DIVISION OF ADULT INSTITUTIONS
Deuel Vocational Institution
23500 Kasson Road
P.O. Box 400
Tracy, CA 95378

8:27

March 27, 2013

Ms. Pamela Creedon
Executive Officer
11020 Sun Center Drive, Suite 200
Rancho Cordova, CA 95670-6114

Dear Ms. Creedon:

The California Department of Corrections and Rehabilitation (CDCR), Deuel Vocational Institution (DVI), is submitting the attached Toxicity Reduction Evaluation (TRE) Report of Final Findings in response to the Central Valley Regional Water Quality Control Board's letter dated July 26, 2012, which requested that DVI initiate a TRE consistent with the Waste Discharge Requirements (WDR) in its Order No. R5-2008-0164. The CDCR hired consultant GHD Inc. to conduct the TRE in accordance with DVI's approved TRE Work Plan (2009). The TRE Report of Final Findings shows evidence that the source of the toxicity is founded in two specific causes: improper laboratory controls and procedures and DVI's Reverse Osmosis Plant (ROP) water treatment plant operational status.

Laboratory Controls and Procedures:

1. Per the institution's WDR, DVI is to use the receiving water control as the diluent unless the receiving water is impaired. Additional testing of the receiving water as part of the TRE investigation showed it to be stimulatory likely due to the fact that the receiving water is an agricultural drain. As such, DVI's laboratory should use laboratory prepared control water as the diluent instead of the receiving water.

2. While not specifically related in this TRE analysis, DVI is not required to perform the chronic toxicity bioassay testing using a 5 dilution series as had been done in prior quarterly testing but instead use 100% effluent for regular quarterly monitoring.

3. Additional testing for the TRE also revealed that algal sticking may have resulted in false positives for toxicity during regular quarterly monitoring and accelerated monitoring. Should a test result indicate toxicity of the effluent and the TUc for the effluent is between 1.33 and 2, DVI should instruct the laboratory to perform a re-suspension step to see if algal sticking is resulting in a false positive for toxicity.

Plant Performance:

1. GHD's review of monthly monitoring reports revealed that electro-conductivity (EC) was above permitted limits during many of the toxic tests and further investigation showed that during those same periods of elevated EC the DVI ROP was off line for maintenance purposes. When collecting samples for chronic toxicity testing, DVI's Waste Water Treatment Plant (WWTP) staff should first identify and verify the operational status of the ROP water. If found to be off line for maintenance, staff should postpone sampling until the unit is back online. However, if a sample must be collected for either regular quarterly monitoring or accelerated and/or TRE monitoring, DVI staff should note this information when reporting the results, particularly if the results indicate toxicity of the effluent.

With the likely sources of toxicity identified, GHD conducted four final Chronic Bioassay tests in an effort to permit DVI to exit the TRE. Samples for the four tests were collected while the ROP was operational to exclude any effects of elevated electro-conductivity and laboratory controls and procedures followed the recommendations above. All test results related to the effluent samples showed a TUc of 1, indicating the absence of toxicity.

Since we have identified the likely sources of toxicity and successfully completed accelerated monitoring with four consecutive passing chronic toxicity bioassays, DVI requests that it be permitted to exit the TRE.

If you should have any further questions or concerns, you may contact me at (209) 830-3932. Thank you for your assistance in this process as it will enhance the CDCR and DVI in maintaining compliance with the program.

Sincerely,

VERN MILLARD
Correctional Plant Manager

cc: DVI Warden (A)
DVI Waste Water Treatment Plant
Fred Cordano
Annette Salazar
Jeff Stanley

Toxicity Reduction Evaluation

Final Findings Report

California Department of
Corrections and Rehabilitation

Deuel Vocational Institution
Wastewater Treatment Plant

March 2013

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Contents

1. Introduction

This report presents the final findings from Deuel Vocational Institution's (DVI) Toxicity Reduction Evaluation (TRE). The TRE was performed with guidance provided in DVI's 2009 TRE Work Plan and the Toxicity Reduction Guidance for Municipal Wastewater Treatment Plants (EPA 1999). The TRE examined data collected from DVI to determine the apparent cause of effluent toxicity, identify any possible source(s), assess control options, recommend corrective actions, and finally, suggestions for strategies to exit the TRE.

1.1 Facility Background

Deuel Vocational Institution operates a wastewater treatment plant (WWTP) to treat wastewater generated at the Institution. The WWTP consists of preliminary treatment, alkalinity adjustment, activated sludge secondary treatment with nitrification, a membrane bioreactor system for tertiary treatment and finally, disinfection by ultraviolet light. After treatment the effluent is passed through a cooling tower to reduce the effluent temperature prior to discharge. The Central Valley Regional Water Quality Control Board (RWQCB) adopted a National Pollutant Discharge Elimination System (NPDES) permit (CA0078093, Order No. R5-2008-0164) for DVI on October 24, 2008 which allows the WWTP to discharge treated effluent to Deuel Drain, an agricultural irrigation drain and waterway of the Sacramento-San Joaquin Delta.

In addition, the NPDES permit for DVI requires the facility to conduct whole effluent toxicity (WET) testing on its WWTP treated effluent at Discharge Point EFF-001. The WET testing includes quarterly acute toxicity and chronic toxicity testing. The acute toxicity testing is required for fathead minnows (Pimephales promelas). The chronic toxicity testing is required for the water flea (Ceriodaphina dubia), fathead minnows (Pimephales promelas) and green algae (Selenastrum capricornutum). The chronic toxicity testing measures sublethal and/or lethal effects to test organisms exposed to an effluent water sample compared to that of organisms exposed to a control water sample. According to DVI's permit, the control sample and effluent diluent is the receiving water, Deuel Drain. The permit also requires that should the receiving water be toxic, DVI is permitted to have the laboratory use the laboratory control water as the diluent. Finally, if the test results show that DVI's WWTP effluent EFF-001 is above their permitted numeric toxicity monitoring trigger as compared against the control with a TUc greater than 1, then they are required to initiate accelerated monitoring. Should at least one accelerated monitoring test result in a TUc greater than 1, the TRE must be initiated.

1.2 Purpose and Need for Report

On 12 January 2012 DVI exceeded the chronic toxicity Trigger level of 1 TUc with a reported result of 1.33 TUc for Selenastrum capricornutum. Accelerated monitoring samples were collected on 2 May, 16 May, 31 May, and 13 June. Additional quarterly testing for Selenastrum was conducted on 5 April and 11 July, and 11 October 2012. GHD was contracted in September to initiate a TRE utilizing the TRE Work Plan (2009) as required by the discharge permit. GHD conducted additional

sampling on 18 October and 25 October as part of the TRE. A summary of the chronic test results for Selenastrum are presented in the following Table 1:

Table 1 – Chronic Selenastrum Bioassay Test Results for EFF-001

Date of Sample	Type of Sample	Diluent	Type of Test	Result as TUc
12 January 2012(1)	24hr Composite	Receiving Water	Quarterly Monitoring	1.33
5 April 2012(1)	24hr Composite	Receiving Water	Quarterly Monitoring	1.33
2 May 2012(1)	24hr Composite	Receiving Water	Accelerated Monitoring	8.00
16 May 2012(1)	24hr Composite	Receiving Water	Accelerated Monitoring	8.00
31 May 2012(1)	24hr Composite	Receiving Water	Accelerated Monitoring	>8.00
13 June 2012(1)	24hr Composite	Receiving Water	Accelerated Monitoring	4.00
11 July 2012(1)	24hr Composite	Receiving Water	Quarterly Monitoring	2.00
11 October 2012(1)	24hr Composite	Receiving Water	Quarterly Monitoring	1.00
18 October 2012(2)	Grab	Receiving Water	TRE Monitoring(3)	1.00(5)
18 October 2012(2)	Grab	Lab Water	TRE Monitoring(3)	1.00(5)
25 October 2012(2)	24hr Composite	Lab Water	TRE Monitoring	1.00
25 October 2012(2)	Grab	Lab Water	TRE Monitoring(4)	1.00

Notes:
1 – Laboratory tests performed by Aquatic Bioassay Consulting, Inc.
2 – Laboratory tests performed by Sierra Foothill Laboratories
3 – Grab samples were conducted instead of 24hr composites for ease of sample gathering and simplification in test result interpretation.
4 – Grab sample on 25 October was taken to compare against composite sample to investigate daily variability in potential toxicity response
5 – Initial TUc was reported as 1.33. re-suspension step was conducted which lowered the TUc to 1.

GHD reviewed annual and accelerated chronic toxicity test results from January of 2012 to October 2012 and confirmed that toxicity was present in DVI's effluent EFF-001. The toxic response begins in January 2012 and increases to a maximum TUc value of greater than 8 in May, and finally decreases to non-toxic levels in October. Based on this information the toxicity was believed to be intermittent and likely seasonal with the greatest toxicity occurring during the spring, coinciding with the agricultural growing season. Since the receiving water is an agricultural drain it is possible that fertilizer, pesticide, or herbicide runoff into the Deuel Drain is at least a partial contributor to the toxicity. To examine the seasonal effects as well as verify WWTP performance first before initiating the Toxicity Identification Evaluation (TIE) portion of the TRE, the TRE follows a two stepped approach:

- Step 1 focuses on the first two elements outlined in the TRE Workplan and the EPA's guidance on conducting Toxicity Reduction Evaluations, namely the Information and Data Acquisition, and Facility Performance Evaluation. This first step focuses efforts on identifying any laboratory data inconsistencies, validation of bioassay test results, treatment performance issues during the period of observed toxicity, and/or environmental conditions

that were present then determine if those conditions continue to persist. If toxic conditions continue to persist then the first phase concludes with identification of actions to correct those conditions.

- Step 2, if warranted, will focus on the remaining steps of the TRE, namely the Toxicity Identification Evaluation followed then by the Toxicity Source Evaluation and finally the Toxicity Control Evaluation. Exhausting possible laboratory variability, treatment performance, or environmental conditions, DVI will begin these final three Evaluations in an effort to identify and control the source of toxicity. Step 2 will only be initiated if Step 1 efforts and actions do not identify and result in the elimination of toxicity. It should be stated that these steps identified here should not be confused with the phases associated with the TIE as outlined in the EPA's TRE guidance.

Step 1 has been completed and the findings are presented in this report.

2. Information and Data Acquisition

2.1 Review of Historical Bioassay Results

GHD reviewed in detail the chronic bioassay test results for both quarterly and accelerated monitoring from 12 January 2012 through 11 October 2012. The first analysis conducted was to examine the relationship between the TUc reported for a given 96 hour cell density for 100% concentration of EFF-001. The TUc value is generated from the test sample EFF-001 cell density as compared to a control sample, whether from receiving water or lab water. Therefore the TUc value is not an absolute value but is instead a numerical ratio of the relationship between the test sample cell density and the control sample cell density and as such can vary in value as a result in variations to both the test cell density and the control sample cell density. For example, the TUc value can be drastically different for identical test sample cell densities if the multiple control sample cell densities against which it is compared are sufficiently different. However, provided that the laboratory control(s) are properly created and monitored, this variability can be identified by employing a second control sample against which both the test sample and the first control sample are compared.

Proceeding on this basis to examine this relationship, the following information presented in Table 2 below was tabulated from the quarterly and accelerated monitoring tests conducted by DVI. When the information in Table 2 is analyzed for correlation between TUc and cell density the pattern that emerges is one of inverse corollary as shown in Figure 1 below: As TUc increases, indicating greater toxicity, cell density effectively decreases.

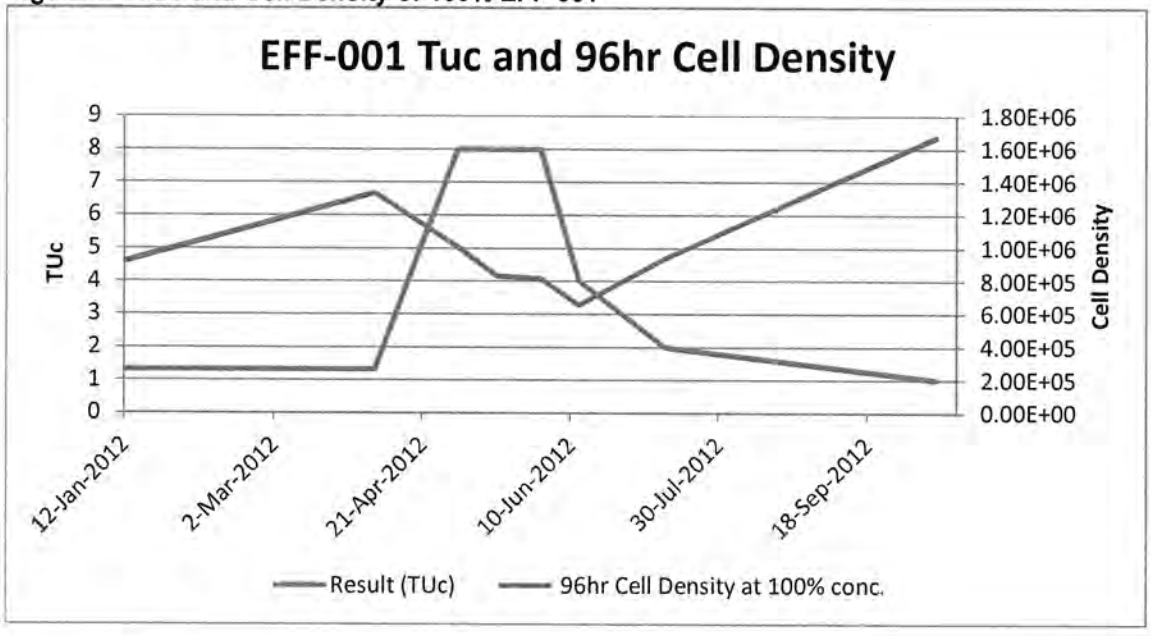
However, two notable exceptions emerge when comparing the results. The first observation was that between 31 May and 13 June there is a simultaneous decrease in both the TUc value from 8 to 4 and in cell density from 8.15E+05 to 6.57E+05 indicating a direct corollary relationship. The second observation was despite having a 35% decrease in cell density from 2 May to 13 June (1.01E+06 compared to 6.57E+05) the TUc value also shows a decrease from 8 to 4. Typically a significant decrease in cell density of that magnitude would likely indicate an increase in toxicity or at the very

least, a TUc value that would be unchanged. Since these measurements for toxicity as reported as TUc are relative to the cell density of a control, this would indicate that the control sample cell density varied significantly during the same time period. Therefore GHD concluded that the receiving water, laboratory water, or laboratory procedures related to the use and application of the controls as possible culprit for the toxicity and warranted further study.

Table 2 – TUc and Cell Density of 100% EFF-001

Date of Sample	TUc	96 hour cell density of 100% Concentration EFF-001
12 January 2012	1.33	9.18E+05
5 April 2012	1.33	1.34E+06
2 May 2012	8.00	1.01E+06
16 May 2012	8.00	8.31E+05
31 May 2012	>8.00	8.15E+05
13 June 2012	4.00	6.57E+05
11 July 2012	2.00	9.38E+05
11 October 2012	1.00	1.67E+06

Figure 1 – TUc and Cell Density of 100% EFF-001



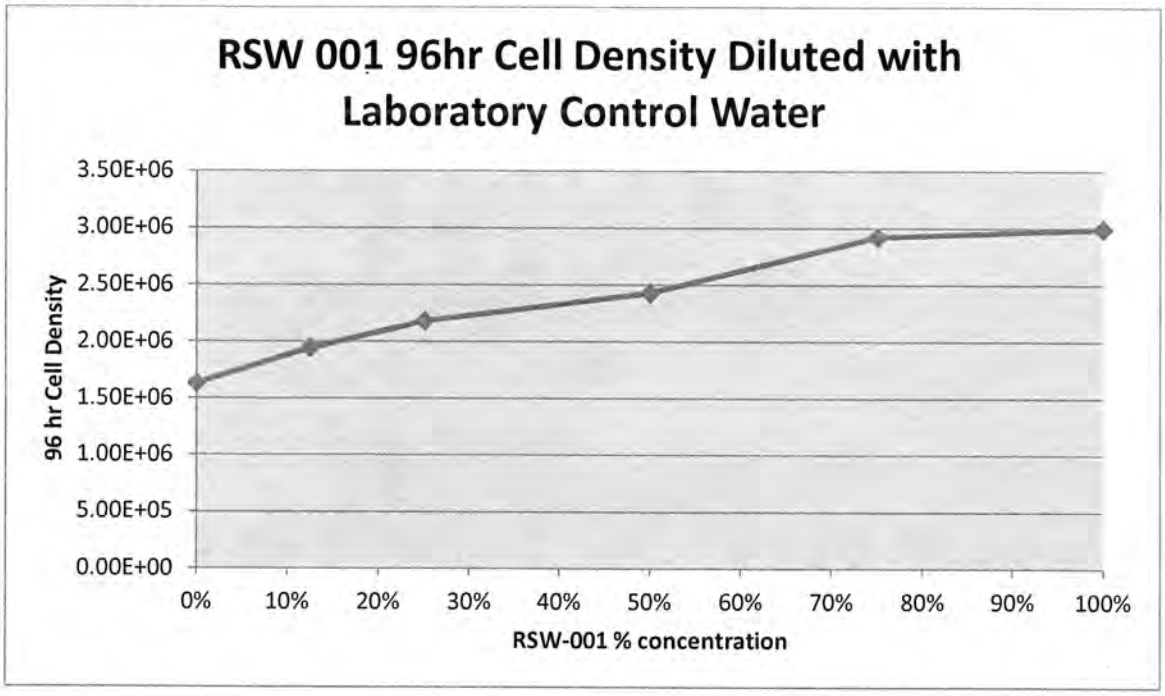
2.2 Analysis and testing of Receiving Water RSW-001

To determine if DVI's receiving water, use of the receiving water as the only apparent control, and/or other laboratory control procedures were possible contributors to the observed toxicity in the quarterly and accelerated monitoring tests, GHD reviewed the same test results from 12 January to 11 October and noted that in all cases water taken from the receiving water RSW-001 was used as the diluent in accordance with their WDR. However, when the entire test results were examined three potential issues where identified. First, regardless of the value reported for the 96 hour cell density for the 100% concentration for RSW-001, RSW-001 was used as the test control in every case; second, in each test report a laboratory control in addition to the receiving water control was not reported by the testing laboratory; and finally, the concentration between the lowest RSW-001 control cell density (1.09E+06 on 11 October) and the highest cell density (2.37E+06 on 31 May) varies by a factor of 2.2. While some variability is expected in the control cell density the fact that it varies by a factor of 2.2 is significant and could contribute to false positive tests for toxicity of the effluent water, particularly if the cell density results of the 100% effluent sample are relatively consistent from test to test. GHD cannot stress enough the use of the laboratory control in addition to the receiving water control when determining when to use and when to exclude the receiving water control.

Based on this finding GHD believes the use of the receiving water as the test control without first analyzing it for toxicity relative to a laboratory control is at least partially to blame for the toxicity results. To attempt to confirm the toxicity of the receiving water, GHD collected a sample of the receiving water on 18 October and had Sierra Foothill Laboratory conduct a 5 dilution series with the receiving water using laboratory prepared control water as both the diluent and control. The results, shown in Figure 2 below, indicate that the receiving water has stimulatory effects likely due to the fact that the receiving water is an agricultural drain. When such effects are present it is recommended that it should neither be used as the diluent nor the sole control.

Since all the bioassay test results appear to only include RSW-001 as the test control, GHD contacted the lab responsible for DVI's bioassay testing, Aquatic Bioassay Consulting, Inc. (ABC) to better understand their procedures and determine if any other controls were being performed but not provided in the report. After discussing with the lab, they do in fact perform laboratory control samples but do not report them in the test results. While ABC should include them as a matter of course, GHD recommends that when sending samples to ABC, DVI should specifically request that the results of the lab prepared controls be included in the test results and, in cases where the receiving water control RSW-001 indicates that it is either stimulatory or toxic in relation to the laboratory prepared control water then the laboratory prepared control be used instead of the receiving water as the reference control. This will greatly reduce if not eliminate false positives for toxicity in situations where the effluent is in fact not toxic but appears falsely toxic relative to the stimulatory effects of the receiving water.

Figure 2 – RSW-001 Chronic Bioassay Results for Selenastrum sampled on 18 October



3. Facility Performance Evaluation

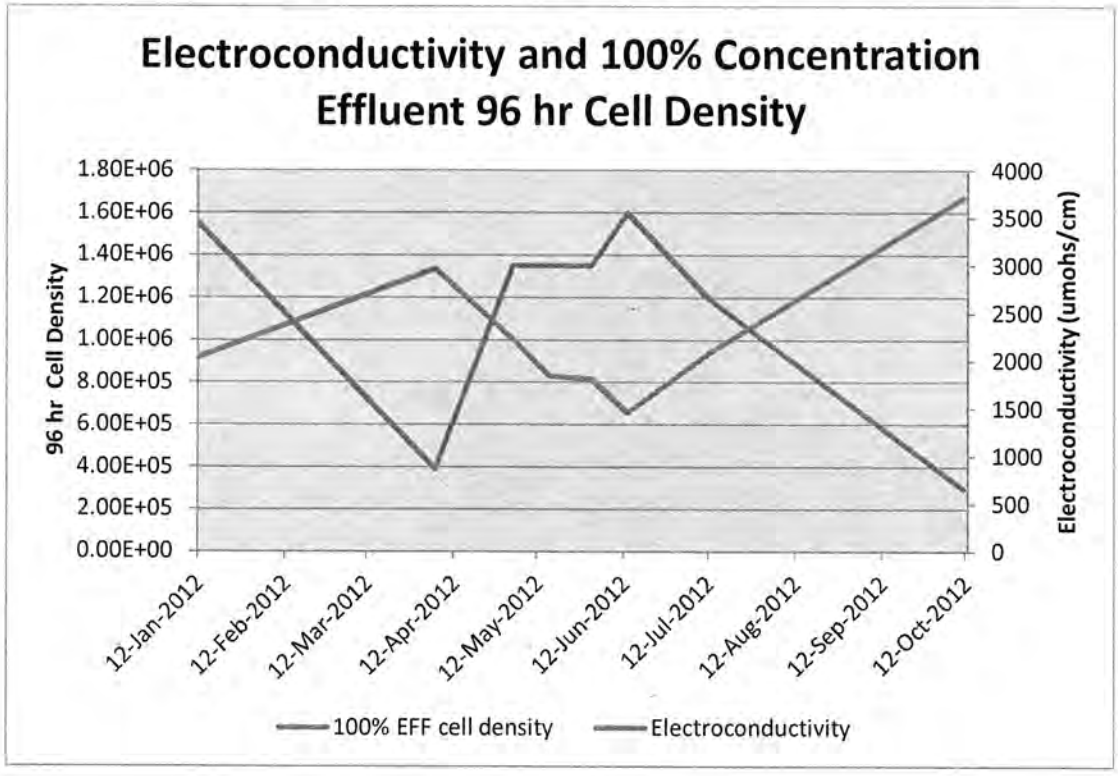
3.1 WWTP Performance Analysis

While the incorrect application of the receiving water as the only test control and the absence of the laboratory prepared control data during quarterly and accelerated monitoring tests from11 January through 12 October is one potential source for the reported toxicity at DVI, it alone does not explain the reduction in 96 hour cell density of the effluent EFF-001 identified in Table 2 from Section 2.1. Even if the proper laboratory controls and procedures had been applied GHD believes that it is likely that several of the bioassay results would have returned as positive for toxicity.

GHD thus began reviewing WWTP performance data in accordance with the TRE Workplan, with special attention focused on monthly reporting data provided as part of DVI's Monitoring and Reporting Program (MRP). A thorough review of the monthly reports indicated that while almost all parameters that DVI is required to report were within permissible limits, one parameter, electro-conductivity (EC) was above permitted limits during many, though not all, toxic tests. However, by

charting the 96hr cell density for 100% effluent along with the EC, an inverse relationship emerges as shown in Figure 3 below:

Figure 3 – 96% Cell Density for 100% EFF-001 along with EC



Based on information provided by DVI WWTP staff, further study indicated that during those same periods of elevated EC the DVI reverse osmosis (RO) plant that is operated as part of the water treatment system was off line for maintenance purposes. Domestic water is supplied to the institution from on-site wells. The well water in turn has elevated constituents – including total dissolved solids, hardness, alkalinity, and other water quality parameters that affect EC – that are removed by the RO process. The RO system is taken off line periodically to perform maintenance. During those maintenance periods the constituents remain in the water which eventually arrives at the WWTP as wastewater influent.

3.2 WWTP Maintenance, Housekeeping, and Chemical Storage

While GHD is confident that the toxicity is explained by both (a) the use of receiving water as the only apparent control and diluent during bioassay testing and (b) sampling for chronic bioassay testing during periods of high EC as the result of the RO plant being off line for maintenance

purposes, GHD performed a review of maintenance, housekeeping, and chemical storage practices at the WWTP as directed by the TRE Workplan. Both review of documentation and discussions with WWTP operations staff were conducted. Findings indicate that the practices used at the WWTP are consistent with best management practices and in keeping with their O&M procedures.

3.3 Additional Bioassay Testing Results

As part of the TRE, GHD also conducted additional chronic toxicity bioassay testing to independently ascertain if toxicity was present by using a different laboratory from the one contracted to perform DVI's testing, and to attempt to narrow and identify the causes or sources of the toxicity. All tests were conducted by Sierra Foothill Laboratory (SFL) and are summarized in the Table 3 below:

Table 3 – Chronic Bioassay Test Results conducted by GHD/SFL 2012

Date of Sample	Type of Sample	Sample	Diluent	Result as TUc
18 October 2012	Grab	Effluent EFF-001	Receiving Water	1.00
18 October 2012	Grab	Effluent EFF-001	Lab Water	1.00
18 October 2012	Grab	Receiving Water	Lab Water	1.00
25 October 2012	24hr Composite	Effluent EFF-001	Lab Water	1.00
25 October 2012	Grab	Effluent EFF-001	Lab Water	1.00

Note that grab samples were conducted in most cases primarily for ease of sample gathering and simplification in test result interpretation. They were not intended to be used in place of quarterly or accelerated monitoring tests which require a 24 hour composite sample as stated in DVI's discharge permit. The one exception was one composite sample collected on 25 October which was compared to a grab sample collected. The purpose of this particular test comparison was to determine if there might be daily variability in the toxicity.

All test results related to the effluent samples being tested indicated a TUc of 1 indicating the absence of toxicity. While the TUc for the receiving water sample diluted with laboratory control water was reported as 1, this specific test showed characteristics of stimulatory effects as reported in Section 2.2. Also of note was that SFL reported that for the two effluent samples collected on 18 October, initial test results reported a TUc=1.33. However, believing that sticking of the algae to the walls of the test container was a likely cause, SFL conducted a second reading after re-suspension which resulted in a TUc=1. The results for the above five bioassays are included in the Appendix.

3.4 TRE Exit Bioassay Testing Results

As part of the TRE, GHD conducted four final Chronic Bioassay tests in an effort to permit DVI to exit the TRE. Samples for the four tests were collected while the RO plant was operational to exclude any effects of elevated electro-conductivity. Sampling was conducted on 17 January, 29 January, 31 January, and 6 February 2013. Each Effluent (EFF-01) sample was a 24 hour composite sample

diluted with laboratory control water. All test results related to the effluent samples showed a TUc of 1, indicating the absence of toxicity. Initial test results from effluent samples collected 29 January and 6 February, showed a TUc of 2 and 1.3 respectively, however SFL conducted a second reading after re-suspension which resulted in a TUc=1. Laboratory test reports for the four bioassays are included in the Appendix.

Concurrent with the EFF-001 sampling, GHD also collected samples of the receiving water RSW-001 on 17 January, 29 January, 31 January, and 6 February 2013 as required by DVI's permit. RSW-001 was subsequently used as a test control in addition to a laboratory prepared control. However, the receiving water was purposely not used as the diluent due to the aforementioned concern of it possibly giving false positive test results. In fact, during sample collection on two of the four testing occasions at the time of sample collection helicopters were observed conducting aerial spraying of the agricultural fields in close proximity to the RSW sampling point.

All tests for exiting the TRE were conducted by Sierra Foothill Laboratory (SFL) and are summarized in Table 4 below. The results for the four bioassays are included in the Appendix.

Table 4 – Chronic Selenastrum Bioassay Test Results for EFF-001

Date of Sample	Type of Sample	Diluent	Type of Test	Result as TUc
17 January 2013	24hr Composite	Lab Water	TRE Monitoring	1.00
29 January 2013	24hr Composite	Lab Water	TRE Monitoring	$1.00_{(1)}$
31 January 2013	24hr Composite	Lab Water	TRE Monitoring	1.00
2 February 2013	24hr Composite	Lab Water	TRE Monitoring	$1.00_{(2)}$

Notes:
1. – Initial TUc was reported as 2. re-suspension step was conducted which lowered the TUc to 1.
2. – Initial TUc was reported as 1.3. re-suspension step was conducted which lowered the TUc to 1.

4. Conclusions and Recommendations

Based on a review of the chronic bioassay test results and WWTP performance from January through October it is highly probable that the source of the toxicity is founded in two specific findings – laboratory controls and procedures and RO plant operational status. Based on these findings GHD recommends the following:

Laboratory Procedures:

1. Per the institution's WDR, specifically Section V.B.7 in Attachment E (MRP), DVI is to use the receiving water control as the diluent unless the receiving water is impaired. With the receiving water known to be stimulatory, likely due to the fact that the receiving water is an agricultural drain and is subject to agricultural run-off, DVI is permitted to use laboratory prepared control water as the diluent instead of the receiving water. Regardless of whether or not the receiving water is toxic/stimulatory, DVI's testing laboratory should be specifically

directed to include test control data from the laboratory prepared control water in addition to the receiving water control when reporting test results to the institution.

2. While not specifically related in this TRE analysis, per the same section identified above (Section V.B.7 in Attachment E) DVI is not required to perform the test using a 5 dilution series and may be performed using 100% effluent for regular quarterly monitoring. Once this TRE has been exited and accelerated and/or TRE monitoring has concluded, DVI staff should instruct the laboratory to conduct the chronic toxicity bioassays using 100% effluent, 100% receiving water, and 100% laboratory control water instead of a 5 dilution series bioassay test. This will cut down on the number of test replicates and therefore cut down on the cost to conduct the test.
3. Finally, should a test result indicate toxicity of the effluent and the TUc for the effluent is between 1.33 and 2, DVI should instruct the laboratory to perform a re-suspension step to see if algal sticking is resulting in a false positive for toxicity.

Plant Performance:

1. When collecting samples for chronic toxicity testing, DVI WWTP staff should first identify and verify the operational status of the RO water plant. If found to be off line for maintenance, staff should postpone sampling until the unit is back online. However, if a sample must be collected for either regular quarterly monitoring or accelerated and/or TRE monitoring DVI staff should note this information when reporting the results, particularly if the results indicate toxicity of the effluent.

With the likely sources of toxicity identified and successful accelerated monitoring with four consecutive passing chronic toxicity bioassays completed by DVI it is recommended that DVI be permitted to exit the TRE.

Farhad, Mohammad@Waterboards

From: Farhad, Mohammad@Waterboards
Sent: Thursday, December 18, 2014 11:54 AM
To: CentralValleySacramento
Subject: FW: Deuel Vocation Institute - TRE
Attachments: Water Board Response Letter from the Warden - 12-17-14.pdf; DVI TRE Action Plan Final 121714.pdf

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From: Pedro Reyes [mailto:pedro.reyes@cdcr.ca.gov]
Sent: Thursday, December 18, 2014 11:39 AM
To: Farhad, Mohammad@Waterboards
Cc: Stanley, Jeff@CDCR; Bettencourt, Miles (Terry)@CDCR; Engleheart, Robert@CDCR; Vasconcellos, Edward@CDCR
Subject: Deuel Vocation Institute - TRE

Good Morning Mr. Farhad,

On behalf of the California Department of Corrections and Rehabilitation, Facilities Management and the Deuel Vocation Institute, attached please find a PDF file containing the TRE as well as a signed letter from Warden Price. The originals will be dropped-off at the Central Valley Regional Water Board late this afternoon or early tomorrow morning. Please let me know if have any questions regarding this email.

Thanks,

Pedro B. Reyes
California Department of Corrections & Rehabilitation
Departmental Construction & Maintenance Supervisor
Facilities Planning, Construction, and Management
Facilities Asset Management Branch
Phone: (916) 255-0516
Fax: (916) 255-3022

DIVISION OF ADULT INSTITUTIONS
DEUEL VOCATIONAL INSTITUTION
P.O. Box 400
Tracy, CA 95378-0004

December 17, 2014

Mr. Mohammad Farhad
Water Resources Control Engineer
NPDES Compliance and Enforcement Unit
Central Valley Regional Water Quality Control Board
11020 Sun Center Dr., Suite 200
Rancho Cordova, CA 95670

Dear Mr. Farhad:

The California Department of Corrections and Rehabilitation is submitting this letter with the attached Toxicity Reduction Evaluation action plan for *Ceriodaphnia Dubia* requirement of the Self-Monitoring Report Review and Notice of Violation, Deuel Vocational Institution, dated November 20, 2014.

If you have any questions or should you need any further information, please contact Miles "Terry" Bettencourt, Correctional Plant Manager II, at (209) 830-3932.

Sincerely,

J. PRICE
Warden (A)
Deuel Vocational Institution
California Department of Corrections and Rehabilitation

Attachment

California Department of Corrections and Rehabilitation

DEUEL VOCATIONAL INSTITUTION

NPDES PERMITTING SUPPORT

(NPDES NO. CA0078093)

TOXICITY REDUCTION EVALUATION ACTION PLAN

FINAL

17 December 2014

DEUEL VOCATIONAL INSTITUTION
NPDES PERMITTING SUPPORT
(NPDES NO. CA0078093)

TOXICITY REDUCTION EVALUATION ACTION PLAN

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1.0 INTRODUCTION

1.1 Summary

The California Department of Corrections and Rehabilitation (CDCR) owns and operates the Wastewater Treatment Facility (WWTF) for the Deuel Vocational Institution (DVI), a California prison facility. DVI is located east of Tracy, CA, in the southwest portion of San Joaquin County. The WWTF currently provides sewerage services for a population of roughly 3,200 persons at one time (POAT), including approximately 2,600 inmates and three separate shifts of prison staff. The WWTF operates under the terms of the Waste Discharge Requirements (WDRs) set forth in a permit (Order R5-2014-0014) issued by the Central Valley Regional Water Quality Control Board (Regional Board). The WDRs also serve as the terms of the federal National Pollutant Discharge Elimination System (NPDES) permit for the DVI facility (NPDES No. CA0078093).

Under the terms of Order No. R5-2014-0014, CDCR is required to conduct regular chronic Whole Effluent Toxicity (WET) monitoring on a quarterly basis for the effluent discharged by the WWTF at Discharge Point No. 001. The WDRs require the discharger to "investigate the causes of, and identify corrective actions to reduce or eliminate effluent toxicity." If the numeric toxicity monitoring trigger is exceeded during regular chronic toxicity monitoring, the discharger "shall initiate accelerated monitoring," which consists of four chronic test conducted every two weeks using the species that exhibited the toxicity. If the results of the accelerated monitoring do not exceed the monitoring trigger, the discharger can stop the accelerated monitoring and return to regular chronic toxicity monitoring.

However, if the discharge exceeds the numeric toxicity monitoring triggers during accelerated monitoring, the discharger is required to cease accelerated monitoring and initiate a Toxicity Reduction Evaluation (TRE) to "investigate the cause(s) of, and identify corrective actions to reduce or eliminate effluent toxicity."

Furthermore, the discharger "shall submit a TRE Action Plan to the Central Valley Water Board including, at minimum:

1. Specific actions the Discharger will take to investigate and identify the cause(s) of toxicity, including a TRE WET monitoring schedule;

2. Specific actions the Discharger will take to mitigate the impact of the discharge and prevent the recurrence of toxicity; and

3. A schedule for these actions."

This TRE Action Plan is designed to address the requirements referenced above.

DEUEL VOCATIONAL INSTITUTION FACILITY LAYOUT

FIGURE 1.1

DEUEL VOCATIONAL INSTITUTION

TOXICITY REDUCTION EVALUATION ACTION PLAN

Dewberry

1.2 Background

In September of 2010, CDCR completed construction of upgrades to their tertiary treatment WWTF and began discharging from the upgraded plant. The upgraded plant utilizes a membrane bioreactor (MBR) process to comply with the effluent limits set forth by Order No. R5-2014-0014. The facility permitted average dry weather flow (ADWF) for the WWTF is 0.62 million gallons per day (mgd) and the facility design flow is 0.70 mgd. Observed average dry weather flows are typically in the range of 0.45 to 0.5 mgd.

The upgraded WWTF system includes preliminary treatment comprised of coarse screening, influent pumping, fine screening, and vortex grit removal. The secondary process includes: biological treatment using anoxic and aeration basins for nitrification and denitrification; four MBR filtration tanks; inline ultraviolet (UV) disinfection; mechanical dewatering using two belt presses, and effluent cooling towers. During the upgrade construction, the WWTF was raised to protect the plant from flooding during a 100-year flood event.

The treated wastewater from the DVI WWTF is discharged at Discharge Point No. 001 to Deuel Drain. Deuel Drain discharges to Paradise Cut, a slough that is a tributary to the San Joaquin River. DVI also discharges industrial storm water from Discharge Point No. 003 to Deuel Drain. DVI's industrial storm water is commingled with groundwater from Discharge Point No. 004 and diverted to effluent holding ponds, where is it retained on DVI property. The geographic location of the DVI facility and discharge sites are shown in Figure 1.1.

1.3 Toxicity Monitoring Provisions

Under the terms of Order No. R5-2014-0014, CDCR is required to conduct regular chronic Whole Effluent Toxicity (WET) monitoring on a quarterly basis for the effluent discharged by the WWTF at Discharge Point No. 001. The numeric toxicity trigger has been defined in Special Provision VI.2.a.iv of the NPDES permit to be 1 TUc (where TUc = 100/NOEC). According to the permit, if the numeric toxicity monitoring trigger is exceeded during regular chronic toxicity monitoring, CDCR "*shall initiate accelerated monitoring*," which consists of four chronic tests conducted every two weeks using the species that exhibited the toxicity. If the results of the accelerated monitoring do not exceed the monitoring trigger, the discharger can stop the accelerated monitoring and return to regular chronic toxicity monitoring.

In addition, if the source(s) of the toxicity is easy to identify (i.e., obvious disruptions in normal plant operations), the discharger can make the "*necessary corrections to the facility*," and continue accelerated monitoring until four consecutive accelerated tests do not meet the monitoring trigger. Upon confirmation that the effluent toxicity has been removed, the discharger may "*cease accelerated monitoring and resume regular chronic toxicity monitoring*."

However, if the discharge exceeds the numeric toxicity monitoring triggers during accelerated monitoring, the discharger is required to cease accelerated monitoring and initiate a Toxicity Reduction Evaluation (TRE) to "*investigate the cause(s) of, and identify corrective actions to reduce or eliminate effluent toxicity*."

1.4 Rationale for TRE Action Plan

A communication to DVI from the Central Valley Regional Water Quality Control Board engineer dated 20 November 2014, noted that Regional Board staff had reviewed the electronic self-monitoring reports (eSMRs) submitted by CDCR for the April 2014, May 2014, June 2014, July 2014, August 2014, September 2014, Second Quarter 2014, and Third Quarter 2014 monitoring periods.

Furthermore, the 20 November 2014 communication included the following statement:

> "*In October 2013, the Discharger's laboratory reported that during the fourth quarter 2013 chronic toxicity test, Ceriodaphnia dubia exhibited higher mortality due to the parent organism not being healthy. The Discharger's laboratory changed its suppliers and the Ceriodaphnia dubia toxicity testing was rescheduled for December 2013. However, the sample exceeded the chronic toxicity trigger again on 19 December 2013. Samples were collected for accelerated monitoring on 6 January 2014, 21 January 2014, 3 February 2014, and 18 February 2014 with reported results of >1 TUc, 1.3 TUc, 1 TUc, and 1 TUc, respectively. In addition, the Discharger exceeded the chronic toxicity trigger for Ceriodaphnia dubia on 28 July 2014 with a reported result of 1.3 TUc*."

Provision VI.C.2.a. of Permit No. R5-2014-0014 states:

> *"If the discharge exceeds the numeric toxicity monitoring trigger during accelerated monitoring established in this Provision, the Discharger is required to initiate a TRE in accordance with an approved TRE Work Plan, and take actions to mitigate the impact of the discharge and prevent recurrence of toxicity."*

Provision VI. C.2.a.iii.c. of Permit No. R5-2014-0014 states:

> "*If the results of any accelerated toxicity test exceeds the monitoring trigger, the Discharger shall cease accelerated monitoring and begin a TRE to investigate the cause(s) of, and identify corrective actions to reduce or eliminate effluent toxicity. Within thirty (30) days of notification by the laboratory of any test result exceeding the monitoring trigger during accelerated monitoring, the Discharger shall submit a TRE Action Plan to the Central Valley Water Board including, at minimum:*
>
> 1. *Specific actions the Discharger will take to investigate and identify the cause(s) of toxicity, including a TRE WET monitoring schedule;*
> 2. *Specific actions the Discharger will take to mitigate the impact of the discharge and prevent the recurrence of toxicity; and*
> 3. *A schedule for these actions*."

The 20 November 2014 communication directed the CDCR to submit a TRE Action Plan consistent with Provision VI.C.2.a.iii.c (as noted above) by 19 December 2014. This TRE Action Plan is designed to address this requirement.

1.5 TRE Objectives

The objectives for a TRE, as defined by the EPA Guidance Document, are:

- *Evaluate the operation and performance of the Publicly Owned Treatment Works (POTW) to identify and correct treatment deficiencies contributing to effluent toxicity (e.g., operations problems, chemical additives, or incomplete treatment);*
- *Identify the compounds causing effluent toxicity;*
- *Trace the effluent toxicants and/or toxicity to their sources (e.g., industrial, commercial, or domestic); and*
- *Evaluate, select, and implement toxicity reduction methods or technologies to control effluent toxicity (i.e., in-plant or pretreatment control options).*

In accordance with the U.S. EPA guidance for Toxicity Reduction Evaluations, the following steps will be utilized to achieve the aforementioned objectives:

- *Validation of Bioassay Results;*
- *Information and Data Acquisition;*
- *Facility Performance Evaluation;*
- *Toxicity Identification Evaluation (TIE);*
- *Toxicity Source Evaluation (TSE);*
- *Toxicity Control Evaluation (TCE); and*
- *Toxicity Control Implementation (TCI).*

2.0 TRE ACTION PLAN COMPONENTS

The intent of this TRE Action Plan is to provide a description of the investigation and evaluation techniques that will be utilized to achieve these steps and the overall TRE objectives, as directed in the 20 November 2014 communication. This will include:

1. *Specific actions the Discharger will take to investigate and identify the cause(s) of toxicity, including a TRE WET monitoring schedule;*
2. *Specific actions the Discharger will take to mitigate the impact of the discharge and prevent the recurrence of toxicity; and*
3. *A schedule for these actions*.

The different sections of the TRE Action Plan reflect the aforementioned steps. The sequential progression of the steps is demonstrated in Figure 2.1, and the steps are discussed in detail in the following sections. The proposed completion schedule is shown in Table 3.1.

2.1 Validation of Bioassay Results

Since the TRE is based on valid bioassay results, the first step of the TRE process will be to verify the validity of the effluent toxicity results. In conjunction with Task 2.2- Information and Data Acquisition, CDCR will review the chronic toxicity results for:

- *Accuracy, variability, consistency and testing conditions;*
- *Procedures for sample collection, transport, and analysis by DVI's contracted lab; and*
- *Proper QA/QC measures.*

First, the procedures for sample collection, sample transport, and analysis by the lab will be reviewed to verify that the proper QA/QC measures were followed. Bioassay results will then be reviewed to confirm that all test acceptability criteria were met. It is important to note that certain test conditions, such as pH, can artificially change due to the testing procedure and potentially increase the toxicity of the sample. Test conditions will be reviewed to verify that toxicity was not erroneously amplified. Historic toxicity data will also be utilized to evaluate the variability and accuracy of the historic and toxicity related WET testing. Finally, validation of the bioassay results will include 10 chronic bioassay for *Ceriodaphnia dubia* through an independent lab for confirmation of toxicity and a results comparison.

2.2 Information and Data Acquisition

In order to conduct a comprehensive investigation into effluent toxicity, it is critical to have all available treatment facility operation and performance data for review. Following are the sources of information that will be reviewed as necessary (and available) for the TRE:

- *Facility design criteria;*
- *Construction drawings and specifications;*
- *List of chemicals used in the facility and their MSDS sheets;*
- *Acute and chronic WET test lab reports;*
- *Self-monitoring reports;*
- *SCADA (supervisory control and data acquisition) files;*
- *Operations, training, and maintenance logs; and*
- *Discussions with facility operators.*

During the site visit, a cursory review indicated that the equipment used at the plant was observed to be in good condition and appropriate for the permitted use and flow capacity.

2.3 Facility Performance Evaluation

All collected facility performance data and operation data will be evaluated for any causal relationships to effluent toxicity. The following sections outline the sequential evaluations that will be performed for this purpose.

2.3.1 Treatment Deficiencies

All available effluent water quality data for the period in question will be analyzed for variability in an effort to identify any deficiencies in treatment that could lead to possible sources of toxicity. Specifically, profiles of effluent water quality data will be evaluated against toxicity testing to determine if any deviations from permit limits were related temporally to toxicity. Further, profiles of the toxicity related effluent data will be compared to historic effluent data to identify deviations in treatment efficacy that could cause toxicity. Alkalinity, pH, hardness, and conductivity are important characteristics that can affect the toxicity of certain compounds. As such, plant data for the period in question will be reviewed to determine if any changes were made to these parameters that may have contributed to toxicity. Influent monitoring data will also be evaluated to pinpoint any irregular concentrations of recalcitrant contaminants that could be correlated to toxicity.

Facility operation and performance for the period in question will also be evaluated against the design criteria. Each major process in the wastewater treatment facility will be evaluated independently for any deviations from the design treatment efficacy. In addition, loads to each process unit will be evaluated to identify any that may be in exceedance of the design capacity. Similar to the effluent water quality analysis, the performance data collected when toxicity was present will be compared against historic facility performance.

