Development of Quality Assurance Guidance for the *Ceriodaphnia dubia* Reproduction Test

The accuracy and comparability of toxicity test information is vital to stormwater and effluent monitoring programs. The SMC has a primary goal of sharing data, and chief amongst the concerns for sharing data is comparability. Data quality is also important for wastewater effluent discharge monitoring programs, as findings of effluent toxicity may lead to costly studies to confirm the toxicity and identify the cause.

A recent toxicity intercalibration study by the SMC identified instances of poor comparability in the *Ceriodaphnia dubia* reproduction test. A sample of laboratory dilution water, prepared in accordance with standardized guidance and expected to be nontoxic, was found to be toxic by some of the participating labs. A repeat of the testing, conducted after modest standardization of test methods, also identified toxicity in the dilution water sample. Furthermore, the relative comparability of the laboratories varied between testing events. The SMC intercalibration study was unable identify the cause of the low comparability and recommended further investigation of this issue.

The goal of this project is to conduct studies to identify laboratory quality assurance practices that will improve comparability of the *C. dubia* reproduction test. The focus of this work is to identify test conditions and procedures that will minimize instances of toxicity when laboratory dilution water is tested by multiple laboratories. Improving the comparability of test results for samples expected to be nontoxic will improve confidence in the use and interpretation of the *C. dubia* reproduction test for effluent and stormwater monitoring. One of the outcomes of this study will be a guidance document that describes test procedures and quality assurance steps to improve toxicity data comparability.

A Technical Advisory Committee (TAC) composed of regulatory agencies (SWRCB, EPA, RWQCB), regulated agencies (stormwater, wastewater), and testing laboratories will be established to guide study design and review interpretation of the results. The proposed study design is composed of four major elements, all using the *C. dubia* reproduction test: 1) identification of factors influencing comparability, 2) optimization of test conditions, 3) confirmation testing, and 4) reporting. Identification of test factors in the first study element will be accomplished by conducting two rounds of testing by multiple laboratories. A set of different types of laboratory dilution water and positive controls will be tested by a large number of laboratories (10 or more) using EPA standard methods. Results from these tests will be used to identify a subset of factors, such as hardness, ionic composition, or culture feeding regime that appear to influence the results between labs.

The second study element (optimization of test conditions) will include targeted studies to identify specific combinations of test conditions that result in the best intra- and interlaboratory comparability of results. Testing will be conducted by a subset of the laboratories participating in the first study element. Results from this study element are expected to identify refinements to the *C. daphnia* test method that improve test comparability.

In the third element, the efficacy of the method refinements during the second element will be evaluated by conducting another round of testing of laboratory dilution water by a large number of laboratories. The results will be compared to those obtained in the first element to document improvements in data comparability.

The final element of the study will consist of preparing a report and database. The report will summarize the study results and describe procedure to improve test quality and comparability. A database containing raw data from the study will be made publicly available to facilitate public review and use of the information.

This project will require 36 months to complete and will be coordinated by SCCWRP. SCCWRP will be responsible for securing laboratories, establishing the TAC, creating the study design, coordinating sample preparation and testing, data evaluation, and reporting. Approximately 50% of project funding will be used to compensate testing laboratories for participating in the study.

Preliminary cost estimates for this project is $700,000. See Table below for costs associated by task.

TASK	**COST**
Identification of Factors Influencing Comparability	$300,000
Optimization of Test Conditions	$100,000
Confirmation Testing	$150,000
Advisory Committee, Data Management and Analysis, Reporting	$150,000
Total	**$700,000**