Appendix A: Response to Comments

UCD AHPL Reponses to Comments provided by Pacific Eco Risk

Report Omissions

In addressing the comments regarding the lack of documentation of the report, the formatting of this annual report was specifically requested by members of SFEI, and this report was written with those specifics in mind. While not required for the end purposes of this report, the UCD-AHPL is happy to provide all requested bench sheets and documentation as listed in the Review.

1. The report is lacking chain of custody records (COC), which prohibits the reviewer from confirming the information presented in Section 2.1, and does not allow the reviewer to determine if all samples were tested or met the holding time limits. ***The report should be revised to include the COCs for this project.***

We have included electronic copies of the COCs in the appendix.

2. The report is lacking all bench sheets from the laboratory. Bench sheets are the datasheets upon which the laboratory analyst records the method required data and observations, such as water quality measurements, and observations of test endpoints (i.e., survival counts, reproduction counts, etc.). Without the bench sheets, it is impossible to determine if the sample holding time limit was met (by comparing to the COCs), and the reviewer is left to assume that what was actually recorded by the staff performing the toxicity tests is accurately presented in the report. The lack of bench sheets in this report is highly unusual, unless this report was for a publication. Given that this report is not a publication, the lack of bench sheets is inconsistent with industry standards and reporting for regulatory programs (e.g., ILRP, NPDES, etc.). ***The report should be revised and should include the actual toxicity test data as appendices.***

We have included the bench sheets as an appendix.

3. The report is lacking statistical analyses worksheets, which along with the lack of bench sheets, does not allow for an evaluation of possible transcription errors (were data from bench sheets appropriately transcribed into statistical software), nor an evaluation as to whether the statistical analyses were properly conducted and properly summarized in the report. ***The report should be revised and should include the actual statistical analyses worksheet as appendices.***

We have included the statistical analysis worksheets.

4. Table 2.2 only includes the hardness, alkalinity and ammonia-nitrogen (and related unionized ammonia calculation) that were collected at the time of sample receipt. However, the table is lacking the other water quality data (i.e., pH, DO and conductivity) that are required in Section 8 of the EPA method manual. Some of these parameters are needed if one wanted to confirm that the proper unionized ammonia value was calculated in Table 2.2. Additionally, these values are necessary to properly qualify an observation of toxic effect, particularly if that affect was associated with observations of low DO. ***The report should be revised to include this important water quality log in data.***

The UCD AHPL records temperature and specific conductance on samples upon receipt. Ammonia-nitrogen is measured within 24-hours of receipt; hardness and alkalinity measurements are recorded within 72-hours of sample receipt. These are the values outlined in Table 2.2 Other water quality measurements such as electrical conductivity, pH, and DO are measured at test initiation for all species. These values are located in the water quality tables at the end of the report (tables 59-100). We calculate unionized ammonia based on the ammonia nitrogen measured at sample receipt, and the temperature, conductivity, and pH of sample waters at test initiation for each individual species. This allows us to make a more accurate measurement, as these water quality values may differ with each test species.

5. The alkalinity for the 10/22/15 *P. promelas* and *C. dubia* control/dilution water is missing from Table 4 with no indication as to why. ***The report should be revised to provide an explanation for this missing value.***

We have made this change.

6. The report is lacking an Executive Summary section that summarizes the work performed, including the summary of the toxicity findings. ***The report should be revised to include a list of tables and figures***.

We assume the reviewer meant to request an Executive Summary, and we have included one.

7. Given that the Delta RMP is such an important program to both the agencies and participating regulated parties, the report should allow interested parties to more quickly obtain data and conclusions from the sites that may be of interest to them. ***The report should be revised to include a list of tables and figures.***

We have included a list of tables and figures.

Recommendations/Questions

1. As pesticides are of critical concern to the Delta RMP, the report should include a section describing the sample handling protocols prior to testing. As is expected form SWAMP-compliant monitoring programs, it is expect that the lab staff vigorously shake sample bottles for ~60 seconds so as to disassociate any loosely adsorbed pesticides (e.g., pyrethroids) from the wall s of the sample container and to equally distribute any suspended or settable solids among the *Ceriodaphnia* and fathead minnow test chambers. ***The report should be revised to include a description of the sample handling procedures as they related to shaking bottles to disassociate loosely adsorbed pesticides.***

We have included this information.