2.3.2 In-House Sources

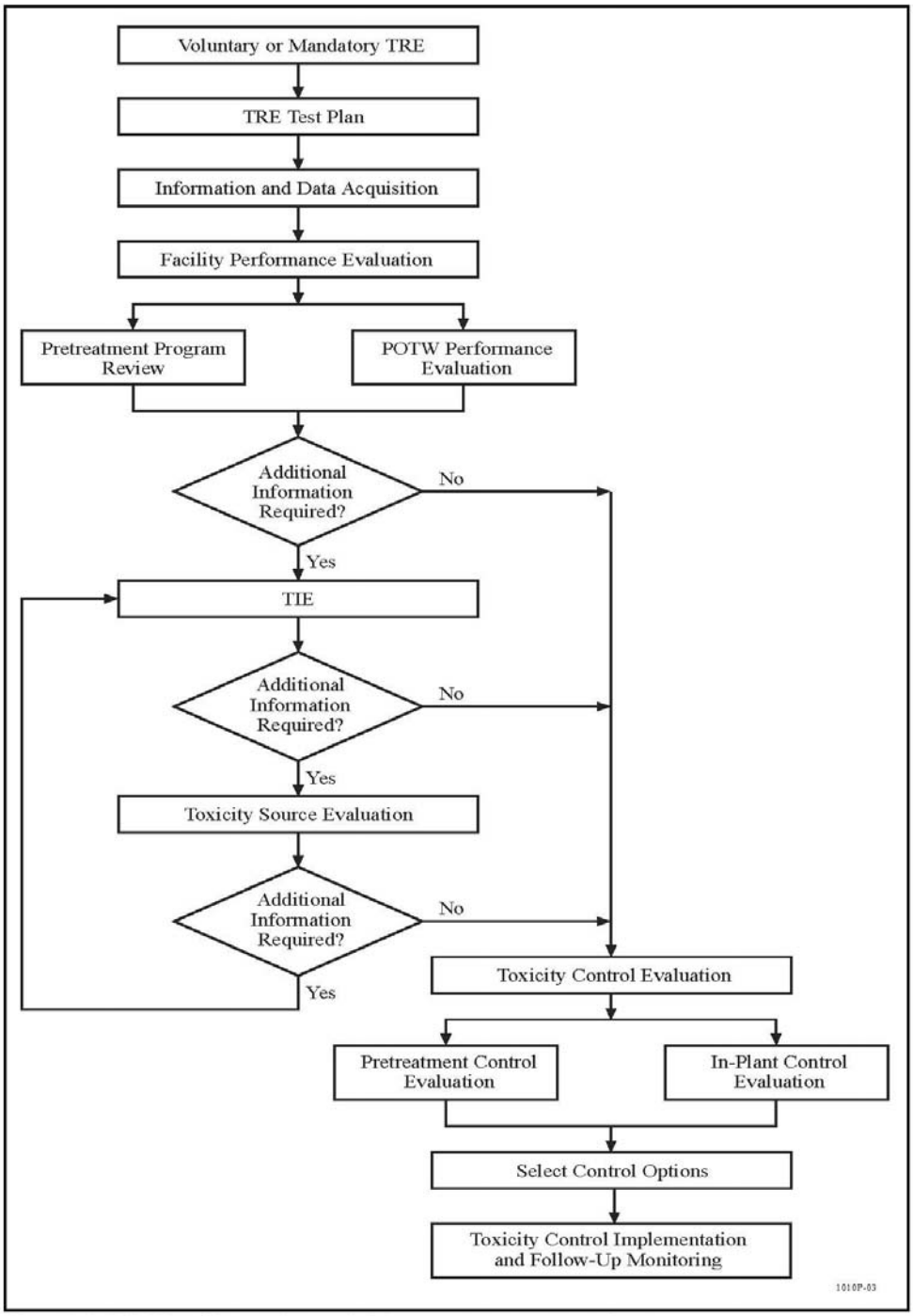
The facility operation data will also be evaluated to identify any potential in-plant sources of toxicity, such as high chemical doses. An inventory of all chemicals stored on-site will be conducted in order to determine if any were erroneously utilized. Chemicals that may be used at the WWTF include (but are not limited to): sodium hypochlorite, citric acid, and polymer. The facility operation data will also be reviewed to identify any potential housekeeping sources of toxicity, such as high or low chemical doses, or low dilution factors. Other sources of chemicals (such as authorized sources of non-storm water discharges), could include: rising groundwater, air conditioning condensate, potable water line flushing, landscape irrigation water, fire hydrant flushing, fire suppression water runoff, building rain gutter runoff and herbicides for weed abatement.

2.3.3 Treatment Modifications

If either a treatment deficiency or an in-house toxicity source is identified, necessary modifications to improve treatment efficacy and eliminate toxicity will be evaluated and implemented before proceeding further in the TRE.

2.3.4 Toxicity Reduction Evaluation Report

Following the above actions, the actual toxicity reduction achieved by the correction of the treatment deficiencies or in-house toxicity sources will be evaluated and documented in a technical report. Upon completion of these studies and subsequent evaluations, the RWQCB will be notified of the results and intended actions.



Courtesy of United States Environmental Protection Agency; "Toxicity Reduction Evaluation Guidance for Municipal Wastewater Treatment Plants."

TRE PROCESS

FIGURE 2.1

DEUEL VOCATIONAL INSTITUTION
TOXICITY REDUCTION EVALUATION ACTION PLAN

2.4 Toxicity Identification Evaluation (if necessary)

2.4.1 Components of a Toxicity Identification Evaluation

The Toxicity Identification Evaluation (TIE) procedure is used to determine the causes of acute and chronic toxicity that cannot be not directly attributable to treatment deficiencies or in-house sources and, as a result, additional investigations are required (Figure 2.1). If deemed necessary, the TIE will be comprised of three phases: (1) toxicant characterization; (2) toxicant identification; and (3) toxicant confirmation. If the TIE is not required, the process will flow directly to the Toxicity Control Evaluation (TCE)

The TIE will utilize toxicity testing to measure changes in the degree of toxicity as an effluent sample is manipulated to eliminate, isolate, or enhance specific classes of constituents (e.g. filterable, organics, metals, etc.). In Phase I, the physical/chemical characteristics of the toxicants will be determined by the manipulations that reduce the toxicity relative to the original effluent sample (EPA 1991a).

Once the physical/chemical properties of the suspect toxicants have been identified, Phase II will be initiated to determine the specific compounds in the effluent sample that contribute to toxicity. For Phase II, the TIE procedure will be utilized with chemical-specific manipulations that separate and concentrate toxicants in order to identify the causative agents (EPA 1993a).

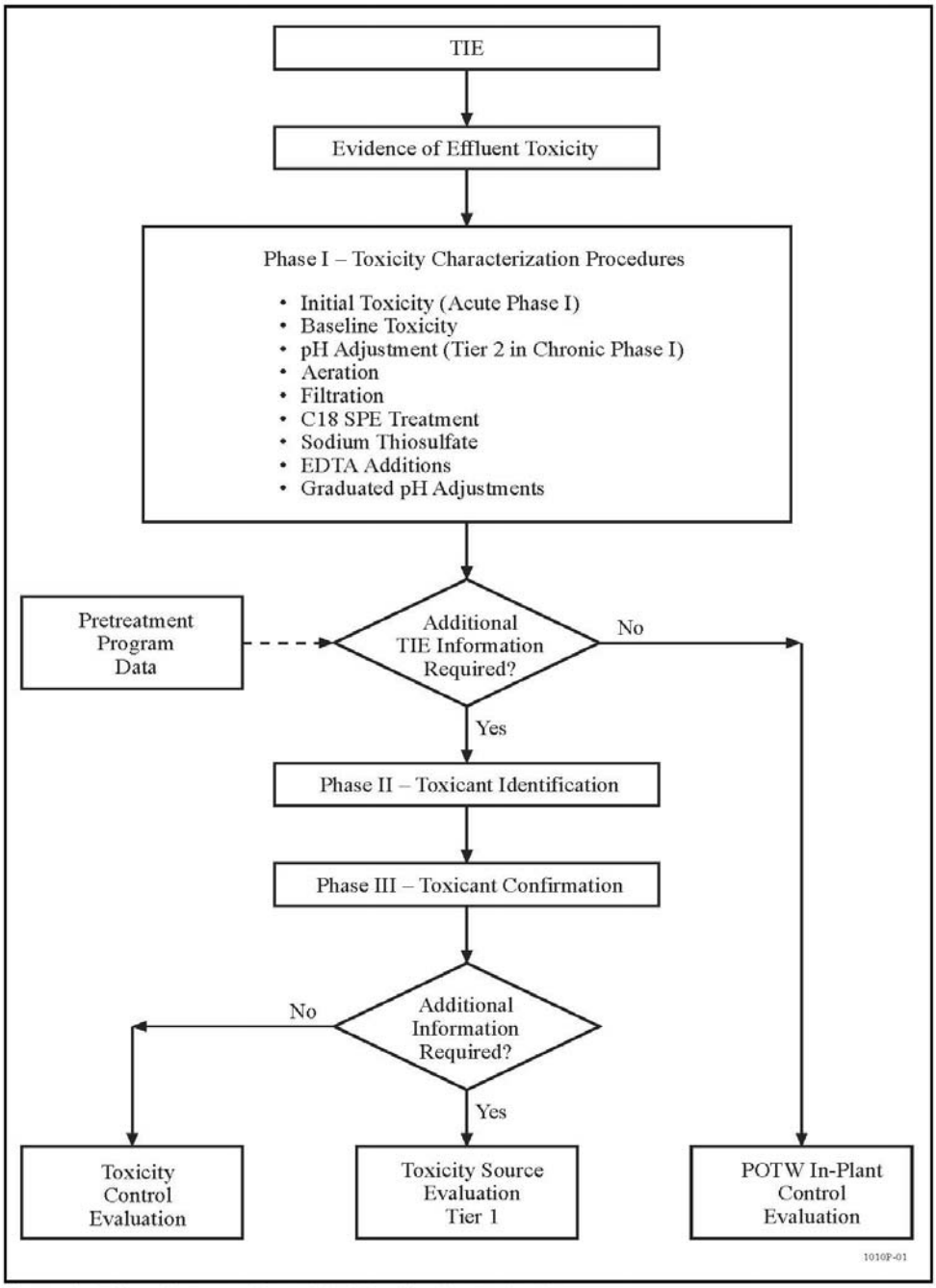
The EPA guidance document for the Phase III toxicity confirmation procedures specifies that the toxicants can be confirmed using correlations between the concentration of the contaminant and the toxicity, mass balances, spiking test samples with the toxicants, test organism symptoms, and species sensitivity (EPA 1993b). Many of the confirmation procedures are conducted during Phase I and II, and the results obtained during those testing phases will be utilized to confirm the suspect toxicants.

2.4.2 Guidance for Toxicity Identification Evaluation Treatments

If a TIE is required, the EPA TIE guidance documents for Phases I, II, and III will serve as the procedural foundation (Figure 2.2) for the TIE and subsequent advanced steps (USEPA 1991, 1993a, 1993b). In addition, CDCR may also rely on the procedures of the contracted bioassay laboratory. In the event that a TIE is required, CDCR will utilize an outside consultant to manage the TIE and the related bioassay work. The consultants will be identified in the TRE action plan that is required for Provision VI.C.2.a.iv.c of the NPDES permit.

In accordance with the EPA guidance documents (EPA 1999), the following modifications may be made to the TIE tests in order to streamline the process:

- *Reduced test volumes;*
- *Shortened test duration; and*
- *Utilize fewer replicates.*



Courtesy of United States Environmental Protection Agency; "Toxicity Reduction Evaluation Guidance for Municipal Wastewater Treatment Plants."

TIE PHASES (IF REQUIRED)

FIGURE 2.2

DEUEL VOCATIONAL INSTITUTION
TOXICITY REDUCTION EVALUATION ACTION PLAN

2.5 Toxicity Source Evaluation (if necessary)

According to the EPA, the goal of the Toxicity Source Evaluation (TSE) is to evaluate the discharges from either direct or indirect sources to determine the source of the toxicant(s) that have been identified through the TIE process as the cause of the effluent toxicity. However, due to the fact that the influent to the DVI wastewater treatment facility is from a single known source, this step will not be necessary and will not be incorporated into the TRE.

2.6 Toxicity Control Evaluation (if necessary)

The goal of the Toxicity Control Evaluation (TCE) is to determine and implement the optimal control strategies to reduce the toxicity to an acceptable level below the permit limit. Criteria that will be used to determine the optimal control options will include (EPA 1999):

- *Efficacy in producing an effluent with a toxicity below the permit limits;*
- *Effect on other parameters and subsequent permit limits;*
- *Capital, operational, and maintenance costs;*
- *Implementation concerns; and*
- *Reliability.*

Because there are no industrial sources, pretreatment will not be analyzed and only in-house control options will be evaluated. Treatability studies may be used to assess whether treatment process optimization or additional treatment processes would more effectively achieve in-house control of toxicity. This information will be included in the Toxicity Reduction Evaluation Reporting if a TIE is not required.

2.7 Toxicity Control Implementation

Once appropriate toxicity control options have been selected either through the TRE Reporting or the TCE process, CDCR will implement the optimal control option and develop a monitoring schedule to confirm the anticipated toxicity reduction. CDCR will consult with the RWQCB about the intended monitoring schedule.

As described in sub-task 2.3.4, if the source of the toxicity has been reasonably identified and the toxicity has been adequately reduced (either through the TRE Reporting or TCE processes), CDCR will conduct the laboratory testing necessary to exit (i.e., four accelerated monitoring tests with results indicating no toxicity), document the findings in the Toxicity Reduction Evaluation Report. CDCR will submit this report to the RWQCB for a recommendation that DVI be permitted to exit the TRE.

If the toxicity has not been adequately reduced following these steps, additional investigations may be required, including investigating sources of potential intermittent or ephemeral toxicity.

3.0 SCHEDULE

This TRE Action Plan is being developed in accordance with Provision VI.C.2.a.iv.c of the NPDES permit. After initiation of the TRE, the RWQCB will be apprised of the ongoing progress and results of each major step throughout the duration of the TRE. The TRE will be conducted sequentially and upon obtaining results that enable identification and reduction of the toxicity, the results will be included in the Toxicity Reduction Evaluation Report. Upon acceptance of the report, the TRE will be terminated with approval from the RWQCB. Table 3.1, below, outlines the proposed schedule for completion of the TRE.

Table 3.1 Proposed Completion Schedule for DVI TRE Action Plan

Task	Interaction With Regional Board	Proposed Schedule
TRE Initiation, including Site Visit and Kick-Off Meeting	N/A	10 December 2014
Validation of Bioassay Results	As necessary and to share findings upon completion.	31 January 2015
Information and Data Acquisition	As necessary and to share findings upon completion.	31 March 2015.
Facility Performance Evaluation	As necessary and to share findings upon completion.	31 March 2015. Termination could depend on treatability testing.
Toxicity Reduction Evaluation Reporting and Exit	As necessary and to share findings upon completion.	31 March 2015. Termination could depend on treatability testing.
Toxicity Identification Evaluation	As necessary and to share findings upon completion.	Initiation dependent on results of prior step. Time scale for completion based on findings.
Toxicity Source Evaluation	As necessary and to share findings upon completion.	Initiation dependent on results of prior step. Time scale for completion based on findings.
Toxicity Control Evaluation	As necessary and to share findings upon completion.	Initiation dependent on results of prior step. Time scale for completion based on findings.
Toxicity Control Implementation	As necessary and to share findings upon completion.	Time scale dependent on control option.
TRE Conclusion	Consulted regarding acceptability of TRE termination.	Can occur at any point in TRE.

4.0 CONCLUSION

This document provides a TRE Action Plan that fulfills the associated requirements in Order No. R5-2008-0164. The major steps for the TRE have been identified as: (1) Validation of Bioassay Results, (2) Information and Data Acquisition, (3) Facility Performance Evaluation, (4) Toxicity Identification Evaluation (TIE), (5) Toxicity Source Evaluation (TSE), (6) Toxicity Control Evaluation (TCE), and (7) Toxicity Control Implementation (TCI). It is important to note that Steps (4) and (5) may not be necessary, and steps (6) and (7) may be included in the Toxicity Reduction Evaluation (TRE) Reporting.

Implementation of this TRE Action Plan is designed to achieve the overall TRE objectives in the timeframe identified in the schedule, including:

- *Identification of the cause(s) of toxicity; and*
- *Measures to mitigate the impact of the discharge and prevent recurrence of toxicity.*

5.0 REFERENCES

1. *Order No. R5-2014-0014, Waste Discharge Requirements for the California Department of Corrections and Rehabilitation Deuel Vocational Institution, San Joaquin County*. California Regional Water Quality Control Board- Central Valley Region. 7 February 2014.

2. *Self-Monitoring Report Review and Notice of Violation, California Department of Corrections, Deuel Vocational Institution, San Joaquin County*. Central Valley Regional Water Quality Control Board. 20 November 2014.

3. *Deuel Vocational Institution, NPDES Permitting Support, Toxicity Reduction Evaluation Work Plan (Final)*. July 2009.

4. *Toxicity Reduction Evaluation Guidance for Municipal Wastewater Treatment Plants*. EPA 833-B-99-002. Office of Wastewater Management. Washington DC. August 1999.

5. *Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures*. Second Edition. EPA 600/6-91/005F. February 1991a.

6. *Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity*. Second Edition. EPA 600/R-92/080. September 1993a.

7. *Methods for Aquatic Toxicity Identification Evaluations: Phase III Toxicity Confirmation Procedures for Samples Exhibiting Acute and Chronic Toxicity*. Second Edition. EPA 600/R-92/081. September 1993b.

8. *Technical Support Document for Water-Quality Based Toxics Control*. EPA 505-2-90-001. Office of Wastewater Management. Washington D.C. March 1991b.

Farhad, Mohammad@Waterboards

From: Daniel Mullins <daniel.mullins@cdcr.ca.gov>
Sent: Monday, April 13, 2015 1:24 PM
To: CentralValleySacramento
Cc: Farhad, Mohammad@Waterboards; Holmes, Kari@Waterboards; Bettencourt, Miles (Terry)@CDCR; Stanley, Jeff@CDCR
Subject: FINAL TRE Tech Memo 4-13-2015
Attachments: FINAL TRE Tech Memo 4-13-2015.pdf

Attached is the Final TRE
If you have any questions feel free to call
Thank You

NPDES #CA0078093
Facility ID 5B390100001

Daniel G Mullins
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Fax-(209) 830-3941

Dewberry

MEMORANDUM

Date: April 13, 2015
To: Jeff Stanley, CDCR
From: Dewberry
Subject: Deuel Vocational Institution
Toxicity Reduction Evaluation

EXECUTIVE SUMMARY

The Deuel Vocational Institution (DVI) wastewater treatment facility (WWTF), NPDES No. CA0078093, exceeded the chronic toxicity limit for *Ceriodaphnia dubia* with a TUc > 1 in December 2013. DVI conducted four accelerated monitoring tests of chronic toxicity during January and February 2014. The first two accelerated monitoring samples exceeded the chronic toxicity limit. The second two accelerated monitoring tests did not exhibit chronic toxicity and thereafter DVI resumed quarterly whole effluent toxicity (WET) testing.

As required by a Notice of Violation (NOV) in November 2014, DVI submitted a Toxicity Reduction Evaluation (TRE) Action Plan in December 2014 and subsequently conducted this TRE to identify causes of chronic toxicity and identify corrective actions to reduce or eliminate effluent toxicity. All permit violations that occurred during 2014 at discharge locations EFF-001 and RSW-002 were evaluated. Probable and potential causes of these violations were identified as summarized in Table ES-1

Table ES-1 2015 TRE Results

Item	Location	Parameter	Violations, number	Probable Source	Potential Source
1	EFF-001	Chronic toxicity	3	High effluent TDS	High HCO3 dose
2	EFF-001	Nitrate + nitrite	3	Hydraulic loading	Anoxic mixer failure
3	RSW-002	pH rise	2	Interpretation error	Algae in stream
4	RSW-002	Temperature rise	5	Low cooling capacity	Transformer failure

Chronic toxicity occurs due to high TDS concentrations in the WWTF effluent. High TDS concentrations (from the brackish groundwater source) discharge from the WWTF when the reverse osmosis (RO) and zero liquid discharge (ZLD) systems which supply drinking water are out of service. Specific recommendations to maintain reliable operation of the RO-ZLD system will be developed in a separate report.

At the WWTF, permit violations have been attributed to wear of the membrane filter modules, limited effluent cooling capacity, and mechanical and electrical equipment failures. To improve reliability, CDCR will replace the membrane filter modules and modify the effluent cooling towers. CDCR will make every effort to keep both the RO-ZLD system and the WWTF in operation.

INTRODUCTION

The CVRWQCB issued a Notice of Violation (NOV) on November 20, 2014 which required CDCR to initiate a toxicity reduction evaluation (TRE) for the Deuel Vocational Institution (DVI) and submit a TRE Action Plan to the CVRWQCB by December 19, 2014. CDCR submitted the required TRE Action Plan on December 17, 2014 in compliance with the NOV.

Prior to July 19, 2013, discharges of industrial storm water from Discharge Points EFF-003 and EFF-004 to the Deuel Drain were regulated under the NPDES permit for the WWTF. These storm water discharge points are currently regulated under the general permit for municipal separate storm sewer systems (MS4s). Violations of the industrial storm water permit (Order 2013-0001-DWQ) for EFF-003 and EFF-004 are not included in this review of the WWTF.

This report presents a review of operation and performance of the DVI wastewater treatment facility, identifies probable and potential causes of the permit violations to the extent possible, and describes corrective actions to reduce or eliminate violations of permit limits.

REVIEW OF 2014 SELF-MONITORING REPORTS

The Central Valley Regional Water Quality Control Board (CVRWQCB) reviewed the electronic self-monitoring reports (eSMRs) submitted by CDCR for the DVI WWTF for each month from April through September 2014 and the Second and Third Quarter 2014 monitoring reports. As summarized in Table 1, CVRWQCB identified several violations of the effluent limitations including effluent nitrate concentrations, excessive surface water temperature increase downstream of the effluent discharge point, and chronic toxicity for *Ceriodaphnia dubia*.

Fourth Quarter 2013 Chronic Toxicity

Chronic toxicity to *Ceriodaphnia dubia* was reported in October 2013. The laboratory that conducted the WET tests (Sierra Foothill Laboratory, Inc.) attributed the *Ceriodaphnia dubia* chronic test failure during October 2013 to the use of unhealthy test organisms. The laboratory retested *Ceriodaphnia dubia* chronic toxicity using fresh test organisms on December 19, 2013. This retest exceeded the chronic toxicity limit for *Ceriodaphnia dubia* with a TUc > 1.

Accelerated Monitoring of Chronic Toxicity First Quarter 2014

During January and February 2014, accelerated monitoring of chronic toxicity for *Ceriodaphnia dubia* was conducted. Accelerated monitoring consisted of four chronic tests conducted every two weeks using the species that exhibited the toxic effects. The chronic toxicity test on January 8, 2014 was used as the first quarter chronic test and also as the first accelerated chronic toxicity test. The first two of these accelerated monitoring samples (January 8 and 21, 2014) exceeded the chronic toxicity limit with results summarized in Table 2. The second two accelerated monitoring tests (February 3 and 13, 2014) did not exhibit any adverse chronic effects on the test

organisms. After the two February accelerated monitoring tests met the chronic toxicity criteria, the quarterly WET testing schedule resumed.

Table 1 – DVI Violations of Effluent Limits During 2014

Monitoring Location	Date	Parameter	Units	Limit	Test Result	Notes
Effluent 1 (EFF-001)	2-14-2014	Nitrate + nitrite	mg/L	10	11	
	8-31-2014	Nitrate + nitrite	mg/L	10	12	
	9-30-2014	Nitrate + nitrite	mg/L	10	12	
	1-08-2014	Chronic toxicity, *C. dubia*	TUc	1	> 1	1st Quarter
	1-21-2014	Chronic toxicity, *C. dubia*	TUc	1	1.3	2nd accelerated
	7-28-2014	Chronic toxicity, *C. dubia*	TUc	1	1.3	3rd Quarter
Receiving Water (RSW-001 & 002)	1-14-2014	Temperature rise	°F	4	5.1	
	1-28-2014	Temperature rise	°F	4	9	
	2-25-2014	Temperature rise	°F	4	7.4	
	3-09-2014	Temperature rise	°F	4	4.7	
	4-10-2014	Temperature rise	°F	4	5	
	2-25-2014	pH	S.U.	0.5	1.0	1.3 in NOV incorrect
	3-18-2014	pH	S.U.	0.5	1.0	

1. TUc = Chronic Toxicity Units = 100/NOEC; (NOEC = No observed effect concentration).
2. Chronic toxicity test on 1-08-2014 was used as the 1st quarter and 1st accelerated toxicity test.
3. TUc > 1 requires accelerated WET testing to begin within 14-days after failure of a quarterly WET test and initiation of a TRE if any accelerated WET test fails.

Table 2 – DVI Accelerated Chronic Toxicity Testing During 2014

Monitoring Location	Date	Parameter	Units	Limit	Test Results	Notes
Effluent 1 (EFF-001)	1-08-2014	Chronic toxicity, *C. dubia*	TUc	1	> 1	1st accelerated
	1-21-2014	Chronic toxicity, *C. dubia*	TUc	1	1.3	2nd accelerated
	2-03-2014	Chronic toxicity, *C. dubia*	TUc	1	1	3rd accelerated
	2-18-2014	Chronic toxicity, *C. dubia*	TUc	1	1	4th accelerated

1. TUc = Chronic Toxicity Units = 100/NOEC; (NOEC = No observed effect concentration).
2. Chronic toxicity test on 1-08-2014 was used as the 1st quarter and 1st accelerated toxicity test.
3. TUc > 1 requires accelerated WET testing to begin within 14-days after failure of a quarterly WET test and initiation of a TRE if any accelerated WET test fails.

TRE Action Plan

The CVRWQCB issued a Notice of Violation (NOV) on November 20, 2014 which stated that CDCR was required to initiate a toxicity reduction evaluation (TRE) after the first accelerated monitoring test result exceeded the toxicity trigger on January 6, 2014. The NOV required DVI to submit a TRE Action Plan by December 19, 2014 and conduct a TRE to identify the cause(s) of the observed toxicity and identify corrective actions to reduce or eliminate effluent toxicity (CVRWQCB, November 20, 2014). In compliance with the NOV, CDCR submited the TRE Action Plan on December 17, 2014.

Second, Third, and Fourth Quarter 2014 Chronic Toxicity

The second quarter 2014 WET test indicated that the effluent was in compliance with the chronic toxicity criteria for all test species. The third quarter 2014 WET tests on July 28, 2014 exceeded the chronic toxicity limit for *Ceriodaphnia dubia*. The fourth quarter 2014 WET test on October 7, 2014 was in compliance with the chronic toxicity limit for all test species.

RESPONSE TO 2014 NOVS

CDCR identified operational conditions which had caused permit violations and provided responses to CVRWQCB to identify the probable causes and corrective actions for the violations of nitrate, temperature, pH, and disinfection byproducts.

Nitrate

Nitrate exceeded the monthly average effluent limit of 10 mg/L in February, August, and September 2014. The effluent nitrate concentrations exceeded the permit limit by 1 to 2 mg/L for each of these violations.

Mechanical Mixer Failures. Two previous nitrate violations during December 2013 and the February 2014 nitrate violation were caused by mechanical failure of 2 of the 4 mixers in the anoxic zone of the denitrification process. CDCR installed two new mixers to restore denitrification capacity. CDCR also purchased spare parts for mixers to allow future mixer mechanical failures to be repaired quickly.

Peak Hydraulic Loading. Nitrate violations during August and September 2014 occurred during maximum day hydraulic loading on the wastewater treatment system. During August and September 2014, wastewater effluent flows increased from an annual average of 0.5 mgd to a daily maximum permitted discharge of 0.62 mgd.

To balance the daily flow variation during high flows in the summer, the operators use the aerated sludge storage tank as a surge or flow equalization tank. The sludge storage tank provides storage for waste sludge to allow intermittent sludge thickening, dewatering, and disposal. To provide storage volume for flow equalization, the water level in the sludge storage tank is reduced during low flows. The portion of the peak flows exceeding the discharge rate is diverted from the aeration basins to the sludge storage tank. After the influent flow rate decreases below the discharge rate, the wastewater stored in the sludge storage tank is pumped back to the MBR process through the anoxic zones.

This operational strategy moderates variations in the hourly influent flow but also transfers dissolved oxygen from the aerated sludge storage tank to the anoxic zones in the MBR process. Dissolved oxygen carried into the anoxic zones would effectively decrease the anoxic contact time and reduce the readily biodegradable soluble BOD5 required for biological denitrification, effectively decreasing denitrification capacity and increasing effluent nitrate concentrations.

Membrane Filter Capacity. The capacity of the membrane filters has been reduced significantly due to membrane fouling and membrane damage due to accumulation of solid debris on the membranes. Lack of mechanical lifting equipment to remove the filter modules from the basins has limited access for maintenance of the air scouring diffusers. This lack of mechanical lifting equipment has resulted in inadequate membrane scouring and reduction of membrane flux capacity.

The membrane modules require a minimum recycle flow of several hundred gpm each to prevent excessive solids accumulation on the membrane surface. During peak flows, additional MBR tanks are placed in operation which increases the total recycle flow and reduces the hydraulic retention time in the anoxic zones. The reduction in the hydraulic retention time in the anoxic zones reduces denitrification capacity and increases effluent nitrate concentrations.

Membrane Filter Upgrades. CDCR plans to replace the existing membrane filters to restore filtration capacity to original design criteria. New membrane filters will allow higher peak hour discharge rates and reduce or eliminate the need for flow equalization in the sludge storage tank, which will enhance denitrification performance. Increased filtration capacity will allow the WWTF to operate with fewer filters during peak flows which will reduce the internal recycle rate, increase hydraulic retention time in the anoxic zones, and maintain denitrification capacity. CDCR plans to install a new hoist or overhead crane system to remove the membrane filters from the basins to provide access for maintenance of the scouring air diffusers.

pH

The effluent pH on February 25 was 7.9 while the surface water upstream and downstream were 7.5 and 8.5, respectively. On March 18, 2014, the effluent pH was 7.9 and the surface water upstream and downstream were 7.3 and 8.3, respectively. On both dates, the DVI effluent pH was mid-way between the upstream and downstream pH values. Although the pH changed by more than 0.5 units in the Deuel Drain on the dates noted, the effluent at a lower pH than the downstream receiving water could not cause the pH increase in the receiving water. These pH violations should be withdrawn because the interpretation of the pH data was erroneous.

Storm water drainage from adjacent agricultural fields is conveyed by the Deuel Drains during the summer irrigation season and during the rainy season in winter. Agricultural fertilizers and pesticides used on the fields wash into Deuel Drains and affect surface water quality throughout the year. Storm water drainage pipes discharge along the Deuel Drains at multiple points including near the EFF-001 location and directly across from the RSW-001 sampling location.

Previous CDCR reports to the CVRWQCB have noted that large areas in the Deuel Drain have had the water surface covered with duck weed while other areas contained algae on the bottom surface. Fertilizers in the storm water from agricultural fields wash into the Deuel Drains and stimulate growth of both duck weed and algae. The duck weed blocks sunlight which prevents algae growth and causes low pH conditions and low dissolved oxygen. The open areas where water is exposed to sunlight, algae grows and pH increases. The observed increase in pH in the

Deuel Drains has been caused by agricultural activities and by other natural conditions unrelated to the DVI facility.

Temperature

The temperature rise in the receiving stream exceeded the 4° F limit on five occasions during 2014. During these events, the cooling towers were operating at the maximum cooling range which reduces the effluent temperature by 20° F.

Cooling Design Range. When the effluent temperature exceeds the receiving stream temperature by more the 24° F, the cooling towers cannot remove enough heat from the water to achieve compliance with the 4° F difference limitation.

Cooling Tower Fan Transformer Failure. The cooling towers have 24-volt cooling fans which require an electrical transformer in each cooling unit to reduce the voltage. When a transformer fails, the cooling fan shuts down and forced air ventilation through the tower ceases. Effluent continues to be pumped through the cooling tower, but lack of forced air ventilation reduces cooling capacity and causes the facility to exceed the effluent temperature limitation (Mullins, 2015).

Cooling System Upgrades and Maintenance. CDCR will modify the cooling tower controls and equipment to increase the cooling range enough to achieve compliance with the temperature rise limitation. CDCR will maintain spare cooling fans and transformers on site to minimize time required to replace failed components in the cooling system.

Whole Effluent Toxicity Testing

DVI is required to conduct both acute and chronic whole effluent toxicity **(**WET) testing on a quarterly basis utilizing three species includinge fathead minnows (*Pimephales promelas*), water fleas (*Ceriodaphnia dubia*), and green algae (*Selenastrum capricornutum*). DVI effluent has exhibited significant acute toxicity sporadically since operation of the WWTF began in 2010.

Chronic Toxicity

Chronic toxicity to *Ceriodaphnia dubia* was reported on December 19, 2013 and on January 8 and 21, 2014. After these incidents, chronic toxicity to *Ceriodaphnia dubia* was reported again on July 28, 2014. The remainder of the evaluation focuses on identification of potential causes of chronic toxicity.

REVIEW OF HISTORICAL TOXICITY

The DVI facility has experienced previous chronic toxicity violations. Previous TRE evaluations have identified potential causes of chronic toxicity and have proposed actions to reduce or eliminate chronic toxicity.

Hydraulic Capacity and Limitations

The previous Order contained effluent limitations prohibiting average dry weather flows or peak wet weather discharge flows from exceeding the treatment plant's design flow of 0.62 mgd. The current Order (4-3-2003) contains a monthly average effluent flow limit of 0.62 mgd. Daily Peak Wet Weather Flow (PWWF) was reported to be 0.783 mgd (4-3-2003) and DVI was unable to consistently comply with this limitation. Monitoring reports from February 2000 to February 2001 showed 136 dates where the discharge was in excess of 0.62 mgd.

On 21 June 2001, CDCR requested a discharge flow increase to 1.0 mgd. Additional receiving water and treatment plant capacity studies were required before this request could be granted. On 7 March 2002, DVI withdrew the request for an increase in the permitted discharge flow, and implemented water conservation measures to reduce the volume of wastewater generated.

Toxicity Reduction Evaluation 2012

During 2012, DVI exceeded the chronic toxicity for the green algae *Selenastrum capricornutum*. DVI conducted accelerated monitoring and performed a TRE (GHD, 2013) as required by the discharge permit. Results of the quarterly and accelerated chronic test results for *Selenastrum* are presented in Table 3. The chronic bioassay test for *Selenastrum* evaluates impacts on growth by comparing the 96 hour cell density of the test sample to the control sample. The TUc value is the ratio of the cell density of the control sample to the cell density of the test sample.

The 2012 TRE used water from the receiving stream (RSW-001) for both the effluent sample diluent and for all control tests. The receiving stream water was subsequently shown to stimulate algae growth compared to a control test using laboratory prepared water. The 2012 TRE results therefore may have included false positive toxicity results due to stimulating algae growth in the control tests. However, the reduction in 96-hour cell density of the effluent samples was considered too large to have been caused by the receiving stream water alone.

Review of the monthly monitoring reports for the DVI WWTF indicated that monitored parameters were within permissible limits, except for electrical conductivity (EC) which was elevated during some of the failed toxicity tests. The 96-hr cell density for the bioassays using 100 percent effluent was shown to be inversely related to EC (i.e. algae cell density decreased when EC increased).

Elevated EC occurred when the DVI reverse osmosis (RO) system that provides drinking water was off line for maintenance. When the RO system was off line, drinking water was supplied from groundwater which has elevated EC due to high concentrations of dissolved minerals. The failed *Selenastrum* toxicity tests were attributed to elevated concentrations of total dissolved solids (TDS), hardness, and alkalinity which increase EC.

Table 3 – Chronic *Selenastrum* Bioassay Results 2012

Date	Laboratory	Type of Monitoring		96 hour cell density	Result, TUc
		Quarterly	TRE		
12 January 2012	ABC	1		9.18E+05	1.33
5 April 2012	ABC	2		1.34E+06	1.33
2 May 2012	ABC		1	1.01E+06	8.00
16 May 2012	ABC		2	8.31E+05	8.00
31 May 2012	ABC		3	8.15E+05	> 8.00
13 June 2012	ABC		4	6.57E+05	4.00
11 July 2012	ABC	3		9.38E+05	2.00
11 October 2012	ABC	4		1.67E+06	1.00
18 October 2012	SFL		TRE (3)		1.00 (3)

1. All Quarterly and Accelerated Monitoring tests conducted on 24hr Composite samples except for 1 Grab sample on 18 October 2012.
2. Laboratories: ABC = Aquatic Bioassay Consulting, Inc., SFL = Sierra Foothill Laboratories
3. Initial TUc was reported as 1.33. Re-suspension of sample reduced TUc to 1.

During January and February 2013, four bioassay tests were conducted on samples while the RO plant was operational which eliminated any effects of elevated electro-conductivity. Each 24 hour composite effluent sample was tested using laboratory control water for dilution. Toxicity was absent for all four test as indicated by TUc of 1. With completion of four consecutive accelerated monitoring tests passing chronic toxicity bioassays and identification of the likely source of toxicity being the identified as high TDS and EC when the (RO) plant was out of service, DVI was permitted to exit this TRE.

VALIDATION OF BIOASSAY RESULTS

The 2014 bioassay tests were performed by Sierra Foothill Laboratory which certified that all test results were in conformance with all applicable Environmental Laboratory Accreditation Program (ELAP) requirements. Sierra Foothill Laboratory is ELAP-accredited by the California State Water Board. The laboratory is qualified to perform WET tests using approved methods which ensure the quality of the analytical data is acceptable for regulatory purposes.

Dewberry Engineers reviewed the quarterly and accelerated chronic toxicity test results reported during 2014 to determine if any anomalies or inconsistencies were evident and which might invalidate the bioassay test results.

First Quarter 2014 WET Test

The first quarter WET test of samples collected on January 6, 2014, shown in Table 4, indicated that *Pimphales promelas* survival was 100 percent for the effluent, receiving water, and control. Growth was not reduced for either the effluent or receiving water compared to the control

Table 4 – First Quarter WET Test 2014, Abbreviated Static-Renewal Chronic Toxicity Tests - Sample Date January 6, 2014

Sample Location	Test Species					
	Pimphales promelas		*Ceriodaphnia dubia*		*Selenastrum capricornutum*	
	7-day % survival	Avg. Dry Wt., mg	6-day % survival	Average young/ female	96-hr cells/mL	
					Unstirred	Shaken 10 minutes
EFF-001	100.0	0.75	80	7.8	1.24	1.99
RSW-001	100.0	0.74	100.0	21.5	4.53	4.86
Control	100.0	0.68	100.0	25.3	1.64	1.62
TUc	1	1	1	> 1	NA	1

Effluent = EFF-001, Receiving Stream = RSW-001, TUc = Chronic Toxicity Unit. Control = Demineralized water (dMW) prepared using 26 percent Evian Spring Water and 74 percent Arrowhead distilled water. Test organisms were cultured in dMW. All tests conducted in 100 percent sample (zero dilution).

Ceriodaphnia dubia survival was not significantly reduced in either the effluent or receiving water, but reproduction was significantly reduced in both the effluent and the receiving water. *Ceriodaphnia dubia* reproduction test result was TUc > 1 which initiated accelerated testing.

The results for *Selenastrum capricornutum* indicated no significant reduction in the 96-hour cell densitities of either the effluent or receiving water. As shown in Table 4, cell densities increased in both the effluent and receiving water compared to the control, indicating that there might be some substance which stimulated algae growth in both the effluent and receiving water. The apparent stimulatory effect is similar to results reported for the 2012 TRE (GHD, 2013), which showed that the receiving water had a stimulatory effect on algae growth. This stimulatory effect was attributed to the impact of agricultural drainage into the receiving water (GHD, 2013). For both 2012 and 2014 results, the apparent stimulatory effect on algae growth in test samples did not have any adverse or toxic effects.

The 2014 laboratory report noted that at termination of the initial test, algae were sticking to the culture flask walls in all of the effluent replicates, resulting in underestimation of algae growth and high variability between replicates. After the initial determination of turbidity, the flasks were shaken for 10 minutes with silica sand added to dislodge and resuspend the cells. Turbidity of the resuspended samples was retested. This cell resuspension procedure had been used previously as reported in the 2012 TRE (GHD, 2013).

Accelerated Chronic Toxicity Tests 2014

Results of the accelerated chronic toxicity tests for *Ceriodaphnia dubia* are summarized in Table 5. The first two accelerated tests indicated chronic toxicity based on reduced reproduction. The second test on January 21 showed improvement compared to the first test on January 8 in terms of increased numbers of young per female and significant effects observed only in the 100 percent effluent series. The second two accelerated tests indicated no chronic toxicity.

Table 5 – Accelerated WET Tests 2014, Definitive Static-Renewal Chronic Toxicity Tests - Sample Date January 6, 2014

Effluent dilution, percent	Test Species *Ceriodaphnia dubia*							
	Test 1		Test 2		Test 3		Test 4	
	January 8, 2014		January 21, 2014		February 3, 2014		February 18, 2014	
	6-day % survival	Average young/ female	6-day % survival	Average young/ female	6-day % survival	Average young/ female	6-day % survival	Average young/ female
100	80.0	7.8	100.0	19.2	100.0	42.6	100.0	39.3
75	100.0	31.0	100.0	37.4	100.0	44.9	100.0	39.9
50	100.0	28.2	100.0	36.4	90.0	40.0	100.0	41.3
25	100.0	24.6	100.0	32.1	100.0	45.0	100.0	38.3
12.5	100.0	21.6	90.0	34.0	100.0	43.1	100.0	35.6
RSW-001	100.0	21.5	100.0	37.2	100.0	41.7	100.0	34.0
Control	100.0	25.3	100.0	35.4	100.0	39.5	100.0	42.0
TUc	1	1.3	1	1.3	1	1	1	1

Effluent = EFF-001, RSW-001 = Receiving Stream, TUc = Chronic Toxicity Unit. Control = Demineralized water (dMW) prepared using 26 percent Evian Spring Water and 74 percent Arrowhead distilled water. Test organisms were cultured in dMW water. All dilutions conducted using the Receiving Stream RSW-001. Solution renewal and feeding with 0.1 mL YCT + 0.1 mL algae conducted daily.

Detailed review of the quarterly and accelerated chronic toxicity tests did not identify any anomalies or inconsistencies which might invalidate the bioassay test results. The chronic toxicity tests were certified by the ELAP accredited laboratory to have followed all approved analytical methods. Sample collection and sample transport procedures were documented on chain of custody forms. Laboratory reference toxicant tests appear to be valid. The quality of the analytical data appears to be acceptable and the reported results appear to be reliable within the limits of WET test method accuracy.

RECEIVING STREAM

Discharge Point 001

The DVI facility discharges effluent to Deuel Drain at Discharge Point 001 (EFF-001) which borders the facility on the east. In addition to effluent from the DVI WWTF, Deuel Drain receives storm water runoff and occasional drainage from agricultural irrigation. Deuel Drain flows into the Paradise Cut. The western end of Paradise Cut discharges to the Old River, which is tributary to the San Joaquin River and Clifton Court Fore bay, a drinking water source for southern California. These waters are located within the Sacramento-San Joaquin Delta. The southern one-third of the Delta is 303(d) listed as an impaired water body for total dissolved solids (TDS)

Effluent Discharge Requirements

Deuel Drain is an ephemeral, effluent dominated stream, with minimal dilution in the vicinity of the discharge. Because dilution is negligible, Basin Plan water quality standards apply at the

outfall to the Deuel Drain. Effluent monitoring locations for the DVI WWTF are listed in Table 6. Discharge requirements for the DVI WWTF are summarized in Tables 7.

Table 6 – Monitoring Station Locations

Station Number	Monitoring Location Description
INF-001	Representative influent sample collected prior to any treatment or return flows.
EFF-001	Representative effluent sample collected downstream of the outfall. Latitude: 37° 45' 02" N Longitude: 121° 19' 35" W
RSW-001	In Deuel Drain, 3,080 feet upstream from Discharge Point 001.
RSW-002	In Deuel Drain, 450 feet downstream from Discharge Point 001.
RSW-003	In Paradise Cut, 900 feet east of the confluence with Deuel Drain.
BIO-001	Representative biosolids sample collected once per year.
SPL-001	Representative sample of municipal water supply.
UVS-001	Representative sample collected upstream of UV disinfection system.
UVS-002	Representative sample collected downstream of the UV disinfection system.

Elimination of Discharge Mixing Zone. Prior to 2003, Deuel Drain was designated as a mixing zone which extended from Discharge Point 001 approximately one mile downstream to the confluence of Deuel Drain with Paradise Cut. This mixing zone had allowed DVI to comply with effluent limitations for temperature and chlorine residual. Elimination of the mixing zone in 2003 required construction of a new effluent cooling system and new UV disinfection system as part of the new DVI WWTF completed in 2010. .

Title 22 Reclamation Criteria. DVI WWTF discharge requirements include tertiary filtration and Title 22 disinfection criteria to allow effluent to be used to irrigate approximately 3 acres of the DVI WWTF site, irrigation of food crops, and for contact recreation. Effluent discharge requirements include an effluent turbidity limitation measured at Monitoring Location UVS-001 shall not exceed 0.2 NTU more than 5 percent of the time within a 24-hour period and 0.5 NTU at any time.

Total Dissolved Solids (TDS) and Electrical Conductivity. Monitoring data shows the discharge from Outfall 001 contains TDS concentrations as high as 2,000 mg/l, and EC levels up to 2400 uS/cm (= umhos/cm). The reported TDS concentrations have increased since startup of the system in 2010, consistent with regional groundwater quality trends.

