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February 26, 2019

Mr. Christopher Bittner
Standards Coordinator
Utah Dept. of Environmental Quality
195 N 1950 W
Salt Lake City, UT 84116

Dr. Gary Belovsky
Environ. Res. Center & Dept. Biol Sci.
University of Notre Dame
Notre Dame, IN 46556

RE: Results of Acute Copper Toxicity to Brine Shrimp

Mr. Bittner / Dr. Belovsky:

Enclosed is a copy of the final report entitled Acute Toxicity of Copper to *Artemia franciscana* under Static-Renewal Test Conditions.

We greatly appreciate this opportunity to provide our services to you. Please do not hesitate to contact us if you have any questions.

Sincerely,

Rami B. Naddy, Ph.D.
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David A. Pillard, Ph.D.
Manager / Senior Toxicologist
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Enclosure:

17001-474-014

University of Notre Dame
Notre Dame, IN

Acute Toxicity of Copper to *Artemia franciscana* Under Static-Renewal Test Conditions

Prepared by:

TRE Environmental Strategies

TRE Environmental Strategies, LLC
100 Racquette Drive, Unit A
Fort Collins, CO 80524

Document No. 17001-474-014

December 2017

Study Title

Acute Toxicity of Copper to *Artemia franciscana* in Laboratory Reconstituted Salt Water Under Static-Renewal Test Conditions

Study Period

Start: December 18, 2017 @ 14:45
End: December 22, 2017 @ 15:00

Performing Laboratory

TRE Environmental Strategies

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Telephone: (970) 416-0916
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Laboratory Project ID

17001-474-014

SUMMARY

Study Directors	Rami B. Naddy, Ph.D. David A. Pillard, Ph.D.
Test Facility	TRE Environmental Strategies, LLC 100 Racquette Drive, Unit A Fort Collins, Colorado 80524 (970) 416-0916
Location of Data	TRE Environmental Strategies, LLC 100 Racquette Drive, Unit A Fort Collins, Colorado 80524 (or an offsite storage location)
Test Substance	Copper chloride $CuCl_2$; Fisher Scientific Lot #033926; TRE #C04-008)
Subject	Static-Renewal Toxicity Test
Test Dates	Initiated: December 18, 2017 @ 1445 Terminated: December 22, 2017 @ 1500
Length of Study	96 Hours
Test Species	*Artemia franciscana*
Source of Organisms	Brine Shrimp Direct (Ogden, UT)
Age of Test Organisms	48 Hours
Test Concentrations	Nominal Copper Concentrations: 0, 288, 412, 588, 840, 1,200 µg Cu/L
Dilution Water	Laboratory Saltwater Reconstituted Water (rGSL; RW #13121) Target: Salinity ~ 120 ppt
Results	96-Hour LC_{50} Based on Measured Copper Concentrations: 766.8 µg total recoverable Cu/L; 95% C.I. (748.9 – 785.1) 299.6 µg dissolved Cu/L; 95% C.I. (295.3 – 303.9)[1]

[1]Use of the dissolved median lethal copper value should be used with caution because dissolved copper values were not found to be a good surrogate of actual copper concentrations

Sponsor and Laboratory Information

Sponsor	Environmental Research Center University of Notre Dame 97 Galvin Life Sciences Center Notre Dame, IN 46556
Project Officer	Gary E. Belovsky, Ph.D. (574) 631-0172
Testing Facility	TRE Environmental Strategies, LLC 100 Racquette Drive, Unit A Fort Collins, Colorado 80524 Fax: (970) 490-2963
Study Directors	Rami B. Naddy, Ph.D. (970) 416-0916 email: naddyrb.tre@gmail.com
	David A. Pillard, Ph.D. (970) 416-0916 email: pillardda.tre@gmail.com

Introduction

This report presents the results of a study conducted to determine the acute toxicity of copper (as copper chloride) to *Artemia franciscana* in a laboratory reconstituted salt water under static-renewal test conditions. The ultimate goal is to use these data to aid in the development of ambient water quality criteria for metals for the protection of species in the Great Salt Lake.

Methods followed the Work Plan for Great Salt Lake Toxicity Test, Version 8, October 23, 2016; Dr. Belovsky, University of Notre Dame, except where noted. The target water used in these studies was prepared to mimic Gilbert Bay water (see work plan; Appendix A), with a salinity of 120 ppt.

All toxicity tests were conducted at TRE (Fort Collins, CO). Chemical confirmation of copper was carried out at ALS Environmental (Kelso, WA; primary analytical laboratory).

METHODS
Test Media

The artificial reconstituted Great Salt Lake (rGSL) water was used in holding and testing conditions for the brine shrimp (but not in hatching). It was prepared as follows with ASTM Type I (Milli-Q®) water (ASTM 2012):

- Crystal Sea marine mix: 50.95 g/L
- Potassium chloride (KCl): 2.99 g/L
- Magnesium sulfate ($MgSO_4$): 6.19 g/L
- Sodium chloride (NaCl): 65.77 g/L

Salts were added to Milli-Q® water in a 20 L carboy and stirred to mix salts. Analytical results for previous batches of this water are reported in the results section. The laboratory reconstituted salt water had an initial salinity of ~120 ppt.

Test Organisms

Test organisms were *Artemia franciscana* nauplii obtained as cysts from Brine Shrimp Direct (Ogden, UT). Brine shrimp were hatched in a 1.0-L separatory funnel containing artificial reconstituted seawater (29 ppt at 29 °C) made using Crystal Sea Marine Mix according to TRE SOP #5104. Hatched nauplii were kept in the pre-test holding conditions for 48 hours before test initiation.