2. Although the authors of the report did not include the statistical analyses and bench sheets in appendices, they spent considerable time entering routine water quality parameters (e.g. DO, conductivity and temperature) for each of the tests that were performed. This data is only important to the interpretation of the toxicity test results when the parameters fall outside of the tolerance limits of the species. Inclusion of such tables in the report is clearly a time consuming, and therefore expensive effort. Furthermore, this data is available via the CEDEN database should a reviewer be interested in evaluating routine test water quality***. It is recommended that this mostly superfluous data be excluded***

from the report and cited as available through CEDEN. Furthermore the repot should be revised to only note when the water quality parameters were beyond the tolerance limits of the species, if corrective measures were required, (e.g,., test aeration), if the parameter draws into question the acceptability of a test (e.g., DO crash associated with mortalities) and if the parameter could explain any observed toxicity (e.g, low conductivity).

We include these tables for easy reference of water quality measurements, and these tables were specifically requested by members of SFEI. These tables also outline important water quality measurements in case one wants to double check the unionized ammonia calculations presented in Table 2.2.

3. The table in Section 2.1 notes the following types of samples were collected: Field QC, FieldQA (Field Blank) and Field QA. Per Section 6.2, the field duplicates were primarily those identified as FIELD QC, however several field duplicates were identified as FIELDQA. Without the COCs in the annual report, reviewers can't determine if this is an error in this table of if the field crew was inconsistently identifying field duplicate samples. ***The report should be updated for consistency.***

We have updated the tables for consistency.

4. Tables 2.3 and 2.4 indicates ND (non detect) for a number of ammonia and/or unionized ammonia values, as well as a footnote for values that are between the MDL and RL. ***The report should be revised to include an indication as to what the MDLs and RLs are for the parameters***.

We have included this information.

5. The first set of samples tested were collected on 7/28/15, which would require the preparation of control/dilution waters for test initiation on 7/29/15. However, Table 4 indicates that the first *C. dubia* control water was prepared on 8/7/15. ***This report should be revised to include the proper date of the control water creation used for the testing initiated on 7/29/15.***

The date of 8/7/15 for the control water used in the C. dubia test for samples collected on 7/29/15 is correct. This control water refers to the C. dubia retest that was initiated on 8/7/16. The test initiated on 7/30/16 did not meet test acceptability, and therefore we did not report that data.

6. There are redundant entries in Table 4. ***The report should be revised to remove the duplicate records***.

We have corrected Table 4.

7. Section 6.6 of the report indicates that glassware or foam plug contamination was identified as the cause of the toxicity observed in a July 28, 2015 sample for *Selenastrum* testing. Section 6.4 (bottle blanks) of the report indicates that the *Selenastrum* growth was significantly lower in the bottle blank sample collected for testing on the same date. The report goes on to note that foam plugs were replaced and the glassware cleaning SOP was updated. However, the February 27, 2016 field blank collected ~ 8 months later produced significantly fewer *C. dubia* offspring than the associated control treatment; an additional field blank was collected during a future event, but "no other follow-up was conducted". These results suggest that the laboratory glassware cleaning corrective action was not remedied, as the sample bottles should be cleaned using the same EPA procedure as the testing glassware. The presence of contaminated sample bottles and laboratory glassware is very disconcerting, as it raises questions about the thoroughness of the laboratory cleaning process, and would draw into

question any finding that a sample was toxic. This is a serious issue, as clean glassware is the fundamental foundation to good laboratory practices, and contaminated glassware draws into question any report of identifying a sample as toxic. The lack of further corrective actions by the laboratory after again having glassware problems ~ 8 months later is of great concern and should be immediately addressed. ***The report should be revised to identify the corrective action performed by the laboratory to address contaminated sample bottles, and the project QA manager should audit the laboratory glassware cleaning SOP and process in the lab (i.e., visit the lab to audit the process).***

We have noted that the potential glassware contamination in the Selenastrum test conducted in July 2015 as such. This may or may not have been the cause of the bottle blank performance in the same S. capricornutum test. However, the reviewer is mistaken in linking a bottle blank result conducted in July 2015, and a field blank significant result in a different species almost a year later. A bottle blank is used to determine whether there is incidental contamination through the cleaning protocols of a laboratory, whereas a field blank is used to determine if there is any incidental contamination during the sample collection process in the field. These are two different mechanisms to measure precision. After the significant result with the field blank sample in the C. dubia test in July of 2016, we included another field blank sample in a future event, and when that field blank sample came back as not significant, we determined that no other follow-up needed to be conducted. However, to further investigate this, we conducted a bottle test with S. capricornutum, which evaluated the difference in algal growth with a brand new bottle from the manufacturer, and a UCD AHPL washed bottle. Both the washed bottle and brand new bottle performed better than the control. Details of this test is presented below.