Table 7 – Effluent Limitations - Discharge Point No. 001 (EFF-001)

Parameter	Units	Average Monthly	Average Weekly	Maximum Daily	Instantaneous	
					Minimum	Maximum
Average Dry Weather Flow	mgd			0.62		
Biochemical Oxygen Demand (5-day @ 20℃)	mg/L lbs/day[2]	10 52	15 78	20 103	-- --	-- --
Total Suspended Solids (TSS)	mg/L lbs/day[2]	10 52	15 78	20 103	-- --	-- --
Copper, Total Recoverable	µg/L	25	--	10	--	--
Cyanide, Total (CN)	µg/L	4.3	--	8.5	--	--
Methylmercury	grams/year					0.021[8]
Mercury, Total interim limit	grams/year					10.6 [9]
Ammonia Nitrogen, Total (N)	mg/L lbs/day[2]	0.7 3.6	-- --	2.2 11.4	-- --	-- --
Nitrate Nitrogen, Total (N)	mg/L	10	--	--	--	--
Nitrite Nitrogen, Total (N)	mg/L	1.0				
pH [2]	Std. units	--	--	--	6.5	8.5
Temperature	ºF	--	--	20/4 [3]	--	--
Total Coliform Organisms	MPN /100 mL	23 any 30-day period	2.2 as 7-day median	--	--	240 at any time
UV dose, hourly average	mJ/cm2				80	
Acute Toxicity	% Survival	--	--	70/90 [1,4]	--	--
Chronic Toxicity	TUc	--	--	1 [5]	--	--

1. Effective 29 March 2014. ORDER R5-2014-0014. NPDES NO. CA0078093.
2. The pH to be depressed below 6.5 nor raised above 8.5.
3. Temperature. In Deuel Drain, the discharge shall not cause the creation of a zone, defined by water temperatures of more than 1ºF above natural receiving water temperature, which exceeds 25 percent of the cross-sectional area of the main river channel at any point.
4. Acute Whole Effluent Toxicity: 70% minimum survival in 96-hour bioassays of undiluted waste for any one bioassay; 90% median for any three consecutive bioassays.
5. Chronic Whole Effluent Toxicity: No chronic toxicity in effluent discharge. Quarterly chronic toxicity tests for the duration of the permit.
6. Diazinon and Chlorpyrifos: Effluent concentrations shall not exceed the sum of 1.0 as identified below:

   Average Monthly Effluent Limitation: SAMEL=CD AVG/0.079 + Cc AVG/0.012 ≤1.0
   CD-avg = average monthly diazinon effluent concentration
   CC-avg = average monthly chlorpyrifos effluent concentration.

   Maximum Daily Effluent Limitation: SMDEL=CD MAX/0.16 + Cc MAX/0.025 ≤1.0
   CD-max = maximum daily diazinon effluent concentration in µg/L.
   CC-max = maximum daily chlorpyrifos effluent concentration in µg/L.
7. Methylmercury: Effluent annual methylmercury load shall not exceed 0.021 grams per calendar year, per the Delta Mercury Control Program. From 30 January 2015 until achieving compliance with final effluent limitations for methylmercury, submit annual progress reports on pollution minimization activities.
8. Interim Effluent Limitations for Mercury total: Effective until 30 December 2030, effluent total annual mercury load per calendar year shall not exceed 10.6 grams/year. This interim effluent limitation shall apply in lieu of the final effluent limitation for methylmercury.

WASTEWATER TREATMENT FACILITY

The DVI wastewater treatment facility (WWTF) began discharging on 22 September 2010. The design capacity of the facility is 0.70 million gallons per day (mgd) and the permitted flow is 0.62 mgd, both as average dry weather flow conditions. The WWTF serves a population of approximately 3,132 inmates plus 1,066 staff. The WWTF process equipment is summarized in Table 8.

The DVI WWTF consists of biological treatment with nitrification and denitrification, four membrane bioreactors (MBRs), and ultraviolet light (UV) disinfection. Effluent (permeate) is discharged from the MBR submerged microfilter modules and thickened mixed liquor suspended solids (MLSS) retained in the MBR basins are returned to the activated sludge basins and an aerated sludge storage basin. Waste solids from the aerated sludge storage basin are dewatered by a belt filter press approximately once per week. The MLSS in the aeration basins increases gradually by 300 to 400 mg/L until some of the suspended solids are removed by belt filter press dewatering. Dewatered waste solids are hauled to offsite disposal.

Table 8 – Wastewater Treatment Equipment

Treatment Units	Units, Number	Capacity, Each, gpm	Capacity, Total, mgd	Type
Headworks				
Coarse screens	2		2.1	Mechanical
Influent pumps (2 + 1 standby)	3	870	2.1	Submersible
Fine screens	2		2.5	Rotary drum
Grit separator	1		2.6	Vortex
Grit cyclonic separator & classifier	1	250		Inclined screw
Bicarbonate feeder (pH adjustment)	1			1 – 40 mg/L feed rate
Aeration basins, gallons each	2	236,000	2.9	
Anoxic zones per basin	2			
Aeration zones per basin	1			
Aeration blowers, scfm	3	1,093		
Aerated sludge storage tank	1			
Membrane bioreactor system			2.9	
Membrane feed pumps, each	4	640		Submerged hollow fiber
Membrane basins, gallons each	4	8,600		
Permeate discharge pumps	4	512		Rotary lobe
Membrane scour blowers, scfm	3	1,710		
Ultraviolet (UV) disinfection	3		4.09	
Effluent cooling towers	3	700	3.02	
Recycled water, tertiary disinfected			1.05	
Distribution pumps	3		1.05	
Bbelt filter press, (1 + 1 standby), each	2	160	0.28	
Emergency generator, kw	1	1,000		

Notes: Inmate population served = 3,132; staff = 1,066.

WASTEWATER TREATMENT PERFORMANCE

Influent BOD5 and TSS

During 2014, influent BOD_5 and TSS concentrations averaged 175 and 296 mg/L, respectively. Influent BOD_5 and TSS concentrations vary widely from day to day. There do not appear to be any seasonal trends in influent BOD_5 concentrations that could potentially upset the wastewater treatment process or affect effluent toxicity.

Influent TSS concentrations are more variable than influent BOD_5 concentrations. TSS concentrations appear to be higher in the winter than in the summer. High TSS concentrations over 700 mg/L were recorded in January, late November, and December. The high TSS concentrations during these winter months appear to coincide with high influent TDS concentrations discussed later in this evaluation.

Effluent BOD5 and TSS

The membrane bioreactors at the DVI WWTF reduce effluent BOD_5 and TSS to annual average concentrations of 0.26 and 0.92 mg/L. The very low effluent concentrations of BOD_5 and TSS appear unlikely to contain any substance that might adversely affect effluent toxicity.

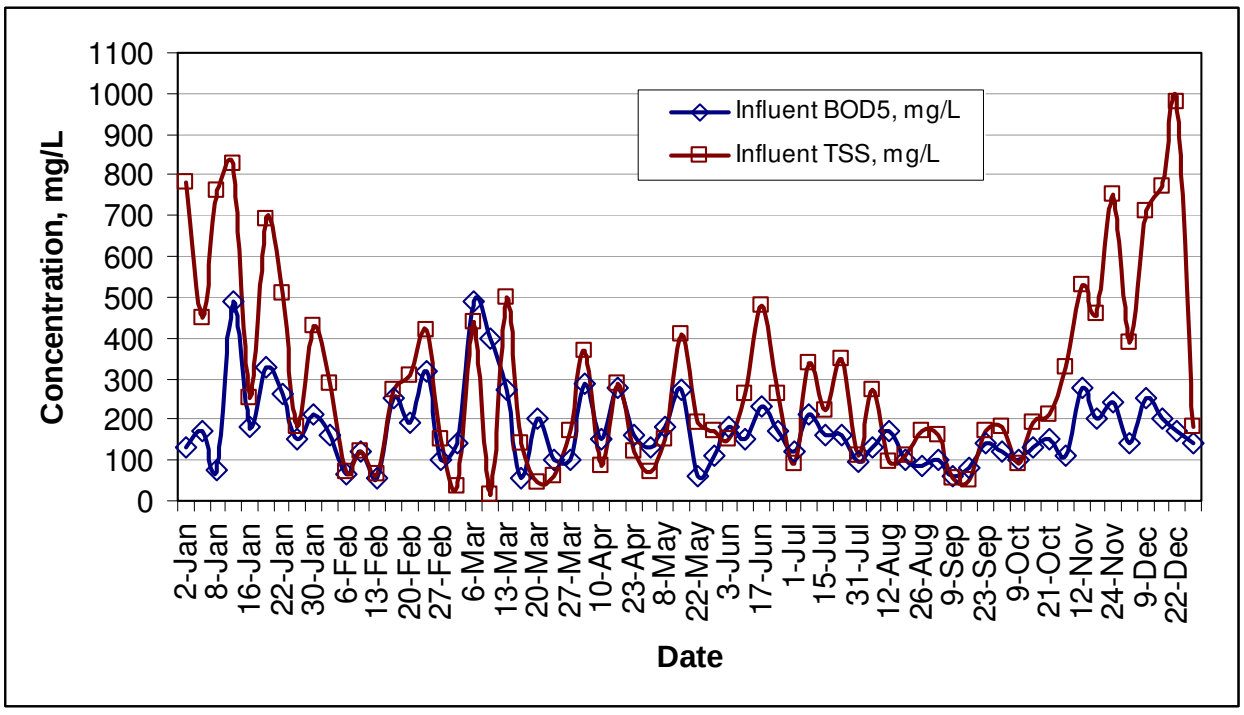

Figure 1 – Wastewater Influent and Effluent BOD5 and TSS Concentrations During 2014

Nitrification Performance

The DVI WWTF consistently achieves complete nitrification (oxidation of ammonia to nitrite) with an average annual effluent ammonia concentration of 0.16 mg/L. The low effluent ammonia concentrations prevent any acute or chronic toxicity due to effluent ammonia.

Wastewater Flows

Wastewater flows for year 2014, shown on Figure 2, average 0.459 and 0.496 mgd for the influent and effluent, respectively. Effluent flows exceed influent flows by 8 percent on average. The difference between influent and effluent flow measurements probably reflects meter accuracy under different hydraulic conditions rather than a real difference in influent and effluent flow rates.

Seasonal Flow Trends. Minimum daily flows occur from November through January. Maximum daily flows occur from late July through September. The seasonal flow increase during summer reflects increased water consumption during hot dry weather. The seasonal flow trends and peak flows do not appear to have potential to upset the wastewater treatment process.

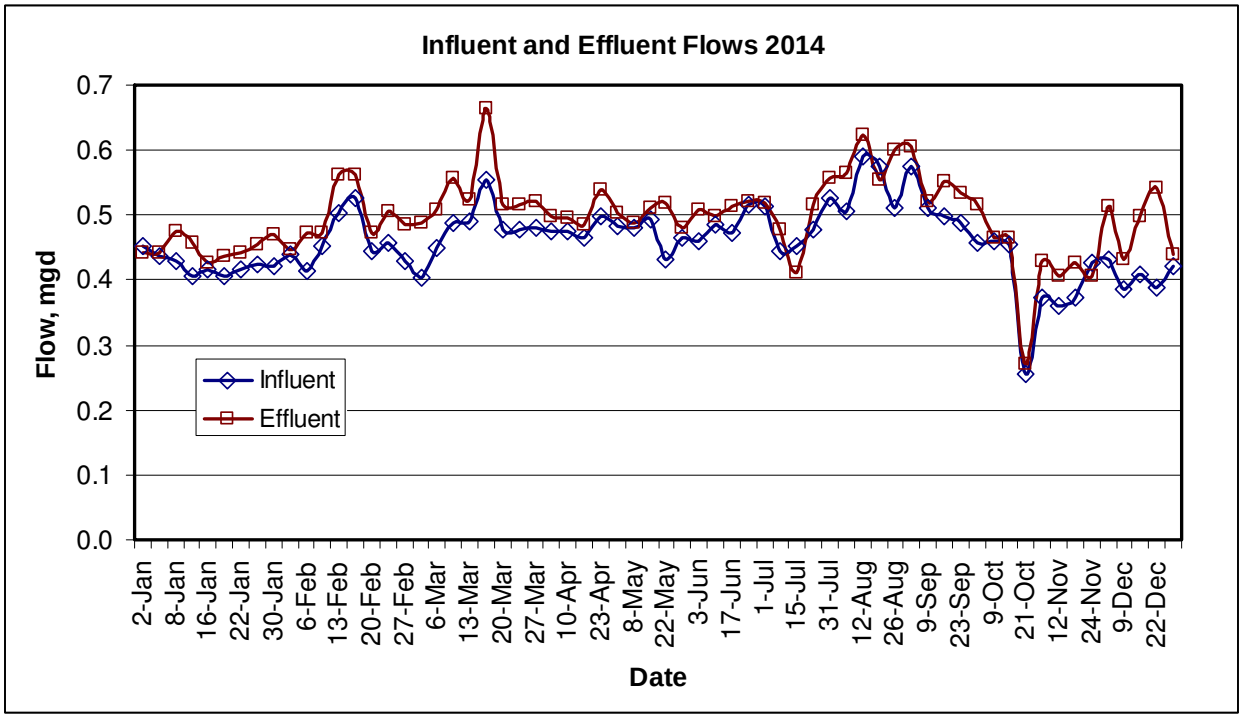

Figure 2 – Wastewater Influent and Effluent Flows During 2014.

Effects of RO System Shut Down. Between October 14 and 21, 2014, influent and effluent flow rates decreased by almost half to 0.26 and 0.27 mgd, respectively. This sudden decrease in wastewater flow rates coincides with shut down of the drinking water reverse osmosis (RO) system on October 18, 2014 for cleaning and maintenance of the brine concentrator (ZLD)

system. During this shut down, DVI used brackish groundwater without reverse osmosis treatment is for water supply. After this change of water sources, drinking water demand and wastewater production appear to have decreased sharply. This reduction reflects DVI efforts to minimize water consumption while the RO-ZLD system was shut down for cleaning and maintenance. This reduction in water consumption might also reflect the response of water users to the change in aesthetic quality of the drinking water due to high TDS concentrations. If so, normal water consumption resumed after a period of acclimation to the changed water quality.

The short term reduction in wastewater hydraulic loads after shut down of the RO system would not be expected to have any adverse effects on the performance of the wastewater treatment system. However, the associated increase in influent and effluent total dissolved solids (TDS) could have affected wastewater treatment system performance and might have affected effluent chronic toxicity.

Total Dissolved Solids and Electrical Conductivity

Electrical conductivity is directly proportional to the TDS concentration in both the drinking water and wastewater. The TDS concentrations and the electrical conductivities in the drinking water and wastewater are also directly related to one another. As shown by Figure 3 and Table 9, an increase or decrease in the drinking water TDS concentration and electrical conductivity is reflected in a similar proportional increase or decrease in the wastewater influent and effluent.

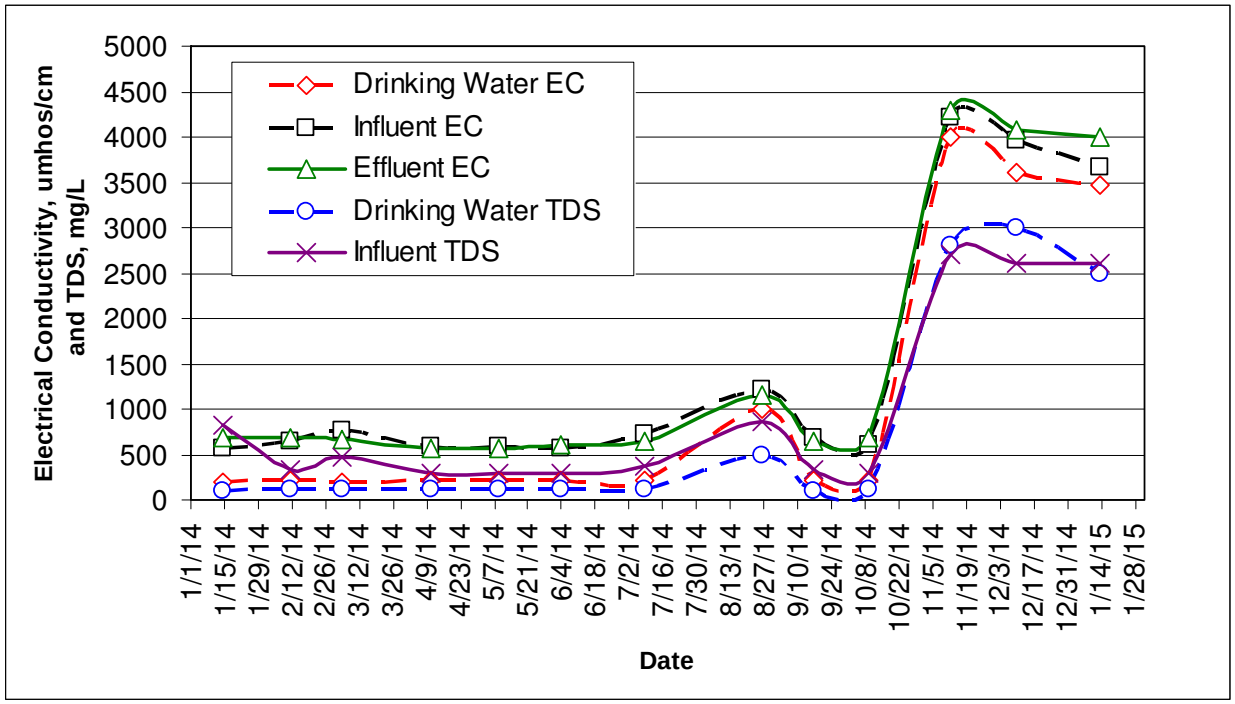

Figure 3 - TDS Concentrations and Electrical Conductivities of DVI Water and Wastewater, 2014

Table 9 – Electrical Conductivity and TDS in Drinking Water, Influent, and Effluent in 2014

Date	Electrical Conductivity, µS/cm @ 25° C			Total Dissolved Solids (TDS), mg/L		
	Drinking Water	Wastewater		Drinking Water	Wastewater	
	SPL-001	Influent-001	Effluent-001	SPL-001	Influent-001	Effluent-001
1/14/2014	199	569	683	100	830	ND
2/11/2014	216	644	682	110	330	ND
3/4/2014	202	766	671	110	480	ND
4/10/2014	215	598	573	110	290	ND
5/8/2014	213	590	572	120	290	ND
6/3/2014	220	567	600	110	290	ND
7/8/2014	219	721	653	110	370	ND
8/26/2014	995	1,215	1,164	500	870	ND
9/16/2014	207	688	638	100	330	ND
10/9/2014	207	601	691	120	300	ND
11/12/2014	4,001	4,210	4,290	2,800	2,700	ND
12/9/2014	3,600	3,970	4,080	3,000	2,600	ND
1/13/2015	3,480	3,660	4,000	2,500	2,600	ND

The wastewater has higher TDS concentrations and electrical conductivities than the drinking water due to salts, minerals, and organic chemicals added during domestic use of the water. Under normal operating conditions, the drinking water contains approximately 110 mg/L of TDS and has an electrical conductivity of approximately 220 µS/cm (= µmhos/cm). The influent wastewater contains approximately 300 mg/L of TDS and has an electrical conductivity of approximately 600 µS/cm.

TDS Removal by Reverse Osmosis Treatment. The reverse osmosis (RO) treatment system that supplies drinking water to DVI removes virtually all of the dissolved solids (TDS) from the brackish groundwater source. Caustic soda and calcium chloride are added to the RO product water to increase hardness, pH, and alkalinity within drinking water quality standards (Carollo Engineers, 2009). Sodium hypochlorite disinfection also slightly increases the mineral contentof the product water.

When the reverse osmosis system is out of service and drinking water reserves from storage are depleted, DVI must use the brackish groundwater source which contains elevated TDS levels to provide water. When brackish groundwater is used for water supply without reverse osmosis treatment, the effluent discharged from the wastewater treatment facility contains the total TDS from the brackish groundwater source plus additional TDS from domestic use of the water.

During 2014, the reverse osmosis system operated consistently from January through October 18, 2014. The reverse osmosis and brine concentrator systems were taken out of service for cleaning and maintenance from October 18, 2014 through March 2015. As shown on Figure 3, the WWTF influent and effluent TDS concentrations and electrical conductivities increased approximately 8-fold after the reverse osmosis system was taken out of service.

Potential Chronic Toxicity from TDS

As shown on Figure 3, the TDS concentrations increased approximately 2-fold during monthly sampling on August 26, 2004 while the RO system was operating. The TDS concentrations increased in the drinking water and in the wastewater, indicating that the RO system was not fully effective during this August sampling event. The period when TDS concentrations increased might have lasted for only several hours or could have extended up to seven weeks between July 8 and August 26, 2004 which are the sampling dates bracketing the higher TDS event.

The chronic toxicity for *Ceriodaphnia dubia* which was observed on July 28, 2014 occurred during the July 8 to August 26 period while the reverse osmosis system was operating less efficiently and might coincide with increased TDS concentrations. If this were the case, the increase in the TDS concentrations in the WWTF effluent during this period might have caused or contributed to the chronic toxicity to *Ceriodaphnia dubia* due to osmotic or ionic imbalances or toxicity of specific ions at elevated concentrations.

Potential TDS Impacts on First Quarter 2014 WET Test

Chronic toxicity to *Ceriodaphnia dubia* was reported during accelerated WET testing on January 8 and 21, 2014 while the RO system was operating. The TDS concentration in the wastewater influent was elevated to 830 mg/L during monthly sampling on January 14. TDS concentrations decreased to normal levels of 330 mg/L by the next monthly monitoring sample on February 11. The TDS concentration in the wastewater influent on January 14 was almost as high as the July-August period (TDS = 870 mg/L) when chronic toxicity to *Ceriodaphnia dubia* was observed again on July 28.

During 2014, the drinking water consistently contained approximately 110 mg/L of TDS, except for August 26 (500 mg/L of TDS) when the RO system appeared to be operating inefficiently and after October 18 when the RO system was shut down. The relatively high TDS concentration of 830 mg/L in the wastewater influent on January 14 appears to be due to higher than average TDS loadings from the DVI facility because the drinking water TDS remained at the normal concentration of 100 mg/L. The higher than average TDS loadings coincide with high wastewater influent TSS concentrations during January which were discussed previously.

Comparison of January and February 2014 Accelerated WET Tests. The January WET test results indicated chronic toxicity to *Ceriodaphnia dubia* when the wastewater influent TDS was elevated to 830 mg/L. After wastewater influent TDS concentrations had decreased to normal levels of 330 mg/L in February (based on the monthly monitoring sample on February 11), the second two accelerated WET tests on February 3 and 13, 2014 did not exhibit chronic toxicity to the test organisms. The reduction of the effluent TDS from 830 mg/L in January to 330 mg/L in February correlates with chronic toxicity observed twice in January but absent in February.

Fourth Quarter 2014 WET Test

The fourth quarter 2014 WET test was in compliance with the acute and chronic toxicity limits for all test species. The absence of effluent toxicity in the fourth quarter WET tests would be expected because the test samples were collected on October 7, 2014 while TDS concentrations were low prior to shut down of the RO system on October 18, 2014.

Chronic Toxicity Comparison - 2012 and 2014

The probable cause of chronic toxicity during 2012 and 2014 was identified as high concentrations of TDS and associated electrical conductivity in the WWTF effluent. The 2012 WET tests indicated chronic toxicity for the green algae *Selenastrum capricornutum* while the 2014 WET tests indicated chronic toxicity for the invertebrate species *Ceriodaphnia dubia.* It is unclear why high TDS concentrations and associated high electrical conductivity did not affect both *Selenastrum capricornutum or Ceriodaphnia dubia* when toxicity was indicted for one or the other of these species. These sensitive species may have been selectively affected by other factors which compound the effects of high TDS concentrations and associated high electrical conductivity.

GROUNDWATER SALINITY

Dissolved Solids in Groundwater

The DVI facility is located in the Tracy Basin which is a division within the San Joaquin Valley. The evapotranspiration rate of 56 inches per year exceeds the average precipitation of 11 to 16 inches per year in the Tracy Basin (California Department of Water Resources, 2005b). The main aquifers within the Tracy Basin are the Late Tertiary to Quaternary Tulare Formation, Older Alluvium, flood basin deposits, and Younger Alluvium. During the dry summers, ground-water extraction rates are high.

Surface water and shallow groundwater drain from the San Joaquin Valley into the Sacramento and San Joaquin Delta. Rivers draining the Sacramento Basin also carry salt into the Delta. Much of the San Joaquin basin relies on water from the Delta, resulting in a net import of salt to the San Joaquin basin (Bennett, Belitz, and Milby Dawson, 2005).

Groundwater in the San Joaquin Valley is increasingly saline due to fertilizers used in commercial agriculture which add salts to the soil. (Most fertilizers are technically nearly 100 percent salts). Plants absorb most of the applied nutrients in fertilizers, but some of these salts remain in the soil and shallow groundwater. High evaporation rates concentrate these dissolved salts in the shallow groundwater. Deep percolation of excess applied irrigation water carries the dissolved salts down into the deep groundwater aquifers. The concentration of dissolved solids generally increases with depth in the San Joaquin Valley. Groundwater quality in the Tracy Basin is shown for several wells in Table 10.

Table 10 – Groundwater Quality in the Tracy Basin

	Well Number				
	TRCY-03	TRCY-07	TRCY-09	TRCY-11	TRCYFP-01
pH	7.5	6.3	7.3	7.9	7.5
TDS	751	414	604	2,740	4,350
Specific conductance, µS/cm at 25℃	1,000	4,180	711	938	5,990
Alkalinity as CaCO3, mg/L	194	139			110
Total hardness, as CaCO3, mg/L	310	1,900	170	160	1200
Chloride, mg/L	102	108	82.1	1,020	2,400
Iron, mg/L		24,500	4,180	8	1,240
Manganese, ug/L		17,100	519	194	2,480
Magnesium, mg/L	26.8	18.3	16.2	213	141
Calcium, mg/L	80.9	38.7	38.5	397	254
Sodium, mg/L	138	77.5	134	240	1,090
Potassium, mg/L	3.17	2.82	3.39	4.2	5.85
Barium, ug/L	25	59	102	44	158
Strontium, ug/L	1,060	2,320	342	664	2,740
Bicarbonate as HCO3 (mg/L, field)	235	169			133
Sulfate, mg/L	248	34	191	750	62.9
Silica, mg/L	23.4	29.4	34.3	45.7	35.1

Source: Bennett, Belitz, and Milby Dawson, 2005, California GAMA Program: Ground-Water Quality Data in the Northern San Joaquin Basin Study Unit: 2005.

The brackish groundwater source for DVI contains 2,600 mg/L of total dissolved solids (TDS). The TDS in the DVI groundwater source, shown in Table 11, includes the major anions of bicarbonate chloride, and sulfate and the major anions of calcium, magnesium, and sodium. The alkalinity of the groundwater is low and consists entirely of bicarbonate. Average water quality in the DVI wells from quarterly groundwater tests in 2013 and 2014 is summarized in Table 12. As shown in Figure 4, Groundwater TDS concentrations in DVI Wells remained relatively constant in 2013 and 2014 while electrical conductivity has shown a steadily declining trend over the same time period.

Table 11 – Average Groundwater Ion Concentrations in DVI Wells

Parameter	Units	RO Design	May 2009	Sept 2009	Mar 2010	Average
pH	Std. units	7.8	7.4	7.6	7.6	7.53
TDS	mg/L	1569	2500	2700	2700	2633
Alkalinity	mg/L	106	27	26	24	26
Barium	mg/L	0.15	0.12	0.12	0.12	0.12
Bicarbonate	mg/L	130	27	26	24	26
Carbonate	mg/L	1.3	--	ND	ND	--
Calcium	mg/L	174	240	220	220	227
Chloride	mg/L	767	1100	900	930	977
Iron	mg/L	ND	0.13	0.18	0.19	0.17
Magnesium	mg/L	87	130	99	110	113
Manganese	mg/L	0.42	0.64	0.61	0.56	1
Potassium	mg/L	6.3	7.7	7.5	7.3	8

Table 11 – Average Groundwater Ion Concentrations in DVI Wells

Parameter	Units	RO Design	May 2009	Sept 2009	Mar 2010	Average
Silica	mg/L	38	18	37	41	32
Sodium	mg/L	249	250	220	240	237
Strontium	mg/L	5.58	--	5.9	6.4	6
Sulfate	mg/L	171	230	220	230	227

Source: Average RO Feed from O&M Manual (Carollo Engineers, 2009).

Table 12 – DVI Average Well Water Quality from Quarterly Groundwater Tests – 2013 & 2014

Year	2013				2014		Average
Quarter	1	2	3	4	1	2	
Date	3/5	4/30	7/23	10/30	3/5	4/30	
Ammonia, Total (N), mg/L	0.22	0.69	0.28	0.24	0.340	0.361	0.36
TDS, mg/L	2180	2,257	1,779	1,815	1,988	2,047	2,011
Nitrate, Total (N), mg/L	0.82	2.37	0.36	0.43	0.46	2.13	1.10
Total Coliform, #/100 mL	46	2.0	4.5	2.0	5.0	2.0	10.3
pH, Std. units	7.52	7.48	7.33	7.27	7.23	7.20	7.34
Electrical Conductivity @ 25 Deg. C, µmhos/cm	2,860	2,666	2,458	2,313	2,173	2,088	2,426

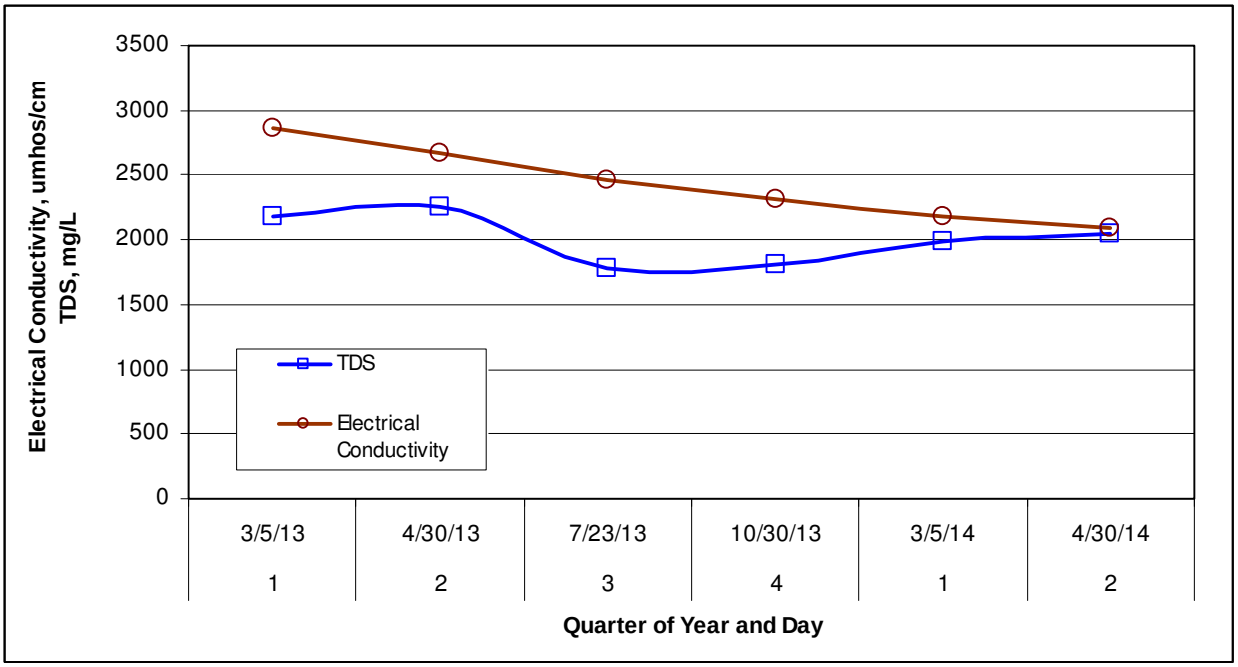

Figure 4 – Groundwater TDS and electrical conductivity in DVI Wells in 2013 and 2014

ION IMBALANCE TOXICITY

Salt concentrations in the internal body fluids of freshwater organisms are higher than in the surrounding water. Passive diffusion causes continuous loss of dissolved minerals from the bodies of freshwater organisms. To maintain biological equilibrium, freshwater organisms continuously excrete water and must actively absorb the ions required for metabolism from the water. If the external ion concentration changes significantly, freshwater organisms must expend more energy to maintain their internal ion balance. The biological stress from chronic exposures to high or low salinity adversely affects endocrine balance, respiration, reproduction, and growth. *Ceriodaphnia dubia* used in WET tests are sensitive to ion imbalance (Ingersoll et al. 1992, Dwyer et al.1992, Mount et al. 1997) which can cause WET test failures.

Freshwater or effluent containing TDS at concentrations above approximately 1,340 mg/L or conductivity above 2,000 µS/cm, can adversely affect freshwater organisms (API 1998, Goodfellow 2000, Society of Environmental Toxicology and Chemistry, 2004). The brackish groundwater source for DVI contains 2,600 mg/L of TDS (Table 11), which is approximately twice the threshold concentration that could adversely affect freshwater organisms.

Several inorganic ions which are normally dissolved in water can cause toxicity when the concentrations or ratios of these ions exceed the normal tolerance range of an organism. Imbalances between major anions and cations can inhibit reproduction of sensitive invertebrate species including *Ceriodaphnia dubia*. The WET test method manual (EPA, 2002) identifies ionic imbalance as a well-known source of interference in the standard chronic toxicity test method.

The toxicity of fresh water with high TDS concentrations varies substantially depending on the specific combination of ions present and the water chemistry. For example, water containing 2,000 mg/L of TDS with chloride as the primary anion is acutely toxic to aquatic life, but the same TDS concentration composed of other ions may be nontoxic. For freshwater, the relative toxicity of single ions in decreasing order is (Mount et al., 1997):

$$K^{+} > HCO^{-3} = Mg^{2+} > Cl^{-} > SO_4^{-2}$$

Sodium salts are the least toxic of all major ions, but the toxicity of sodium salts varies depending on the associated anions. Water containing multiple cations (positively charged ions) tends to be less toxic than similar solutions that contain only one cation. TDS toxicity tends to decrease as the water hardness increases (Mount et al., 1997). The presence of simple dissolved organic compounds reduces the biological stress caused by ionic imbalance in the water.

Sulfate Toxicity

High sulfate concentrations cause acute toxicity to *Ceriodaphnia dubia* due to osmotic imbalance. The acute toxicity of sulfate to *Ceriodaphnia dubia* was assessed in 2008 to support updates of Illinois aquatic life criteria for sulfate (Iowa Department of Natural Resources, February 2009). The mean LC50 sulfate concentrations in moderately hard reconstituted water

(MHRW) are summarized in Table 13. Sulfate toxicity was affected by the chloride concentration and hardness of the water. Increasing hardness, tested at six levels ranging from 100 to 600 mg/L and at constant Ca:Mg ratios, decreased the toxicity of sodium sulfate (LC50s) in a linear fashion for *Ceriodaphnia dubia*. Sulfate toxicity decreased progressively (LC50s increased) for *Ceriodaphnia dubia* when chloride was increased from 100 to 500 mg/L.

Table 13 – Acute Sulfate Toxicity to *Ceriodaphnia dubia*

Sulfate LC50, mg/L	Hardness, mg/L (as $CaCO_3$)
2,050	100
2,526	
2,946	300
3,516	484

Source: Soucek, 2005.

British Columbia developed ambient water quality criteria for sulfate based on recent studies of sulfate toxicity over a range of water hardness for several freshwater aquatic organisms. Table 14 summarizes the acute and chronic sulfate toxicities for *Ceriodaphnia dubia* (BC Ministry of Environment, 2013). Chronic toxicity occurred at lower sulfate concentrations than acute toxicity. Organisms that survive the initial shock of acute exposure can therefore persist indefinitely at the same sulfate concentration.

The groundwater source at DVI contains approximately 230 mg/L of sulfate and 1,000 mg/L of chloride. The sulfate concentration is more than 6 times lower than the British Columbia acute sulfate toxicity LC50 at a hardness of 320 mg/L. This comparison indicates that sulfate toxicity is unlikely to be a potential cause of effluent toxicity at DVI.

Table 14 – British Columbia Estimates of Sulfate Toxicity to *Ceriodaphnia dubia*

Water Hardness, mg/L(as $CaCO_3$)	Survival Endpoint LC50		Reproduction Endpoint IC50	
	Benchmark dose, mg/L	95% Confidence Interval	Benchmark dose, mg/L	95% Confidence Interval
40	809	612 – 1,071	468	217 – 1009
80	1,282	962 – 1,708	1,119	911– 1,374
160	1,531	1,189 – 1,972	1,263	1,253 – 1,273
320	1,580	1,236 – 2,019	717	343 – 1,498

Source: Elphick et al. (2011). Benchmark dose (BMD) calculated by model averaging based on sulfate toxicity tests and water hardness.

Chloride Toxicity

Ceriodaphnia dubia are very susceptible to stress induced by chlorides. The brackish groundwater source for DVI contains approximately 1,000 mg/L of chloride which might cause or contribute to effluent chronic toxicity.

EPA established national water quality criteria for chloride for aquatic life protection based on sodium chloride toxicity (USEPA, 2010). However, potassium, calcium, and magnesium chlorides are more acutely toxic to aquatic organisms than sodium chloride. Canadian water quality guidelines established lower criteria for chloride toxicity to freshwater organisms based on tests with calcium chloride and sodium chloride.

Acute chloride toxicity for *Ceriodaphnia dubia* is shown in Table 16 from several sources. The acute chloride toxicity for the 48-hour LC50 test ranges from 1,080 mg/L to 1,356 mg/L. Variability of the LC50 concentration for chloride toxicity depends on water hardness, sulfate concentrations, and amount of other ions present in the test sample.

The groundwater source at DVI contains approximately 1,000 mg/L of chloride. When the reverse osmosis treatment system is out of service, the wastewater from DVI contains the TDS from the groundwater source plus additional TDS from domestic use of the water. Without reverse osmosis treatment of the groundwater, the chloride concentration in the wastewater from DVI exceeds the U.S. and Canadian national acute and chronic aquatic life criteria. In addition, the chloride concentration in the DVI wastewater may approach the 48-h LC50 for *Ceriodaphnia dubia*. Therefore chloride might be a potential cause of or contributor to failures of the chronic whole effluent toxicity test for *Ceriodaphnia dubia*.

Table 15 – Current National Standards for Chloride Freshwater Aquatic Life Criteria

Country	Chloride		Reference
	Acute, mg/L	Chronic, mg/L	
United States	860	230	USEPA 2010, National Recommended Water Quality Criteria.
Canada	640	120	Canadian Water Quality Guidelines for the Protection of Aquatic Life: Chloride. (Canadian Council of Ministers of the Environment, 2011)

Table 16 – Chloride Acute and Chronic Toxicity to *Ceriodaphnia dubia*

Concentration, mg/L			Exposure Time (hours)	Reference
LC50 Chloride	NaCl	Sulfate		
1,652	2,724		24	Cowgill and Milazzo, 1990
1,400	2,308		96	Cowgill and Milazzo, 1990
1,596	2,630		96	Wisconsin State Laboratory of Health1995
1,080			48	Canadian Council of Ministers of the Environment, 2011
1,356		25	48	USEPA, 2008
1,192		600	48	USEPA, 2008

Potassium and Magnesium Chloride

Potassium and magnesium chloride (KCl and MgCl2) salts are more toxic than calcium chloride and sodium chloride (CaCl2 and NaCl) (Mount et al1997). The toxic effects of calcium chloride and sodium chloride are likely due to the chloride anion. Toxic effects of potassium and magnesium chloride are due to the potassium and magnesium cations, rather than the chloride anion. Potassium toxicity is greater at low sodium background concentrations Toxicities of both sodium and magnesium salts are higher at low calcium background concentrations. The approximate order of chloride salt toxicity to freshwater organisms is:

KCl > MgCl2 > CaCl2 > NaCl

The DVI groundwater concentrations of potassium are relatively low (8 mg/L) which makes potassium chloride toxicity an unlikely cause of WET test failures. Magnesium concentrations in the DVI source water are approximately half of the sodium concentrations which makes magnesium chloride toxicity unlikely to cause of WET test failures at DVI.

Bicarbonate

Multiple acute toxicity studies have calculated the LC_{50} of bicarbonate between 699 and 827 mg/L. The relative order of toxicity for bicarbonate, sulfate and chloride is:

$$HCO^{-3} > SO_4^{-2} > Cl^-$$

Table 17 – Bicarbonate Acute Toxicity to *Ceriodaphnia dubia*

Bicarbonate LC50, mg/L	Source
699	Mount et al., 1997
740	Harper et al., 2014
827	Johnson, 2014

Significantly increasing the ratio of alkalinity (bicarbonate) to hardness is known to inhibit reproduction in *Ceriodaphnia dubia*. Normally, the ratio between alkalinity and hardness is approximately 1:1. Sodium bicarbonate impairs *C. dubia* reproduction with an IC10 of 340 mg/L.

The DVI groundwater concentrations of bicarbonate are low and the DVI WWTF effluent bicarbonate concentrations are probably as well due to consumption of carbonate during biological nitrification. Therefore, bicarbonate appears unlikely to cause of WET test failures at DVI.

Effect of Ion Combinations

The toxicity of TDS depends on the specific combinations and concentrations of each ion. When different ions are combined, the toxicity of the mixture may be less than or greater than the sum of the toxicities of the individual ions in the mixture. For example, NaCl and $CaCl_2$ had similar toxicities based on the chloride anion, but combinations of these salts were significantly less toxic than either salt alone (Mount et al., 1997).

Although chloride ion toxicity appears to be more likely at DVI than toxicity due to other ions, the combined effect of multiple ions might be greater than toxicity of chloride alone. The brackish groundwater source for DVI contains 2,600 mg/L of TDS, which is approximately twice the threshold concentration that could adversely affect freshwater organisms.

Hardness

The current discharge permit for DVI indicates that effluent hardness for Discharge Point 001 ranged from 564 mg/L to 590 mg/L, based on five samples from 2010 to 2013. Based on these samples, the permit used a default minimum effluent hardness of 400 mg/L to calculate discharge limits for metals. Relatively high effluent hardness would decrease ion toxicity for sulfate and other ions.

SUMMARY AND CONCLUSIONS

During the TRE, the source of chronic toxicity in the effluent discharged to the Deuel Drain and all permit violations that had occurred at effluent discharge point EFF-001 or during 2014 were evaluated including:

1. Chronic toxicity (EFF-001)
2. High effluent nitrate concentrations (EFF-001)
3. Receiving water (Deuel Drain) pH increase of more than 0.5 units (RSW-002)
4. Excessive surface water temperature increase below the effluent discharge (RSW-002)

The TRE identified probable and potential causes of these violations as summarized in Table 18 and recommended corrective actions to prevent similar future occurrences.

Table 18 – 2015 TRE Results

Item	Location	Parameter	Number of Violations	Probable Source	Other Potential Source
1	EFF-001	Chronic toxicity	3	High effluent TDS	High HCO3 dose
2	EFF-001	Nitrate + nitrite	3	Hydraulic loading	Anoxic mixer failure
3	RSW-002	pH rise	2	Interpretation error	Algae in stream
4	RSW-002	Temperature rise	5	Low cooling capacity	Instrument failure

Proposed Actions to Improve Reliability

High TDS concentrations (from the brackish groundwater source) cause chronic toxicity in the WWTF effluent when the reverse osmosis (RO) and zero liquid discharge (ZLD) systems which supply drinking water are out of service. To keep the RO-ZLD system in operation, new standby equipment will be installed for critical components. Specific equipment upgrades to maintain reliable operation of the RO-ZLD system will be developed in a separate report.

To improve reliability at the WWTF, as needed CDCR will replace equipment including the membrane filter modules. CDCR plans to modify operations to balance peak hydraulic loads. CDCR plans to modify the cooling tower controls and equipment to increase the cooling range to achieve compliance with the temperature rise limitation.

CDCR will develop a spare parts inventory for critical equipment for both the RO-ZLD system and the WWTF to minimize time needed for repairs and to minimize delays for long-lead time delivery and procurement procedures. Proposed new and modified equipment and spare parts for critical equipment are summarized in Table 19.

Table 19 – Equipment Replacement and On-site Spare Parts Storage

WWTF Equipment	Capacity	Function
Replace MBR filters	2.9 mgd	Restore original capacity
New hoist for filter removal		Routine MBR filter maintenance
Effluent cooling upgrades	20^{O} F decrease	Increase cooling range, modify controls
Spare parts on-site storage		Critical equipment and parts ready to install

Conclusions

CDCR constructed and operates a technically complex drinking water system which includes reverse osmosis (RO) and a prototype zero liquid discharge (ZLD) system. This RO-ZLD system allows brackish groundwater, the only available raw water source, to be used beneficially. The RO-ZLD system is the prime source of wastewater effluent chronic toxicity due to the operation and maintenance challenges of the equipment. CDCR will continue to operate and maintain this water treatment system while also complying with all environmental and water quality criteria.

This TRE identified the probable sources of toxicity and all NPDES permit violations which occurred during 2014. CDCR will strive to eliminate all of the sources of toxicity and other violations identified in this TRE. Whereas chronic toxicity has not recurred since the end of accelerated monitoring in February 2015, and the probable sources of toxicity have been identified, and CDCR has committed to eliminate these sources of toxicity as well as other permit compliance issues, DVI should be permitted to exit the TRE.

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APPENDIX A

Toxicity Criteria and Terminology

Acute Toxicity Criteria: 0.3 toxicity unit (0.3 TUa).

Chronic Toxicity Criteria: 1.0 toxicity unit (1.0 TUc).

NOAEC: Highest tested concentration of an effluent or a toxicant at which no adverse effects are observed on the aquatic test organisms at a specific time of observation.

LC50: Lethal Concentration that is the point estimate of the toxicant concentration that would be lethal to 50% of the test organisms during a specific period, usually 48 or 96 hours.

IC25: The inhibition concentration that is a point estimate of the toxicant concentration that would cause a 25% reduction in a nonlethal biological measurement of the test organisms, such as reproduction or growth.

Toxic Unit Acute (TUa): A dimensionless mathematical conversion of LC50 into a regulatory limit. TUa = 100/LC50.

Toxic Unit Chronic (TUc): A dimensionless mathematical conversion of an NOEC or NOEL into a regulatory limit. TUc = 100/NOEC.

