CASA Toxicity Work Plan

Objective

The purpose of this study is to quantify the false positive error rate (i.e. the erroneous identification of a non-toxic sample as toxic) of the *Ceriodaphnia dubia* survival and reproduction bioassay (USEPA test method 1002.0) using statistical endpoints currently supported by the USEPA.

Study Methods

Science Advisory Panel:

An expert panel shall be convened from Table 1. This panel will review and provide edits and comments regarding the final study plans, data interpretations, and conclusions.

Principal Investigator:

A Principal Investigator shall be contracted to generate a final study plan, select a referee laboratory, and evaluate data. Using minimum TAC (per method manual), the investigator shall evaluate the tests and calculate statistical endpoints (i.e. NOEC, EC/IC25, and TST) using each tested concentration as the critical concentration. Based on this evaluation, the investigator shall calculate the false positive error rate for each statistical endpoint for each critical concentration. The Principal Investigator, with input from the Science Advisory Panel, shall prepare a final report summarizing the study, its findings. Such a final report shall contain appendices including replicate and summary data for each bioassay.

Referee Laboratory:

The Principal Investigator shall select and contract a referee laboratory. The referee laboratory shall be responsible to prepare and distribute all samples.

Sample and Bioassay Details:

Synthetic dilution waters are used in bioassays when the goal is to estimate the absolute chronic toxicity of a sample. This study shall use five synthetic dilution water samples (blanks) to quantify the likelihood of identifying a non-toxic sample as toxic (false positive). The following synthetic dilution water blanks will be used in the study:

x Moderately Hard Reconstituted DI Water (EPA Manual Formulation) x Dilute mineral water (Perrier and DI water to moderately hard) x Hard Reconstituted DI Water (EPA Manual Formulation) x Very Hard Reconstituted DI Water

(EPA Manual Formulation) x Hard Reconstituted DI Water ("Duluth" Modified EPA Manual Formulation)

Twenty certified laboratories will analyze each sample. Either TNI or California ELAP certification shall be a sufficient demonstration of capabilities. A mix of academic, commercial, and agency sector laboratories will be selected from a pre-populated list (Table 2) with a preference towards labs either within or near California. Participating laboratories shall receive all samples in one batch and have 30days to complete testing. Each laboratory shall use their routine dilution water for a control and all dilutions. Each sample shall be used for initiation and all renewals of a multi-concentration (20%, 40%, 60%, 80%, and 100%/undiluted sample) bioassay. Laboratories shall have 7-days from completion of testing to submit all raw data, bench sheets, and COCs to the Principal Investigator or their designee.

Table 1. Advisory Panel Candidates

Name	Affiliation	State	Sector
Teresa Norberg-King	USEPA ORD	Minnesota	Government
Jerry Diamond	Tetra-Tech	Maryland	Private
Rami Naddy	TRE Environmental	Colorado	Private
Dan Gallagher	Virginia Tech	Virginia	Academic
Bill Goodfellow	Exponent	Virginia	Private
Jim Oris	Miami University	Ohio	Academic
Donald Mount	Asci-ETL	Minnesota	Private

Table 2. Laboratory List

Lab	City	State	Sector
Aquatec Environmental Inc	Williston	Vermont	Commercial
Aquatics Laboratories, LLC	Lexington	Kentucky	Commercial
Bio-Analytical Laboratories	Doyline	Louisiana	Commercial
Biological Monitoring Incorporated	Blacksburg	Virginia	Commercial
Coastal Bioanalysts, Inc.	Gloucester	Virginia	Commercial
EA Engineering, Science, and Technology - Ecotoxicology Laboratory	Hunt Valley	Maryland	Commercial
Environmental Resources Management Aquatic Toxicology Lab	Holland	Michigan	Commercial
Environmental Science Corporation	Mt. Juliet	Tennessee	Commercial
ENVIROSYSTEMS INC	Hampton	New Hampshire	Commercial
James R. Reed & Associates	Newport News	Virginia	Commercial
Mountain River Toxicology Inc.	Norton	Virginia	Commercial
NEW ENGLAND BIOASSAY	Manchester	Connecticut	Commercial
Northeast Ohio Regional Sewer District Analytical Services	Cuyahoga Heights	Ohio	Agency
R E I Consultants - Main Laboratory & Corporate Headquarters	Beaver	West Virginia	Commercial
Ramboll Environ US Corporation	Brentwood	Tennessee	Commercial
Sage Environmental Consulting L.P.	Oklahoma City	Oklahoma	Commercial
Shealy Consulting, LLC	Batesburg-Leesville	South Carolina	Commercial
South Valley Water Reclamation	West Jordan	Utah	Commercial
State Hygienic Laboratory at the Univ. of Iowa - Ankeny	Ankeny	Iowa	Research
TRE Environmental Strategies, LLC	Fort Collins	Colorado	Commercial
Water & Environmental Testing Incorporated	American Fork	Utah	Commercial
UC Davis ATL	Davis	California	Research
UC Davis Granite Canyon	Carmel	California	Research
Pacific EcoRisk	Fairfield	California	Commercial
Nautilus Environmental	San Diego	California	Commercial
Aquatic Bioassay & Consulting	Ventura	California	Commercial
MBC Analytical	Costa Mesa	California	Commercial
AQUA-Science	Davis	California	Commercial
EPA-ORD	Duluth	Minnesota	Research
EPA-Region 9	Richmond	California	Research

City of Los Angeles	Los Angeles	California	Agency
East Bay MUD	Oakland	California	Agency
City of San Jose	San Jose	California	Agency

Ceriodaphnia False Positive Study Design Review

Study Review Panel:

A three-expert study design review panel shall be selected from the following experts:

Name	Affiliation	State	Sector
Teresa Norberg-King	USEPA ORD	Minnesota	Government
Jerry Diamond	Tetra-Tech	Maryland	Private
Rami Naddy	TRE Environmental	Colorado	Private
Dan Gallagher	Virginia Tech	Virginia	Academic
Bill Goodfellow	Exponent	Virginia	Private
Jim Oris	Miami University	Ohio	Academic
Donald Mount	Asci-ETL	Minnesota	Private
Steve Bay	SCCWRP	California	Research

Teresa Norberg-King would be requested to chair the committee. Either Dan Gallagher or Jim Oris would meet the need for an evaluation of the statistical robustness. The third panel member would be an at large member, selected from the list.

Charge to the Panel:

In addition to evaluating whether the study plan appears sufficient to answer the study question, reviewers are asked to specifically address questions based on the following study plan expert:

> "Twenty certified laboratories will analyze each sample resulting in the analysis of 100 blank samples. Either TNI or California ELAP certification shall be a sufficient demonstration of capabilities. A mix of academic, commercial, and agency sector laboratories will be selected from a pre-populated list (Table 2) with a preference towards labs either within or near California. Participating laboratories shall receive all samples in one batch and have 30-days to complete testing."

- Does the panel believe that 100 blanks would be sufficient to accurately quantify a false positive rate? Could this be done with fewer samples?

- Does the panel believe that a blend of industry sectors is important to the study? Agency laboratories might be less costly and commercial laboratories may be most readily available.
- Prioritizing laboratories in or near California would potentially help reduce costs; would this regional prioritization negatively impact the study?