Treatment	***Mean Cell Density ($x10^6$)***	***SE***	***%CV***
Glass Distilled Control	*1.509*	*0.068*	*9.03*
GD in Brand New Bottle	*1.913*	*0.057*	*5.98*
GD in Washed Bottle	*1.846*	*0.053*	*5.77*

8. Although issues of contaminated glassware and stoppers identified by the laboratory for the July 29, 2015 *Selenastrum* test, and quality control issues were identified in Section 6.6 of the report, Table 15 summarizes the *Selenastrum* test results for the July 29, 2015 testing and still identified several samples as toxic. ***The report should be revised to add a footnote to this table that would question the conclusion that the samples were toxic given the contaminated glassware/plugs***.

We have indicated that the Mokelumne and SJR @ Buckley sites toxicity is likely due to glassware contamination.

9. Table 25 identifies the conductivity control, 510SACC3A and 510SOL010 samples as being toxic to *Ceriodaphnia* reproduction. However there is no indication if the conductivity was low for the two samples, so the reviewer can't determine if conductivity or another driver was the cause of the toxicity for the two samples. The same situation occurs in Tables 41 (February 18, 2016 testing), 48 (April 20, 2016 testing), 54 (June 16, 2016) and 57 (July 14, 2016 testing). ***The reports should be revised to identify any statistical comparisons that were performed against samples with a low conductivity (i.e., were low conductivity samples toxic when compared to the conductivity control?).***

We have included in the text when we include a low conductivity control to help clarify this statement.

10. The second sentence of Section 9.0 (Summary) indicates "During this period there were twenty-seven instances of observed toxicity, outlined below in Table 103". ***The report should be revised to add the total number of tests performed that resulted in an outcome of 27 instances in toxicity***.

We have included this information in the text.

Quality Control Issues

1. Table 2.3 provides the hardness and alkalinity values for the control/dilution waters for each species. It is expected that the hardness and alkalinity of the control water for the fathead minnow (ROEPAMH) and *Ceriodaphnia* test (L1650) will comply with the requirements for moderately hard water. Section 7.2.3.1 of the EPA manual indicates that moderately hard water should have a hardness of 80-100 mg/L and alkalinity of 57-64 mg/L. However, multiple alkalinity values for the fathead minnow control water were outside of the expected range for a properly prepared moderately hard control water. Similarly, the 11/9/15 control water batch used for *Ceriodaphnia* testing exceeded the range specified in the EPA manual. ***The report should be revised to identify such quality control errors.***

We have included this information.

2. The test temperature for Selenastrum is recommend to be 25°C +/- 1°C, yet the test initiation *Selenastrum* test temperature in Tables 64, 68, and 84 indicate that the testing was initiated between ~22.0-23.0°C. Other *Selenastrum* tests were initiated at the recommended temperature. The recommended temperature for the fathead minnow test is also 25°C +/- 1°C, yet the test temperature in Table 73 indicate that the test temperature ranged from ~ 22.1-24.0°C. Although the test temperature is a recommendation in the EPA manual, and the lab met the requirement that the temperature not deviate by >3°C over the course of the test for all but the *Selenastrum* tests initiated on February 18, 2016, the lab should be more consistent in the performance of their testing. Such a range of temperatures over multiple tests suggest that either there were equipment issues (e.g., incubator) or staff performance problems, both of which should be addressed by the Lab's QA Manager/Officer. ***The lab should assure that the test temperatures comply with those noted in the EPA manual.***

The UCD AHPL does comply with those requirements listed in the EPA manual. Due to the short-term changes in temperature when the chamber door is open, fluctuation in temperature is to be expected during a chronic toxicity test. As the S. capricornutum and fathead minnow tests in question met all test acceptability criteria, and the temperature fluctuation did meet US EPA requirements of not deviating by more than 3°C, these data are considered reliable.

3. The test temperature data in Table 67 indicates that the temperature did not deviate at all from 24.0°C during the entire 6-8 day *Ceriodaphnia* tests initiated on 9-24-15. This is very hard to believe, as even the best temperature controlled rooms or water bath systems do not maintain the temperature this well. ***The data should be reviewed for accuracy and revised (at least in CETIS).***

The C. dubia test initiated on 9/24/15 was conducted at AQUA-Science in Davis, CA. They have two different methods in which they record temperatures: with a TidBit data logger, as well as a constant temperature wheel recorder. These thermometers are calibrated quarterly, and are certified to be reliable and constant. The room in which the C. dubia tests are conducted is very well insulated, and the door is kept shut at all times. Water chemistry is measured in this room. Per the protocol in USEPA

(2002), temperature is recommended to be 25±1°C, and for this protocol, AQUA-Science records temperature to the nearest whole number integer. Therefore, the temperature reported for this test is considered reliable and accurate.