Hardness: Sum of calcium and magnesium concentrations, both expressed as calcium carbonate, in milligrams per liter. Hardness mitigate the toxicity of many metals to aquatic life. (Standard Methods).

APPENDIX B

DVI Influent & Effluent Data 2014

Date	Flow, mgd		BOD5, mg/L		TSS, mg/L		Ammonia, Total (N), mg/L	Nitrate, Total (N), mg/L
	Influent	Effluent	Influent	Effluent	Influent	Effluent	Effluent	Effluent
2-Jan	0.453	0.441	130	0	780	0		
6-Jan	0.437	0.442	170	2.8	450	0	0.14	
8-Jan	0.429	0.475	76	0	760	2.6		
14-Jan	0.406	0.456	490	0	830	0.8	0.26	
16-Jan	0.417	0.427	180	2	250	0.8		
20-Jan	0.407	0.438	330	2	690	0	0.28	
22-Jan	0.417	0.441	260	0	510	1.4		
28-Jan	0.424	0.454	150	0	180	0	0.10	
30-Jan	0.421	0.470	210	0	430	10		
3-Feb	0.440	0.448	160	0	290	4.6	0.21	
6-Feb	0.415	0.473	68	0	70	0		
11-Feb	0.453	0.473	120	0	120	0	0.14	
13-Feb	0.503	0.562	56	0	66	0.8		
18-Feb	0.526	0.562	250	0	270	0	0.10	
20-Feb	0.444	0.472	190	0	310	0.6		
25-Feb	0.458	0.506	320	0	420	5.1	0.18	
27-Feb	0.430	0.485	100	0	150	0		
4-Mar	0.404	0.487	140	0	36	0	0.14	
6-Mar	0.450	0.508	490	0	440	0		
11-Mar	0.489	0.557	400	0	14	2	0.07	
13-Mar	0.490	0.525	270	0	500	3.8		
18-Mar	0.556	0.664	56	0	140	1.4	0.07	
20-Mar	0.477	0.516	200	0	43	1.6		
25-Mar	0.477	0.515	99	0	62	0.8	0.10	
27-Mar	0.481	0.522	100	0	170	0		
1-Apr	0.476	0.499	290	0	370	0	0.14	
10-Apr	0.475	0.496	150	0	86	0	0.14	
15-Apr	0.464	0.484	280	0	290	1	0.21	
23-Apr	0.498	0.538	160	0	120	0.3	0.14	
29-Apr	0.483	0.503	130	0	71	0.7	0.18	
8-May	0.480	0.488	180	0	150	0	0.21	
13-May	0.492	0.512	270	0	410	0	0.10	
22-May	0.433	0.519	59	0	190	0	0.18	
27-May	0.466	0.481	110	3.0	170	1.0	0.18	
3-Jun	0.459	0.508	180	2.6	150	0	0.10	
10-Jun	0.485	0.498	150	2.2	260	0	0.24	
17-Jun	0.473	0.513	230	0.0	480	0	0.00	

DVI Influent & Effluent Data 2014

Date	Flow, mgd		BOD5, mg/L		TSS, mg/L		Ammonia, Total (N), mg/L	Nitrate, Total (N), mg/L
	Influent	Effluent	Influent	Effluent	Influent	Effluent	Effluent	Effluent
24-Jun	0.515	0.521	170	0.0	260	0	0.32	
1-Jul	0.512	0.518	120	0	89	4.5	0.1	
8-Jul	0.446	0.478	210	2	340	0	0	
15-Jul	0.452	0.412	160	0	220	0	0	
22-Jul	0.477	0.517	160	0	350	0	0	
31-Jul	0.526	0.557	98	0	110	2.7	0	
4-Aug	0.506	0.565	130	0	270	0	0	
12-Aug	0.591	0.623	170	0	97	1.8	0	
19-Aug	0.574	0.556	100	0	110	1.1	0	
26-Aug	0.512	0.601	87	0	170	0	0	
2-Sep	0.574	0.606	100	0	160	1.7	0	
9-Sep	0.512	0.522	63	0	58	0	0	
16-Sep	0.499	0.552	80	0	51	0	0	
23-Sep	0.488	0.534	140	0	170	0	0	
30-Sep	0.456	0.516	120	0	180	0	0	
9-Oct	0.460	0.465	100	0	92	0	0	
14-Oct	0.456	0.465	130	0	190	0	0	
21-Oct	0.255	0.271	150	0	210	0	0	
4-Nov	0.374	0.428	110	0	330	1.4	0	
12-Nov	0.359	0.407	280	0	530	1.8	0	
18-Nov	0.373	0.427	200	0	460	1.2	0	
24-Nov	0.426	0.407	240	0	750	1.2	0	
2-Dec	0.433	0.514	140	0	390	1.3	0	
9-Dec	0.385	0.432	250	0	710	0	0	
16-Dec	0.408	0.498	200	0	770	1.1	0	
22-Dec	0.390	0.543	170	0	980	0	0	
29-Dec	0.421	0.439	140	0	180	0	0	
Annual Avg	**0.459**	**0.496**	**175**	**0.26**	**296**	**0.92**	**0.079**	

APPENDIX C

Groundwater Test 1st Quarter – March 5, 2013

Well Number	GW-001	GW-002	GW-003	GW-004	GW-005	GW-006	Average
Ammonia, Total (N), mg/L	0.119	0.284	0.202	0.503	0.144	0.084	0.223
TDS, mg/L	2370	1830	1930	1950	2200	2800	2,180
Nitrate, Total (N), mg/L	0.99	2.68	0.38	0.17	0.13	0.57	0.82
Total Coliform, #/100 mL	2	8	220	38.9	2	8	46.5
pH, Std. units	7.37	7.64	7.44	7.68	7.32	7.66	7.52
Electrical Conductivity @ 25 Deg. C, µmhos/cm	3351	2763	2877	2542	3014	2613	2,860

Groundwater Test 2nd Quarter – April 30, 2013

Well Number	GW-001	GW-002	GW-003	GW-004	GW-005	GW-006	Average
Ammonia, Total (N), mg/L	0.428	0.947	0.541	1.25	0.38	0.604	0.692
TDS, mg/L	2700	1830	1910	2220	2260	2620	2,257
Nitrate, Total (N), mg/L	12.8	0.81	0.47		0.16		2.37
Total Coliform, #/100 mL	2	2	2	2	2	2	2.0
pH, Std. units	7.32	7.59	7.42	7.6	7.31	7.64	7.48
Electrical Conductivity @ 25 Deg. C, µmhos/cm	2851	2673	2490	2577	2956	2450	2,666

Groundwater Test 3rd Quarter – July 23, 2013

Well Number	GW-001	GW-002	GW-003	GW-004	GW-005	GW-006	Average
Ammonia, Total (N), mg/L	ND	0.243	0.145	0.814	0.104	0.085	0.278
TDS, mg/L	2330	1690	585	1580	1910	2580	1,779
Nitrate, Total (N), mg/L	1.18	0.34	0.3	0.14	0.17		0.36
Total Coliform, #/100 mL	2	2	17	2	2	2	4.5
pH, Std. units	7.3	7.4	7.1	7.5	7.2	7.5	7.33
Electrical Conductivity @ 25 Deg. C, µmhos/cm	2634	2551	2440	2631	2430	2060	2,458

Groundwater Test 4th Quarter – October 30, 2013

Well number	GW-001	GW-002	GW-003	GW-004	GW-005	GW-006	Average
Ammonia, Total (N), mg/L	0.2	0.233	0.448	0.18	0.277	0.113	0.242
TDS, mg/L	2080	1520	1290	1950	1650	2400	1,815
Nitrate, Total (N), mg/L	1.26	0.37	0.15	0.66		0.13	0.43
Total Coliform, #/100 mL	2	2	2	2	2	2	2.0
pH, Std. units	7.3	7.3	7.1	7.3	7.2	7.4	7.27
Electrical Conductivity @ 25 Deg. C, µmhos/cm	2491	2493	2270	2450	2070	2104	2,313

Groundwater Test 1st Quarter – February 10, 2014

Well Number	GW-001	GW-002	GW-003	GW-004	GW-005	GW-006	Average
Ammonia, Total (N), mg/L	ND	0.41	0.16	0.731	0.286	0.115	0.340
TDS, mg/L	1580	1550	1890	2350	1940	2620	1,988
Nitrate, Total (N), mg/L	1.31	0.18	0.53	0.09	0.18	0.49	0.46
Total Coliform, #/100 mL	2	ND	ND	ND	ND	8	5.0
pH, Std. units	7.2	7.2	7.1	7.3	7.2	7.4	7.23
Electrical Conductivity @ 25 Deg. C, µmhos/cm	2106	2290	2003	2293	2140	2203	2,173

Groundwater Test 2nd Quarter – June 16, 2014

Well Number	GW-001	GW-002	GW-003	GW-004	GW-005	GW-006	Average
Ammonia, Total (N), mg/L	ND	ND	ND	0.361	ND	ND	0.361
TDS, mg/L	2380	1710	1840	1900	1740	2710	2,047
Nitrate, Total (N), mg/L	7.04	0.82	ND	0.52	ND	0.15	2.13
Total Coliform, #/100 mL	< 2	2	< 2		< 2	< 2	2.0
pH, Std. units	7.2	7.2	7.1	7.3	7.1	7.3	7.20
Electrical Conductivity @ 25 Deg. C, µmhos/cm	1870	2040	2083	2190	2255	2092	2,088

Farhad, Mohammad@Waterboards

From: Daniel Mullins <daniel.mullins@cdcr.ca.gov>
Sent: Friday, April 24, 2015 1:25 PM
To: Farhad, Mohammad@Waterboards
Cc: Bettencourt, Miles (Terry)@CDCR
Subject: FW: Alpha Labs: Draft results of the toxicity tests performed on the Deuel effluent samples collected 4/13, 4/15, and 4/17, 2015

Below is the Draft Results for the Acute and Chronic sampled on April 13, 2015, all passed but the Ceriodaphnia Reproduction, the RO plant went on line April 23, 2015

Daniel G Mullins
Water & Wastewater Treatment Plant Supervisor
Deuel Vocational Institution
23500 kasson Rd Tracy CA 95376
Office-(209) 835-4141 x5897
Fax-(209) 830-3941

From: David Pingatore [mailto:david@alpha-labs.com]
Sent: Thursday, April 23, 2015 5:00 PM
To: 'Padrick Anderson'
Cc: Mullins, Daniel@CDCR
Subject: RE: Alpha Labs: Draft results of the toxicity tests performed on the Deuel effluent samples collected 4/13, 4/15, and 4/17, 2015

Great – thank you.

From: Padrick Anderson [mailto:panderson@pacificecorisk.com]
Sent: Thursday, April 23, 2015 4:45 PM
To: David Pingatore
Cc: Stephen Clark
Subject: Alpha Labs: Draft results of the toxicity tests performed on the Deuel effluent samples collected 4/13, 4/15, and 4/17, 2015

Hello David,

The acute and chronic toxicity tests of the Deuel Vocational Institution effluent samples collected 4/13, 4/15, and 4/17 have terminated. Due to the high conductivity of the effluent (~4200 µS/cm), we also set up conductivity controls with each chronic test to assess if the conductivity could be contributing to toxicity. The results are as follows:

Acute Fathead Minnow
Survival (%)
Lab Water Control = 100%
100% effluent = 100%

NOEC = 100% effluent
TUa (100/NOEC) = 1

Chronic *Selenastrum capricornicum*

Algal Growth (cells/mL x 10^6)
Lab Water Control = 7.57
Conductivity Control = 6.36
100% effluent = 7.53

NOEC = 100% effluent
TUc (100/NOEC) = 1

Chronic *Ceriodaphnia dubia*
Survival (%)
Lab Water Control = 100%
Conductivity Control = 100%
12.5% effluent = 90%
25% effluent = 100%
50% effluent = 100%
75% effluent = 100%
100% effluent = 100%

NOEC = 100% effluent
EC25 = >100% effluent
EC50 = >100% effluent
TUc (100/NOEC) = 1.0
TUc (100/EC25) = <1.0
TUc (100/EC50) = <1.0

Reproduction (mean neonates/female)
Lab Water Control = 38.3
Conductivity Control = 16.8
12.5% effluent = 27.9
25% effluent = 34.1
50% effluent = 33.6
75% effluent = 21.3
100% effluent = 10.6

NOEC = 50% effluent
IC25 = 57.5% effluent
IC50 = 80% effluent
TUc (100/NOEC) = 2
TUc (100/IC25) = 1.7
TUc (100/IC50) = 1.3

Chronic Fathead Minnow
Survival (%)
Lab Water Control = 90%
Conductivity Control = 95%
100% effluent = 100%

NOEC = 100% effluent
TUc (100/NOEC) = 1.0

Mean Biomass (mg)
Lab Water Control = 0.46
Conductivity Control = 0.57
100% effluent = 0.60

NOEC = 100% effluent
TUc (100/NOEC) = 1.0

Please let my colleague Stephen Clark or me know if you have any questions.

Thank you,

Padrick Anderson
Senior Aquatic Ecotoxicologist
Pacific EcoRisk
2250 Cordelia Road
Fairfield, CA 94534
(707) 207-7775
(707) 207-7916 (fax)

Farhad, Mohammad@Waterboards

From: Mullins, Daniel@CDCR <Daniel.Mullins@cdcr.ca.gov>
Sent: Thursday, September 03, 2015 12:06 PM
To: WB-RB5S-CentralValleySacramento
Cc: Rodriguez, Jaime (DVI)@CDCR; Farhad, Mohammad@Waterboards; Holmes, Kari@Waterboards; Vasconcellos, Edward@CDCR
Subject: Chronic three species Prelim
Attachments: Bioassy prelim 1.pdf

On August 10, 2015 the RO Water Treatment plant shut down for maintenance, as required by the Cleanup and abatement order R5-2015-0703 in the event the RO plant is taken off line for more than seven days and beginning on the eighth day after the RO plant is taken off-line, the discharger shall conduct three species chronic toxicity testing, to determine whether the effluent is contributing chronic toxicity to the receiving water due to RO plant being off line. Attached is a preliminary for the three species chronic toxicity testing sampled on August 17, 2015. The final report will be forwarded and attached to the monthly eSMR report for August 2015.

Date of Title of Submittal	September 3, 2015 – Monthly Report R5-2014-0014-01
Regulatory Program	NPDES
Unit	Compliance and Enforcement
Regulated Party (Discharger)	California Department of Corrections and Rehabilitation
Facility Name	Deuel Vocational Institution
County	San Joaquin

Daniel G Mullins
Water & Wastewater Treatment Plant Supervisor
Deuel Vocational Institution
23500 kasson Rd Tracy CA 95376
Office-(209) 835-4141 x5897
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Mullins, Daniel@CDCR

From: David Pingatore <david@alpha-labs.com>
Sent: Monday, August 24, 2015 2:11 PM
To: Mullins, Daniel@CDCR; 'Violet Renick'
Subject: FW: DVI Final Effluent Prelim Results

Hi Daniel, please see below:

From: Violet Renick [mailto:violet@nautilusenvironmental.com]
Sent: Monday, August 24, 2015 1:59 PM
To: David Pingatore
Subject: DVI Final Effluent Prelim Results

Hi David,

I am beginning to review the DVI toxicity test data, and wanted to give you a heads up that there is a significant effect in the effluent sample for the green algae toxicity test (TUc > 1). Additionally, it looks as though there will also be a significant hit in the effluent sample for the chronic water flea test (which ends tomorrow). The chronic fathead minnow and water flea tests will end tomorrow and I will gather and analyze that data as soon as I can.

At this point the fathead minnow acute and chronic tests look as though there are no significant effects. I will keep you posted on the remaining results. And as far as I can see, the concurrent reference toxicant tests look valid.

Keep in mind these analyses are preliminary at this stage; the data have not yet been through our secondary QC review. However, I will let you know if anything changes as soon as I know.

One last thing - do you know if DVI has a completed and/or approved TRE work plan? If it exists and you have access to it, could you please forward it my way so that I can be prepared for future toxicity testing scenarios in case of repeated toxicity?

Please let me know how you would like to proceed, and if would like to discuss the results at any point.

Thanks!

Violet

--
Violet Renick, Ph.D.
Environmental Scientist
Nautilus Environmental
4340 Vandever Avenue
San Diego, California 92120

Office: (858) 587-7333 x206
Cell: (619) 807-6019

Farhad, Mohammad@Waterboards

From: Wyels, Wendy@Waterboards
Sent: Wednesday, September 09, 2015 2:44 PM
To: Holmes, Kari@Waterboards; Farhad, Mohammad@Waterboards
Subject: FW: Chronic three species Prelim
Attachments: Bioassy prelim 1.pdf

From: Daniel Mullins [mailto:daniel.mullins@cdcr.ca.gov]
Sent: Wednesday, September 09, 2015 2:26 PM
To: Wyels, Wendy@Waterboards
Cc: Rodriguez, Jaime (DVI)@CDCR
Subject: FW: Chronic three species Prelim

Here is the preliminary for the Acute and chronic toxicity sampled on August 17, 2015, I am waiting for the final results as indicated in attached email.

Daniel G Mullins
Water & Wastewater Treatment Plant Supervisor
Deuel Vocational Institution
23500 kasson Rd Tracy CA 95376
Office-(209) 835-4141 x5897
Fax-(209) 830-3941

From: Mullins, Daniel@CDCR
Sent: Thursday, September 03, 2015 12:06 PM
To: WB-RB5S-CentralValleySacramento
Cc: Rodriguez, Jaime (DVI)@CDCR; Farhad, Mohammad@Waterboards (Mohammad.Farhad@waterboards.ca.gov); Kari R. Holmes (kari.holmes@waterboards.ca.gov); Vasconcellos, Edward@CDCR
Subject: Chronic three species Prelim

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Date of Title of Submittal	September 3, 2015 – Monthly Report R5-2014-0014-01
Regulatory Program	NPDES
Unit	Compliance and Enforcement
Regulated Party (Discharger)	California Department of Corrections and Rehabilitation
Facility Name	Deuel Vocational Institution
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Mullins, Daniel@CDCR

From: David Pingatore <david@alpha-labs.com>
Sent: Monday, August 24, 2015 2:11 PM
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Subject: FW: DVI Final Effluent Prelim Results

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Subject: DVI Final Effluent Prelim Results

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At this point the fathead minnow acute and chronic tests look as though there are no significant effects. I will keep you posted on the remaining results. And as far as I can see, the concurrent reference toxicant tests look valid.

Keep in mind these analyses are preliminary at this stage; the data have not yet been through our secondary QC review. However, I will let you know if anything changes as soon as I know.

One last thing - do you know if DVI has a completed and/or approved TRE work plan? If it exists and you have access to it, could you please forward it my way so that I can be prepared for future toxicity testing scenarios in case of repeated toxicity?

Please let me know how you would like to proceed, and if would like to discuss the results at any point.

Thanks!

Violet

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Cell: (619) 807-6019

Farhad, Mohammad@Waterboards

From: Daniel Mullins <daniel.mullins@cdcr.ca.gov>
Sent: Friday, September 11, 2015 8:13 AM
To: Farhad, Mohammad@Waterboards; Holmes, Kari@Waterboards; Wyels, Wendy@Waterboards
Subject: additional toxicity screening
Attachments: DVI - Alpha Analytics EC Control Quote.pdf

We spoke about this additional EC screening when the RO plant is down, since this additional cost is not on the state contract with the lab the only way I can get this done is if there is a change in the discharge requirements, just need an email so I can submit to procurement for approval to conduct this screening next time the RO plant is down.
See attached
Thanks

Daniel G Mullins
Water & Wastewater Treatment Plant Supervisor
Deuel Vocational Institution
23500 kasson Rd Tracy CA 95376
Office-(209) 835-4141 x5897
Fax-(209) 830-3941

Nautilus Environmental

September 10, 2015

Mr. David Pingatore
Alpha Analytical Laboratories, Inc.
6398 Dougherty Road, Suite 35
Dublin, CA 94568
Submitted via email to: david@alpha-labs.com

SUBJECT: Updated Quote for Deuel Vocational Institution – Chronic Water Flea Toxicity Testing

Thank you for the opportunity to provide an additional quote in support of the Deuel Vocational Institution (DVI) National Pollutant Discharge Elimination System (NPDES) whole effluent toxicity (WET) testing program. We have observed reduced reproductive output of the water flea (*Ceriodaphnia dubia*) in effluent samples with electrical conductivity (EC) greater than 2,000 µmhos/cm. Therefore, we propose including an EC control treatment alongside standard chronic water flea tests in circumstances where DVI effluent EC is greater than 2,000 µmhos/cm. This additional treatment would be prepared by increasing the EC of laboratory control water to match that of the effluent (± 10 percent), with the goal of demonstrating that comparable rates of decreased water flea reproduction could be linked to increased EC.

We have included below unit test costs for standard water flea tests and tests including the additional EC control treatment at an additional 20 percent of test cost.

Test Species	Scope of Testing	Unit Test Cost
Chronic Testing *Ceriodaphnia dubia*	Standard design: Laboratory Control and 100% effluent	$975
Chronic Testing *Ceriodaphnia dubia*	Modified design: Laboratory Control, EC Control, and 100% effluent	$1,170

If you have any questions please let us know. Thank you for your consideration.

Violet Renick

Violet Renick, Environmental Scientist
violet@nautilusenvironmental.com
(858) 587-7333 x206

California
4340 Vandever Avenue
San Diego, CA 92120
858-587-7333
fax: 858-587-3961

British Columbia
8664 Commerce Court
Burnaby, British Columbia
V5A 4N7
604-420-8773
fax: 604-603-9381

Farhad, Mohammad@Waterboards

From: Mullins, Daniel@CDCR <Daniel.Mullins@cdcr.ca.gov>
Sent: Friday, September 11, 2015 9:21 AM
To: Wyels, Wendy@Waterboards; Farhad, Mohammad@Waterboards; Holmes, Kari@Waterboards
Subject: RE: additional toxicity screening
Attachments: Bioassy prelim 1.pdf

Page 8. 2. B.
We did sample on the 8th day for the Acute and Chronic Toxicity, and the prelim showing a toxicity for the Algae and the water flea. The cleanup and abatement order does not state any further action if the test shows a toxicity.
The failures are most likely due to Electrical conductivity according to the past Toxic Reduction Evaluation conclusions, our contract lab did recommend an additional screening that can be done to verify Electrical conductivity is the toxicity.
I would like to know if there is any further action necessary on our part here at the wastewater treatment plant when there is a toxicity failure while the RO water treatment plant is down?
Thanks

Daniel G Mullins
Water & Wastewater Treatment Plant Supervisor
Deuel Vocational Institution
23500 kasson Rd Tracy CA 95376
Office-(209) 835-4141 x5897
Fax-(209) 830-3941

From: Wyels, Wendy@Waterboards [mailto:Wendy.Wyels@waterboards.ca.gov]
Sent: Friday, September 11, 2015 8:17 AM
To: Mullins, Daniel@CDCR; Farhad, Mohammad@Waterboards; Holmes, Kari@Waterboards
Subject: RE: additional toxicity screening

Daniel,
This is required by the Cleanup and Abatement Order (attached). It's been in effect since March 3, 2015.

Wendy

From: Daniel Mullins [mailto:daniel.mullins@cdcr.ca.gov]
Sent: Friday, September 11, 2015 8:13 AM
To: Farhad, Mohammad@Waterboards; Holmes, Kari@Waterboards; Wyels, Wendy@Waterboards
Subject: additional toxicity screening

We spoke about this additional EC screening when the RO plant is down, since this additional cost is not on the state contract with the lab the only way I can get this done is if there is a change in the discharge requirements, just need an email so I can submit to procurement for approval to conduct this screening next time the RO plant is down.
See attached
Thanks

Daniel G Mullins
Water & Wastewater Treatment Plant Supervisor
Deuel Vocational Institution
23500 kasson Rd Tracy CA 95376
Office-(209) 835-4141 x5897

Fax-(209) 830-3941

Mullins, Daniel@CDCR

From: David Pingatore <david@alpha-labs.com>
Sent: Monday, August 24, 2015 2:11 PM
To: Mullins, Daniel@CDCR; 'Violet Renick'
Subject: FW: DVI Final Effluent Prelim Results

Hi Daniel, please see below:

From: Violet Renick [mailto:violet@nautilusenvironmental.com]
Sent: Monday, August 24, 2015 1:59 PM
To: David Pingatore
Subject: DVI Final Effluent Prelim Results

Hi David,

I am beginning to review the DVI toxicity test data, and wanted to give you a heads up that there is a significant effect in the effluent sample for the green algae toxicity test (TUc > 1). Additionally, it looks as though there will also be a significant hit in the effluent sample for the chronic water flea test (which ends tomorrow). The chronic fathead minnow and water flea tests will end tomorrow and I will gather and analyze that data as soon as I can.

At this point the fathead minnow acute and chronic tests look as though there are no significant effects. I will keep you posted on the remaining results. And as far as I can see, the concurrent reference toxicant tests look valid.

Keep in mind these analyses are preliminary at this stage; the data have not yet been through our secondary QC review. However, I will let you know if anything changes as soon as I know.

One last thing - do you know if DVI has a completed and/or approved TRE work plan? If it exists and you have access to it, could you please forward it my way so that I can be prepared for future toxicity testing scenarios in case of repeated toxicity?

Please let me know how you would like to proceed, and if would like to discuss the results at any point.

Thanks!

Violet

--
Violet Renick, Ph.D.
Environmental Scientist
Nautilus Environmental
4340 Vandever Avenue
San Diego, California 92120

Office: (858) 587-7333 x206
Cell: (619) 807-6019

Farhad, Mohammad@Waterboards

From: Wyels, Wendy@Waterboards
Sent: Thursday, September 17, 2015 9:52 AM
To: WB-RB5S-CentralValleySacramento
Subject: FW: Toxicity Final report
Attachments: 18-25 August 2015 Acute and Chronic 15H1526BIO.pdf

---

From: Daniel Mullins [mailto:daniel.mullins@cdcr.ca.gov]
Sent: Tuesday, September 15, 2015 7:56 AM
To: Farhad, Mohammad@Waterboards; Holmes, Kari@Waterboards; Wyels, Wendy@Waterboards
Cc: Rodriguez, Jaime (DVI)@CDCR; Vasconcellos, Edward@CDCR
Subject: Toxicity Final report

Date of Title of Submittal	August 18-25, 2015
Regulatory Program	NPDES R5-2014-0014-01
Unit	Compliance and Enforcement
Regulated Party (Discharger)	California Department of Corrections and Rehabilitation
Facility Name	Deuel Vocational Institution
County	San Joaquin

A series of freshwater bioassay tests using green algae (*Raphidocelis subcapitata*), fathead minnow (*Pimephales promelas*), and water flea (*Ceriodaphnia dubia*) was performed on samples collected from the California Department of Corrections and Rehabilitation (CDCR) Deuel Vocational Institution (DVI) wastewater treatment facility. Tests were conducted following the shut-down of the reverse osmosis (RO) water treatment plant on August 10, 2015. Testing was conducted according to the Cleanup and Abatement Order R5-2015-0704 (RWQCB 2015) and National Pollutant Discharge Elimination System (NPDES) Permit No. CA0078093, Order No. R5-2014-0014 (RWQCB 2014). Testing was conducted at Nautilus Environmental (Nautilus) between August 18 and 25, 2015.

Significant decreases in green algae growth (34.0 percent effect) and water flea reproduction (60.6 percent effect) were observed in the undiluted final effluent relative to the lab control. However, no significant effects to water flea survival or fathead minnow growth or survival in the acute and chronic tests were observed.

Daniel G Mullins
Water & Wastewater Treatment Plant Supervisor
Deuel Vocational Institution
23500 kasson Rd Tracy CA 95376
Office-(209) 835-4141 x5897
Fax-(209) 830-3941

Nautilus Environmental

Toxicity Testing Results for Deuel Vocational Institution

Test Period: August 18 – 25, 2015

Prepared for: Alpha Analytical Laboratories, Inc.
6398 Dougherty Road, Suite 35
Dublin, CA 94568

Prepared by: Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120
(858) 587-7333

Data Quality Assurance:

- Nautilus Environmental is accredited in accordance with NELAP by the State of Oregon Environmental Laboratory Accreditation Program (Certificate No. 4053-002). It is also certified by the State of California Water Resources Control Board Environmental Laboratory Accreditation Program (Certificate No. 1802) and the State of Washington Department of Ecology (Lab ID C552). Specific fields of testing applicable to each accreditation are available upon request.
- All data have been reviewed and verified.
- All test results have met minimum test acceptability criteria under their respective EPA protocols, unless otherwise noted in this report.
- All test results have met internal Quality Assurance Program requirements.

Verified by: _____ **Date:** 9/9/2015

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San Diego, California 92120
858.587.7333
fax: 858.587.3961

British Columbia
8664 Commerce Court
Burnaby, British Columbia
V5A 4N7
604-420-8773
fax: 604-603-9381

INTRODUCTION

A series of freshwater bioassay tests using green algae (*Raphidocelis subcapitata*), fathead minnow (*Pimephales promelas*), and water flea (*Ceriodaphnia dubia*) was performed on samples collected from the California Department of Corrections and Rehabilitation (CDCR) Deuel Vocational Institution (DVI) wastewater treatment facility. Tests were conducted following the shut-down of the reverse osmosis (RO) water treatment plant on August 10, 2015. Testing was conducted according to the Cleanup and Abatement Order R5-2015-0704 (RWQCB 2015) and National Pollutant Discharge Elimination System (NPDES) Permit No. CA0078093, Order No. R5-2014-0014 (RWQCB 2014). Testing was conducted at Nautilus Environmental (Nautilus) between August 18 and 25, 2015.

MATERIALS AND METHODS

Test Material

Test material for the acute fathead minnow test included one grab sample of wastewater effluent collected from DVI on August 17, 2015. A 24-hour composite effluent sample for initiation of chronic tests was also collected on August 17, 2015. Additional composite samples were collected on August 19 and 21, 2015, and were used for renewal of the chronic fathead minnow and water flea tests. Collection was conducted under the direction of Mr. David Pingatore of Alpha Analytical Laboratories, Inc., and the samples were shipped overnight to Nautilus. A summary of the sample collection and receipt times is provided in Table 1.

Table 1. Sample Collection and Receipt Information

Sample ID	Method of Collection	Sample Collection Dates, Times	Sample Receipt Dates, Times
Final Effluent	Grab	8/17/2015, 08:35	8/18/2015, 10:26
	24-hour Composite	8/17/2015, 08:30 8/19/2015, 10:51 8/21/2015, 10:40	8/18/2015, 10:26 8/20/2015, 08:56 8/22/2015, 08:53

Upon arrival at Nautilus, an aliquot was drawn from each sample to measure temperature, pH, dissolved oxygen (DO), conductivity, alkalinity, and hardness. Testing was initiated the day the samples were received and the remaining sample volume was stored in the dark at 4°C until used for daily renewals. Sample receipt information is provided in Appendix A, and copies of the chain of custody (COC) forms are presented in Appendix B.

Test Methods

Chronic toxicity testing was conducted according to USEPA (2002a). Acute toxicity testing was conducted according to procedures presented by USEPA (2002b).

Green Algae Toxicity Test Specifications

Test Period:	8/18/2015, 15:15 – 8/22/2015, 13:00
Test Organism:	*Raphidocelis subcapitata (*green algae; *aka Selenastrum capricornutum)*
Test Organism Source; Age:	In-house culture; 5 days
Control Water[a,b]:	EPA diluted mineral water (80% deionized water and 20% Perrier mineral water)
Test Concentrations[a,b]:	100 percent sample and lab control
Test Acceptability Criteria:	Mean control density of ≥ 1 x 10^6 cells/mL;
	≤ 20% variability among control replicates (coefficient of variation, CV);
	Percent minimum significant difference (PMSD) must be between 9.1 and 29
Protocol Used:	EPA/821/R-02-013 (2002a)
Statistical Analysis Software:	CETIS™, version 1.8.7.20

[a] Macro- and micronutrient solutions were added to sample and control waters to ensure any observed decreases in algal growth were due to toxic constituents present in the sample rather than nutrient deficiency.

[b] Sample and control water were 0.45-µm filtered prior to testing; an unfiltered, undiluted sample portion was also tested concurrently for comparison purposes.

Note: Filtered and unfiltered 100 percent sample blanks (not inoculated with *Raphidocelis*) were also tested to determine if native algae or particulate material present in the sample might be competing with *Raphidocelis* for nutrients, light, and/or space.

Fathead Minnow Chronic Toxicity Test Specifications

Test Period:	8/18/2015, 14:05 – 8/25/2015, 12:15
Test Organism:	*Pimephales promelas* (fathead minnow)
Test Organism Source; Age:	Aquatic Biosystems, Inc. (Fort Collins, CO); 1 day old
Control Water:	EPA diluted mineral water
Test Concentrations:	100 percent sample and lab control
Test Acceptability Criteria:	Mean control survival of ≥ 80 percent;
	Mean control biomass of ≥ 0.25 mg per organism;
	PMSD for biomass must be between 12 and 30
Protocol Used:	EPA/821/R-02-013 (2002a)
Statistical Analysis Software:	CETIS™, version 1.8.7.20

Water Flea Chronic Toxicity Test Specifications

Test Period:	8/18/2015, 14:55 – 8/25/2015, 12:00
Test Organism:	*Ceriodaphnia dubia* (water flea)
Test Organism Source; Age:	In-house culture; <8 h
Control Water:	EPA diluted mineral water
Test Concentrations:	100 percent sample and lab control
Test Acceptability Criteria:	Mean control survival of ≥ 80 percent; 60 percent of surviving females in the control must produce ≥ 3 broods of offspring; Total offspring produced per surviving female must average ≥ 15 (only the first 3 broods are used for calculating reproduction) in the control; PMSD for reproduction must be between 13 and 47
Protocol Used:	EPA/821/R-02-013 (2002a)
Statistical Analysis Software:	CETIS™, version 1.8.7.20

Fathead Minnow Acute Toxicity Test Specifications

Test Period:	8/18/2015, 13:05 – 8/22/2015, 11:40
Test Organism:	*Pimephales promelas* (fathead minnow)
Test Organism Source; Age:	Aquatic Biosystems, Inc. (Fort Collins, CO); 6 days old
Control Water:	EPA diluted mineral water
Test Concentrations:	100 percent sample and lab control
Test Acceptability Criterion:	Mean control survival of ≥ 90 percent
Protocol Used:	EPA/821/R-02-012 (2002b)
Statistical Analysis Software:	CETIS™, version 1.8.7.20

All statistical endpoints reported for compliance purposes were calculated using the Comprehensive Environmental Toxicity Information System™ (CETIS) by Tidepool Scientific Software according to flowchart specifications provided in USEPA method guidance. Organism performance in each sample was compared to performance observed in laboratory control exposures. A No Observed Effect Concentration (NOEC), Lowest Observed Effect Concentration (LOEC), and acute or chronic toxic units (TU_a/TU_c), as appropriate, were calculated for all tests.

RESULTS AND DISCUSSION

Significant decreases in green algae growth (34.0 percent effect) and water flea reproduction (60.6 percent effect) were observed in the undiluted final effluent relative to the lab control. However, no significant effects to water flea survival or fathead minnow growth or survival in the acute and chronic tests were observed. A summary of statistical results for the acute and chronic bioassays is presented in Table 2. Detailed test results for the bioassays are presented in Table 3. Raw data and statistical analyses are presented in full in Appendix C.

Table 2. Summary of Statistical Results

Species & Test Endpoint	NOEC (% effluent)	LOEC (% effluent)	Toxic Units (TU_a/TU_c)
Green Algae			
Growth (Cell Density)	< 100	100	> 1.0
Fathead Minnow			
Acute Survival	100	> 100	1.0
Chronic Survival	100	> 100	1.0
Chronic Growth (Biomass)	100	> 100	1.0
Water Flea			
Chronic Survival	100	> 100	1.0
Chronic Reproduction	< 100	100	> 1.0

NOEC = The highest concentration tested that caused No Observed Effect to the test organisms.

LOEC = The Lowest Observed Effect Concentration.

TU_a = Acute Toxic Units (TU_a): 100 ÷ LC_{50}. Note: a TU_c of 1.0 indicates no toxicity was observed.

TU_c = Chronic Toxic Units (TU_c): 100 ÷ NOEC. Note: a TU_c of 1.0 indicates no toxicity was observed.

Table 3. Summary of Toxicity Test Results

Test Concentration (%)	Green Algae[a]	Fathead Minnow			Water Flea	
	Cell Density (10^6 cells/mL)	Mean Survival: Acute (%)	Mean Survival: Chronic (%)	Mean Biomass (mg)	Mean Survival (%)	Mean Reproduction (# neonates/org)
Lab Control	3.11	97.5	97.5	0.482	100	23.4
100	**2.05**	100	97.5	0.501	77.8	**9.22**

Results in **bold** indicate a statistically significant decrease compared to the lab control.

[a] The lab control and effluent sample were 0.45-µm filtered prior to use in the green algae test. An unfiltered effluent sample was also tested for comparison purposes and was significantly different from the lab control; mean cell density was 2.21 x10^6 cells/mL.

QUALITY ASSURANCE

The samples were received within the appropriate temperature range, and all tests were initiated within the required 36-hour holding time. The laboratory controls met all minimum test acceptability requirements. Statistical analyses followed standard USEPA flowchart selections and were deemed reliable. The PMSD values for fathead minnow growth and green algae cell density were below the lower limit for test acceptability. A low PMSD indicates low variability in the data set and may result in statistical analysis being oversensitive in detecting a difference from the control. The statistical results were evaluated to ensure that proper NOEC and LOEC values were reported according to EPA 2000.

Reference Toxicant Testing

Concurrent reference toxicant tests met all minimum test acceptability requirements. All PMSD values were within the acceptable ranges for each species, with one exception. The PMSD value for the chronic green algae test growth endpoint was below the lower limit for test acceptability. The statistical results were evaluated to ensure that proper NOEC and LOEC values were reported according to EPA 2000. Additionally, the calculated effect concentration values for all reference toxicant tests were within two standard deviations of the historical means, indicating typical organism sensitivity to copper.

Reference toxicant test results are summarized in Table 4 and are presented in full in Appendix D. A list of laboratory qualifier codes used for data recording can be found in Appendix E.

Table 4. Summary of Reference Toxicant Test Results

Species & Test Endpoint	IC/EC/LC_{50} (µg/L copper)	Historical mean ± 2 SD (µg/L copper)	CV (%)
Green Algae			
Chronic Growth (Cell Density)	48.4	50.2 ± 10.2	10.1
Fathead Minnow			
Acute Survival	32.1	45.4 ± 37.0	40.7
Chronic Survival	60.8	81.5 ± 69.6	42.7
Chronic Growth (Biomass)	59.1	82.2 ± 50.6	30.8
Water Flea			
Chronic Survival	35.4	36.0 ± 8.23	11.4
Chronic Reproduction	40.1	35.9 ± 6.90	9.62

IC/EC/LC_{50} = The concentration expected to cause an inhibition/adverse effect/lethal effect to 50 percent of the test organisms.
Historical Mean = The mean IC/EC/LC_{50} from the laboratory's previous 20 tests, plus or minus two standard deviations (SD).
CV = Coefficient of Variation.

REFERENCES

RWQCB. 2014. Waste Discharge Requirements for the California Department of Corrections and Rehabilitation, Deuel Vocational Institution. National Pollutant Discharge Elimination System. California Regional Water Quality Control Board, Central Valley Region. Order No. R5-2014-0014, NPDES No. CA0078093. Effective Date: March 29, 2014.

RWQCB. 2015. Cleanup and Abatement Order for the California Department of Corrections and Rehabilitation, Deuel Vocational Institution. California Regional Water Quality Control Board, Central Valley Region. Order No. R5-2015-0704.

Tidepool Scientific Software. 2000-2013. CETIS Comprehensive Environmental Toxicity Information System Software, Version 1.8.7.20.

USEPA. 2000. Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination System. United States Environmental Protection Agency Office of Wastewater Management (EPA-833-R-00-003).

USEPA. 2002a. Methods for Measuring the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Fourth Edition. United States Environmental Protection Agency Office of Water, Washington DC (EPA-821-R-02-013).

USEPA. 2002b. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Fourth Edition. United States Environmental Protection Agency Office of Water, Washington DC (EPA-821-R-02-012).

Appendix A
Sample Information

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

Client: Deuel Vocational Institution / Alpha Analytical Laboratories, Inc
Sample ID: ~~ERF-001~~ Final Effluent ⑤
Test ID No(s).: 1508-S067 to -S070

Sample Check-In Information

Sample (A, B, C):	A	B	C	Grab Sample
Log-in No. (15-xxxx):	0743	0744	0760	0742
Sample Collection Date & Time:	8/17/15 0830	8/19/15 1051	8/21/15 1040	8/17/15 0835
Sample Receipt Date & Time:	8/18/15 1020	8/20/15 0856	8/22/15 ~~0853~~	8/18/15 1020
Number of Containers & Container Type:	1, 4L cubi	1, 4L cubi	1, 4L cubi	1, 4L cubi
Approx. Total Volume Received (L):	4L	~4L	~4L	4L
Check-in Temperature (°C)	1.0	4.0	5.0	1.0
Temperature OK? [1]	(Y) N	(Y) N	(Y) N	(Y) N
DO (mg/L)	10.8	10.6	12.5	11.2
pH (units)	7.67	7.41	7.45	7.84
Conductivity (µS/cm)	3880	3820	4170	3910
Salinity (ppt)	2.1	2.1	2.2	2.2
Alkalinity (mg/L) [2]	102	100	107	114
Hardness (mg/L) [2, 3]	>1000	>1000	>1000	>1000
Total Chlorine (mg/L)	0.02	~~0.05~~ <0.02	<0.02	0.02
Technician Initials	ALB	AG	BL	ALB

Sample Description:
A: light yellow, clear, odorless, light debris
B: colorless, clear, odorless, no debris
C: light yellow color, clear, odorless, no debris
Grab: light yellow, clear, odorless, light debris

COC Complete (Y/N)?
A Y B Y C Y

Filtration? (Y) N
Pore Size: 0.45 um for Selenastrum
(Organisms) or (Debris) (green algae)

Salinity Adjustment? Y (N)

Test:	Source:	Target ppt:
Test:	Source:	Target ppt:
Test:	Source:	Target ppt:

pH Adjustment? Y (N)

	A	B	C
Initial pH:			
Amount of HCl added:			
Final pH:			

Cl_2 Adjustment? Y (N)

	A	B	C
Initial Free Cl_2:			
STS added:			
Final Free Cl_2:			

Sample Aeration? Y (N)

	A	B	C
Initial D.O.			
Duration & Rate			
Final D.O.			

Subsamples for Additional Chemistry Required? Y (N)
NH3 Other _____
Tech Initials A____ B____ C____

QC Check: VCR 8/24/15
Final Review: KBB 8/25/15

Fathead minnow acute & chronic

Test Performed: Water flea, Green algae (Control)/Dilution Water: (8:2) / Lab SW / Lab ART Other: _____
Alkalinity: 79 Hardness or Salinity: 81
Additional Control? Y (N) = _____ Alkalinity: _____ Hardness or Salinity: _____

Test Performed: _____ Control/Dilution Water: 8:2 / Lab SW / Lab ART Other: _____
Alkalinity: _____ Hardness or Salinity: _____
Additional Control? Y N = _____ Alkalinity: _____ Hardness or Salinity: _____

Test Performed: _____ Control/Dilution Water: 8:2 / Lab SW / Lab ART Other: _____
Alkalinity: _____ Hardness or Salinity: _____
Additional Control? Y N = _____ Alkalinity: _____ Hardness or Salinity: _____

Notes: [1] Temperature of sample should be 0-6°C, if received more than 24 hours past collection time.
[2] mg/L as CaCO3, [3] Measured for freshwater samples only, NA = Not Applicable

Additional Comments: ⑤ COS VCR 8/18/15

Appendix B
Chain-of-Custody Information

Alpha Analytical Laboratories Inc.
e-mail: clientservices@alpha-labs.com

Laboratory & Corporate: 208 Mason Street, Ukiah, CA 95482
707-468-0401 Fax: 707-468-5267

Service Center & Micro Lab: 6398 Dougherty Rd, Ste 35, Dublin, CA 94568
925-828-6226 Fax: 925-828-6309

Chain of Custody Record

Reports and Invoices will be delivered by email in .pdf format.

Lab No._____ Page 1 of 1

Report to:
Company: CDCR - Deuel Vocational Institution
Attn: Daniel Mullins
Address: 23500 Kasson Road, Tracy, CA 95376
Phone/Fax: 209-835-4141 ext 5897
Email Addresses: daniel.mullins@cdcr.ca.gov

Invoice to (if different):
Company: (month end consolidated billing)
Address:
Phone/Fax:

Project Info for Report:
Project ID: Chronic & Acute Bioassay
Project Number:

Please contact David Pingatore with any questions about COC completion

Samplers Signature: Daniel G Mullins
Print: Daniel G Mullins

Sample Identification	Sampled: Date	Sampled: Time	Container: 1 Gallon Cub	Container:	Container:	Container: 500 mL	Container: Soil Jar	Preservative: Na2S2O3	Preservative: HNO3	Preservative: H2SO4	Preservative: Other	Preservative: None	Matrix: Waste Water	Matrix: Grab	Matrix: Composite	Total Number of Containers	DVI Acute FH Minnow non-renewal		DVI Chronic FH Minnow 100% Effluent	DVI Chronic Green Algae 100% Effluent	DVI Chronic Water Flea 100% Effluent		DVI Ref Toxicant Report - FH Minnow	DVI Ref Toxicant Report - Green Algae	DVI Ref Toxicant Report - Water Flea		
Final Effluent Grab	8/17/15	08:35	X									X	X	X		1	X										
Final Effluent Comp	8/17/15	08:30	X									X	X		X	1			X	X	X		X	X	X		
Set Comp Sampler 8/16/15 08:30																											

Analyses Requested

TAT
10 days ●
RUSH:
5 days ○
48 hours ○
Other: ____days ○

Lab Approval Required For Rush TATs

Sample Notes (lab use only)
Temperature: ____ deg. C
Shipment Method:
Custody Seals: Y / N

Sample Notes or CDPH Source Numbers:
Relinquish Temp 13.0 °C
Relinquish Temp 2.0 °C

Receipt temp °C
1.0
1.0

Relinquished by:	Received by:	Date:	Time:
Daniel G Mullins	Jay SOB COC	8/17/15	925
	Ann Perrin	8/18/15	1020

CDPH Write On EDT Transmission? ○ Yes ● No
State System Number:
If "Y" please enter the Source Number(s) in the column above

CA Geotracker EDF Report? ○ Yes ● No
Global ID: Sampling Company Log Code:
EDF to (Email Address):

Travel and Site Time: | Mileage: | Misc. Supplies:

NAMMS ID: 15-0742 to 0743

SUBCONTRACT ORDER

Alpha Analytical Laboratories, Inc.

