depths less than 0.1 m as part of SWAMP routine monitoring, which was conducted during all months of the year (Cassandra Lamerdin, Moss Landing Marine Laboratories, personal communication). The average temperature for data collected between 2001 and 2010 was 15.8°C, considerably lower than the standard test temperature (23°C). Exposing amphipods at the 15°C test temperature assesses toxicity at a more environmentally relevant temperature for California surface waters (Anderson et al., 2012), and suggests that the potential for surface water toxicity is likely underestimated based on assessing toxicity at the standard protocol temperature of 23°C.

This memo is the result of on-going SWAMP Round Table discussions of the regulatory implications of using low temperature test data for listing waterbodies as impaired under U.S. EPA 303(d) listing criteria. The purpose of this memo is to provide guidance for data users as SPoT and other SWAMP monitoring programs continue to generate low temperature test results. The initial focus is on data generated for tests with *H. azteca*, but the principles apply to tests with other standard test organisms.

DATA USAGE

Data from the reduced temperature tests are reported as part of SPoT routine monitoring activities to the SWAMP database, and ultimately to CEDEN. Within the SWAMP or CEDEN databases, tests conducted with test temperature modifications are clearly qualified in the "treatment", "concentration", and "unit treatment" data fields. Data from these databases are available for use in regulatory actions through the Integrated Reporting process, and beneficial use assessments. These data may be used to include selected water bodies on the federal Clean Water Act, Section 303(d) List of impaired water segments, but there is some question about whether using data from modified U.S. EPA tests are appropriate for 303(d) listing. The specific issue is whether conducting the test at a non-standard temperature precludes using the resulting data for regulatory applications.

U.S. EPA (2000) Section 1.3.6 states, "Altering the procedures described in this manual may alter bioavailability and produce results that are not directly comparable with results of acceptable procedures. Comparison of results obtained using modified versions of these procedures might provide useful information concerning new concepts and procedures for conducting sediment tests with aquatic organisms… If tests are conducted with procedures different from those described in this manual, additional tests are required to determine comparability of results". This section suggests that testing at 15°C would be acceptable depending on the objectives of the study and as long as there are previous experiments demonstrating that test acceptability criteria have been met, and that the test can be used routinely. SPoT data from 15°C tests are submitted to the SWAMP database only if test acceptability criteria have been met as defined in the method and in the SWAMP QAPrP (SWAMP, 2008).

The comparability of the results between the two temperatures is the objective of running these tests in tandem. The test organisms respond differently to pyrethroids at colder temperatures. This has been thoroughly demonstrated with previously published studies (Anderson et al., 2008; Holmes et al., 2008; Harwood et al., 2009; Weston et al., 2009).

WORKGROUP RECOMMENDATION

The method of testing *H. azteca* at colder temperatures was originally developed as a TIE treatment to provide an additional line of evidence for the potential cause of toxicity. The cold temperature treatment provides supporting evidence for the cause of toxicity in tests conducted at standard temperatures. Therefore, the toxicity work group recommends that data from toxicity tests conducted at colder temperatures be interpreted as additional lines of evidence for regulatory decisions in tests where the data can be compared to data from tests conducted at the standard temperature. Whenever possible, toxicity data should be used in combination with pyrethroid concentration data to determine the potential for pyrethroid impairment of sediment in a water segment.

All tests must have met established test acceptability criteria. These include the criteria that control survival is equal to or greater that 80% and that demonstrable control growth is observed (test acceptability criteria for *H. azteca* (U.S. EPA, 2000)).

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SUGGESTED CITATION

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