Reporting Errors

1. Section 2.1 indicated that an asterisk (*) in the table reflects samples that were received at the laboratory within two hours of sample collection, which precludes the 0-6°C sample receiving temperature criterion. However, there is no such "two hour" criterion in the EPA method manual (EPA-821-R-02-013). In regards to samples shipped to off-site facilities, Section 8.5.7.1 of the manual indicates:

"*Samples collected for off-site toxicity testing are to be chilled to 0-6°C during or immediately after collection, and shipped iced to the performing laboratory. Sufficient ice should be placed with the sample in the shipping container to ensure that ice will still be present when the sample arrives at the laboratory and is unpacked*."

This section simply requires that the samples that are received the day of collection should be received with sufficient ice to assure that they are being chilled from the time of collection to the time of receipt at the lab. Unless the project QAPP has a more stringent requirement that the EPA method manual, and given that the table in Section 2.1 suggests that all sample sere received the day of sample collection, ***the report should be revised to remove any indication that samples outside of the 0-6°C required some form of a special notation.***

We have made this change.

2. Table 4.0 indicates that the organism age for *C. dubia* is <24 hr. This is only one of two requirements for the age of *C. dubia* in the testing manual. The other requirement is that the organism be within 8 hours of age. ***The report should be revised to identify if the age of C. dubia at test initiation were <24 h and within 8 hr of age.***

We have made this change.

UCD AHPL Responses to Comments provided by CH2M

1. Some, but not all tables list the sample ID and the site name. It might be helpful to list a more recognizable/simpler ID than the current alpha-numeric listed in Table 1 that is also used in the subsequent tables (e.g., 'Hood' instead of 510SACCHOD and 510SACC3A).

We have made this change to clarify the sites. These new sample IDs are referenced in Table 1.

2. Many samples did not meet the target temp upon receipt at the lab. Add a section discussing corrective actions to meet the goals/criterion where not met during this round of sampling (e.g., ore ice to help sample temps <6°C upon receipt; sample labelling to clarify the type of field QC sample). Other issues described under Deviation follow-up might fit under this corrective action section.

This issue was also discussed by another reviewer. In the comments, they pointed out the following:

"Section 2.1 indicated that an asterisk (*) in the table reflects samples that were received at the laboratory within two hours of sample collection, which precludes the 0-6°C sample receiving temperature criterion. However, there is no such "two hour" criterion in the EPA method manual (EPA-821-R-02-013). In regards to samples shipped to off-site facilities, Section 8.5.7.1 of the manual indicates:

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This section simply requires that the samples that are received the day of collection should be received with sufficient ice to assure that they are being chilled from the time of collection to the time of receipt at the lab. Unless the project QAPP has a more stringent requirement that the EPA method manual, and given that the table in Section 2.1 suggests that all sample sere received the day of sample collection, ***the report should be revised to remove any indication that samples outside of the 0-6°C required some form of a special notation."***

With this in mind, we removed indications that follow-up was required.

3. Clarify the type of "Field QC" as field blanks or dups in tables.

We have made this change.

4. Define abbreviations – upon first use (e.g., ROEPAMH and L1650).

We have made this change.

5. Clarify that algae tox control were not conducted in "Distilled water" (Table 4). Rather, indicate what DI was amended with or even simply that was amended.

The controls we use for our alga tests are with distilled water. However, we do amend the distilled water. We have changed the text to reflect that the distilled water is amended with nutrients.

6. Flag results in all tables whenever TAC or reference tox were not met, PMSD out of bounds, or if other deviations (per section 6.6) should be considered in the interpretation of results.

We do not report tests that do not meet test acceptability criteria. However, we made notes where applicable, if results should be considered suspect. Reference toxicant charts have qualifiers where necessary.

7. Identify which ref tox results correspond with each test/test dates. Ref tox figures only show an arbitrary number whereas tables of tox endpoints have a date.

We have made this change.

8. Please check units in Table 8 or explain why the endpoint units change from µS/cm to g/L in C.d. March 2016 ref tox tests.

We have clarified this reason in the report.