15H1526

SENDING LABORATORY:	RECEIVING LABORATORY:
Alpha Analytical Laboratories, Inc.	Nautilus Environmental
208 Mason St.	4340 Vandever Avenue
Ukiah, CA 95482	San Diego, CA 92120
Phone: (707)468-0401	Phone :(858) 587-7333
Fax: (707)468-5267	Fax: -
Project Manager: David S. Pingatore	**Terms: Net 30**

Analysis	Due	Expires	Comments
15H1526-01 Final Effluent Grab [Water] Sampled 08/17/15 08:35 Pacific			
DVI Acute FH Minnow Non-Renew	08/31/15 12:00	08/18/15 20:35	
Containers Supplied:			
Other (A)			
15H1526-02 Final Effluent Comp [Water] Sampled 08/17/15 08:30 Pacific			
DVI Ref Toxicant Report Water Flea	08/31/15 12:00	08/18/15 20:30	
DVI Ref Toxicant Report Green Alg	08/31/15 12:00	08/18/15 20:30	
DVI Ref Toxicant Report FH Minno	08/31/15 12:00	08/18/15 20:30	
DVI Chronic Water Flea 100% Effl	08/31/15 12:00	08/18/15 20:30	
DVI Chronic Green Algae 100% Ef	08/31/15 12:00	08/18/15 20:30	
DVI Chronic FH Minnow 100% Eff	08/31/15 12:00	08/18/15 20:30	
Containers Supplied:			
Other (A)			

☐ *Report to State* (N/A)

System Name: _____ **Employed by:** _____

User ID: _____ **Sampler:** _____

System Number: _____

08/17/15		Golden State Overnite	08/17/15
Released By	Date	Received By	Date
650 08/18/15			1024
Released By	Date	Received By	Date

Chain of Custody Record

Alpha Analytical Laboratories Inc.
e-mail: clientservices@alpha-labs.com

Laboratory & Corporate: 208 Mason Street, Ukiah, CA 95482
707-468-0401 Fax: 707-468-5267

Service Center & Micro Lab: 6398 Dougherty Rd, Ste 35, Dublin, CA 94568
925-828-6226 Fax: 925-828-6309

Reports and Invoices will be delivered by email in .pdf format.

Lab No._____ Page 1 of 1

Please contact David Pingatore with any questions about COC completion

Report to:	Invoice to (if different):	Project Info for Report:
Company: CDCR - Deuel Vocational Institution	Company: (month end consolidated billing)	Project ID: Chronic & Acute Bioassay
Attn: Daniel Mullins		
Address: 23500 Kasson Road Tracy, CA 95376	Address:	Project Number:
Phone/Fax: 209-835-4141 ext 5897	Phone/Fax:	
Email Addresses: daniel.mullins@cdcr.ca.gov		

Samplers Signature: [signature]

Print: Daniel G Mullins

Sample Identification	Sampled: Date	Sampled: Time	Container: 1 Gallon Cube	Container: Sterile bacti	Container: 1 liter Poly	Container: 500 mL	Container: Soil jar	Preservative: Na2S2O3	Preservative: HNO3	Preservative: H2SO4	Preservative: Other	Preservative: None	Matrix: Waste Water	Matrix: Grab	Matrix: Composite	:Total Number of Containers	DVI Acute FH Minnow non-renewal		DVI Chronic FH Minnow 100% Effluent	DVI Chronic Green Algae 100% Effluent	DVI Chronic Water Flea 100% Effluent		DVI Ref Toxicant Report - FH Minnow	DVI Ref Toxicant Report - Green Algae	DVI Ref Toxicant Report - Water Flea			Sample Notes or CDPH Source Numbers:
Final Effluent Comp	8/19/15	~~09:30~~	X									X	X		X	1			X	X	X		X	X	X			Relinquish Temp____ °C
Set Comp Sampler		10:51																										Relinquish Temp____ °C
8/18/2015 09:30																												

TAT: 10 days ● RUSH: 5 days ○ 48 hours ○ Other: ____days ○

Lab Approval Required For Rush TATs

Sample Notes (lab use only)

Temperature: ____ deg. C

Shipment Method:

Custody Seals: Y / N

Relinquished by:	Received by:	Date:	Time:
[signature]	[signature]	8/19/15	10:51
[signature] BELL			
[signature]	[signature]	8/20/15	0856

CDPH Write On EDT Transmission? ○ Yes ● No

State System Number: _____

If "Y" please enter the Source Number(s) in the column above

CA Geotracker EDF Report? ○ Yes ● No

Global ID: Sampling Company Log Code:

EDF to (Email Address):

Travel and Site Time: Mileage: Misc. Supplies:

Nautilus ID: 15-0748

3 C / 4.0

Alpha Analytical Laboratories Inc.
e-mail: clientservices@alpha-labs.com

Laboratory & Corporate: 208 Mason Street, Ukiah, CA 95482
707-468-0401 Fax: 707-468-5267

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925-828-6226 Fax: 925-828-6309

Chain of Custody Record

Reports and Invoices will be delivered by email in .pdf format.

Lab No._____ Page___of___

Report to:	Invoice to (if different):	Project Info for Report:
Company: CDCR - Deuel Vocational Institution	Company: (month end consolidated billing)	Project ID: Chronic & Acute Bioassay
Attn: Daniel Mullins		
Address: 23500 Kasson Road Tracy, CA 95376	Address:	Project Number:
Phone/Fax: 209-835-4141 ext 5897	Phone/Fax:	
Email Addresses: daniel.mullins@cdcr.ca.gov		

Please contact David Pingatore with any questions about COC completion

Samplers Signature: DG

Print: Daniel G Mullins

Sample Identification	Sampled: Date	Sampled: Time	Container: 1 Gallon Cube	Container: Sterile bacti	Container: 1 liter Poly	Container: 500 mL	Container: Soil jar	Preservative: Na2S2O3	Preservative: HNO3	Preservative: H2SO4	Preservative: Other	Preservative: None	Matrix: Waste Water	Matrix: Grab	Matrix: Composite	Total Number of Containers	DVI Acute FH Minnow non-renewal		DVI Chronic FH Minnow 100% Effluent	DVI Chronic Green Algae 100% Effluent	DVI Chronic Water Flea 100% Effluent		DVI Ref Toxicant Report - FH Minnow	DVI Ref Toxicant Report - Green Algae	DVI Ref Toxicant Report - Water Flea		
Final Effluent Comp	8/21/15	10:40	X									X	X		X	1			X	X	X		X	X	X		
Set Comp Sampler																											
8/20/2015 10:00																											

Analyses Requested	TAT	Lab Approval Required For Rush TATs	Sample Notes (lab use only)
	10 days ●		Temperature:
	RUSH: 5 days ○		____ deg. C
	48 hours ○		Shipment Method:
	Other: ___days ○		Custody Seals: Y / N

Sample Notes or CDPH Source Numbers:

Relinquish Temp 5.0 °C

Relinquish Temp____ °C

0C

5.0

Relinquished by:	Received by:	Date:	Time:
		8/21/15	1046
	Ben Kerr (Nautilus)	8/22/15	~~0853~~ 0855

Nautilus ID: 15-0760

@018 VCR 8/24/15

CDPH Write On EDT Transmission? ○ Yes ● No

State System Number:

If "Y" please enter the Source Number(s) in the column above

CA Geotracker EDF Report? ○ Yes ● No

Global ID: Sampling Company Log Code:

EDF to (Email Address):

Travel and Site Time: Mileage: Misc. Supplies:

Appendix C
Raw Data and Statistical Analysis

Green Algae 96-hour Chronic Growth

CETIS Summary Report

Selenastrum Growth Test — **Nautilus Environmental (CA)**

Batch ID:	18-9924-9647	**Test Type:**	Cell Growth	**Analyst:**	
Start Date:	18 Aug-15 15:15	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Nutrient Enriched 8:2
Ending Date:	22 Aug-15 13:00	**Species:**	Selenastrum capricornutum	**Brine:**	Not Applicable
Duration:	94h	**Source:**	In-House Culture	**Age:**	5d

Control: QA/CBB 8/25/15

Sample ID:	03-9234-0593	**Code:**	15-0743	**Client:**	Alpha Analytical Laboratories
Sample Date:	17 Aug-15 08:30	**Material:**	Effluent Sample	**Project:**	
Receive Date:	18 Aug-15 10:26	**Source:**	Deuel Vocational Institution		
Sample Age:	31h (1 °C)	**Station:**	Final Effluent		

Batch Note: 101 = Unfiltered 100 % effluent

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
02-6094-4637	Cell Density	<100	100	NA	8.41%	>1	Equal Variance t Two-Sample Test

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
02-6094-4637	Cell Density	Control CV	0.03194	NL - 0.2	Yes	Passes Acceptability Criteria
02-6094-4637	Cell Density	Control Resp	3.10E+6	1.00E+6 - NL	Yes	Passes Acceptability Criteria
02-6094-4637	Cell Density	PMSD	0.08413	0.091 - 0.29	Yes	Below Acceptability Criteria Q16

Cell Density Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	3.105E+6	2.947E+6	3.262E+6	2.977E+6	3.219E+6	4.958E+4	9.916E+4	3.19%	0.0%
100		4	2.050E+6	1.652E+6	2.448E+6	1.744E+6	2.355E+6	1.249E+5	2.498E+5	12.19%	33.97%
101		4	2.208E+6	1.900E+6	2.516E+6	1.984E+6	2.456E+6	9.672E+4	1.934E+5	8.76%	28.89%

Cell Density Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	2.977E+6	3.219E+6	3.116E+6	3.106E+6
100		2.033E+6	2.355E+6	1.744E+6	2.068E+6
101		2.184E+6	1.984E+6	2.207E+6	2.456E+6

Q16: However, the LOEC of 100 is appropriate because the percent effect is 33.97 which is well above the lower-bound PMSD limit of 9.1 and considered significant according to EPA 2000.

CETIS Analytical Report

Selenastrum Growth Test — **Nautilus Environmental (CA)**

Analysis ID:	02-6094-4637	**Endpoint:**	Cell Density	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Aug-15 12:33	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes

Batch Note: 101 = Unfiltered 100 % effluent

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Untransformed	NA	C > T	NA	NA	8.41%	Fails cell density

Equal Variance t Two-Sample Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		100*	7.846	1.943	3E+05	6	0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	2.223941E+12	2.223941E+12	1	61.56	0.0002	Significant Effect
Error	2.16775E+11	36129170000	6			
Total	2.440716E+12		7			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	6.348	47.47	0.1633	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9464	0.6451	0.6745	Normal Distribution

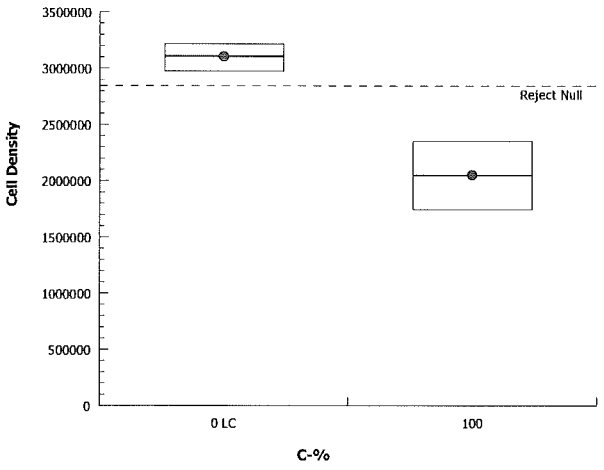
Cell Density Summary

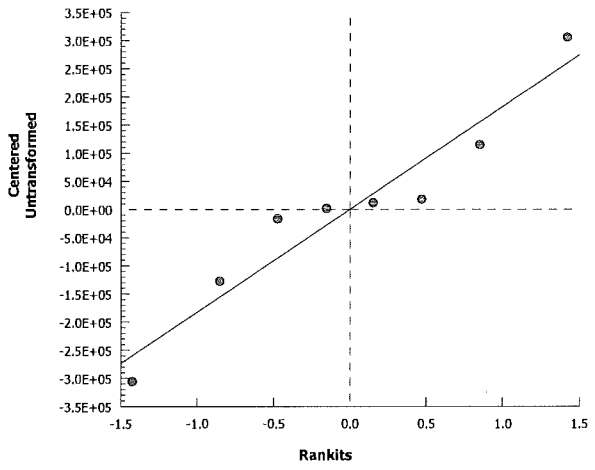
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	3.105E+6	2.947E+6	3.262E+6	3111000	2.977E+6	3.219E+6	4.958E+4	3.19%	0.0%
100		4	2.050E+6	1.652E+6	2.448E+6	2051000	1.744E+6	2.355E+6	1.249E+5	12.19%	33.97%

Cell Density Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	2.977E+6	3.219E+6	3.116E+6	3.106E+6
100		2.033E+6	2.355E+6	1.744E+6	2.068E+6

Graphics





CETIS Analytical Report

Test Code: 1508-S070 | 17-9434-6872

Selenastrum Growth Test — Nautilus Environmental (CA)

Analysis ID:	16-7208-4978	**Endpoint:**	Cell Density	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Aug-15 12:34	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes

Batch Note: 101 = Unfiltered 100 % effluent

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Untransformed	NA	C > T	NA	NA	6.8%	Fails cell density

Equal Variance t Two-Sample Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		101*	8.251	1.943	2E+05	6	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.608321E+12	1.608321E+12	1	68.07	0.0002	Significant Effect
Error	1.417577E+11	23626290000	6			
Total	1.750079E+12		7			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	3.805	47.47	0.3014	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9576	0.6451	0.7867	Normal Distribution

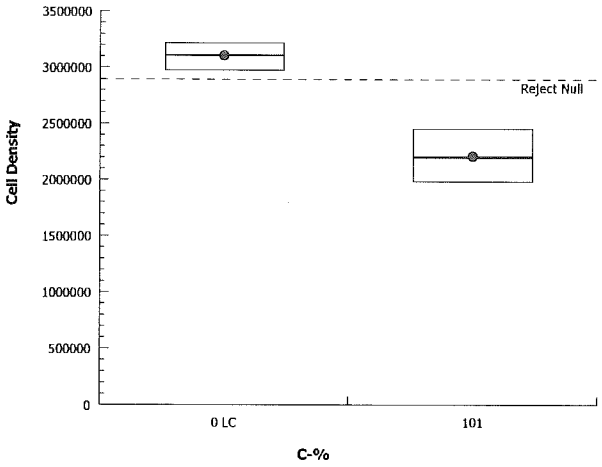
Cell Density Summary

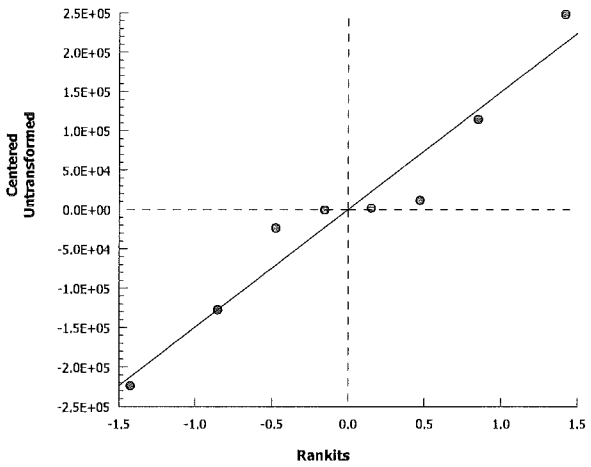
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	3.105E+6	2.947E+6	3.262E+6	3111000	2.977E+6	3.219E+6	4.958E+4	3.19%	0.0%
101		4	2.208E+6	1.900E+6	2.516E+6	2196000	1.984E+6	2.456E+6	9.672E+4	8.76%	28.89%

Cell Density Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	2.977E+6	3.219E+6	3.116E+6	3.106E+6
101		2.184E+6	1.984E+6	2.207E+6	2.456E+6

Graphics





Analyst: NR QA: KB 8/25/15

Fluorometric & Microscopic Determination of Cell Density
Turner Fluorometer Model TD-700

Test Species: *S. capricornutum*

Client : DVI / Alpha Analytical Laboratories **Start Date/Time:** 8/18/2015 1515

Sample ID: ~~EFF-001~~ Final Effluent **End Date/Time:** 8/22/2015 1300

Test No: 1508-S070 **Analyst:** BK

Random Number	Cell Density (fluorometric) (cells/ml *10^5)	Dilution	Cell Density (microscopic) (cells/ml *10^4)
Blank		NA	
Cal Check 1 (NEW, Solid)	0.00, 2.33		
25	20.33 *		
26	32.19 *		
27	23.55 *		
28	19.84 *		
29	31.06 *		
30	29.77 *		
31	17.44 *		
32	22.07 *		
33	20.68 *		
34	24.56 *		
35	21.84 *		
36	31.16 *		
Cal Check 2 (NEW, Solid)			
100% filtered blank	0.00		
100% unfiltered blank	0.06		

* Flasks scraped to loosen cells.

Comments: AQA VCR 8/24/15

QC Check: VCR 8/24/15 **Final Review:** KBO 8/25/15

CETIS Test Data Worksheet

Selenastrum Growth Test — **Nautilus Environmental (CA)**

Start Date: 18 Aug-15 | **Species:** Selenastrum capricornutum | **Sample Code:** 15- 0743
End Date: 22 Aug-15 | **Protocol:** EPA/821/R-02-013 (2002) | **Sample Source:** Deuel Vocational Institution
Sample Date: 17 Aug-15 | **Material:** Effluent Sample | **Sample Station:** (A) Eff-001 (Composite) Final Effluent

C-%	Code	Rep	Pos	Cell Density	Absorbance	Biomass	Chlorophyll a	Notes
0	LC	1	30					
0	LC	2	26					
0	LC	3	36					
0	LC	4	29					
100		1	25					
100		2	27					
100		3	31					
100		4	33					
101		1	35					
101		2	28					
101		3	32					
101		4	34					

106% Unfiltered (A)

(A) BK QR 8/18/15

Freshwater Chronic Bioassay

Water Quality Measurements
Algal Growth Inhibition

Client : DVI / Alpha Analytical Laboratories

Test Species: *S. capricornutum*

Sample ID: ~~EFF-001~~ Final Effluent

Start Date/Time: 8/18/2015 1515

Sample Log No.: 15- 0743

End Date/Time: 8/22/2015 1300

Dilutions made by: BS

Test No: 1508-S070

	Initial Readings				Final Readings	
Concentration (%)	D.O. (mg/L)	Conductivity (umhos-cm)	Alkalinity (mg/L)	Hardness (mg/L)	D.O. (mg/L)	Conductivity (umhos-cm)
Lab Control (8:2)	6.6	276	86	96	9.6	195
100	6.5	4070	120	1081	8.8	3850
100 unfiltered	8.2	4130	132	1116	8.7	3940

		0 Hour	24 Hour	48 Hour	72 Hour	96 Hour
pH/Temperature (°C):	LC	8.11 / 24.9	8.38 / 25.0	8.55 / 24.4	9.17 / 24.5	9.46 / 24.6
pH/Temperature (°C):	100	7.69 / 25.0	8.29 / 25.5	8.34 / 25.1	8.70 / 24.6	9.08 / 25.1
pH/Temperature (°C):	100unfilt	7.60 / 25.3	8.31 / 25.5	8.41 / 25.2	8.73 / 25.0	9.11 / 24.9
pH/Temperature (°C):		/	/	/	/	/
pH/Temperature (°C):		/	/	/	/	/
pH/Temperature (°C):		/	/	/	/	/
pH/Temperature (°C):		/	/	/	/	/
	Technician:	MG	ALB	BK	AG	EG

Comments: @ QS VCR 8/24/15

QC Check: VCR 8/24/15

Final Review: KBB 8/25/15

Freshwater Chronic Bioassay **Algal Growth Inhibition Worksheet**

Client/Sample ID : Deuel Vocational Institution / Alpha Analytical Laboratories **Test Species:** *S. capricornutum*

Test No: 1508-5070 **Start Date/Time:** 8/18/2015 1515

Analyst: PA **End Date/Time:** 8/22/2015 1300

Culture Used (circle one): (Nutrient Enriched DI Water (NEW)) / Very Hard Water (VHW)

Date Stock Culture Started: 7/13/15 Culture Age: 5 d

Culture subsample inspected for algal cell health? MS (initials) bacteria/invasive algal species present? Y (N)

Stock Cell Density Measurements: 41.55
42.40
42.26
42.35
42.03

Mean: 42.08

(mean no. * 100,000)/(500,000) = x (dilution factor): 8.4

Prepare inoculum according to the dilution factor. This yields a solution with the desired cell density of 500,000 cells/ml.

dil. factor 8.4

-1.0 part Sele stock = 15 ml

7.4 part(s) NEW = 111 ml

Inoculate 1 ml into 3 initial count flasks containing 50 ml of NEW, stir and count on the hemacytometer. Flasks should contain a final density of 10,000 cells/ml ± 10%.

Inoculum Cell Density Confirmation Counts: 1
0
2

Mean: 1

Location in Environmental Chamber (All replicates in each test must be on the same shelf; do not split up tests among shelves):

Shelf Number	Measured Light Intensity Range (must be between 360 and 440 ft-c)	Random Number Range
(A) 17	371 — 428	25-36
(B) 29	368 — 423	1-24
3		
4		
5		
6		

Are lights on 24 hour cycle? (Y) / N

Comments: (A) Q16 8/19/15

QC Check: VCR 8/24/15 Final Review: KBB 8/25/15

Fathead Minnow 7-day Chronic Survival and Growth

CETIS Summary Report

Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental (CA)**

Batch ID:	16-8432-6451	**Test Type:**	Growth-Survival (7d)	**Analyst:**	
Start Date:	18 Aug-15 14:05	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Not Applicable
Ending Date:	25 Aug-15 12:15	**Species:**	Pimephales promelas	**Brine:**	Not Applicable
Duration:	6d 22h	**Source:**	Aquatic Biosystems, CO	**Age:**	1d

Sample ID:	03-3432-2103	**Code:**	15-0743	**Client:**	Alpha Analytical Laboratories
Sample Date:	17 Aug-15 08:30	**Material:**	Effluent Sample	**Project:**	
Receive Date:	18 Aug-15 10:26	**Source:**	Deuel Vocational Institution		
Sample Age:	30h (1 °C)	**Station:**	Final Effluent		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
06-4552-9980	7d Survival Rate	100	>100	NA	7.07%	1	Wilcoxon Rank Sum Two-Sample Test
06-2944-9820	Mean Dry Biomass-mg	100	>100	NA	8.09%	1	Equal Variance t Two-Sample Test

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
06-4552-9980	7d Survival Rate	Control Resp	0.975	0.8 - NL	Yes	Passes Acceptability Criteria
06-2944-9820	Mean Dry Biomass-mg	Control Resp	0.482	0.25 - NL	Yes	Passes Acceptability Criteria
06-2944-9820	Mean Dry Biomass-mg	PMSD	0.08089	0.12 - 0.3	Yes	Below Acceptability Criteria Q16

7d Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.975	0.8954	1	0.9	1	0.025	0.05	5.13%	0.0%
100		4	0.975	0.8954	1	0.9	1	0.025	0.05	5.13%	0.0%

Mean Dry Biomass-mg Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.482	0.4364	0.5276	0.444	0.507	0.01434	0.02867	5.95%	0.0%
100		4	0.5008	0.4561	0.5454	0.466	0.529	0.01404	0.02808	5.61%	-3.89%

7d Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1	1	1	0.9
100		0.9	1	1	1

Mean Dry Biomass-mg Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	0.501	0.476	0.507	0.444
100		0.466	0.491	0.517	0.529

Q16: However the sample outperformed the control, therefore the PMSD requirements do not apply in this case.

Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental (CA)**

Analysis ID: 06-4552-9980 **Endpoint:** 7d Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 28 Aug-15 11:46 **Analysis:** Nonparametric-Two Sample **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Angular (Corrected)	NA	C > T	NA	NA	7.07%	Passes 7d survival rate

Wilcoxon Rank Sum Two-Sample Test

Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		100	18	NA	2	6	0.7857	Exact	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0	0	1	0	1.0000	Non-Significant Effect
Error	0.039839	0.006639833	6			
Total	0.039839		7			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	1	47.47	1.0000	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.5659	0.6451	<0.0001	Non-normal Distribution

7d Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	0.975	0.8954	1	1	0.9	1	0.025	5.13%	0.0%
100		4	0.975	0.8954	1	1	0.9	1	0.025	5.13%	0.0%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	1.371	1.242	1.501	1.412	1.249	1.412	0.04074	5.94%	0.0%
100		4	1.371	1.242	1.501	1.412	1.249	1.412	0.04074	5.94%	0.0%

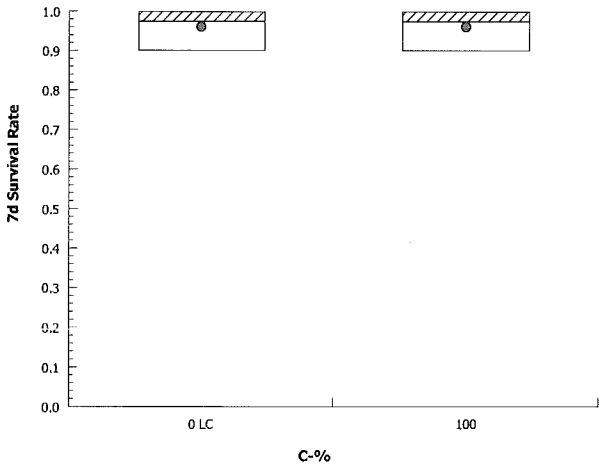
7d Survival Rate Detail

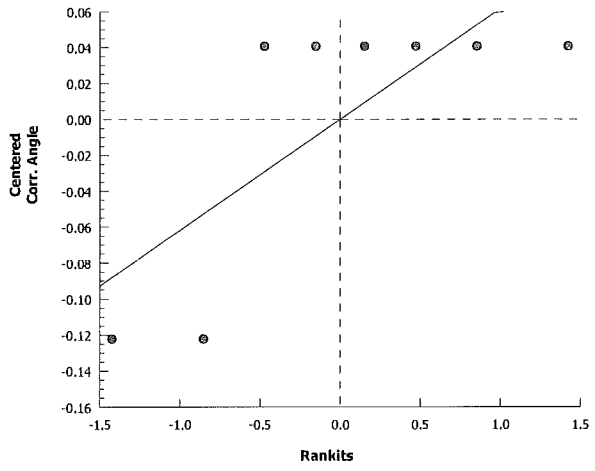
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1	1	1	0.9
100		0.9	1	1	1

Angular (Corrected) Transformed Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1.412	1.412	1.412	1.249
100		1.249	1.412	1.412	1.412

Graphics





Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental (CA)**

Analysis ID: 06-2944-9820 **Endpoint:** Mean Dry Biomass-mg **CETIS Version:** CETISv1.8.7
Analyzed: 28 Aug-15 11:46 **Analysis:** Parametric-Two Sample **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Untransformed	NA	C > T	NA	NA	8.09%	Passes mean dry biomass-mg

Equal Variance t Two-Sample Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		100	-0.9345	1.943	0.039	6	0.8069	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.000703122	0.000703122	1	0.8733	0.3861	Non-Significant Effect
Error	0.004830742	0.0008051237	6			
Total	0.005533864		7			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	1.043	47.47	0.9733	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.882	0.6451	0.1969	Normal Distribution

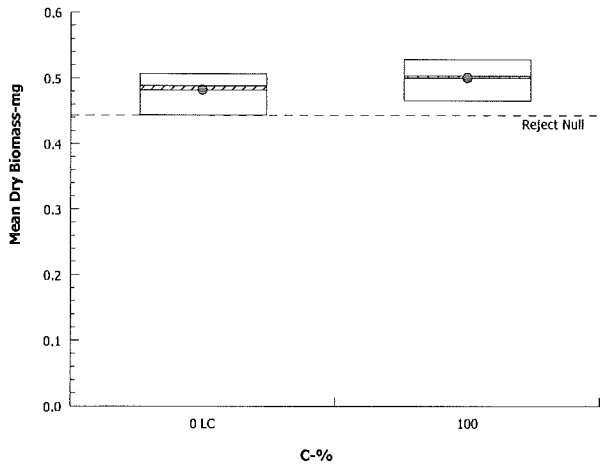
Mean Dry Biomass-mg Summary

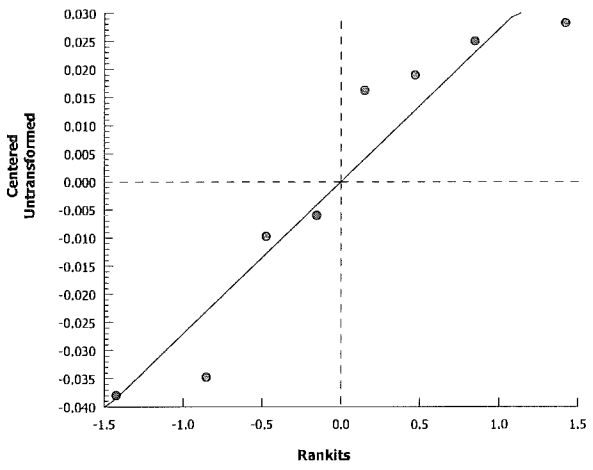
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	0.482	0.4364	0.5276	0.4885	0.444	0.507	0.01434	5.95%	0.0%
100		4	0.5008	0.4561	0.5454	0.504	0.466	0.529	0.01404	5.61%	-3.89%

Mean Dry Biomass-mg Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	0.501	0.476	0.507	0.444
100		0.466	0.491	0.517	0.529

Graphics





Freshwater Chronic Bioassay | **Larval Fish Survival**

Client: Deuel Vocational Institution / Alpha Analytical Laboratories

Test Species: *P. promelas*

Sample ID: Final Effluent

Start Date/Time: 8/18/2015 1405

Test No.: 1508-S068

End Date/Time: 8/25/2015 1215

Conc. (%)	Rep.	Rand #	Test Day / No. Organisms Alive								Percent Survival
			0	1	2	3	4	5	6	7	
Lab Control	a	7	10	10	10	10	10	10	10	10	100
(8:2)	b	5	10	10	10	10	10	10	10	10	100
	c	1	10	10	10	10	10	10	10	10	100
	d	3	10	10	10	10	10	10	10	9	90
100%	a	4	10	10	9	9	9	9	9	9	90
	b	8	10	10	10	10	10	10	10	10	100
	c	6	10	10	10	10	10	10	10	10	100
	d	2	10	10	10	10	10	10	10	10	100

Initial Counts QC'd by: EG

Rand # QC: KB

	0	1	2	3	4	5	6	7
Tech Initials	BK	EG	ALB	ALB	BK	EG	CH	MM
Time	1405	1130	1330	1420	1200	1055	1145	1215

Time Fed (day):	0	1	2	3	4	5	6
morning:	/	0807	0830	0860	0715	0800	0830
midday:	/	1210	1230	1235	1120	1200	1210
evening:	1530	1600	1520	1500	1515	1500	1615

Drying Oven Info

Tare wt. Initials/Date: BJ / 8/24/15

Date/Time in: Ⓐ 8/25/15 / 1240

Date/Time out: 8/27/15 1346

Temp (°C): 58.0 Q1

QC Check: VCR 8/28/15

Final Review: KB 8/28/15

Comments: Ⓐ Q18 MM 8/25/15

Freshwater Chronic Bioassay

Larval Fish Weights

Client: Deuel Vocational Institution / Alpha Analytical Laboratories **Test Species:** *Pimephales promelas*

Sample ID: Final Effluent **Start Date/Time:** 8/18/2015 1405

Test No.: 1508-S068 **End Date/Time:** 8/25/2015 1215

Conc. (µg/L)	Rep.	pan weight (mg)	pan + fish weight (mg)	organism weight (mg)
Lab Control	a	22.60	27.61	5.01
	b	22.80	27.56	4.76
	c	22.11	27.18	5.07
	d	22.64	27.08	4.44
100	a	22.18	26.84	4.66
	b	22.81	27.72	4.91
	c	22.41	27.58	5.17
	d	21.36	26.65	5.29
	a			
	b			
	c			
	d			
	a			
	b			
	c			
	d			
	a			
	b			
	c			
	d			
	a			
	b			
	c			
	d			
Tech Initials:		BJ	AG	
Date/Time:		8/24/2015 1641	8/27/15 1340	

QC Check: KB 8/28/15

Final Review:

Freshwater Chronic Bioassay **Water Quality Measurements**

Client: Deuel Vocational Institution / Alpha Analytical Laboratories **Test Species:** *P. promelas*

Sample ID: Final Effluent **Start Date/Time:** 8/18/2015 1405

Test No: 1808-S068 **End Date/Time:** 8/25/2015 1215

Concentration	Lab Control (8:2)							
Day	0	1	2	3	4	5	6	7
Initial								
pH	8.19	8.21	8.19	8.17	8.16	8.21	8.29	
DO (mg/L)	8.3	7.6	7.5	7.8	7.6	7.5	7.8	
Cond. (µmhos/cm)	193	1816	186	185	196	193	193	
Temp (°C)	24.9	24.8	24.6	24.2	25.1	24.9	24.2	
Final								
pH		8.01	7.98	7.94	7.87	7.79	7.84	7.85
DO (mg/L)		6.8	6.4	6.3	6.1	5.8	6.4	6.4
Temp (°C)		25.2	25.1	24.9	25.3	24.9	24.9	25.5

Concentration	100%							
Day	0	1	2	3	4	5	6	7
Initial								
pH	7.41	7.64	7.38	7.35	7.42	7.51	7.50	
DO (mg/L)	8.6	8.9	8.7	9.0	8.6	8.6	8.9	
Cond. (µmhos/cm)	4060	3910	3990	3980	4410	(A) 4310 ~~4240~~	4350	
Temp (°C)	24.3	26.0	25.5	24.3	25.2	24.3	24.1	
Final								
pH		7.98	7.90	7.80	7.71	7.75	7.77	7.87
DO (mg/L)		6.8	6.5	6.3	6.0	5.7	6.2	6.2
Temp (°C)		26.3	26.1	25.2	25.3	24.9	25.3	25.4

Concentration								
Day	0	1	2	3	4	5	6	7
Initial								
pH								
DO (mg/L)								
Cond. (µmhos/cm)								
Temp (°C)								
Final								
pH								
DO (mg/L)								
Temp (°C)								

	0	1	2	3	4	5	6	7
Analysts: Initial:	EG	ALB	BK	AG	EG	BK	CH	—
Final:	—	ALB	BK	AG	EG	BK	CH	ALB
Dilutions made by:	KB	AW	ALB	ALB	BK	EG	CH	—
Sample Used (A, B, C):	A	A	B	B	C	C	C	—

Comments: (A) BK QAS 8/23/15

Animal Source/Date Received: ABS | 8/18/15 **Animal Age at Initiation:** 1 day

Sample Log-In Numbers: A: 15-0743 B: 15-0744 C: 15-0760

QC Check: VCR 8/28/15 **Final Review:** KBB 8/28/15

Water Flea 7-day Chronic Survival and Reproduction

CETIS Summary Report

Ceriodaphnia 7-d Survival and Reproduction Test — **Nautilus Environmental (CA)**

Batch ID:	06-1370-6976	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	
Start Date:	18 Aug-15 14:55	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Not Applicable
Ending Date:	25 Aug-15 12:00	**Species:**	Ceriodaphnia dubia	**Brine:**	Not Applicable
Duration:	6d 21h	**Source:**	In-House Culture	**Age:**	< 8h

Sample ID:	15-2615-3883	**Code:**	15-0743	**Client:**	Alpha Analytical Laboratories
Sample Date:	17 Aug-15 08:30	**Material:**	Effluent Sample	**Project:**	
Receive Date:	18 Aug-15 10:26	**Source:**	Deuel Vocational Institution		
Sample Age:	30h (1 °C)	**Station:**	Final Effluent		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
14-6919-3642	7d Survival Rate	100	>100	NA	NA	1	Fisher Exact Test
06-5842-9159	Reproduction	<100	100	NA	34.4%	>1	Equal Variance t Two-Sample Test

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
14-6919-3642	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
06-5842-9159	Reproduction	Control Resp	23.4	15 - NL	Yes	Passes Acceptability Criteria
06-5842-9159	Reproduction	PMSD	0.3438	0.13 - 0.47	Yes	Passes Acceptability Criteria

7d Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	10	1	1	1	1	1	0	0	0.0%	0.0%
100		9	0.7778	0.4388	1	0	1	0.147	0.441	56.69%	22.22%

Reproduction Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	10	23.4	14.22	32.58	0	38	4.058	12.83	54.85%	0.0%
100		9	9.222	5.016	13.43	0	15	1.824	5.472	59.34%	60.59%

7d Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Lab Control	1	1	1	1	1	1	1	1	1	1
100		0		1	1	1	1	1	0	1	1

Reproduction Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Lab Control	30	30	26	31	29	4	32	0	14	38
100		0		12	15	11	13	11	0	12	9

CETIS Analytical Report

Test Code: 1508-S069 | 07-9744-9290

Ceriodaphnia 7-d Survival and Reproduction Test — **Nautilus Environmental (CA)**

Analysis ID:	14-6919-3642	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	25 Aug-15 16:39	**Analysis:**	Single 2x2 Contingency Table	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	Test Result
Untransformed		C > T	NA	NA	Passes 7d survival rate

Fisher Exact Test

Control	vs	C-%	Test Stat	P-Value	P-Type	Decision(α:5%)
Lab Control		100	0.2105	0.2105	Exact	Non-Significant Effect

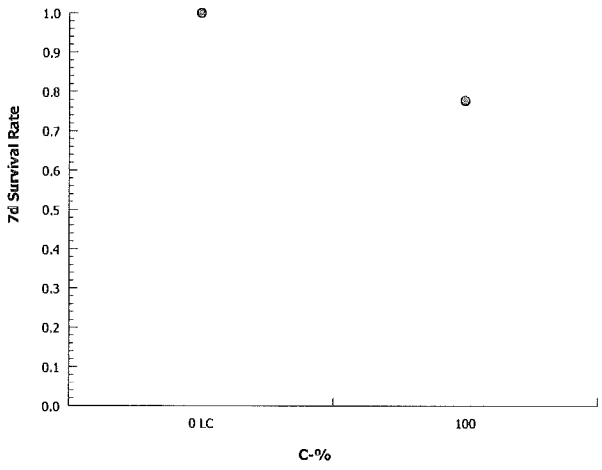
Data Summary

C-%	Control Type	NR	R	NR + R	Prop NR	Prop R	%Effect
0	Lab Control	10	0	10	1	0	0.0%
100		7	2	9	0.7778	0.2222	22.22%

7d Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Lab Control	1	1	1	1	1	1	1	1	1	1
100		0	1	1	1	1	1	0	1	1	

Graphics



 Analyst: VCR QA: KBB 8/26/15

CETIS Analytical Report

Test Code: 1508-S069 | 07-9744-9290

Ceriodaphnia 7-d Survival and Reproduction Test — **Nautilus Environmental (CA)**

Analysis ID:	06-5842-9159	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.8.7
Analyzed:	25 Aug-15 16:39	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Untransformed	NA	C > T	NA	NA	34.4%	Fails reproduction

Equal Variance t Two-Sample Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		100*	3.066	1.74	8.044	17	0.0035	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	952.1497	952.1497	1	9.4	0.0070	Significant Effect
Error	1721.956	101.2915	17			
Total	2674.105		18			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	5.501	7.339	0.0250	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8732	0.8605	0.0164	Normal Distribution

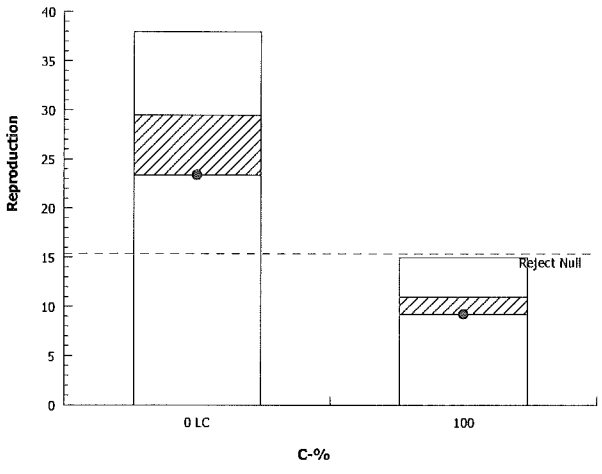
Reproduction Summary

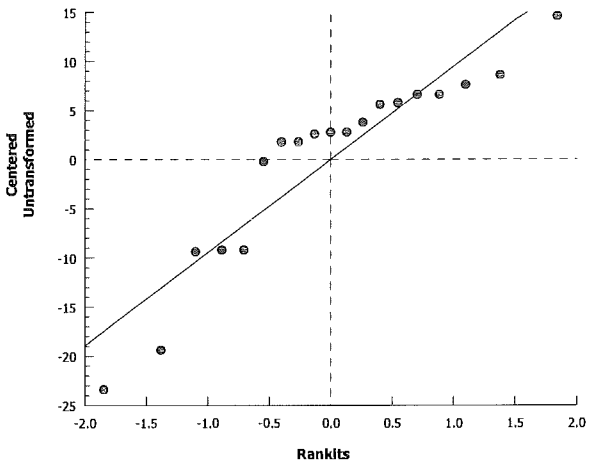
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	10	23.4	14.22	32.58	29.5	0	38	4.058	54.85%	0.0%
100		9	9.222	5.016	13.43	11	0	15	1.824	59.34%	60.59%

Reproduction Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Lab Control	30	30	26	31	29	4	32	0	14	38
100		0	12	15	11	13	11	0	12	9	

Graphics





 Analyst: VCR QA: KBD 8/26/15

Freshwater Chronic Bioassay | **Daphnid Survival and Reproduction Datasheet**

Test Species: ***C. dubia***

Client/Sample ID: Alpha Analytical Laboratories / Deuel Vocational Institution / Final Effluent **Start Date/Time:** 8/18/2015 1455

Test Number: Ⓐ ~~Final Effluent~~ 1508-S069 **End Date/Time:** 8/25/2015 1200

Conc.	Rep	Rand #	Daily Reproduction/ Survival 1	2	3	4	5	6	7	8	Total	QC
LC	1	19	0	0	0	6	11	13	0		30	
	2	12	0	0	0	5	12	0	13		30	13
	3	18	0	0	0	3	10	0	13		26	
	4	16	0	0	0	6	11	0	14		31	
	5	14	0	0	0	5	9	0	15		29	
	6	7	0	0	0	3	0	0	1*		4	
	7	1	0	0	0	4	11	0	17		32	
	8	8	0	0	0	0	@~~0~~	0	0		0	
	9	13	0	0	0	0	5	3	6*		14	
	10	3	0	0	0	7	11	0	20		38	
		Tech:	AD	EG	AG	AD	CH	AW	CH			AG

$\bar{x}$ = 23.4

Conc.	Rep	Rand #	Daily Reproduction/ Survival 1	2	3	4	5	6	7	8	Total	QC
100%	1	9	0	0	0	0	0/d	-	-		0/d	
	2	17	0	0	0	2	0/Q8	-	-		Q8	
	3	6	0	0	0	4	0	8	0		12	
	4	10	0	0	0	4	0	7	4*		15	
	5	20	0	0	0	2	4	0	5		11	
	6	5	0	0	0	4	3	0	6*		13	
	7	2	0	0	0	4	0	Ⓑ80	7		11	
	8	15	0/d	—	—	—	-	-	-		0/d	
	9	11	0	0	0	2	0	8	2		12	2
	10	4	0	0	0	0	3	6	0		9	

Conc.	Rep	Rand #	Daily Reproduction/ Survival 1	2	3	4	5	6	7	8	Total	QC
	1											
	2											
	3											
	4											
	5											
	6											
	7											
	8											
	9											
	10											

Neonates for each replicate were blocked across concentrations at test initiation

Rep:	1	2	3	4	5	6	7	8	9	10
Board:	99	—	—	—	—	—	—	7	100	7
Cup:	3	6	7	10	40	47	49	57	2	31
Rand # QC:	AW		Initiated By:	AW			Verified By:	AG		

Time Fed/Test Solution Renewed (day):

(0) 1455 (1) 1200 (2) 1325 (3) 1200 (4) 1110 (5) 1200 (6) 1125 (7) —

Notes: d = dead; M = male; LIP = lost in progress; B = 4th brood (only the first 3 broods are included in total)

* = dead neonates observed, but only live neonate counts recorded Ⓐ CH QA 9/23/15 Ⓑ QA VCR 8/25/15

Ⓑ AW QA 8/24/15

QC Check: VCR 8/25/15 **Final Review:** VCR 8/26/15

Freshwater Chronic Bioassay **Water Quality Measurements**

Client: Deuel Vocational Institution / Alpha Analytical Laboratories **Test Species:** *C.dubia*

Sample ID: Final Effluent **Start Date/Time:** 8/18/2015 1455

Test No: 1508-S069 **End Date/Time:** 8/25/2015 1200

Concentration	LC (8:2)							
Day	0	1	2	3	4	5	6	7
Initial								
pH	8.18	8.23	8.19	8.22	8.17	8.20	8.29	
DO (mg/L)	8.3	7.4	7.9	7.7	7.6	7.6	7.8	
Cond. (µmhos/cm)	192	187	188	185	194	193	194	
Temp (°C)	24.8	24.4	24.4	24.9	24.1	24.2	24.0	
Final								
pH		8.26	8.19	8.21	8.28	8.27	8.18	8.19
DO (mg/L)		9.0	7.9	7.9	8.1	8.3	7.6	8.0
Temp (°C)		24.5	25.4	24.8	25.1	25.0	25.0	24.0

Concentration	100%							
Day	0	1	2	3	4	5	6	7
Initial								
pH	7.45	7.67	7.44	7.53	7.50	7.57	7.58	
DO (mg/L)	8.7	8.4	8.8	8.6	8.6	8.5	8.6	
Cond. (µmhos/cm)	4020	3900	4000	3980	4380	4320	4380	
Temp (°C)	25.0	24.7	24.6	25.0	24.7	24.3	24.2	
Final								
pH		8.17	8.15	8.22	8.22	8.21	8.16	8.16
DO (mg/L)		9.0	7.9	8.0	8.2	8.2	7.7	7.7
Temp (°C)		24.5	25.4	24.8	25.1	25.0	25.0	24.0

Concentration								
Day	0	1	2	3	4	5	6	7
Initial								
pH								
DO (mg/L)								
Cond. (µmhos/cm)								
Temp (°C)								
Final								
pH								
DO (mg/L)								
Temp (°C)								

	0	1	2	3	4	5	6	7
Analysts: Initial:	EG	ALB	BK	AG	EG	BK	CH	–
Final:	–	ALB	EG	AG	AD	CH	AW	
Dilutions made by:	KB	AG	ALB	ALB	BK	EG	CH	–
Sample Used (A, B, C):	A	A	B	B	C	C	C	–

Comments: @BK QB 8/18/15

Animal Source/Date Received: Internal / ~~8 hrs~~ N/A **Animal Age at Initiation:** < 8 hrs

Sample Log-in Numbers: A: 15-0743 B: 15-0748 C: 15-0760

QC Check: VR 8/25/15 **Final Review:** KB 8/26/15

Nautilus Environmental. 4340 Vandever Avenue. San Diego, CA 92120.

Fathead Minnow 96-hour Survival

CETIS Summary Report

Fathead Minnow 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Batch ID:	17-6963-2862	**Test Type:**	Survival (96h)	**Analyst:**	
Start Date:	18 Aug-15 13:05	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Not Applicable
Ending Date:	22 Aug-15 11:40	**Species:**	Pimephales promelas	**Brine:**	Not Applicable
Duration:	95h	**Source:**	Aquatic Biosystems, CO	**Age:**	6d

Sample ID:	16-3203-4743	**Code:**	15-0742	**Client:**	Alpha Analytical Laboratories
Sample Date:	17 Aug-15 08:35	**Material:**	Effluent Sample	**Project:**	
Receive Date:	18 Aug-15 10:26	**Source:**	Deuel Vocational Institution		
Sample Age:	28h (1 °C)	**Station:**	Final Effluent		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
08-3965-7521	96h Survival Rate	100	>100	NA	5.2%	1	Wilcoxon Rank Sum Two-Sample Test

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
08-3965-7521	96h Survival Rate	Control Resp	0.975	0.9 - NL	Yes	Passes Acceptability Criteria

96h Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.975	0.8954	1	0.9	1	0.025	0.05	5.13%	0.0%
100		4	1	1	1	1	1	0	0	0.0%	-2.56%

96h Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1	1	0.9	1
100		1	1	1	1

Fathead Minnow 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Analysis ID: 08-3965-7521 **Endpoint:** 96h Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 24 Aug-15 12:18 **Analysis:** Nonparametric-Two Sample **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Angular (Corrected)	NA	C > T	NA	NA	5.2%	Passes 96h survival rate

Wilcoxon Rank Sum Two-Sample Test

Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		100	20	NA	1	6	1.0000	Exact	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.003319917	0.003319917	1	1	0.3559	Non-Significant Effect
Error	0.0199195	0.003319917	6			
Total	0.02323942		7			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	1	13.75	0.3559	Equal Variances
Variances	Levene Equality of Variance	9	13.75	0.0240	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.7065	0.6451	0.0027	Non-normal Distribution

96h Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	0.975	0.8954	1	1	0.9	1	0.025	5.13%	0.0%
100		4	1	1	1	1	1	1	0	0.0%	-2.56%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	1.371	1.242	1.501	1.412	1.249	1.412	0.04074	5.94%	0.0%
100		4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	-2.97%

96h Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1	1	0.9	1
100		1	1	1	1

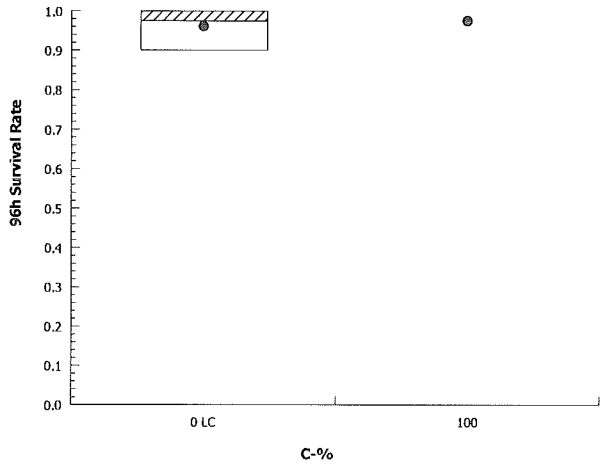
Angular (Corrected) Transformed Detail

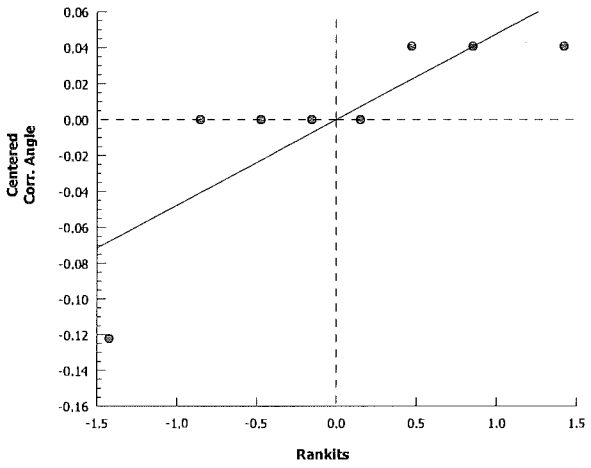
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1.412	1.412	1.249	1.412
100		1.412	1.412	1.412	1.412

Fathead Minnow 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Analysis ID:	08-3965-7521	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Aug-15 12:18	**Analysis:**	Nonparametric-Two Sample	**Official Results:**	Yes

Graphics





96-hour Freshwater Acute Bioassay
Static-Renewal Conditions

Water Quality Measurements
& Test Organism Survival

Client: Deuel Vocational Institution / Alpha Analytical Laboratories
Sample ID: Final Effluent.
Test No.: 1508-S067

Test Species: *P. promelas*
Start Date/Time: 8/18/2015 1305
End Date/Time: ~~8/25/2015~~ 1140 Ⓐ 8/22/2015

Tech Initials	0	24	48	72	96
Counts:	EG	ALB	VCR	EG	EG
Readings:	EG	ALB	BK	AG	EG
Dilutions made by:	KB		ALB		

Concentration (%)	Rep	Number of Live Organisms					Conductivity (µmhos/cm)					Temperature (°C)					Dissolved Oxygen (mg/L)					pH (units)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Lab Control	A	10	10	10	10	10	193	189	i 186	187	199	20.2	20.5	i 20.0	20.5	20.4	8.3	7.8	i 8.1	7.7	7.8	8.20	8.00	i 8.19	7.99	7.95
(8:2)	B	10	10	10	10	10			f 194					f 20.1					f 6.6					f 7.91		
	C	10	10	10	10	9																				
	D	10	10	10	10	10											Q14									
100%	A	10	10	10	10	10	4090	3870	i 3940	3890	4100	20.0	20.4	i 19.7	20.4	20.4	9.3	7.9	i 8.8	8.2	7.8	7.68	7.94	i 7.51	7.85	7.55
	B	10	10	10	10	10			f 3900					f 20.0					f 7.2					f 8.00		
	C	10	10	10	10	10																				
	D	10	10	10	10	10																				
	A								i					i					i					i		
	B								f					f					f					f		
	C																									
	D																									
	A								i					i					i					i		
	B								f					f					f					f		
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	A								i					i					i					i		
	B								f					f					f					f		
	C																									
	D																									
	A								i					i					i					i		
	B								f					f					f					f		
	C																									
	D																									

Initial Counts QC'd by: KB

Animal Source/Date Received: ABS 8/18/15 **Age at Initiation:** 6d

Feeding Times	0	24	48	72	96
AM:	--	--	0830	--	--
PM:	--	--	--	--	--

Comments: i = initial reading in fresh test solution, f = final reading in test chamber prior to renewal
Organisms fed prior to initiation, circle one (y) / n) Ⓐ Q18 VCR 8/24/15

QC Check: VCR 8/24/15

Final Review: KB 8/25/15

Appendix D
Reference Toxicant Test Data

Green Algae 96-hour Chronic Growth

CETIS Summary Report

Selenastrum Growth Test — **Nautilus Environmental (CA)**

Batch ID:	04-5856-5310	**Test Type:**	Cell Growth	**Analyst:**	
Start Date:	18 Aug-15 15:15	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Nutrient Enriched Water
Ending Date:	22 Aug-15 13:00	**Species:**	Selenastrum capricornutum	**Brine:**	Not Applicable
Duration:	94h	**Source:**	In-House Culture	**Age:**	5d

Sample ID:	13-7586-0740	**Code:**	150818scrt	**Client:**	Internal
Sample Date:	18 Aug-15	**Material:**	Copper chloride	**Project:**	
Receive Date:	18 Aug-15	**Source:**	Reference Toxicant		
Sample Age:	15h	**Station:**	Copper Chloride		

Batch Note: Q16: However, the percent effect in the LOEC (37.5 ug/L) was 29.74 percent, which is above the lower bound PMSD limit of 9.1 and is therefore considered significant according to EPA 2000.

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
21-2657-2186	Cell Density	18.8	37.5	26.55	8.87%		Steel Many-One Rank Sum Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
12-8425-2408	Cell Density	IC25	31.27	18.68	42.96		Linear Interpolation (ICPIN)
		IC50	48.37	43.69	52.41		

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
12-8425-2408	Cell Density	Control CV	0.07032	NL - 0.2	Yes	Passes Acceptability Criteria
21-2657-2186	Cell Density	Control CV	0.07032	NL - 0.2	Yes	Passes Acceptability Criteria
12-8425-2408	Cell Density	Control Resp	2.71E+6	1.00E+6 - NL	Yes	Passes Acceptability Criteria
21-2657-2186	Cell Density	Control Resp	2.71E+6	1.00E+6 - NL	Yes	Passes Acceptability Criteria
21-2657-2186	Cell Density	PMSD	0.0887	0.091 - 0.29	Yes	Below Acceptability Criteria Q16

Cell Density Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	2.711E+6	2.407E+6	3.014E+6	2.509E+6	2.890E+6	9.531E+4	1.906E+5	7.03%	0.0%
9.4		4	2.734E+6	2.617E+6	2.851E+6	2.628E+6	2.795E+6	3.681E+4	7.361E+4	2.69%	-0.87%
18.8		4	2.317E+6	2.000E+6	2.634E+6	2.118E+6	2.516E+6	9.969E+4	1.994E+5	8.61%	14.53%
37.5		4	1.905E+6	1.594E+6	2.215E+6	1.736E+6	2.170E+6	9.770E+4	1.954E+5	10.26%	29.74%
75		4	3.075E+4	1.940E+4	4.210E+4	2.300E+4	3.900E+4	3.568E+3	7.136E+3	23.21%	98.87%
150		4	3.250E+3	-1.502E+3	8.002E+3	0.000E+0	7.000E+3	1.493E+3	2.986E+3	91.88%	99.88%

Cell Density Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	2.856E+6	2.509E+6	2.588E+6	2.890E+6
9.4		2.628E+6	2.795E+6	2.746E+6	2.768E+6
18.8		2.516E+6	2.458E+6	2.118E+6	2.175E+6
37.5		1.736E+6	1.782E+6	2.170E+6	1.930E+6
75		3.400E+4	3.900E+4	2.700E+4	2.300E+4
150		0.000E+0	2.000E+3	4.000E+3	7.000E+3

Selenastrum Growth Test — **Nautilus Environmental (CA)**

Analysis ID: 21-2657-2186 **Endpoint:** Cell Density **CETIS Version:** CETISv1.8.7
Analyzed: 24 Aug-15 12:53 **Analysis:** Nonparametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	8.87%	18.8	37.5	26.55	

Steel Many-One Rank Sum Test

Control	vs	C-µg/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		9.4	18	10	0	6	0.8333	Asymp	Non-Significant Effect
		18.8	11	10	0	6	0.0805	Asymp	Non-Significant Effect
		37.5*	10	10	0	6	0.0417	Asymp	Significant Effect
		75*	10	10	0	6	0.0417	Asymp	Significant Effect
		150*	10	10	0	6	0.0417	Asymp	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	3.254888E+13	6.509776E+12	5	326.2	<0.0001	Significant Effect
Error	3.592207E+11	19956710000	18			
Total	3.29081E+13		23			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	34.66	15.09	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.9429	0.884	0.1891	Normal Distribution

Cell Density Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	2.711E+6	2.407E+6	3.014E+6	2722000	2.509E+6	2.890E+6	9.531E+4	7.03%	0.0%
9.4		4	2.734E+6	2.617E+6	2.851E+6	2757000	2.628E+6	2.795E+6	3.681E+4	2.69%	-0.87%
18.8		4	2.317E+6	2.000E+6	2.634E+6	2317000	2.118E+6	2.516E+6	9.969E+4	8.61%	14.53%
37.5		4	1.905E+6	1.594E+6	2.215E+6	1856000	1.736E+6	2.170E+6	9.770E+4	10.26%	29.74%
75		4	3.075E+4	1.940E+4	4.210E+4	30500	2.300E+4	3.900E+4	3.568E+3	23.21%	98.87%
150		4	3.250E+3	-1.502E+3	8.002E+3	3000	0.000E+0	7.000E+3	1.493E+3	91.88%	99.88%

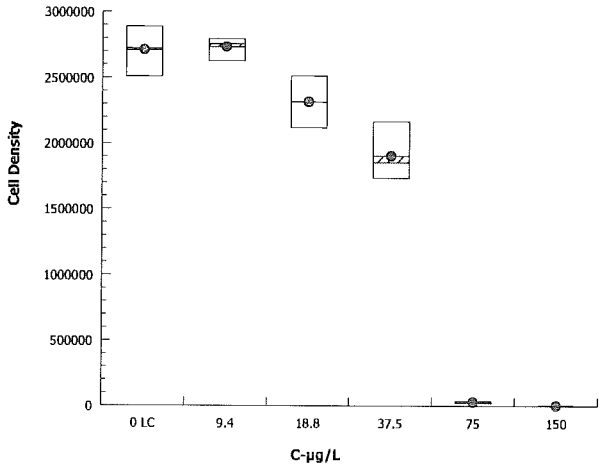
Cell Density Detail

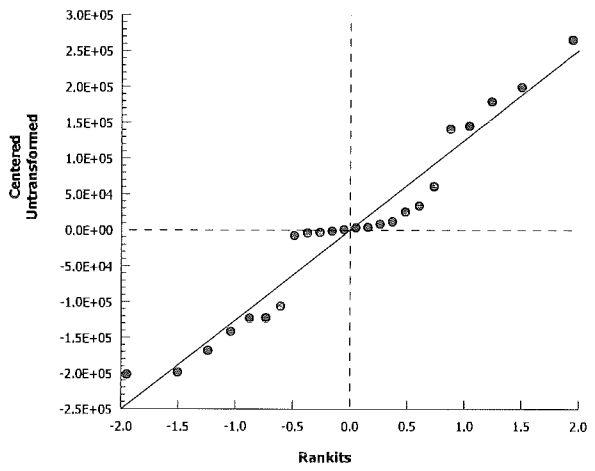
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	2.856E+6	2.509E+6	2.588E+6	2.890E+6
9.4		2.628E+6	2.795E+6	2.746E+6	2.768E+6
18.8		2.516E+6	2.458E+6	2.118E+6	2.175E+6
37.5		1.736E+6	1.782E+6	2.170E+6	1.930E+6
75		3.400E+4	3.900E+4	2.700E+4	2.300E+4
150		0.000E+0	2.000E+3	4.000E+3	7.000E+3

Selenastrum Growth Test — **Nautilus Environmental (CA)**

Analysis ID:	21-2657-2186	**Endpoint:**	Cell Density	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Aug-15 12:53	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Selenastrum Growth Test — **Nautilus Environmental (CA)**

Analysis ID:	12-8425-2408	**Endpoint:**	Cell Density	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Aug-15 12:54	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1641674	1000	Yes	Two-Point Interpolation

Point Estimates

Level	µg/L	95% LCL	95% UCL
IC25	31.27	18.68	42.96
IC50	48.37	43.69	52.41

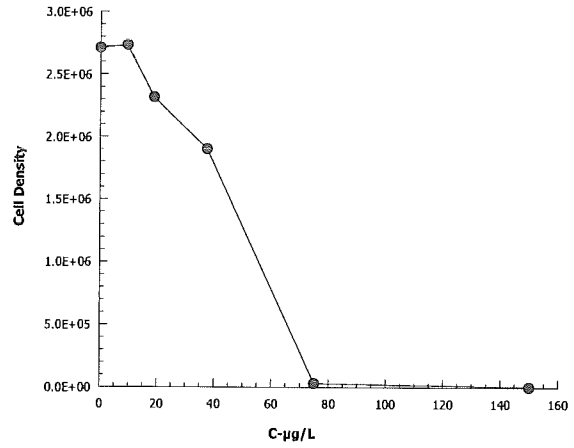
Cell Density Summary

			Calculated Variate						
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	2.711E+6	2.509E+6	2.890E+6	9.531E+4	1.906E+5	7.03%	0.0%
9.4		4	2.734E+6	2.628E+6	2.795E+6	3.681E+4	7.361E+4	2.69%	-0.87%
18.8		4	2.317E+6	2.118E+6	2.516E+6	9.969E+4	1.994E+5	8.61%	14.53%
37.5		4	1.905E+6	1.736E+6	2.170E+6	9.770E+4	1.954E+5	10.26%	29.74%
75		4	3.075E+4	2.300E+4	3.900E+4	3.568E+3	7.136E+3	23.21%	98.87%
150		4	3.250E+3	0.000E+0	7.000E+3	1.493E+3	2.986E+3	91.88%	99.88%

Cell Density Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	2.856E+6	2.509E+6	2.588E+6	2.890E+6
9.4		2.628E+6	2.795E+6	2.746E+6	2.768E+6
18.8		2.516E+6	2.458E+6	2.118E+6	2.175E+6
37.5		1.736E+6	1.782E+6	2.170E+6	1.930E+6
75		3.400E+4	3.900E+4	2.700E+4	2.300E+4
150		0.000E+0	2.000E+3	4.000E+3	7.000E+3

Graphics

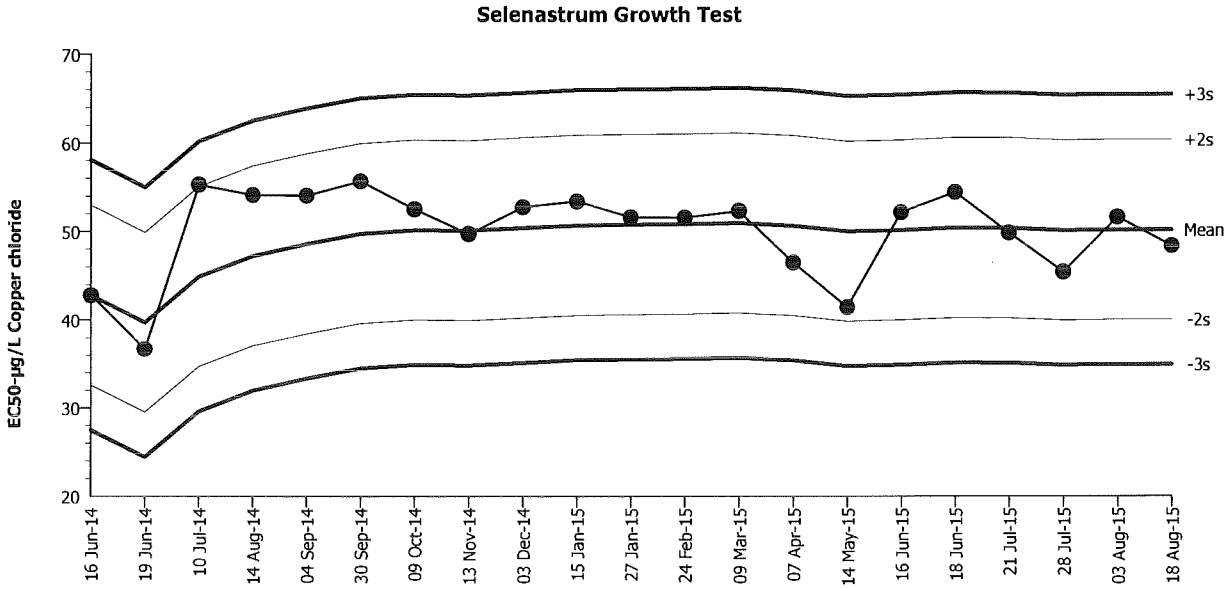


CETIS QC Plot

Report Date: 01 Sep-15 14:25 (1 of 1)

Selenastrum Growth Test — **Nautilus Environmental (CA)**

Test Type:	Cell Growth	**Organism:**	Selenastrum capricornutum (Green	**Material:**	Copper chloride
Protocol:	EPA/821/R-02-013 (2002)	**Endpoint:**	Cell Density	**Source:**	Reference Toxicant-REF



Mean:	50.22	**Count:**	20	**-2s Warning Limit:**	40.04	**-3s Action Limit:**	34.94
Sigma:	5.092	**CV:**	10.10%	**+2s Warning Limit:**	60.4	**+3s Action Limit:**	65.5

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2014	Jun	16	15:10	42.79	-7.428	-1.459			10-1755-7810	15-6584-0145
2			19	13:00	36.69	-13.53	-2.657	(-)		15-7341-7331	10-2337-8161
3		Jul	10	16:00	55.31	5.094	1			19-1696-7868	15-9662-7047
4		Aug	14	15:00	54.15	3.926	0.7709			02-2999-5939	14-8521-4748
5		Sep	4	15:55	54.08	3.86	0.7581			08-9694-1534	20-3830-1721
6			30	15:15	55.7	5.476	1.075			09-5264-3988	04-8584-3476
7		Oct	9	16:40	52.52	2.303	0.4523			09-5509-4099	01-3950-5623
8		Nov	13	15:40	49.76	-0.4634	-0.09101			01-8291-9026	09-2622-6392
9		Dec	3	15:05	52.78	2.555	0.5018			13-1752-8006	07-0151-0293
10	2015	Jan	15	15:50	53.42	3.202	0.6289			12-7590-3897	05-6189-7569
11			27	15:45	51.65	1.427	0.2802			16-2613-5448	09-6828-4812
12		Feb	24	16:30	51.6	1.381	0.2712			09-4362-6615	18-0710-1129
13		Mar	9	19:00	52.35	2.133	0.4189			12-0803-4267	18-0043-2497
14		Apr	7	15:45	46.52	-3.701	-0.7269			17-2347-9095	04-5950-9207
15		May	14	17:00	41.47	-8.753	-1.719			08-4681-1156	08-3785-2634
16		Jun	16	16:00	52.19	1.971	0.387			06-7469-4638	04-3734-1008
17			18	16:15	54.47	4.249	0.8344			04-0368-6277	08-2233-2128
18		Jul	21	13:15	49.89	-0.3328	-0.06535			11-2288-6753	07-8077-7240
19			28	15:30	45.44	-4.783	-0.9393			21-3289-4850	14-5475-0351
20		Aug	3	13:50	51.64	1.416	0.278			16-2718-2287	10-0061-6945
21			18	15:15	48.37	-1.848	-0.3629			04-1917-1411	12-8425-2408

Fluorometric & Microscopic Determination of Cell Density
Turner Fluorometer Model TD-700

Test Species: *S. capricornutum*

Client : Internal

Start Date/Time: 8/18/2015 1515

Sample ID: $CuCl_2$

End Date/Time: 8/22/2015 1300

Test No: 150818scrt

Analyst: BK

Random Number	Cell Density (fluorometric) (cells/ml *10^5)	Dilution	Cell Density (microscopic) (cells/ml *10^4)
Blank		NA	
Cal Check 1 (NEW, Solid)	0.00, 2.34		
1	26.28		
2	27.95		
3	17.82		
4	0.39		
5	0.27		
6	0.34		
7	21.70		
8	21.75		
9	25.88		
10	27.46		
11	27.68		
12	19.30		
Cal Check 2 (NEW, Solid)	0.00, 2.33		
13	0.23		
14	17.36		
15	25.09		
16	25.16		
17	24.58		
18	0.02		
19	0.00		
20	28.90		
21	21.18		
22	0.07		
23	28.56		
24	0.04		

Comments:

QC Check: VCR 8/24/15

Final Review: KBB 8/25/15

CETIS Test Data Worksheet

Selenastrum Growth Test — **Nautilus Environmental (CA)**

Start Date: 18 Aug-15 | **Species:** Selenastrum capricornutum | **Sample Code:** 150818scrt
End Date: 22 Aug-15 | **Protocol:** EPA/821/R-02-013 (2002) | **Sample Source:** Reference Toxicant
Sample Date: 18 Aug-15 | **Material:** Copper chloride | **Sample Station:** Copper Chloride

C-µg/L	Code	Rep	Pos	Cell Density	Absorbance	Biomass	Chlorophyll a	Notes
0	LC	1	23					
0	LC	2	15					
0	LC	3	9					
0	LC	4	20					
9.4		1	1					
9.4		2	2					
9.4		3	10					
9.4		4	11					
18.8		1	16					
18.8		2	17					
18.8		3	21					
18.8		4	8					
37.5		1	14					
37.5		2	3					
37.5		3	7					
37.5		4	12					
75		1	6					
75		2	4					
75		3	5					
75		4	13					
150		1	19					
150		2	18					
150		3	24					
150		4	22					

QL: 75

Freshwater Chronic Bioassay

Water Quality Measurements
Algal Growth Inhibition

Client : Internal **Test Species:** *S. capricornutum*

Sample ID: $CuCl_2$ **Start Date/Time:** 8/18/2015 1515

Test No: 150818scrt **End Date/Time:** 8/22/2015 1300

Dilutions made by: MG

High conc. made (µg/L):	150
Vol. Cu stock added (mL):	16.9
Final Volume (mL):	1000
Cu stock concentration (µg/L):	8850

	Initial Readings				Final Readings	
Concentration (µg/L)	**D.O. (mg/L)**	**Conductivity (umhos-cm)**	**Alkalinity (mg/L)**	**Hardness (mg/L)**	**D.O. (mg/L)**	**Conductivity (umhos-cm)**
Lab Control	6.2	(A) ~~92.7~~ 93	11	14	9.8	(A) ~~87.4~~ 87
9.4	6.8	(A) ~~92.5~~ 93	11	15	9.9	(A) ~~88.6~~ 89
18.8	6.6	(A) ~~92.7~~ 93	–	–	9.6	(A) ~~91.3~~ 91
37.5	6.4	(A) ~~92.5~~ 93	10	17	9.4	(A) ~~90.1~~ 90
75	6.5	(A) ~~92.5~~ 93	–	–	8.1	91
150	6.4	(A) ~~92.3~~ 92	12	17	7.9	90

		0 Hour	24 Hour	48 Hour	72 Hour	96 Hour
pH/Temperature (°C):	LC	7.41 / 24.4	7.77 / 24.6	8.05 / 24.5	9.73 / 24.7	9.84 / 24.6
pH/Temperature (°C):	9.4	7.42 / 24.7	7.64 / 24.8	8.02 / 24.6	9.78 / 24.9	9.80 / 25.1
pH/Temperature (°C):	18.8	7.44 / 24.5	7.65 / 24.6	7.99 / 24.3	9.68 / 25.2	9.68 / 25.0
pH/Temperature (°C):	37.5	7.48 / 24.7	7.62 / 25.0	7.91 / 24.9	9.44 / 25.2	9.51 / 25.1
pH/Temperature (°C):	75	7.45 / 24.8	7.61 / 25.0	7.77 / 24.9	8.76 / 25.1	8.65 / 25.2
pH/Temperature (°C):	150	7.42 / 25.4	7.57 / 24.6	7.68 / 24.6	8.35 / 25.1	8.37 / 24.9
	Technician:	MG	ALB	BK	AG	EG

Comments: (A) BK QB 8/22/15

QC Check: VCR 8/24/15 **Final Review:** KB 8/25/15

Freshwater Chronic Bioassay **Algal Growth Inhibition Worksheet**

Client/Sample ID : Internal / CuCl2 **Test Species:** *S. capricornutum*

Test No: 150818scrt. **Start Date/Time:** 8/18/2015 1515

Analyst: PA **End Date/Time:** 8/22/2015 1300

Culture Used (circle one): (Nutrient Enriched DI Water (NEW)) / Very Hard Water (VHW)

Date Stock Culture Started: 8/13/15 Culture Age: 5 d

Culture subsample inspected for algal cell health? WS (initials) bacteria/invasive algal species present? Y (N)

Stock Cell Density Measurements: 41.35
42.40
42.26
42.35
42.03

Mean: 42.08

(mean no. * 100,000)/(500,000) = x (dilution factor): 8.4

Prepare inoculum according to the dilution factor. This yields a solution with the desired cell density of 500,000 cells/ml.

dil. factor 8.4

-1.0 part Sele stock = 15 ml

7.4 part(s) NEW = 111 ml

Inoculate 1 ml into 3 initial count flasks containing 50 ml of NEW, stir and count on the hemacytometer. Flasks should contain a final density of 10,000 cells/ml ± 10%.

Inoculum Cell Density Confirmation Counts: 1
0
2

Mean: 1

Location in Environmental Chamber (All replicates in each test must be on the same shelf; do not split up tests among shelves):

Shelf Number	Measured Light Intensity Range (must be between 360 and 440 ft-c)	Random Number Range
(A) 17	371 — 428	25-36
(B) 29	368 — 423	1-24
3		
4		
5		
6		

Are lights on 24 hour cycle? (Y) / N

Comments: (A) Q18 & 8/19/15

QC Check: VCR 8/24/15 Final Review: KCB 8/25/15

Fathead Minnow 7-day Chronic Survival and Growth

CETIS Summary Report

Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental (CA)**

Batch ID:	14-8068-4698	**Test Type:**	Growth-Survival (7d)	**Analyst:**	
Start Date:	18 Aug-15 14:15	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Diluted Mineral Water (8:2)
Ending Date:	25 Aug-15 11:40	**Species:**	Pimephales promelas	**Brine:**	Not Applicable
Duration:	6d 21h	**Source:**	Aquatic Biosystems, CO	**Age:**	1d

Sample ID:	05-1153-4250	**Code:**	150818pprt	**Client:**	Internal
Sample Date:	18 Aug-15	**Material:**	Copper chloride	**Project:**	
Receive Date:	18 Aug-15	**Source:**	Reference Toxicant		
Sample Age:	14h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
09-5306-5785	7d Survival Rate	15	30	21.21	12.0%		Dunnett Multiple Comparison Test
01-7331-4989	96h Survival Rate	15	30	21.21	12.6%		Dunnett Multiple Comparison Test
04-7679-9598	Mean Dry Biomass-mg	15	>15	NA	22.0%		Equal Variance t Two-Sample Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
06-1808-0046	7d Survival Rate	EC50	60.8	47.49	77.83		Trimmed Spearman-Kärber
08-6213-2861	96h Survival Rate	EC50	73.44	57.47	93.86		Trimmed Spearman-Kärber
13-7861-7654	Mean Dry Biomass-mg	IC25	28.49	14.08	54.46		Linear Interpolation (ICPIN)
		IC50	59.13	21.52	97.82		

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
06-1808-0046	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
09-5306-5785	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
04-7679-9598	Mean Dry Biomass-mg	Control Resp	0.4683	0.25 - NL	Yes	Passes Acceptability Criteria
13-7861-7654	Mean Dry Biomass-mg	Control Resp	0.4683	0.25 - NL	Yes	Passes Acceptability Criteria
04-7679-9598	Mean Dry Biomass-mg	PMSD	0.2199	0.12 - 0.3	Yes	Passes Acceptability Criteria

7d Survival Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	1	1	1	1	1	0	0	0.0%	0.0%
15		4	0.875	0.6748	1	0.7	1	0.06292	0.1258	14.38%	12.5%
30		4	0.675	0.5227	0.8273	0.6	0.8	0.04787	0.09574	14.18%	32.5%
60		4	0.5	0.275	0.725	0.4	0.7	0.07071	0.1414	28.28%	50.0%
120		4	0.375	0.2227	0.5273	0.3	0.5	0.04787	0.09574	25.53%	62.5%
240		4	0.025	0	0.1046	0	0.1	0.025	0.05	200.0%	97.5%

96h Survival Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	1	1	1	1	1	0	0	0.0%	0.0%
15		4	0.925	0.7727	1	0.8	1	0.04787	0.09574	10.35%	7.5%
30		4	0.725	0.6454	0.8046	0.7	0.8	0.025	0.05	6.9%	27.5%
60		4	0.575	0.3032	0.8468	0.4	0.8	0.08539	0.1708	29.7%	42.5%
120		4	0.4	0.2701	0.5299	0.3	0.5	0.04082	0.08165	20.41%	60.0%
240		4	0.125	0	0.2773	0	0.2	0.04787	0.09574	76.59%	87.5%

Mean Dry Biomass-mg Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.4683	0.4242	0.5123	0.448	0.509	0.01384	0.02768	5.91%	0.0%
15		4	0.4645	0.3017	0.6273	0.333	0.563	0.05116	0.1023	22.03%	0.8%
30		4	0.3385	0.1412	0.5358	0.232	0.498	0.062	0.124	36.63%	27.71%
60		4	0.231	0.07851	0.3835	0.148	0.344	0.04791	0.09583	41.48%	50.67%
120		4	0.1088	0.05568	0.1618	0.072	0.153	0.01668	0.03335	30.67%	76.78%
240		4	0.00775	-0.01691	0.03241	0	0.031	0.00775	0.0155	200.0%	98.34%

Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental (CA)**

7d Survival Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1	1	1	1
15		0.7	0.9	0.9	1
30		0.7	0.6	0.8	0.6
60		0.5	0.4	0.7	0.4
120		0.3	0.3	0.5	0.4
240		0	0	0.1	0

96h Survival Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1	1	1	1
15		0.8	1	0.9	1
30		0.7	0.7	0.8	0.7
60		0.6	0.5	0.8	0.4
120		0.4	0.3	0.5	0.4
240		0.2	0	0.1	0.2

Mean Dry Biomass-mg Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	0.509	0.455	0.461	0.448
15		0.333	0.437	0.563	0.525
30		0.375	0.232	0.498	0.249
60		0.277	0.155	0.344	0.148
120		0.105	0.072	0.105	0.153
240		0	0	0.031	0

Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental (CA)**

Analysis ID: 01-7331-4989 **Endpoint:** 96h Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 28 Aug-15 11:51 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	12.6%	15	30	21.21	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		15	1.384	2.407	0.203	6	0.2672	CDF	Non-Significant Effect
		30*	4.637	2.407	0.203	6	0.0005	CDF	Significant Effect
		60*	6.462	2.407	0.203	6	<0.0001	CDF	Significant Effect
		120*	8.618	2.407	0.203	6	<0.0001	CDF	Significant Effect
		240*	12.54	2.407	0.203	6	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	3.089178	0.6178355	5	43.25	<0.0001	Significant Effect
Error	0.2571435	0.01428575	18			
Total	3.346321		23			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	2.35	4.248	0.0828	Equal Variances
Variances	Levene Equality of Variance	2.927	4.248	0.0418	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9435	0.884	0.1952	Normal Distribution

96h Survival Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	1	1	1	1	1	1	0	0.0%	0.0%
15		4	0.925	0.7727	1	0.95	0.8	1	0.04787	10.35%	7.5%
30		4	0.725	0.6454	0.8046	0.7	0.7	0.8	0.025	6.9%	27.5%
60		4	0.575	0.3032	0.8468	0.55	0.4	0.8	0.08539	29.7%	42.5%
120		4	0.4	0.2701	0.5299	0.4	0.3	0.5	0.04082	20.41%	60.0%
240		4	0.125	0	0.2773	0.15	0	0.2	0.04787	76.59%	87.5%

Angular (Corrected) Transformed Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	0.0%
15		4	1.295	1.061	1.529	1.331	1.107	1.412	0.07348	11.35%	8.28%
30		4	1.02	0.9279	1.112	0.9912	0.9912	1.107	0.029	5.69%	27.75%
60		4	0.8658	0.5784	1.153	0.8357	0.6847	1.107	0.09033	20.87%	38.68%
120		4	0.6836	0.5499	0.8173	0.6847	0.5796	0.7854	0.04201	12.29%	51.59%
240		4	0.352	0.121	0.5829	0.3927	0.1588	0.4636	0.07256	41.23%	75.07%

96h Survival Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1	1	1	1
15		0.8	1	0.9	1
30		0.7	0.7	0.8	0.7
60		0.6	0.5	0.8	0.4
120		0.4	0.3	0.5	0.4
240		0.2	0	0.1	0.2

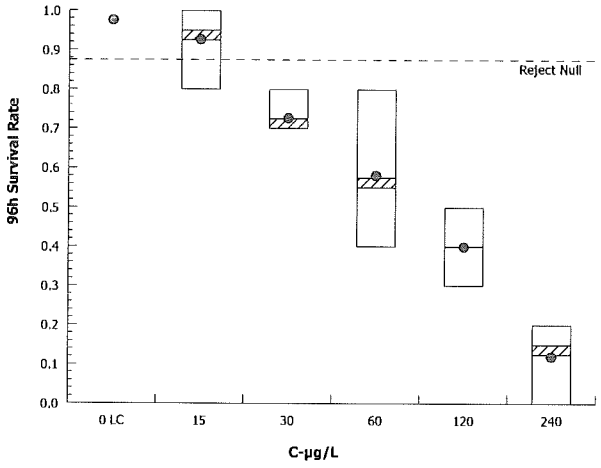
Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental (CA)**

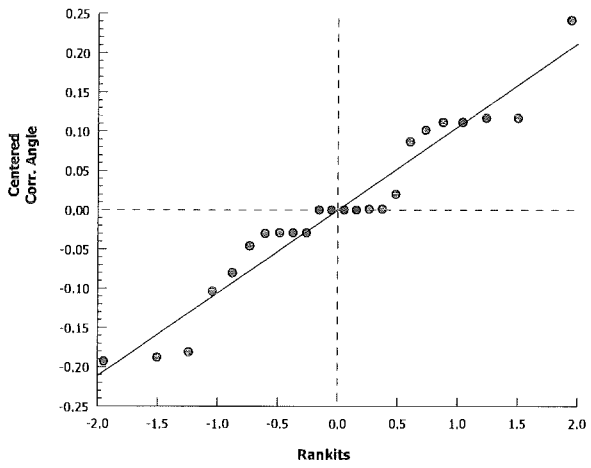
Analysis ID: 01-7331-4989 **Endpoint:** 96h Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 28 Aug-15 11:51 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Angular (Corrected) Transformed Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1.412	1.412	1.412	1.412
15		1.107	1.412	1.249	1.412
30		0.9912	0.9912	1.107	0.9912
60		0.8861	0.7854	1.107	0.6847
120		0.6847	0.5796	0.7854	0.6847
240		0.4636	0.1588	0.3218	0.4636

Graphics





Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental (CA)**

Analysis ID: 08-6213-2861 **Endpoint:** 96h Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 28 Aug-15 11:51 **Analysis:** Trimmed Spearman-Kärber **Official Results:** Yes

Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	12.50%	1.866	0.05326	73.44	57.47	93.86

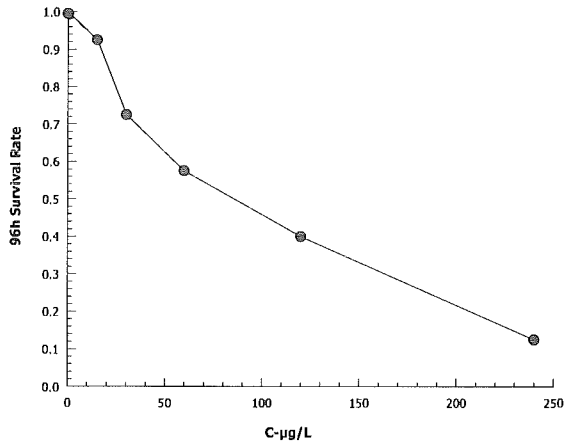
96h Survival Rate Summary

			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	4	1	1	1	0	0	0.0%	0.0%	40	40
15		4	0.925	0.8	1	0.04787	0.09574	10.35%	7.5%	37	40
30		4	0.725	0.7	0.8	0.025	0.05	6.9%	27.5%	29	40
60		4	0.575	0.4	0.8	0.08539	0.1708	29.7%	42.5%	23	40
120		4	0.4	0.3	0.5	0.04082	0.08165	20.41%	60.0%	16	40
240		4	0.125	0	0.2	0.04787	0.09574	76.59%	87.5%	5	40

96h Survival Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1	1	1	1
15		0.8	1	0.9	1
30		0.7	0.7	0.8	0.7
60		0.6	0.5	0.8	0.4
120		0.4	0.3	0.5	0.4
240		0.2	0	0.1	0.2

Graphics



Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental (CA)**

Analysis ID: 09-5306-5785 **Endpoint:** 7d Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 28 Aug-15 11:51 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	12.0%	15	30	21.21	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		15	2.306	2.407	0.195	6	0.0604	CDF	Non-Significant Effect
		30*	5.49	2.407	0.195	6	0.0001	CDF	Significant Effect
		60*	7.728	2.407	0.195	6	<0.0001	CDF	Significant Effect
		120*	9.323	2.407	0.195	6	<0.0001	CDF	Significant Effect
		240*	14.98	2.407	0.195	6	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	3.724634	0.7449269	5	56.85	<0.0001	Significant Effect
Error	0.2358813	0.01310452	18			
Total	3.960515		23			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	0.9958	4.248	0.4480	Equal Variances
Variances	Levene Equality of Variance	1.64	4.248	0.2003	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9445	0.884	0.2056	Normal Distribution

7d Survival Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	1	1	1	1	1	1	0	0.0%	0.0%
15		4	0.875	0.6748	1	0.9	0.7	1	0.06292	14.38%	12.5%
30		4	0.675	0.5227	0.8273	0.65	0.6	0.8	0.04787	14.18%	32.5%
60		4	0.5	0.275	0.725	0.45	0.4	0.7	0.07071	28.28%	50.0%
120		4	0.375	0.2227	0.5273	0.35	0.3	0.5	0.04787	25.53%	62.5%
240		4	0.025	0	0.1046	0	0	0.1	0.025	200.0%	97.5%

Angular (Corrected) Transformed Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	0.0%
15		4	1.225	0.9485	1.502	1.249	0.9912	1.412	0.08699	14.2%	13.22%
30		4	0.9676	0.7999	1.135	0.9386	0.8861	1.107	0.05269	10.89%	31.47%
60		4	0.7865	0.5566	1.016	0.7351	0.6847	0.9912	0.07223	18.37%	44.3%
120		4	0.6573	0.5003	0.8144	0.6322	0.5796	0.7854	0.04935	15.01%	53.45%
240		4	0.1995	0.06986	0.3292	0.1588	0.1588	0.3218	0.04074	40.84%	85.87%

7d Survival Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1	1	1	1
15		0.7	0.9	0.9	1
30		0.7	0.6	0.8	0.6
60		0.5	0.4	0.7	0.4
120		0.3	0.3	0.5	0.4
240		0	0	0.1	0

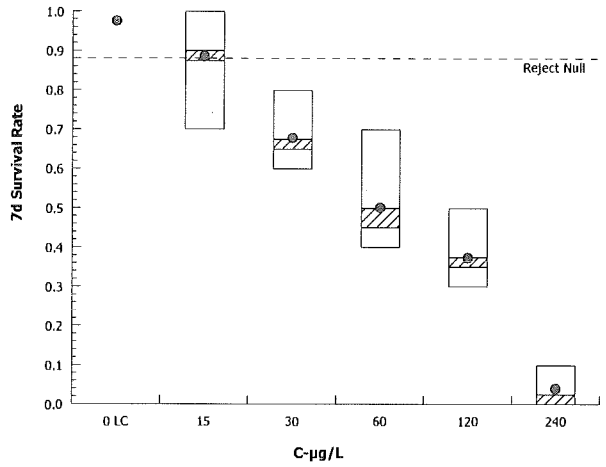
Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental (CA)**

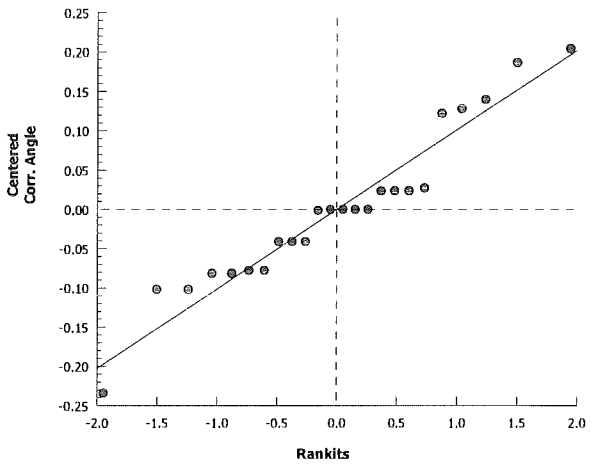
Analysis ID:	09-5306-5785	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	28 Aug-15 11:51	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Angular (Corrected) Transformed Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1.412	1.412	1.412	1.412
15		0.9912	1.249	1.249	1.412
30		0.9912	0.8861	1.107	0.8861
60		0.7854	0.6847	0.9912	0.6847
120		0.5796	0.5796	0.7854	0.6847
240		0.1588	0.1588	0.3218	0.1588

Graphics





Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental (CA)**

Analysis ID:	06-1808-0046	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	28 Aug-15 11:51	**Analysis:**	Trimmed Spearman-Kärber	**Official Results:**	Yes

Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	12.50%	1.784	0.05362	60.8	47.49	77.83

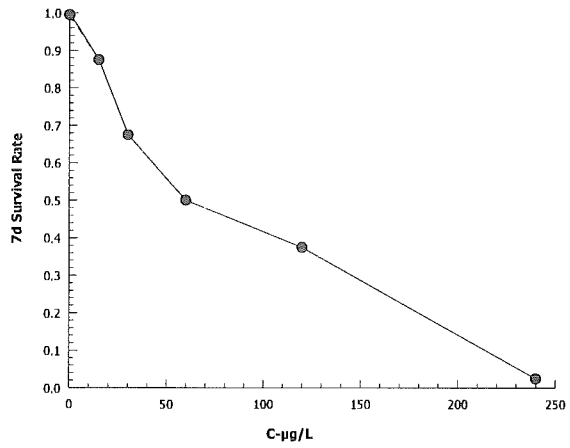
7d Survival Rate Summary

			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	4	1	1	1	0	0	0.0%	0.0%	40	40
15		4	0.875	0.7	1	0.06292	0.1258	14.38%	12.5%	35	40
30		4	0.675	0.6	0.8	0.04787	0.09574	14.18%	32.5%	27	40
60		4	0.5	0.4	0.7	0.07071	0.1414	28.28%	50.0%	20	40
120		4	0.375	0.3	0.5	0.04787	0.09574	25.53%	62.5%	15	40
240		4	0.025	0	0.1	0.025	0.05	200.0%	97.5%	1	40

7d Survival Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1	1	1	1
15		0.7	0.9	0.9	1
30		0.7	0.6	0.8	0.6
60		0.5	0.4	0.7	0.4
120		0.3	0.3	0.5	0.4
240		0	0	0.1	0

Graphics



Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental (CA)**

Analysis ID:	04-7679-9598	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	28 Aug-15 11:51	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Untransformed	NA	C > T	NA	NA	22.0%	Passes mean dry biomass-mg

Equal Variance t Two-Sample Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		15	0.07075	1.943	0.103	6	0.4729	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	2.812551E-05	2.812551E-05	1	0.005006	0.9459	Non-Significant Effect
Error	0.03370972	0.005618286	6			
Total	0.03373785		7			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	13.66	47.47	0.0592	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9401	0.6451	0.6122	Normal Distribution

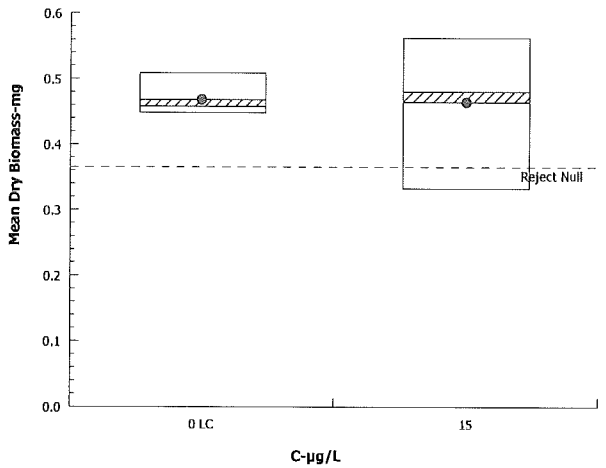
Mean Dry Biomass-mg Summary

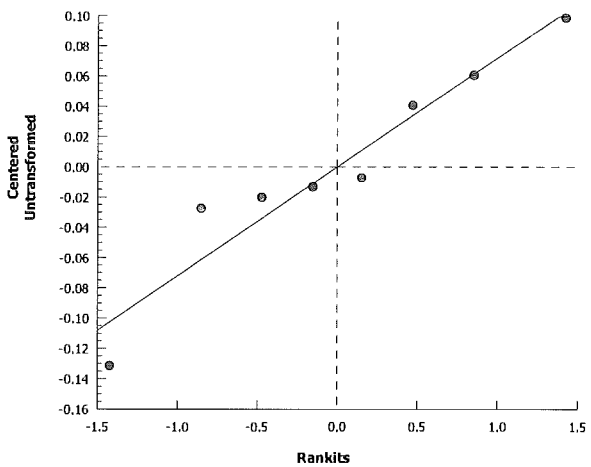
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	0.4683	0.4242	0.5123	0.458	0.448	0.509	0.01384	5.91%	0.0%
15		4	0.4645	0.3017	0.6273	0.481	0.333	0.563	0.05116	22.03%	0.8%

Mean Dry Biomass-mg Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	0.509	0.455	0.461	0.448
15		0.333	0.437	0.563	0.525

Graphics





CETIS Analytical Report

Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental (CA)**

Analysis ID:	13-7861-7654	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	28 Aug-15 11:51	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	734910	1000	Yes	Two-Point Interpolation

Point Estimates

Level	µg/L	95% LCL	95% UCL
IC25	28.49	14.08	54.46
IC50	59.13	21.52	97.82

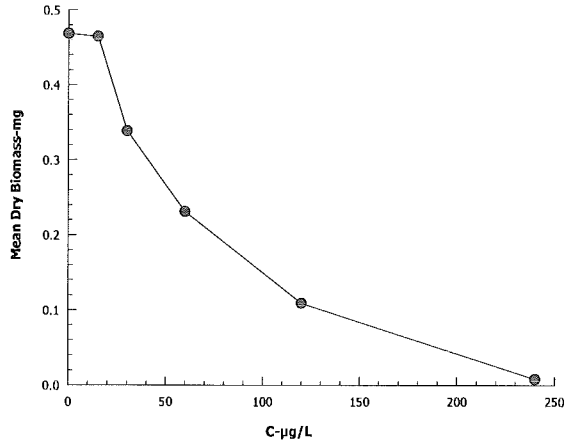
Mean Dry Biomass-mg Summary

			Calculated Variate						
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.4683	0.448	0.509	0.01384	0.02768	5.91%	0.0%
15		4	0.4645	0.333	0.563	0.05116	0.1023	22.03%	0.8%
30		4	0.3385	0.232	0.498	0.062	0.124	36.63%	27.71%
60		4	0.231	0.148	0.344	0.04791	0.09583	41.48%	50.67%
120		4	0.1088	0.072	0.153	0.01668	0.03335	30.67%	76.78%
240		4	0.00775	0	0.031	0.00775	0.0155	200.0%	98.34%

Mean Dry Biomass-mg Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	0.509	0.455	0.461	0.448
15		0.333	0.437	0.563	0.525
30		0.375	0.232	0.498	0.249
60		0.277	0.155	0.344	0.148
120		0.105	0.072	0.105	0.153
240		0	0	0.031	0

Graphics

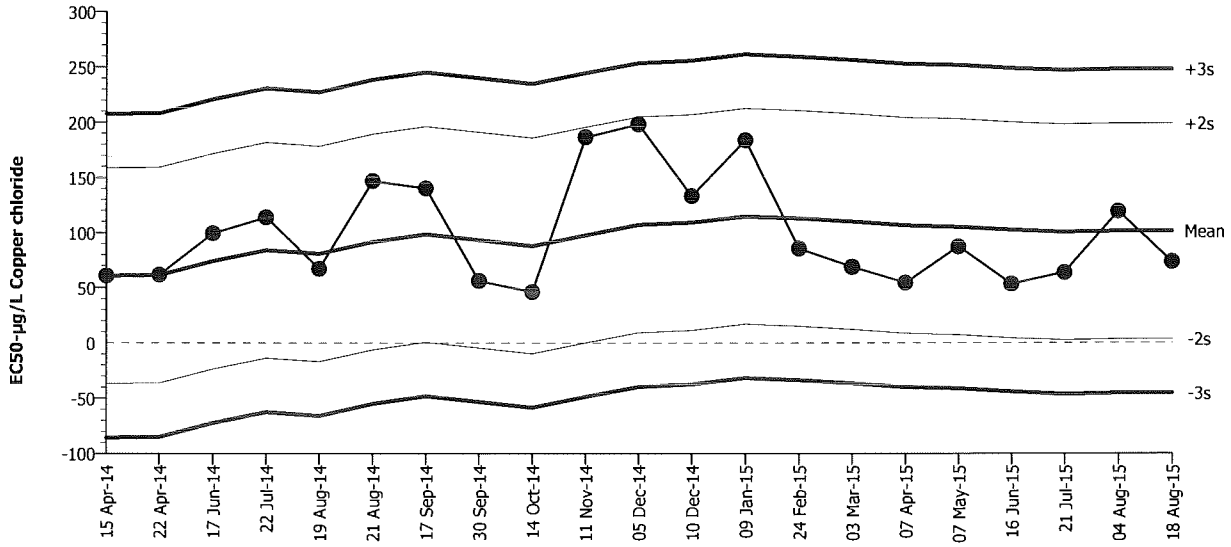


CETIS QC Plot

Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental (CA)**

Test Type:	Growth-Survival (7d)	**Organism:**	Pimephales promelas (Fathead Minn	**Material:**	Copper chloride
Protocol:	EPA/821/R-02-013 (2002)	**Endpoint:**	96h Survival Rate	**Source:**	Reference Toxicant-REF

Fathead Minnow 7-d Larval Survival and Growth Test



Mean:	101.3	**Count:**	20	**-2s Warning Limit:**	3.598	**-3s Action Limit:**	-45.27
Sigma:	48.87	**CV:**	48.20%	**+2s Warning Limit:**	199.1	**+3s Action Limit:**	247.9

Quality Control Data

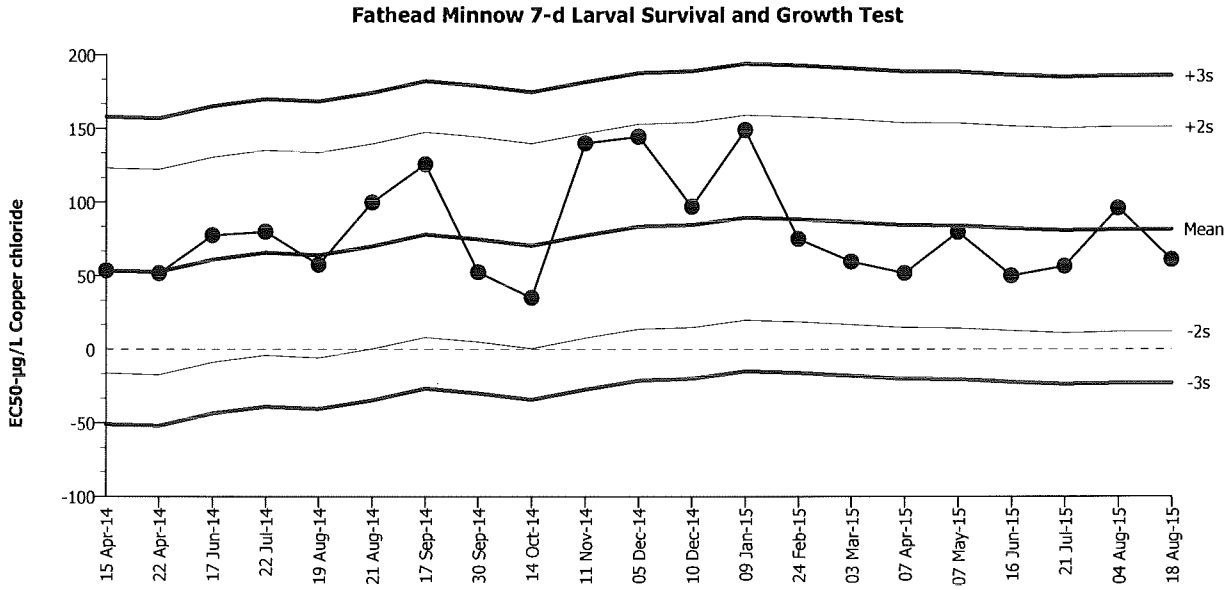
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2014	Apr	15	14:45	61.01	-40.29	-0.8244			11-2472-8616	13-8506-5156
2			22	14:35	61.85	-39.45	-0.8072			11-1031-0903	16-8546-4454
3		Jun	17	12:15	99.42	-1.88	-0.03847			06-7743-9282	06-9631-0541
4		Jul	22	14:50	113.8	12.47	0.2552			06-9902-9007	03-0373-8950
5		Aug	19	12:30	67.07	-34.23	-0.7004			09-8915-2714	01-3754-0139
6			21	15:40	146.7	45.42	0.9293			10-3404-1908	06-3758-1952
7		Sep	17	16:00	140	38.68	0.7916			17-5335-6618	15-2069-5815
8			30	16:40	56.14	-45.16	-0.9241			13-3817-1933	12-0745-1286
9		Oct	14	14:30	46.06	-55.24	-1.13			12-6672-8689	20-6427-1949
10		Nov	11	12:30	186.5	85.23	1.744			00-8166-7036	02-1996-5448
11		Dec	5	15:40	198.3	97.05	1.986			19-0595-5096	12-7143-9259
12			10	16:15	133.1	31.85	0.6517			05-2629-0227	05-1591-6898
13	2015	Jan	9	14:25	183.8	82.54	1.689			09-7026-0964	12-6561-2284
14		Feb	24	13:50	85.57	-15.73	-0.3219			06-5445-6391	09-4282-0742
15		Mar	3	15:40	68.86	-32.44	-0.6638			21-4330-3782	10-5231-9623
16		Apr	7	14:35	54.7	-46.6	-0.9536			01-4418-5519	03-5517-6206
17		May	7	15:30	87.25	-14.05	-0.2875			11-2951-1958	05-9733-5003
18		Jun	16	15:00	53.34	-47.96	-0.9813			14-6429-1311	02-4456-9436
19		Jul	21	13:45	63.81	-37.49	-0.7671			06-3455-6123	05-7711-2942
20		Aug	4	15:15	119.4	18.06	0.3696			03-4525-3853	10-5365-0381
21			18	14:15	73.44	-27.86	-0.57			09-4164-3186	08-6213-2861

CETIS QC Plot

Report Date: 28 Aug-15 11:56 (1 of 1)

Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental (CA)**

Test Type:	Growth-Survival (7d)	**Organism:**	Pimephales promelas (Fathead Minn	**Material:**	Copper chloride
Protocol:	EPA/821/R-02-013 (2002)	**Endpoint:**	7d Survival Rate	**Source:**	Reference Toxicant-REF



Mean:	81.52	**Count:**	20	**-2s Warning Limit:**	11.9	**-3s Action Limit:**	-22.91
Sigma:	34.81	**CV:**	42.70%	**+2s Warning Limit:**	151.1	**+3s Action Limit:**	185.9

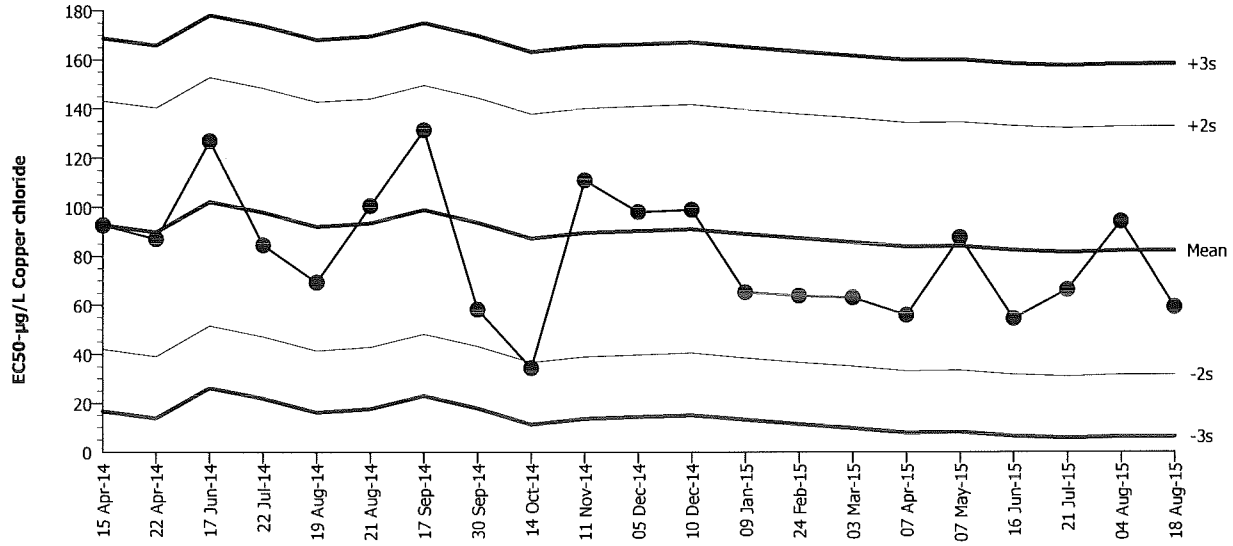
Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2014	Apr	15	14:45	53.45	-28.07	-0.8063			11-2472-8616	01-9744-5903
2			22	14:35	51.59	-29.93	-0.8598			11-1031-0903	13-4565-2338
3		Jun	17	12:15	77.34	-4.184	-0.1202			06-7743-9282	03-4494-9671
4		Jul	22	14:50	79.74	-1.783	-0.05121			06-9902-9007	13-9366-0197
5		Aug	19	12:30	57.29	-24.23	-0.696			09-8915-2714	18-8642-4310
6			21	15:40	99.75	18.23	0.5237			10-3404-1908	01-6998-1515
7		Sep	17	16:00	125.7	44.16	1.268			17-5335-6618	01-3008-4842
8			30	16:40	52.33	-29.19	-0.8385			13-3817-1933	19-6315-2769
9		Oct	14	14:30	34.96	-46.56	-1.338			12-6672-8689	14-0140-7538
10		Nov	11	12:30	139.9	58.39	1.677			00-8166-7036	09-0534-4786
11		Dec	5	15:40	144.5	62.95	1.808			19-0595-5096	12-6262-3079
12			10	16:15	96.95	15.43	0.4433			05-2629-0227	17-6271-7071
13	2015	Jan	9	14:25	149	67.5	1.939			09-7026-0964	11-9519-6628
14		Feb	24	13:50	74.87	-6.651	-0.1911			06-5445-6391	08-4817-6008
15		Mar	3	15:40	59.55	-21.97	-0.6313			21-4330-3782	19-3347-3198
16		Apr	7	14:35	51.65	-29.87	-0.858			01-4418-5519	20-8487-3271
17		May	7	15:30	79.46	-2.056	-0.05908			11-2951-1958	19-6509-1046
18		Jun	16	15:00	49.92	-31.6	-0.9077			14-6429-1311	16-2737-6690
19		Jul	21	13:45	56.44	-25.08	-0.7205			06-3455-6123	12-4190-1918
20		Aug	4	15:15	95.98	14.46	0.4154			03-4525-3853	07-3979-9146
21			18	14:15	60.8	-20.72	-0.5953			09-4164-3186	06-1808-0046

Fathead Minnow 7-d Larval Survival and Growth Test **Nautilus Environmental (CA)**

Test Type:	Growth-Survival (7d)	**Organism:**	Pimephales promelas (Fathead Minn	**Material:**	Copper chloride
Protocol:	EPA/821/R-02-013 (2002)	**Endpoint:**	Mean Dry Biomass-mg	**Source:**	Reference Toxicant-REF

Fathead Minnow 7-d Larval Survival and Growth Test



Mean:	82.17	**Count:**	20	**-2s Warning Limit:**	31.53	**-3s Action Limit:**	6.205
Sigma:	25.32	**CV:**	30.80%	**+2s Warning Limit:**	132.8	**+3s Action Limit:**	158.1

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2014	Apr	15	14:45	92.65	10.48	0.4138			11-2472-8616	13-1864-2221
2			22	14:35	86.99	4.817	0.1902			11-1031-0903	19-4178-8305
3		Jun	17	12:15	127	44.82	1.77			06-7743-9282	17-6062-6189
4		Jul	22	14:50	84.56	2.387	0.09429			06-9902-9007	16-9453-8667
5		Aug	19	12:30	69.36	-12.81	-0.506			09-8915-2714	12-9213-1784
6			21	15:40	100.5	18.33	0.7238			10-3404-1908	04-2864-0035
7		Sep	17	16:00	131.4	49.21	1.943			17-5335-6618	16-3792-2926
8			30	16:40	58.21	-23.96	-0.9464			13-3817-1933	13-6598-1511
9		Oct	14	14:30	34.21	-47.96	-1.894			12-6672-8689	01-5069-5081
10		Nov	11	12:30	110.9	28.77	1.136			00-8166-7036	15-0727-8192
11		Dec	5	15:40	98.07	15.9	0.628			19-0595-5096	19-6852-3882
12			10	16:15	98.95	16.78	0.6627			05-2629-0227	09-2076-0050
13	2015	Jan	9	14:25	65.25	-16.92	-0.6683			09-7026-0964	06-8044-1821
14		Feb	24	13:50	63.84	-18.33	-0.7239			06-5445-6391	00-5276-5594
15		Mar	3	15:40	62.96	-19.21	-0.7586			21-4330-3782	21-2581-7352
16		Apr	7	14:35	55.94	-26.23	-1.036			01-4418-5519	18-5461-6477
17		May	7	15:30	87.64	5.47	0.2161			11-2951-1958	18-7801-0518
18		Jun	16	15:00	54.48	-27.69	-1.094			14-6429-1311	00-2803-0740
19		Jul	21	13:45	66.22	-15.95	-0.6301			06-3455-6123	03-9457-6462
20		Aug	4	15:15	94.18	12.01	0.4742			03-4525-3853	18-4986-6260
21			18	14:15	59.13	-23.04	-0.91			09-4164-3186	13-7861-7654

Freshwater Chronic Bioassay

Larval Fish Survival

Client: Internal **Test Species:** *P. promelas*

Sample ID: $CuCl_2$ **Start Date/Time:** 8/18/2015 1415

Test No.: 150818pprt **End Date/Time:** 8/25/2015 1140

Conc. (__µg/L__)	Rep.	Rand #	Test Day / No. Organisms Alive								Percent Survival
			0	1	2	3	4	5	6	7	
	a	13	10	10	10	10	10	10	10	10	100
Lab Control	b	4	10	10	10	10	10	10	10	10	100
	c	18	10	10	10	10	10	10	10	10	100
	d	22	10	10	10	10	10	10	10	10	100
	a	23	10	10	9	8	8	8	8	7	70
15	b	16	10	10	10	10	10	10	9	9	90
	c	14	10	10	9	9	9	9	9	9	90
	d	15	10	10	10	10	10	10	10	10	100
	a	20	10	10	9	9	7	7	7	7	70
30	b	6	10	9	Ⓐ10 1	7	7	7	6	6	60
	c	19	10	10	9	9	8	8	8	8	80
	d	12	10	10	8	8	7	6	6	6	60
	a	10	10	10	9	9	6	6	6	5	50
60	b	17	10	10	7	7	5	4	4	4	40
	c	21	10	9	9	9	8	7	7	7	70
	d	8	10	9	5	5	4	4	4	4	40
	a	5	10	7	7	7	4	4	3	3	30
120	b	7	10	8	7	5	3	3	3	3	30
	c	1	10	9	6	4	5	5	5	5	50
	d	3	10	10	8	7	4	4	4	4	40
	a	24	10	6	Ⓐ5	3	2	1	1	0	0
240	b	11	10	0	—	—	—	—	—	—	0
	c	2	10	3	3	1	1	1	1	1	10
	d	9	10	5	4	4	2	1	1	0	0
Rand # QC: VCR		Tech Initials	BK	EG	ALB	AUB	BK	EG	CH	MM	
Initial Count QC: AG		Time	1415	1105	1336	1440	1210	1055	1150	1140	

Time Fed (day):	0	1	2	3	4	5	6
morning:	✓	0807	0830	0800	0715	0800	0830
midday:	✓	1210	1230	1235	1120	1200	1210
evening:	1530	1600	1520	1500	1515	1500	1615

Drying Oven Info

Tare wt. Initials/Date: BJ 8/24/15

Date/Time in: 8/25/15 1240

Date/Time out: 8/27/15 1340

Temp (°C): 58.0 Q1

QC Check: VCR 8/28/15

Final Review: KB 8/28/15

Comments: Ⓐ ALD Q18 8/20/15

Nautilus Environmental. 4340 Vandever Avenue. San Diego, CA 92120.

Freshwater Chronic Bioassay

Larval Fish Weights

Client: Internal **Test Species:** *Pimephales promelas*

Sample ID: $CuCl_2$ **Start Date/Time:** 8/18/2015 1415

Test No.: 150818pprt **End Date/Time:** 8/25/2015 1140

Conc. (µg/L)	Rep.	pan weight (mg)	pan + fish weight (mg)	organism weight (mg)
Lab Control	a	23.08	28.17	5.09
	b	23.20	27.75	4.55
	c	22.82	27.43	4.61
	d	21.88	26.36	4.48
15	a	22.38	25.71	3.33
	b	22.22	26.59	4.37
	c	21.29	26.92	5.63
	d	22.31	27.56	5.25
30	a	21.08	24.83	3.75
	b	22.63	24.95	2.32
	c	22.66	27.64	4.98
	d	22.42	24.91	2.49
60	a	22.13	24.90	2.77
	b	21.40	22.95	1.55
	c	22.24	25.68	3.44
	d	22.18	23.66	1.48
120	a	22.88	23.93	1.05
	b	22.75	23.47	0.72
	c	23.84	24.89	1.05
	d	21.92	23.45	1.53
240	a	0.00	0.00	0.00
	b	0.00	0.00	0.00
	c	22.37	22.68	0.31
	d	0.00	0.00	0.00
Tech Initials:		BJ	AG	
Date/Time:		8/24/2015 1632	8/27/15 1340	

QC Check: VCR 8/28/15

Final Review: KB 8/28/15

Freshwater Chronic Bioassay

Water Quality Measurements

Client: Internal

Test Species: *P. promelas*

Sample ID: $CuCl_2$

Start Date/Time: 8/18/2015 1415

Test No: 150818pprt

End Date/Time: 8/25/2015 1140

Concentration	Lab Control							
Day	0	1	2	3	4	5	6	7
Initial								
pH	8.21	8.21	8.19	8.19	8.13	8.19	8.17	
DO (mg/L)	8.4	7.6	7.4	7.7	7.3	7.5	7.6	
Cond. (µmhos/cm)	192	187	187	185	198	195	195	
Temp (°C)	24.6	~~24.9~~ 24.6	24.3	24.5	24.3	24.2	24.8	
Final							7.93	
pH		8.04	7.99	7.84	7.79	7.81	~~7.94~~	7.94
DO (mg/L)		6.8	6.2	6.0	6.6	6.2	6.7	6.8
Temp (°C)		25.3	25.0	24.6	25.0	24.8	24.7	24.5

Concentration	60 µg/L							
Day	0	1	2	3	4	5	6	7
Initial								
pH	8.17	8.20	8.20	8.23	8.12	8.17	8.19	
DO (mg/L)	8.3	7.6	7.4	7.8	7.5	7.7	7.8	
Cond. (µmhos/cm)	193	186	186	185	197	194	193	
Temp (°C)	24.7	24.7	24.5	24.5	24.3	24.2	24.7	
Final								
pH		8.11	8.02	7.91	7.96	7.85	7.93	7.93
DO (mg/L)		7.1	6.7	6.4	6.1	6.1	6.3	6.5
Temp (°C)		25.3	25.2	25.1	25.2	25.0	25.3	25.3

Concentration	15 µg/L							
Day	0	1	2	3	4	5	6	7
Initial								
pH	8.19	8.20	8.20	8.23	8.12	8.18	8.18	
DO (mg/L)	8.3	7.6	7.4	7.8	7.5	7.6	7.8	
Cond. (µmhos/cm)	193	187	187	185	198	195	193	
Temp (°C)	24.8	24.9	24.4	24.6	24.4	24.2	24.7	
Final							7.94	
pH		8.12	7.99	7.92	7.93	7.83	~~7.89~~	8.01
DO (mg/L)		7.0	6.5	6.6	6.4	6.0	6.7	6.8
Temp (°C)		25.1	25.1	24.6	25.0	24.8	24.7	25.1

Concentration	120 µg/L							
Day	0	1	2	3	4	5	6	7
Initial								
pH	8.17	8.20	8.21	8.23	8.09	8.17	8.19	
DO (mg/L)	8.3	7.6	7.5	7.7	7.6	7.6	7.8	
Cond. (µmhos/cm)	193	186	186	185	197	194	194	
Temp (°C)	24.7	24.5	24.5	24.4	24.4	24.3	24.6	
Final								
pH		8.12	8.04	7.93	7.97	7.85	8.03	8.10
DO (mg/L)		7.1	6.8	6.6	6.5	6.5	6.9	6.9
Temp (°C)		25.3	25.1	25.2	25.2	24.9	25.0	25.3

Concentration	30 µg/L							
Day	0	1	2	3	4	5	6	7
Initial								
pH	8.18	8.21	8.20	8.25	8.13	8.19	8.19	
DO (mg/L)	8.3	7.6	7.5	7.7	7.5	7.6	7.8	
Cond. (µmhos/cm)	192	186	186	185	198	194	193	
Temp (°C)	24.5	24.8	24.4	24.6	24.3	24.2	24.7	
Final								
pH		8.12	8.00	7.94	7.99	7.81	7.96	7.97
DO (mg/L)		7.1	6.4	6.5	6.7	6.2	6.7	6.7
Temp (°C)		25.1	25.0	24.8	24.7	24.6	25.2	25.2

Concentration	240 µg/L							
Day	0	1	2	3	4	5	6	7
Initial								
pH	8.14	8.17	8.20	8.24	8.06	8.18	8.19	
DO (mg/L)	8.1	7.6	7.5	7.5	7.6	7.6	7.8	
Cond. (µmhos/cm)	193	185	185	185	197	194	193	
Temp (°C)	24.6	24.4	24.6	24.6	24.4	24.2	24.4	
Final								
pH		8.15	8.04	8.03	8.03	8.00	8.12	8.13
DO (mg/L)		7.4	6.8	7.1	7.1	6.1	7.3	7.3
Temp (°C)		24.8	25.0	24.9	24.7	24.8	24.8	24.9

Animal Source/Date Received: ABS / 8/18/15

Animal Age at Initiation: 1 day

Cu Stock Concentration (µg/L): 85,900

	0	1	2	3	4	5	6	7
Analysts: Initial:	EG	ALB	BK	AG	EG	BK	CH	–
Final:	–	ALB	BK	AG	EG	BK	CH	ALB
Dilutions made by:	KB	AW	ALB	ALB	ALB	EG	CH	–
High conc. made (µg/L):	240	240	240	240	240	240	240	–
Vol. Cu stock added (mL):	5.6	5.4	5.6	5.6	5.6	5.6	5.6	–

Added to Final Volume = 2000 mL

Comments: @ ALB 8/19/15 Q18 @ CH Q18 8/24/15

QC Check: VCR 8/28/15

Final Review: KB 8/28/15

Water Flea 7-day Chronic Survival and Reproduction

CETIS Summary Report

Ceriodaphnia 7-d Survival and Reproduction Test — **Nautilus Environmental (CA)**

Batch ID:	05-4331-4620	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	
Start Date:	12 Aug-15 13:45	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Diluted Mineral Water (8:2)
Ending Date:	19 Aug-15 16:20	**Species:**	Ceriodaphnia dubia	**Brine:**	Not Applicable
Duration:	7d 3h	**Source:**	In-House Culture	**Age:**	<24h

Sample ID:	17-8675-7909	**Code:**	150812cdrt	**Client:**	Internal
Sample Date:	12 Aug-15	**Material:**	Copper chloride	**Project:**	
Receive Date:	12 Aug-15	**Source:**	Reference Toxicant		
Sample Age:	14h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
14-2079-9635	7d Survival Rate	25	50	35.36	NA		Fisher Exact Test
17-2745-3967	Reproduction	25	>25	NA	30.1%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
17-0191-1313	7d Survival Rate	EC50	35.36	25	50		Binomial/Graphical
21-0487-8039	Reproduction	IC25	32.53	31.89	33.36		Linear Interpolation (ICPIN)
		IC50	40.06	38.78	41.72		

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
14-2079-9635	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
17-0191-1313	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
17-2745-3967	Reproduction	Control Resp	16.5	15 - NL	Yes	Passes Acceptability Criteria
21-0487-8039	Reproduction	Control Resp	16.5	15 - NL	Yes	Passes Acceptability Criteria
17-2745-3967	Reproduction	PMSD	0.3007	0.13 - 0.47	Yes	Passes Acceptability Criteria

7d Survival Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	10	1	1	1	1	1	0	0	0.0%	0.0%
12.5		10	1	1	1	1	1	0	0	0.0%	0.0%
25		10	1	1	1	1	1	0	0	0.0%	0.0%
50		10	0	0	0	0	0	0	0		100.0%
100		10	0	0	0	0	0	0	0		100.0%
200		10	0	0	0	0	0	0	0		100.0%

Reproduction Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	10	16.5	10.85	22.15	4	26	2.496	7.892	47.83%	0.0%
12.5		10	25	22	28	19	33	1.325	4.19	16.76%	-51.52%
25		10	25.5	22.95	28.05	21	32	1.128	3.567	13.99%	-54.55%
50		10	3.8	1.568	6.032	1	9	0.9866	3.12	82.1%	76.97%
100		10	0	0	0	0	0	0	0		100.0%
200		10	0	0	0	0	0	0	0		100.0%

Ceriodaphnia 7-d Survival and Reproduction Test — **Nautilus Environmental (CA)**

7d Survival Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Lab Control	1	1	1	1	1	1	1	1	1	1
12.5		1	1	1	1	1	1	1	1	1	1
25		1	1	1	1	1	1	1	1	1	1
50		0	0	0	0	0	0	0	0	0	0
100		0	0	0	0	0	0	0	0	0	0
200		0	0	0	0	0	0	0	0	0	0

Reproduction Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Lab Control	26	11	17	21	5	24	21	23	4	13
12.5		25	27	19	33	25	23	27	24	19	28
25		26	23	21	27	32	22	28	29	22	25
50		1	2	9	5	1	1	9	5	2	3
100		0	0	0	0	0	0	0	0	0	0
200		0	0	0	0	0	0	0	0	0	0

CETIS Analytical Report

Ceriodaphnia 7-d Survival and Reproduction Test — **Nautilus Environmental (CA)**

Analysis ID: 14-2079-9635 **Endpoint:** 7d Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 02 Sep-15 13:02 **Analysis:** Single 2x2 Contingency Table **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	NOEL	LOEL	TOEL	TU
Untransformed		C > T	NA	NA	25	50	35.36	

Fisher Exact Test

Control	vs	C-µg/L	Test Stat	P-Value	P-Type	Decision(α:5%)
Lab Control		12.5	1	1.0000	Exact	Non-Significant Effect
		25	1	1.0000	Exact	Non-Significant Effect

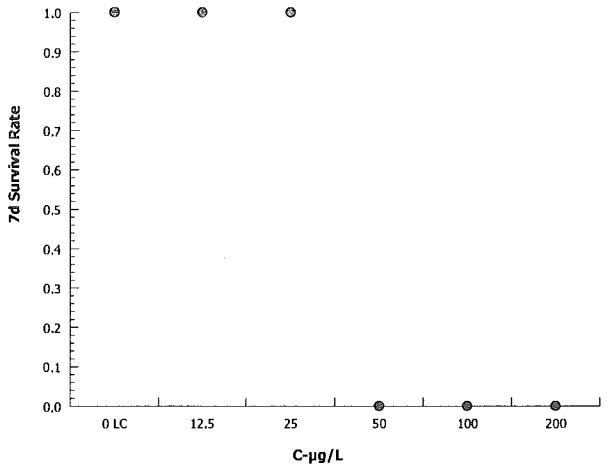
Data Summary

C-µg/L	Control Type	NR	R	NR + R	Prop NR	Prop R	%Effect
0	Lab Control	10	0	10	1	0	0.0%
12.5		10	0	10	1	0	0.0%
25		10	0	10	1	0	0.0%
50		0	10	10	0	1	100.0%
100		0	10	10	0	1	100.0%
200		0	10	10	0	1	100.0%

7d Survival Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Lab Control	1	1	1	1	1	1	1	1	1	1
12.5		1	1	1	1	1	1	1	1	1	1
25		1	1	1	1	1	1	1	1	1	1
50		0	0	0	0	0	0	0	0	0	0
100		0	0	0	0	0	0	0	0	0	0
200		0	0	0	0	0	0	0	0	0	0

Graphics



CETIS Analytical Report

Ceriodaphnia 7-d Survival and Reproduction Test — **Nautilus Environmental (CA)**

Analysis ID: 17-0191-1313 **Endpoint:** 7d Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 02 Sep-15 13:02 **Analysis:** Binomial Method **Official Results:** Yes

Binomial/Graphical Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	0.00%	1.548	0	35.36	25	50

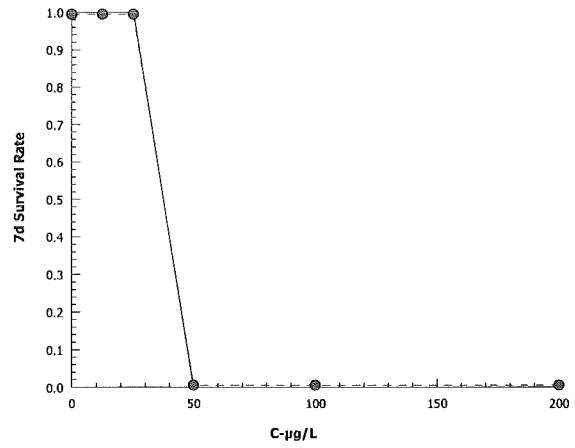
7d Survival Rate Summary

			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	10	1	1	1	0	0	0.0%	0.0%	10	10
12.5		10	1	1	1	0	0	0.0%	0.0%	10	10
25		10	1	1	1	0	0	0.0%	0.0%	10	10
50		10	0	0	0	0	0		100.0%	0	10
100		10	0	0	0	0	0		100.0%	0	10
200		10	0	0	0	0	0		100.0%	0	10

7d Survival Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Lab Control	1	1	1	1	1	1	1	1	1	1
12.5		1	1	1	1	1	1	1	1	1	1
25		1	1	1	1	1	1	1	1	1	1
50		0	0	0	0	0	0	0	0	0	0
100		0	0	0	0	0	0	0	0	0	0
200		0	0	0	0	0	0	0	0	0	0

Graphics



CETIS Analytical Report

Ceriodaphnia 7-d Survival and Reproduction Test — **Nautilus Environmental (CA)**

Analysis ID:	17-2745-3967	**Endpoint:** Reproduction	**CETIS Version:** CETISv1.8.7
Analyzed:	02 Sep-15 13:02	**Analysis:** Parametric-Control vs Treatments	**Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	30.1%	25	>25	NA	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		12.5	-3.422	1.997	4.962	18	0.9999	CDF	Non-Significant Effect
		25	-3.623	1.997	4.962	18	1.0000	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	511.6667	255.8333	2	8.292	0.0016	Significant Effect
Error	833	30.85185	27			
Total	1344.667		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	6.409	9.21	0.0406	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9756	0.9031	0.7011	Normal Distribution

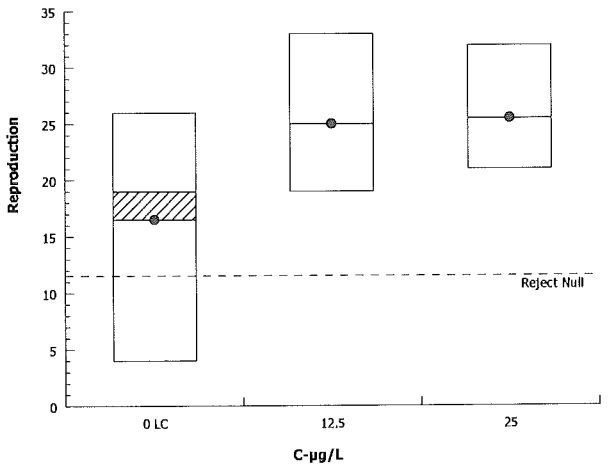
Reproduction Summary

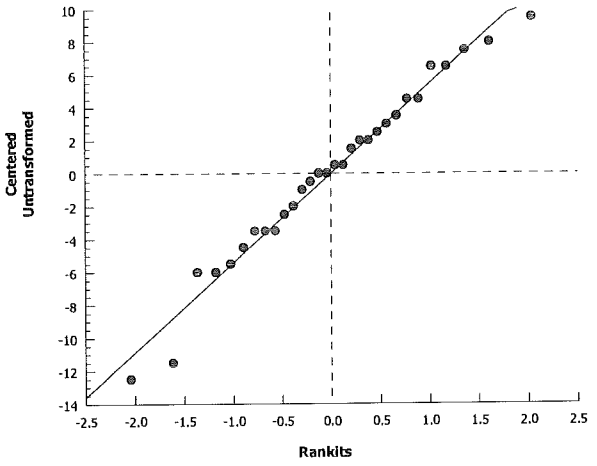
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	10	16.5	10.85	22.15	19	4	26	2.496	47.83%	0.0%
12.5		10	25	22	28	25	19	33	1.325	16.76%	-51.52%
25		10	25.5	22.95	28.05	25.5	21	32	1.128	13.99%	-54.55%

Reproduction Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Lab Control	26	11	17	21	5	24	21	23	4	13
12.5		25	27	19	33	25	23	27	24	19	28
25		26	23	21	27	32	22	28	29	22	25

Graphics





CETIS Analytical Report

Ceriodaphnia 7-d Survival and Reproduction Test — **Nautilus Environmental (CA)**

Analysis ID:	21-0487-8039	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Sep-15 13:03	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	626309	1000	Yes	Two-Point Interpolation

Point Estimates

Level	µg/L	95% LCL	95% UCL
IC25	32.53	31.89	33.36
IC50	40.06	38.78	41.72

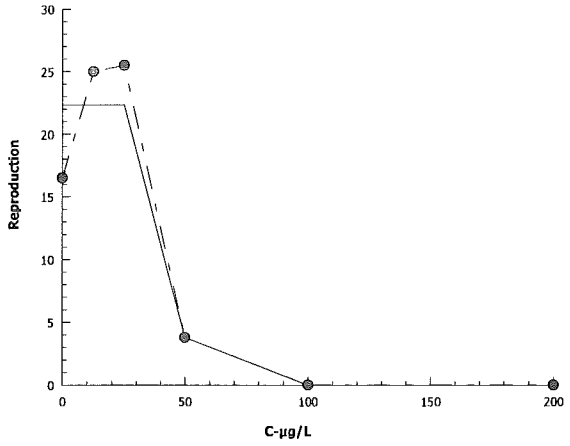
Reproduction Summary

			Calculated Variate						
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	10	16.5	4	26	2.496	7.892	47.83%	0.0%
12.5		10	25	19	33	1.325	4.19	16.76%	-51.52%
25		10	25.5	21	32	1.128	3.567	13.99%	-54.55%
50		10	3.8	1	9	0.9866	3.12	82.1%	76.97%
100		10	0	0	0	0	0		100.0%
200		10	0	0	0	0	0		100.0%

Reproduction Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Lab Control	26	11	17	21	5	24	21	23	4	13
12.5		25	27	19	33	25	23	27	24	19	28
25		26	23	21	27	32	22	28	29	22	25
50		1	2	9	5	1	1	9	5	2	3
100		0	0	0	0	0	0	0	0	0	0
200		0	0	0	0	0	0	0	0	0	0

Graphics



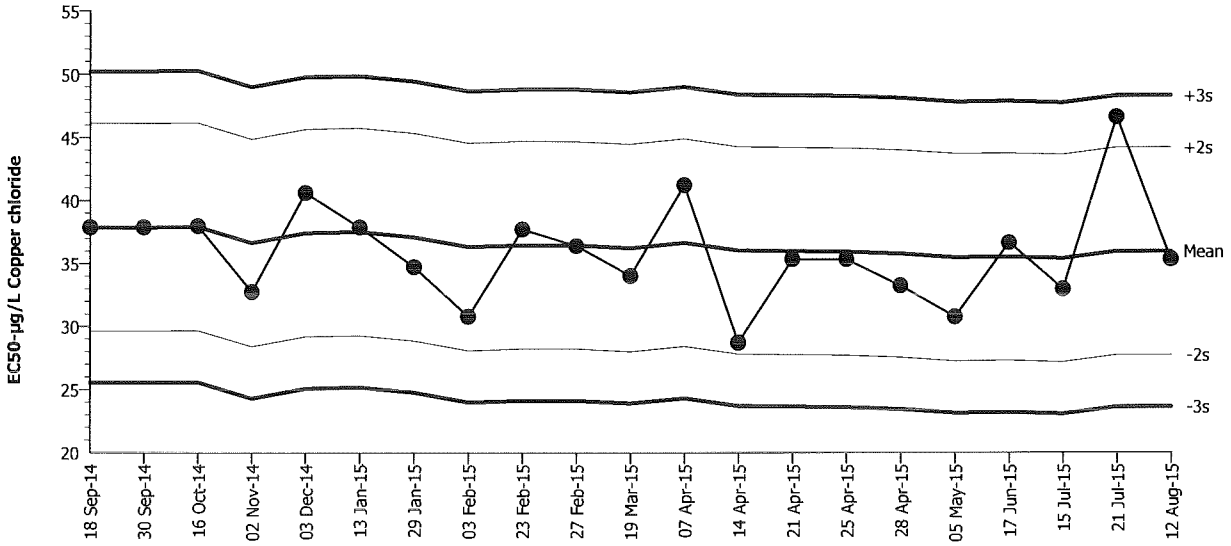
CETIS QC Plot

Report Date: 02 Sep-15 13:04 (1 of 1)

Ceriodaphnia 7-d Survival and Reproduction Test — **Nautilus Environmental (CA)**

Test Type:	Reproduction-Survival (7d)	**Organism:**	Ceriodaphnia dubia (Water Flea)	**Material:**	Copper chloride
Protocol:	EPA/821/R-02-013 (2002)	**Endpoint:**	7d Survival Rate	**Source:**	Reference Toxicant-REF

Ceriodaphnia 7-d Survival and Reproduction Test



Mean:	35.98	**Count:**	20	**-2s Warning Limit:**	27.75	**-3s Action Limit:**	23.64
Sigma:	4.115	**CV:**	11.40%	**+2s Warning Limit:**	44.21	**+3s Action Limit:**	48.33

Quality Control Data

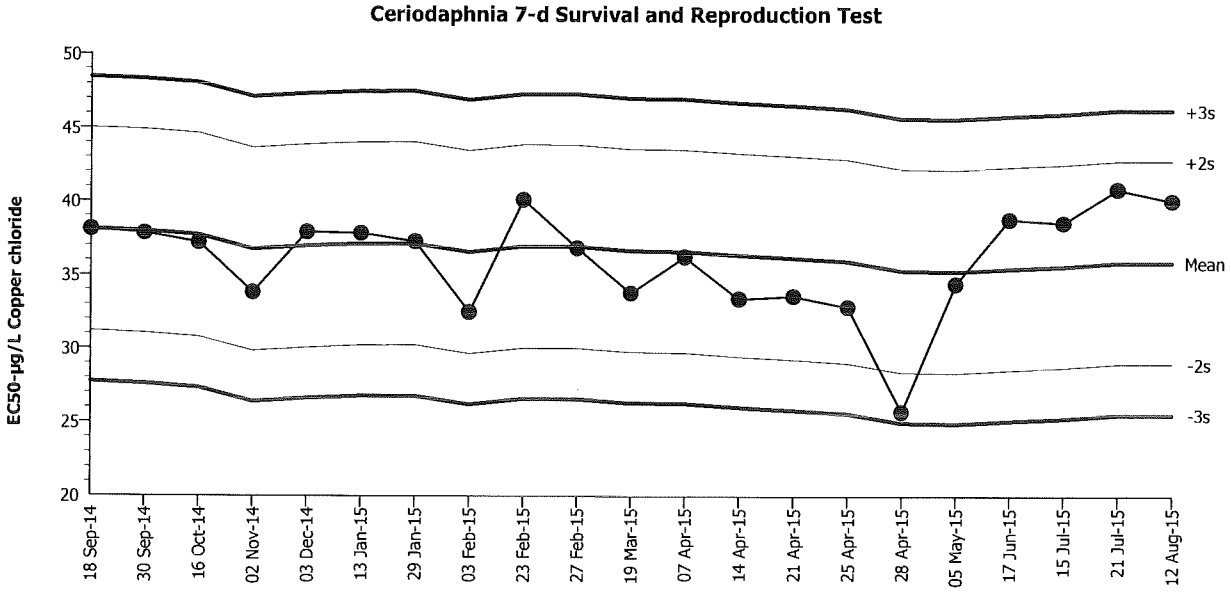
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2014	Sep	18	16:30	37.89	1.913	0.4649			10-3919-7113	00-7527-5489
2			30	16:30	37.89	1.913	0.4649			17-7360-1623	12-6592-0929
3		Oct	16	15:35	37.98	2.004	0.4869			13-1839-5388	13-1471-2526
4		Nov	2	20:30	32.73	-3.245	-0.7887			14-0063-8882	16-8982-6987
5		Dec	3	17:00	40.61	4.633	1.126			07-1595-8853	01-9960-3482
6	2015	Jan	13	13:10	37.89	1.913	0.4649			12-1862-1567	17-5368-1907
7			29	14:30	34.72	-1.264	-0.3071			03-4351-1917	01-9946-4833
8		Feb	3	14:35	30.78	-5.201	-1.264			00-7232-3197	04-8551-5217
9			23	15:50	37.73	1.749	0.4251			00-7659-2286	02-1377-0074
10			27	15:50	36.39	0.4113	0.09996			20-9761-2342	13-1133-1780
11		Mar	19	14:30	34.02	-1.96	-0.4764			11-9819-1081	06-5785-8726
12		Apr	7	17:15	41.24	5.263	1.279			14-2414-0720	05-0075-5359
13			14	15:15	28.72	-7.263	-1.765			08-1687-0117	04-8656-1115
14			21	16:50	35.36	-0.6247	-0.1518			08-8249-0219	06-7338-3548
15			25	14:55	35.36	-0.6247	-0.1518			11-0221-5046	01-9496-3153
16			28	15:00	33.26	-2.722	-0.6615			10-3693-9244	14-6082-6538
17		May	5	14:30	30.78	-5.201	-1.264			06-6041-8297	04-6423-6416
18		Jun	17	16:00	36.67	0.689	0.1674			11-2834-8346	14-3874-1583
19		Jul	15	16:10	32.99	-2.992	-0.7272			03-2688-9700	05-5389-5032
20			21	15:20	46.65	10.67	2.593	(+)		09-0472-0460	08-1505-5240
21		Aug	12	13:45	35.36	-0.6247	-0.1518			18-3540-4492	17-0191-1313

CETIS QC Plot

Report Date: 02 Sep-15 13:04 (1 of 1)

Ceriodaphnia 7-d Survival and Reproduction Test — **Nautilus Environmental (CA)**

Test Type:	Reproduction-Survival (7d)	**Organism:**	Ceriodaphnia dubia (Water Flea)	**Material:**	Copper chloride
Protocol:	EPA/821/R-02-013 (2002)	**Endpoint:**	Reproduction	**Source:**	Reference Toxicant-REF



Mean:	35.88	**Count:**	20	**-2s Warning Limit:**	28.98	**-3s Action Limit:**	25.53
Sigma:	3.451	**CV:**	9.62%	**+2s Warning Limit:**	42.78	**+3s Action Limit:**	46.23

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2014	Sep	18	16:30	38.1	2.22	0.6433			10-3919-7113	02-3573-6056
2			30	16:30	37.83	1.948	0.5646			17-7360-1623	11-7251-9566
3		Oct	16	15:35	37.19	1.306	0.3785			13-1839-5388	19-9710-5289
4		Nov	2	20:30	33.82	-2.065	-0.5984			14-0063-8882	06-0137-8585
5		Dec	3	17:00	37.91	2.028	0.5877			07-1595-8853	16-3719-7733
6	2015	Jan	13	13:10	37.83	1.946	0.5638			12-1862-1567	02-0313-2763
7			29	14:30	37.27	1.389	0.4025			03-4351-1917	15-0162-2619
8		Feb	3	14:35	32.47	-3.405	-0.9867			00-7232-3197	15-2197-9470
9			23	15:50	40.13	4.248	1.231			00-7659-2286	12-8533-1650
10			27	15:50	36.85	0.9703	0.2812			20-9761-2342	16-4125-6138
11		Mar	19	14:30	33.77	-2.107	-0.6106			11-9819-1081	18-4577-2481
12		Apr	7	17:15	36.24	0.3583	0.1038			14-2414-0720	13-2569-6583
13			14	15:15	33.4	-2.479	-0.7185			08-1687-0117	10-1752-2895
14			21	16:50	33.59	-2.286	-0.6625			08-8249-0219	20-5661-3872
15			25	14:55	32.85	-3.027	-0.8773			11-0221-5046	18-1887-3965
16			28	15:00	25.7	-10.18	-2.949	(-)		10-3693-9244	20-0691-0269
17		May	5	14:30	34.41	-1.468	-0.4255			06-6041-8297	15-5089-2029
18		Jun	17	16:00	38.79	2.915	0.8446			11-2834-8346	04-5457-1024
19		Jul	15	16:10	38.57	2.687	0.7786			03-2688-9700	00-1051-9288
20			21	15:20	40.85	4.971	1.44			09-0472-0460	04-9280-3401
21		Aug	12	13:45	40.06	4.183	1.212			18-3540-4492	21-0487-8039

Freshwater Chronic Bioassay

Daphnid Survival and Reproduction Datasheet
Test Species: *C. dubia*

Client/Sample ID: Internal/$CuCl_2$

Test No: 1508~~11~~ 12 Ⓐ cdrt

Start Date/Time: 8/~~11~~ 12 @ /2015 1345

End Date/Time: 8/~~18~~ 19 Ⓑ /2015 1020

Conc.	Rep	Rand #	Daily Reproduction/ Survival 1	2	3	4	5	6	7	8	Total	QC
LC	1	20	0	0	0	6	5	7	8		26	
	2	14	0	0	0	4	7	0	0		11	
	3	13	8	0	3	6	0	3	5		17	
	4	33	0	0	0	6	8	7	~~4~~ 10	21	~~31~~	10
	5	9	0	0	0	1	4	0	0		5	
	6	37	0	0	0	4	7	13	0		24	
	7	49	6	0	3	9	0	9	7	21	~~28~~ Ⓒ	
	8	50	0	0	5	0	11	5	~~23~~		23	
	9	39	0	0	0	1	0	3	0		4	
	10	6	0	0	0	6	7	0	0		13	
		Tech:	EG	ALB	EG	AW	AW	AW	AD			EG
										Mean neonates/surviving female (for TAC):	16.5	

Conc.	Rep	Rand #	Daily Reproduction/ Survival 1	2	3	4	5	6	7	8	Total	QC
50 µg/L	1	28	0	0	0	1/d	–	–	–		1/d	
	2	15	0	0	0	2*	0*/d	–	–		2/d	
	3	36	0	0	3	6	0/d				Ⓒ ~~9~~ 9/d	
	4	11	0	0	0	5	0/d				5/d	
	5	18	0	0	0	1*	0/d		all dead		1/d	
	6	22	0	0	0	1*	0/d				1/d	
	7	1	0	0	4	5*	0/d				Ⓒ 9 ~~5~~/d	
	8	55	0	0	0	5	0/d				5/d	
	9	21	0	0	0	2*	0/d				2/d	
	10	48	0	0	0	3	0/d				3/d	

Conc.	Rep	Rand #	Daily Reproduction/ Survival 1	2	3	4	5	6	7	8	Total	QC
12.5 µg/L	1	24	0	0	0	5	10	10	0		25	
	2	2	0	0	0	5	8	14	0		27	
	3	34	0	0	4	5	0	10	14/B		19	
	4	4	0	0	0	6	11	16	B		33	
	5	54	0	0	0	4	9	12	B		25	
	6	31	0	0	0	4	8	11	B		23	
	7	23	0	0	5	9	0	13	B		27	16
	8	40	0	0	0	5	9	10	10/B		24	
	9	45	0	0	0	5	7	7	B		19	
	10	59	0	0	0	5	8	15	B		28	

Conc.	Rep	Rand #	Daily Reproduction/ Survival 1	2	3	4	5	6	7	8	Total	QC
100 µg/L	1	57	0/d	–	–	–	–	–	–		0/d	
	2	17	0	0	0/d	–	–	–	–		0/d	
	3	26	0	0/d	–	–	–	–	–		0/d	
	4	47	0/d	–	–	–	–	–	–		0/d	
	5	58	0	0/d	–	–	–	–	–	–	0/d	
	6	38	0	0	0	0/d	–	–	–	–	0/d	
	7	5	0	0/d	–	–	–	–	–	–	0/d	
	8	16	0/d	–	–	–	–	–	–		0/d	
	9	43	0	0	0/d	–	–	–	–		0/d	
	10	35	0/d	–	–	–	–	–	–		0/d	

Conc.	Rep	Rand #	Daily Reproduction/ Survival 1	2	3	4	5	6	7	8	Total	QC
25 µg/L	1	51	0	0	0	5	10	11	0*		26	
	2	60	0	0	0	5	9	9	0		23	
	3	19	0	0	5	6	0	10	B		21	
	4	7	0	0	0	6	10	11	0		27	
	5	30	0	0	0	3	5	8	Ⓓ ~~3~~ 16		32	16
	6	29	0	0	0	4	8	10	12/B		22	
	7	25	0	0	5	8	0	15	B		28	
	8	46	0	0	0	6	8	15	10/B		29	
	9	3	0	0	0	2	9	11	B		22	
	10	27	0	0	0	5	9	11	B		25	

Conc.	Rep	Rand #	Daily Reproduction/ Survival 1	2	3	4	5	6	7	8	Total	QC
200 µg/L	1	41	0/d								0/d	
	2	52	0/d								0/d	
	3	32	0/d								0/d	
	4	8	0/d								0/d	
	5	42	0/d			all dead					0/d	
	6	10	0/d								0/d	
	7	56	0/d								0/d	
	8	12	0/d								0/d	
	9	44	0/d								0/d	
	10	53	0/d								0/d	

Neonates for each replicate were blocked across concentrations at test initiation

Rep:	1	2	3	4	5	6	7	8	9	10
Board:	97 →									→
Cup:	20	23	24	30	32	33	39	45	49	55

Rand # QC: EG **Initiated By:** EG **Verified By:** AG

Notes: d = dead; M = male; LIP = lost in progress;
B = 4th brood (only the first 3 broods are included in total)
* = dead neonates observed, but only live neonate counts recorded

Time Fed/Test Solution Renewed (day): (0) 1345 (1) 1030 (2) 1310 (3) 1130 (4) 1345 (5) 1130 (6) 1245 (7) —

Comments: Ⓐ AG Q18 8/17/15 Ⓑ AD Q18 8/19/15 Ⓒ Q18 VCR 8/26/15 Ⓓ KFP Q18 9/2/15

QC Check: VCR 8/26/15 **Final Review:** KFP 9/2/15

Freshwater Chronic Bioassay

Client: Internal
Sample ID: $CuCl_2$
Test No: 150811 12 cdrt (A)

Test Species: *C. dubia*
Start Date/Time: 8/11 12 (A) 2015 1345
End Date/Time: 8/18 19 (B) 15 1620

Water Quality Measurements

Concentration	Lab Control							
Day	0	1	2	3	4	5	6	7
Initial								
pH	8.23	8.21	8.20	8.11	8.24	8.18	8.16	8.18 (D)
DO (mg/L)	8.0	8.1	8.4	8.2	8.0	7.9	8.2	7.6 (D)
Cond. (µmhos/cm)	188	192	192	190	194	191	191	200
Temp (°C)	24.7	24.0	24.0	24.0	24.3	24.5	24.8	25.0
Final								
pH		8.41	8.20	8.26	8.31	8.27	8.15	8.20
DO (mg/L)		7.6	8.4	8.2	7.4	8.1	8.0	8.1
Temp (°C)		25.6	26.2	25.3	25.6	24.2	24.6	24.3

Concentration	50 µg/L							
Day	0	1	2	3	4	5	6	7
Initial								
pH	8.21	8.16	8.25	8.14	8.14	8.19		
DO (mg/L)	8.1	8.1	8.0	8.3	8.0	7.9		
Cond. (µmhos/cm)	187	191	190	188	190	190		
Temp (°C)	24.7	24.0	24.4	24.2	24.7	24.8		
Final								
pH		8.34	8.25	8.29	8.32	8.25		
DO (mg/L)		8.1	8.3	8.3	7.7	8.1		
Temp (°C)		25.6	25.2	25.3	25.6	24.2		

Concentration	12.5 µg/L							
Day	0	1	2	3	4	5	6	7
Initial								
pH	8.22	8.21	8.20	8.14	8.22	8.20	8.19	8.20
DO (mg/L)	8.0	8.1	8.5	8.2	8.0	7.9	8.1 (C)	7.6 (D)
Cond. (µmhos/cm)	187	191	191	189	194	194	191	195
Temp (°C)	25.1	24.0	24.2	24.1	24.6	24.7	24.8	25.0
Final								
pH		8.37	8.20	8.29	8.31	8.26	8.18	8.20
DO (mg/L)		8.1	8.3	8.3	7.7	7.9	7.9	8.0
Temp (°C)		25.6	26.2	25.3	25.6	24.2	24.6	24.3

Concentration	100 µg/L							
Day	0	1	2	3	4	5	6	7
Initial								
pH	8.18	8.15	8.24	8.14	8.08			
DO (mg/L)	8.0	8.1	8.4	8.4	8.0			
Cond. (µmhos/cm)	187	190	189	187	190			
Temp (°C)	24.8	24.3	24.6	24.0	24.7			
Final								
pH		8.33	8.25	8.29	8.34			
DO (mg/L)		8.1	8.4	8.2	8.0			
Temp (°C)		25.6	25.2	25.3	25.6			

Concentration	25 µg/L							
Day	0	1	2	3	4	5	6	7
Initial								
pH	8.21	8.22	8.20	8.14	8.20	8.20	8.20	8.19 (E)
DO (mg/L)	8.0	8.0	8.5	8.2	8.0	7.9	8.4	7.6
Cond. (µmhos/cm)	188	192	191	189	194	191	191	189
Temp (°C)	25.5	24.2	24.2	24.1	24.9	24.8	24.9	25.1
Final								
pH		8.37	8.20	8.30	8.33	8.20	8.18	8.21
DO (mg/L)		8.3	8.3	8.3	7.7	8.1	7.9	8.0
Temp (°C)		25.6	26.2	25.3	25.6	24.2	24.6	24.3

Concentration	200 µg/L							
Day	0	1	2	3	4	5	6	7
Initial								
pH	8.18	8.14						
DO (mg/L)	8.0	8.0						
Cond. (µmhos/cm)	185	191						
Temp (°C)	25.0	24.5						
Final								
pH		8.30						
DO (mg/L)		8.0						
Temp (°C)		25.6						

	0	1	2	3	4	5	6	7
Analysts: Initial:	ALB	CH	ALB	JW	AG	AW	EG	(B) ~~AD~~ —
Final:	—	EG	ALB	EG	AW	AW	EG	AD
Dilutions made by:	ALB	AW	AW	ALB	AG	ICB	EG	(B) ~~AD~~ —
High conc. made (µg/L):	200	200	100	100	100	50	25	~~25~~
Vol. Cu stock added (mL):	1.0	9.0	4.5	4.5	4.5	2.3	1.13	~~1.13~~

Added to Final Volume = 400 mL

Animal Source/Date Received: Internal/ N/A
Animal Age at Initiation: <24 hrs

Cu Stock Concentration (µg/L): 8,850

Comments: (A) ~~AD~~ QIB 8/11/15 (B) QIB VCR 8/26/15

QC Check: VCR 8/26/15

Final Review: KFF 9/2/15

Fathead Minnow 96-hour Acute Survival

CETIS Summary Report

Fathead Minnow 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Batch ID:	06-3457-2565	**Test Type:**	Survival (96h)	**Analyst:**	
Start Date:	18 Aug-15 13:10	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Diluted Mineral Water (8:2)
Ending Date:	22 Aug-15 11:45	**Species:**	Pimephales promelas	**Brine:**	Not Applicable
Duration:	95h	**Source:**	Aquatic Biosystems, CO	**Age:**	6d

Sample ID:	19-2881-3490	**Code:**	150818ppra	**Client:**	Internal
Sample Date:	18 Aug-15	**Material:**	Copper chloride	**Project:**	
Receive Date:	18 Aug-15	**Source:**	Reference Toxicant		
Sample Age:	13h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
04-6281-9629	96h Survival Rate	<15	15	NA	11.4%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
11-1489-1006	96h Survival Rate	EC50	32.09	24.22	42.53		Trimmed Spearman-Kärber

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
04-6281-9629	96h Survival Rate	Control Resp	0.975	0.9 - NL	Yes	Passes Acceptability Criteria
11-1489-1006	96h Survival Rate	Control Resp	0.975	0.9 - NL	Yes	Passes Acceptability Criteria

96h Survival Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.975	0.8954	1	0.9	1	0.025	0.05	5.13%	0.0%
15		4	0.7	0.475	0.925	0.5	0.8	0.07071	0.1414	20.2%	28.21%
30		4	0.55	0.3446	0.7554	0.4	0.7	0.06455	0.1291	23.47%	43.59%
60		4	0.225	0.1454	0.3046	0.2	0.3	0.025	0.05	22.22%	76.92%
120		4	0.15	0.05813	0.2419	0.1	0.2	0.02887	0.05774	38.49%	84.62%
240		4	0	0	0	0	0	0	0		100.0%

96h Survival Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1	1	1	0.9
15		0.8	0.7	0.5	0.8
30		0.7	0.5	0.4	0.6
60		0.2	0.2	0.3	0.2
120		0.1	0.2	0.2	0.1
240		0	0	0	0

Fathead Minnow 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Analysis ID: 04-6281-9629 **Endpoint:** 96h Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 24 Aug-15 12:24 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	11.4%	<15	15	NA	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		15*	4.944	2.356	0.178	6	0.0003	CDF	Significant Effect
		30*	7.074	2.356	0.178	6	<0.0001	CDF	Significant Effect
		60*	11.63	2.356	0.178	6	<0.0001	CDF	Significant Effect
		120*	12.95	2.356	0.178	6	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	2.501997	0.6254993	4	54.79	<0.0001	Significant Effect
Error	0.1712427	0.01141618	15			
Total	2.67324		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	3.112	13.28	0.5393	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9656	0.866	0.6609	Normal Distribution

96h Survival Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	0.975	0.8954	1	1	0.9	1	0.025	5.13%	0.0%
15		4	0.7	0.475	0.925	0.75	0.5	0.8	0.07071	20.2%	28.21%
30		4	0.55	0.3446	0.7554	0.55	0.4	0.7	0.06455	23.47%	43.59%
60		4	0.225	0.1454	0.3046	0.2	0.2	0.3	0.025	22.22%	76.92%
120		4	0.15	0.05813	0.2419	0.15	0.1	0.2	0.02887	38.49%	84.62%
240		4	0	0	0	0	0	0	0		100.0%

Angular (Corrected) Transformed Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	1.371	1.242	1.501	1.412	1.249	1.412	0.04074	5.94%	0.0%
15		4	0.9977	0.7563	1.239	1.049	0.7854	1.107	0.07587	15.21%	27.24%
30		4	0.8368	0.6273	1.046	0.8357	0.6847	0.9912	0.06584	15.74%	38.97%
60		4	0.4926	0.4004	0.5849	0.4636	0.4636	0.5796	0.029	11.77%	64.07%
120		4	0.3927	0.2623	0.5231	0.3927	0.3218	0.4636	0.04096	20.86%	71.36%
240		4	0.1588	0.1588	0.1588	0.1588	0.1588	0.1588	0	0.0%	88.42%

96h Survival Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1	1	1	0.9
15		0.8	0.7	0.5	0.8
30		0.7	0.5	0.4	0.6
60		0.2	0.2	0.3	0.2
120		0.1	0.2	0.2	0.1
240		0	0	0	0

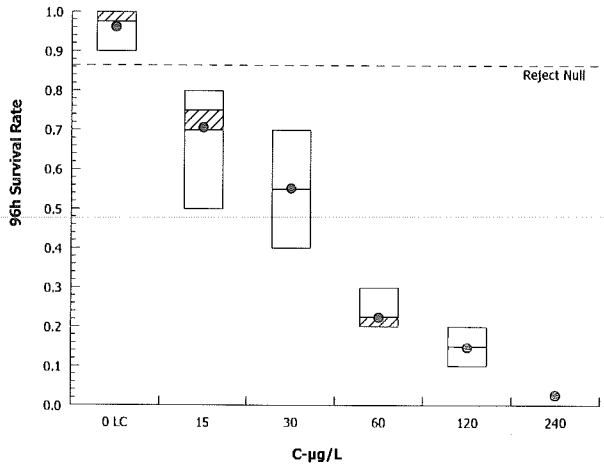
Fathead Minnow 96-h Acute Survival Test — **Nautilus Environmental (CA)**

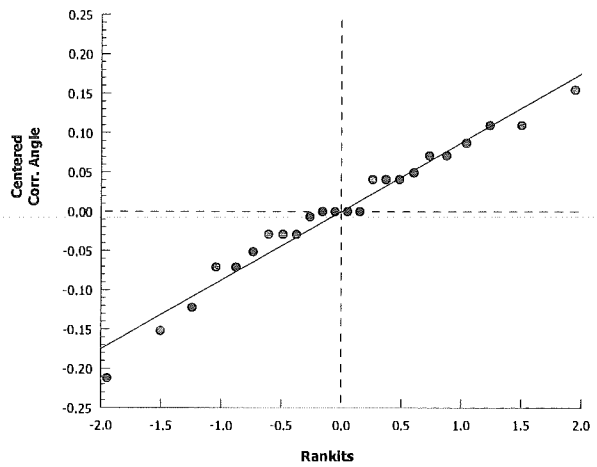
Analysis ID:	04-6281-9629	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Aug-15 12:24	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Angular (Corrected) Transformed Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1.412	1.412	1.412	1.249
15		1.107	0.9912	0.7854	1.107
30		0.9912	0.7854	0.6847	0.8861
60		0.4636	0.4636	0.5796	0.4636
120		0.3218	0.4636	0.4636	0.3218
240		0.1588	0.1588	0.1588	0.1588

Graphics





CETIS Analytical Report

Test Code: 150818ppra | 05-4894-2965

Fathead Minnow 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Analysis ID:	11-1489-1006	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Aug-15 12:24	**Analysis:**	Trimmed Spearman-Kärber	**Official Results:**	Yes

Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.025	28.21%	1.506	0.06114	32.09	24.22	42.53

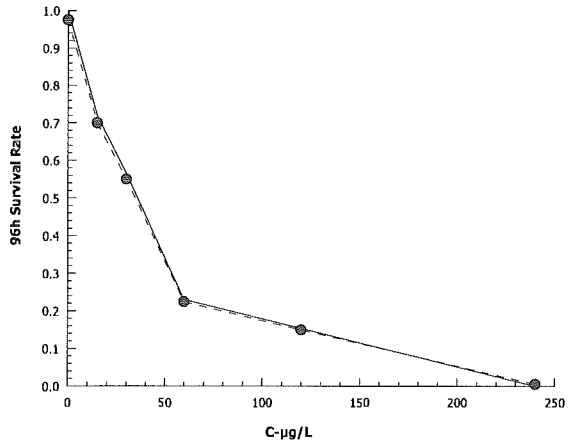
96h Survival Rate Summary

			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	4	0.975	0.9	1	0.025	0.05	5.13%	0.0%	39	40
15		4	0.7	0.5	0.8	0.07071	0.1414	20.2%	28.21%	28	40
30		4	0.55	0.4	0.7	0.06455	0.1291	23.47%	43.59%	22	40
60		4	0.225	0.2	0.3	0.025	0.05	22.22%	76.92%	9	40
120		4	0.15	0.1	0.2	0.02887	0.05774	38.49%	84.62%	6	40
240		4	0	0	0	0	0		100.0%	0	40

96h Survival Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1	1	1	0.9
15		0.8	0.7	0.5	0.8
30		0.7	0.5	0.4	0.6
60		0.2	0.2	0.3	0.2
120		0.1	0.2	0.2	0.1
240		0	0	0	0

Graphics

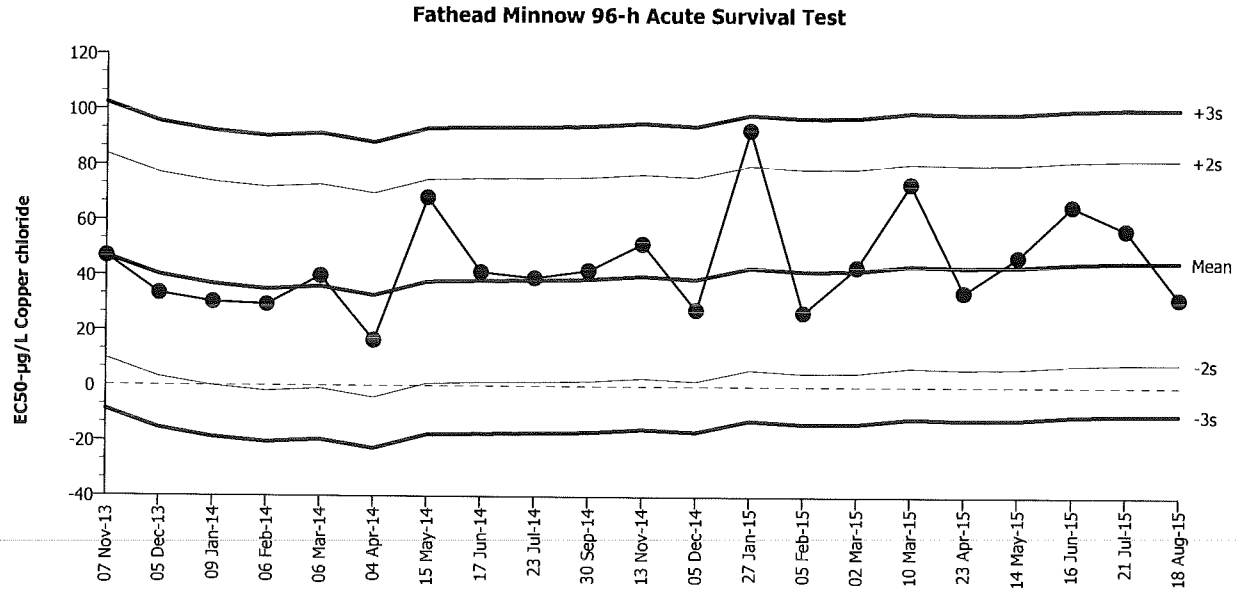


 Analyst: VCR QA: KBB 8/25/15

CETIS QC Plot

Fathead Minnow 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Test Type:	Survival (96h)	**Organism:**	Pimephales promelas (Fathead Minn	**Material:**	Copper chloride
Protocol:	EPA/821/R-02-012 (2002)	**Endpoint:**	96h Survival Rate	**Source:**	Reference Toxicant-REF



Mean:	45.41	**Count:**	20	**-2s Warning Limit:**	8.429	**-3s Action Limit:**	-10.06
Sigma:	18.49	**CV:**	40.70%	**+2s Warning Limit:**	82.39	**+3s Action Limit:**	100.9

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2013	Nov	7	13:00	46.88	1.47	0.07948			00-9091-1171	05-3514-4061
2		Dec	5	13:35	33.43	-11.98	-0.6478			03-5498-7590	18-6731-8860
3	2014	Jan	9	14:30	30.36	-15.05	-0.8139			02-9671-4557	13-7041-2345
4		Feb	6	14:55	29.54	-15.87	-0.8582			01-4932-9675	02-7759-7858
5		Mar	6	16:00	39.82	-5.587	-0.3022			15-7137-9679	11-6247-2364
6		Apr	4	17:55	16.56	-28.85	-1.56			13-2870-2407	20-2803-8732
7		May	15	16:00	68.33	22.92	1.239			18-7494-5068	09-4299-8323
8		Jun	17	13:25	41.31	-4.1	-0.2217			05-7169-9299	04-2830-7951
9		Jul	23	11:40	39.34	-6.073	-0.3285			15-5022-7021	13-4007-2355
10		Sep	30	15:05	42.04	-3.374	-0.1825			02-2426-0140	19-6371-2028
11		Nov	13	15:20	51.72	6.308	0.3412			19-4779-2607	18-9797-8784
12		Dec	5	14:40	27.84	-17.57	-0.9503			05-7934-6283	15-5144-9047
13	2015	Jan	27	14:05	92.96	47.55	2.571	(+)		19-2603-7396	13-4290-6809
14		Feb	5	0:00	26.9	-18.51	-1.001			17-5331-9354	20-6831-1307
15		Mar	2	12:50	43.42	-1.992	-0.1077			14-4829-6636	02-1391-8116
16			10	14:40	73.57	28.16	1.523			20-7997-6449	09-3501-0518
17		Apr	23	14:15	34.29	-11.12	-0.6012			06-7611-5819	12-9351-9341
18		May	14	15:10	47.2	1.791	0.09685			19-7359-3994	06-7541-8732
19		Jun	16	15:30	65.64	20.23	1.094			20-8767-5723	20-5489-9980
20		Jul	21	13:40	57.04	11.63	0.6291			18-9160-5144	11-5950-8811
21		Aug	18	13:10	32.09	-13.32	-0.7202			05-4894-2965	11-1489-1006

96-hour Freshwater Acute Bioassay
Static-Renewal Conditions

Water Quality Measurements & Test Organism Survival

Client: Internal
Sample ID: $CuCl_2$
Test No.: 150818ppra

Test Species: *P. promelas*
Start Date/Time: 8/18/2015 1310
End Date/Time: 8/22/2015 1145

	Tech Initials				
	0	24	48	72	96
Counts:	EG	ALB	BK	EG	EG
Readings:	EG	ALB	BK	AG	EG
Dilutions made by:	KB	—	ALB	—	—
High conc. made (µg/L):	240	--	240	--	--
Vol. Cu stock added (mL):	5.6	--	5.6	--	--
Final Volume (mL):	2000	--	2000	--	--

Cu stock concentration (µg/L): 85,900

Concentration µg/L	RAND #	Number of Live Organisms					Conductivity (µmhos/cm)					Temperature (°C)					Dissolved Oxygen (mg/L)					pH (units)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Lab Control	19	10	10	10	10	10	191	191	i 185	187	198	20.6	20.5	i 19.7	20.2	20.6	8.5	7.8	i 8.1	8.2	7.9	8.20	8.03	i 8.21	8.01	7.99
	4	10	10	10	10	10			f 191					f 20.1					f 7.0					f 7.93		
	21	10	10	10	10	10																				
	24	10	10	10	10	9																				
15	11	10	10	10	10	8	191	192	i 185	187	201	20.6	20.4	i 19.8	20.2	20.6	8.5	7.9	i 8.2	8.1	8.1	8.19	8.09	i 8.20	8.05	8.03
	13	10	10	9	7	7			f 193					f 20.1					f 7.0					f 7.93		
	20	10	10	8	5	5																				
	10	10	10	10	9	8																				
30	18	10	10	9	7	7	191	192	i 185	187	206	20.6	20.4	i 19.8	20.1	20.2	8.5	7.9	i 8.2	8.3	8.2	8.19	8.07	i 8.21	8.12	8.11
	3	10	10	8	5	5			f 194					f 19.9					f 7.5					f 7.93		
	8	10	10	7	5	4																				
	15	10	10	8	6	6																				
60	2	10	9	5	4	2	190	189	i 185	187	199	20.7	20.3	i 19.8	20.3	20.2	8.5	8.0	i 8.2	8.1	8.3	8.18	8.08	i 8.20	8.13	8.16
	14	10	7	3	2	2			f 192					f 20.1					f 7.4					f 7.92		
	22	10	10	7	5	3																				
	16	10	9	4	3	2																				
120	12	10	8	1	1	1	190	201	i 184	189	200	20.8	20.3	i 19.7	20.4	20.3	8.5	8.0	i 8.1	8.1	8.2	8.17	8.05	i 8.19	8.12	8.13
	6	10	7	2	2	2			f 199					f 20.0					f 7.5					f 7.95		
	1	10	8	~~2~~ 3	2	2																				
	23	10	9	~~4~~ 3 @	3	1																				
240	9	10	7	0			193	194	i 184	-	-	20.6	20.5	i 19.9	-	-	8.6	8.0	i 8.2	-	-	8.13	8.06	i 8.19	-	-
	5	10	6	0					f 195					f 19.9					f 7.8					f 7.97		
	7	10	9	0																						
	17	10	5	0																						

Rand # QC: VCR
Initial Count QC: KB

Animal Source/Date Received: ABS 8/18/15
Age at Initiation: 6d

Feeding Times					
	0	24	48	72	96
AM:			0830		
PM:					

Comments: i = initial reading in fresh test solution, f = final reading in test chamber prior to renewal
Organisms fed prior to initiation, circle one (y) / n
@ BK QC 8/20/15

QC Check: VCR 8/24/15.
Final Review: KB 8/25/15

Appendix E
Laboratory Qualifier Codes

Glossary of Qualifier Codes:

Q1 - Temperatures out of recommended range; corrective action taken and recorded in Test Temperature Correction Log

Q2 - Temperatures out of recommended range; no action taken, test terminated same day

Q3 - Sample aerated prior to initiation or renewal due to dissolved oxygen (D.O.) levels below 6.0 mg/L

Q4 - Test aerated; D.O. levels dropped below 4.0 mg/L

Q5 - Test initiated with aeration due to an anticipated drop in D.O.

Q6 - Airline obstructed or fell out of replicate and replaced; drop in D.O. occurred

Q7 - Salinity out of recommended range

Q8 - Spilled test chamber/ Unable to recover test organism(s)

Q9 - Inadequate sample volume remaining, 50% renewal performed

Q10 - Inadequate sample volume remaining, no renewal performed

Q11 - Sample out of holding time; refer to QA section of report

Q12 - Replicate(s) not initiated; excluded from data analysis

Q13 - Survival counts not recorded due to poor visibility or heavy debris

Q14 - D.O. percent saturation was checked and was ≤ 110%

Q15 - Did not meet minimum test acceptability criteria. Refer to QA section of report.

Q16 - Percent minimum significant difference (PMSD) was below the lower bound limit for acceptability. This indicates that statistics may be over-sensitive in detecting a difference from the control due to low variability in the data set.

Q17 - Percent minimum significant difference (PMSD) was above the upper bound limit for acceptability. This indicates that statistics may be under-sensitive in detecting a difference from the control due to high variability in the data set.

Q18 - Incorrect Entry

Q19 - Illegible Entry

Q20 - Miscalculation

Q21 - Other (provide reason in comments section)

Q22 - Greater than 10% mortality observed upon receipt and/or in holding prior to test initiation. Organisms acclimated to test conditions at Nautilus and ultimately deemed fit to use for testing.

Q23 - Test organisms received at a temperature greater than 3°C outside the recommended test temperature range. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate tests upon the day of arrival. Organisms were acclimated to the appropriate test conditions upon receipt and prior to test initiation.

Q24 - Test organisms received at salinity greater than 3 ppt outside of the recommended test salinity range. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate tests upon the day of arrival. Organisms were acclimated to the appropriate test conditions upon receipt and prior to test initiation.

Alpha Analytical Laboratories Inc.
e-mail: clientservices@alpha-labs.com

Laboratory & Corporate: 208 Mason Street, Ukiah, CA 95482
707-468-0401 Fax: 707-468-5267

Service Center & Micro Lab: 6398 Dougherty Rd, Ste 35, Dublin, CA 94568
925-828-6226 Fax: 925-828-6309

Chain of Custody Record

Reports and Invoices will be delivered by email in .pdf format.

Lab No.__________ Page_____ of_____

Report to:	Invoice to (if different):	Project Info for Report:
Company: CDCR - Deuel Vocational Institution	Company: (month end consolidated billing)	Project ID: Chronic & Acute Bioassay
Attn: Daniel Mullins		
Address: 23500 Kasson Road, Tracy, CA 95376	Address:	Project Number:
Phone/Fax: 209-835-4141 ext 5897	Phone/Fax:	
Email Addresses: daniel.mullins@cdcr.ca.gov		

Samplers Signature: [signature]

Print: Daniel G Mullins

Please contact David Pingatore with any questions about COC completion

TAT: 10 days ● | RUSH: 5 days ○ | 48 hours ○ | Other: ____days ○

Lab Approval Required For Rush TATs

Sample Notes (lab use only): Temperature: [illegible] deg. C | Shipment Method: | Custody Seals: Y / N

Sample Identification	Sampled: Date	Sampled: Time	Container: 1 Gallon Cube	Sterile bacti	1 liter Poly	500 mL	Soil jar	Preservative: Na2S2O3	HNO3	H2SO4	Other	None	Matrix: Waste Water	Grab	Composite	Total Number of Containers	DVI Acute FH Minnow non-renewal		DVI Chronic FH Minnow 100% Effluent	DVI Chronic Green Algae 100% Effluent	DVI Chronic Water Flea 100% Effluent		DVI Ref Toxicant Report - FH Minnow	DVI Ref Toxicant Report - Green Algae	DVI Ref Toxicant Report - Water Flea			Sample Notes or CDPH Source Numbers:
Final Effluent Comp	8/21/15	10:40	X									X	X		X	1			X	X	X		X	X	X			Relinquish Temp 7.0 °C
Set Comp Sampler																												Relinquish Temp_____ °C
8/20/2015 10:05																												

Relinquished by:	Received by:	Date:	Time:
[signature]	[signature]	8/21/15	1046
[signature]	SUB COC		
	[signature]	8/21/15	2000
[signature]	Lab [signature]	8/21/15	2240

CDPH Write On EDT Transmission? ○ Yes ● No

State System Number: __________

If "Y" please enter the Source Number(s) in the column above

CA Geotracker EDF Report? ○ Yes ● No

Global ID: Sampling Company Log Code:

EDF to (Email Address):

Travel and Site Time: | Mileage: | Misc. Supplies:

Farhad, Mohammad@Waterboards

From: Farhad, Mohammad@Waterboards
Sent: Wednesday, March 23, 2016 3:30 PM
To: WB-RB5S-CentralValleySacramento
Subject: FW: 2016 1st qtr Acute and Chronic Prelim
Attachments: 2016 1st qtr Acute and Chronic Prelim.pdf

From: Daniel Mullins [mailto:daniel.mullins@cdcr.ca.gov]
Sent: Wednesday, March 23, 2016 11:14 AM
To: Farhad, Mohammad@Waterboards
Cc: Barlay, Alfred@CDCR
Subject: 2016 1st qtr Acute and Chronic Prelim

Attached is the 2016 1st quarter Acute and Chronic Prelim, the water flea reproduction was significantly reduced in the 100 percent effluent relative to the control, the RO plant shut down on February 26, 2016 at 4:00 PM and started back up on Monday February 29, 2016 at 2:00 PM, the shutdown caused an elevated EC in the wastewater plant influent, we started the composite sampler for the Chronic on February 28, 2016, I was on vacation the week of March 14, 2016 and received this email this week, I have scheduled the accelerated to start the week of March 28, 2016.

If you have any questions feel free to contact me
Thanks

NPDES #CA0078093
Facility ID 5B390100001

Daniel G Mullins
Water & Wastewater Treatment Plant Supervisor
Deuel Vocational Institution
23500 kasson Rd Tracy CA 95376
Office-(209) 835-4141 x5897
Fax-(209) 830-3941

Mullins, Daniel@CDCR

From: Violet Renick <violet@nautilusenvironmental.com>
Sent: Tuesday, March 15, 2016 4:21 PM
To: Mullins, Daniel@CDCR; David Pingatore
Subject: Preliminary results of Q1 toxicity testing for DVI

Hi Daniel and David,

I hope that you have both been doing well since we last spoke. I wanted to let you know that I have finished a preliminary review and analysis of the toxicity test data for samples collected on February 29, 2016, from Deuel Vocational Institution. There were no statistically significant effects in the green algae, or chronic or acute fathead minnow tests.

There was no statistically significant effect in water flea survival, however water flea reproduction was significantly reduced in the 100 percent effluent relative to the control. The conductivity was quite elevated in this sample (~2,000 uS/cm) so it is possible that this contributed to the reproductive toxicity that we observed in this round of testing. However, as we have discussed in the past it is not possible to definitively determine if this was the cause of toxicity.

The data should be reviewed by the end of the week and I should be able to complete and share the report by next week.

In the meantime, please do not hesitate to let me know if you have any questions.

Many thanks,

Violet

--
Violet Renick, Ph.D.
Environmental Scientist
Nautilus Environmental
4340 Vandever Avenue
San Diego, California 92120

Office: (858) 587-7333 x206
Cell: (619) 807-6019

Farhad, Mohammad@Waterboards

From: Daniel Mullins <daniel.mullins@cdcr.ca.gov>
Sent: Monday, May 02, 2016 11:33 AM
To: Farhad, Mohammad@Waterboards
Subject: TRE Action Plan Green Algae
Attachments: DVI-TRE Action Plan 04-26-2016.pdf

Here is the TRE Action Plan for the Green Algae failures, I will upload on the April eSMR due by the 1st of June 2016.

Daniel G Mullins
Water & Wastewater Treatment Plant Supervisor
Deuel Vocational Institution
23500 kasson Rd Tracy CA 95376
Office-(209) 835-4141 x5897
Fax-(209) 830-3941

April 26, 2016

Ms. Pamela Creedon
Executive Officer
Central Valley Water Quality Control Board
11020 Sun Center Drive, #200
Rancho Cordova, California 95670-6114

Re: Toxicity Reduction Evaluation (TRE) Action Plan for Deuel Vocational Institution

Dear Ms. Creedon,

This TRE Action Plan is submitted as required by Section VI.C.2.iv.c of WDR Order No. R5-2014-0014-1 when a numeric toxicity monitoring trigger is exceeded during Accelerated Chronic WET Monitoring.

Accelerated Chronic Toxicity Monitoring

The Acute and Chronic Toxicity test results during the 4th quarter of 2015, sampled on October 19, 2015, showed a toxicity of >1 to *Selenastrum capricornutum* (green algae) in the undiluted whole effluent at the DVI WWTP discharge EFF-001. Accelerated chronic toxicity monitoring for *Selenastrum capricornutum* was conducted as shown in Table 1.

Table 1 – DVI Accelerated Chronic Toxicity Testing During 4Q 2015 and 1Q 2016

Accelerated Test	Date	Test Organism	Growth reduction, percent	TUc Limit	TUc Test Results	Chronic toxicity, pass/fail
1	11-18-2015	*Selenastrum capricornutum*	31.8	1	> 1	Fail
2	12-02-2015	*Selenastrum capricornutum*	29.94	1	> 1	Fail
3	12-16-2015	*Selenastrum capricornutum*	0	1	1	Pass
4	12-30-2015	*Selenastrum capricornutum*	0	1	1	Pass
5	01-13-2016	*Selenastrum capricornutum*	43.4	1	> 1	Fail
6	01-26-2016	*Selenastrum capricornutum*	59.5	1	> 1	Fail

1. TUc = Chronic Toxicity Units = 100/NOEC; (NOEC = No observed effect concentration).
2. TUc > 1 requires accelerated WET testing to begin within 14-days after failure of a quarterly WET test and initiation of a TRE if any accelerated WET test fails.
3. Monitoring Location at Effluent 1 (EFF-001).

TRE Tasks and Schedule

Results of the accelerated chronic toxicity monitoring require DVI to conduct a TRE. DVI will initiate a TRE in accordance with the DVI TRE Workplan, dated July 2009, and the current discharge permit. Dewberry Architects and Engineers will perform the tasks for the DVI TRE following the schedule shown in Table 2.

Table 2 – DVI TRE Tasks and Schedule

Task	Description	Duration, weeks	Completion Date
1	Complete and Submit Action Plan	3	29 April 2016
2	Validation of Bioassay Results	12	27 June 2016
3	Information and Data Acquisition	20	22 August 2016
4	Facility Performance Evaluation	20	22 August 2016
5	Toxicity Identification Evaluation	20	22 August 2016
6	Toxicity Reduction Evaluation Report and Exit	28	17 September 2016

Completion Date based on CDCR authorization to begin TRE work on April 4, 2016.

I certify under penalty of law that this document and all attachments were prepared under my direction or supervision in accordance with a system designated to assure that qualified personnel properly gather and evaluate the information submitted. Based on my inquiry of the person or persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is, to the best of my knowledge and belief, true accurate, and complete. I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations.

Sincerely,

JAIME RODRIGUEZ, CHIEF ENGINEER I

Note: Per Standard Provisions, Reporting sections V.B.2 and V.B.3, the LRO must be a principal executive officer or ranking elected official of the Discharger's agency, or a duly authorized representative that meets the intent of 40 CFR 122.22(b)(2).