The food source for the *A. franciscana* was the salt water alga *Dunaliella viridis*. *D. viridis* was cultured at ~10°C with 16:8 h light: dark photoperiod under constant aeration. The media used to culture *D. viridis* consisted of the following added to Milli-Q® water:

- Morton's Water Softener Salt (80 ml/L)
- Crystal Sea marine mix (53.3 ml/L)

The media was mixed well and filtered (Buchner funnel) through a 110-mm Whatman® #4 filter paper and sterilized using a pressure cooker. Nutrients (1-3 ml) were added to the *D. viridis* cultures 1-2X per week. The nutrient solution consisted of Milli-Q® water (80 ml), blue solution (10 ml), and P/N solution (10 ml). The Blue solution consisted of 41.7 g/L Hydrosol, 27.5 g/L calcium nitrate, and 22.1 g/L ammonium nitrate dissolved in Milli-Q® water. The P/N (phosphorus/nitrogen) solution consists of 8.79 g/L monopotassium phosphate and 20.0 g/L ammonium nitrate dissolved in Milli-Q® water.

Pre-Test Conditions

In the pre-test holding conditions 200 < 24-h old brine shrimp nauplii were placed in 50 ml of 120 ppt rGSL seawater in a 80-ml Pyrex beaker and fed *D. viridis* at a concentration of 100 µg/L Chl*a* (chlorophyll a) at the beginning of the holding period (no additional food was added during the pre-test holding period). The solutions were gently aerated during the holding period. Four batches of brine shrimp (e.g., 200 organisms each) were prepared and held in this manner to ensure a sufficient number of organisms for the toxicity test. After 48-h, the organisms were transferred to the test chambers using a block design (e.g., organisms from the first chamber were used for all 'A' replicates, etc.).

Test Conditions

The chemical used in testing was copper chloride ($CuCl_2$-$2H_2O$; Fisher Scientific, Lot #033926, TRE # C04-008). Individual test solutions were prepared by addition of the appropriate volume of the copper stock solution (365 mg/L as Cu) to rGSL water. Algae (*D. viridis*) were added at a concentration of 145 µg/L Chl*a* along with the metal stock solution and rGSL to a total volume of 1.0 L. This volume was sufficient for biological and analytical sampling. The solutions were allowed to equilibrate for ~3 hours prior to use in testing.

After the equilibration period, the solutions were shaken to re-suspend any settled algae / precipitate to ensure homogenous distribution to test chambers for testing and for analytical sampling. Approximately 150 ml of solution was poured into each test chamber (n = 5). Test chambers consisted of 12 ounce Pro-Kal® polypropylene cups[1]. Twenty 48-h old brine shrimp nauplii were placed in each test chamber, and test chambers were placed in a randomized design in a temperature-controlled water bath (20℃). The photoperiod was 16:8 hour light:dark using fluorescent lighting.

The study consisted of a 96-h exposure period in which *A. franciscana* were exposed to different copper concentrations. The test solutions were renewed after 48 h[2] with test solutions that had been equilibrated with *D. viridis* (same feeding rate as at test initiation) for ~ 3-h. Surviving organisms were verified at 48 h (test solution renewal) and 96 h (organisms were not handled on days 1 and 3 so surviving organisms on these days were estimates). While organism growth was included in the work plan, it was determined not to be required for these studies, and therefore only survival was measured.

[1] Polypropylene test chambers were used instead of HDPE test chambers as mentioned in the work plan
[2] The work plan mentioned renewals every 24-h although preliminary analytical work indicated that 48-h renewals were sufficient for analytical and biological needs; therefore the latter renewal cycle was adopted.

Further detail is provided in Table 1 below.

Table 1. Additional Test Conditions in the Toxicity Test

Type	96-h Static-Renewal Acute (renewal at 48-h; see deviation to work plan)
Test Endpoints	Mortality (no response to stimulus); see deviation to work plan regarding growth endpoint
Test Concentrations (nominal)	0 (control), 288, 412, 588, 840, and 1200 µg Cu/L
Quality Criterion	≥90% control survival
Analytical Confirmation	Test initiation (new): Dissolved and total recoverable samples for each treatment Test renewal (new and old): Dissolved and total recoverable samples for each treatment Test termination (old): Dissolved and total recoverable samples for each treatment
Copper Analyses	ICPMS (EPA Method 200.7) ALS Environmental – Primary Analytical Laboratory
Statistical Analyses	96-h median lethal concentrations were determined using CETIS 2014

Reference Toxicant

Three reference toxicant studies were performed with *A. franciscana* to determine the relative sensitivity of the organism. A summary of the results of the reference toxicant tests is presented in the results section below.

RESULTS

The initial characteristics of the rGSL water used in this experiment are reported in Table 2.

Table 2. Initial Dilution/Control Water Characterization

Batch No.	pH	Hard. (mg/L)[a]	Alk. (mg/L)[a]	Spec. Cond. (µS/cm)	Salinity (ppt)
13121	8.5	11,800[c]	111[c]	176,400	118

[a] As $CaCO_3$
[b] Total residual chlorine
[c] Measured in rGSL batch #13090 (12/07/17)

The batch of rGSL water was not analyzed for dissolved and total recoverable metals as in previous batches due to the consistency in results in earlier studies. Refer to the two previous studies that reported the dissolved and total recoverable metals for the rGSL water (see TRE report #s: 14001-474-012 and 14001-472-018). A summary of the analyses can also be found in Appendix B.

The range of water quality parameters measured during the toxicity test is provided in the table below (Table 3). Overall, the pH, dissolved oxygen, conductivity, and temperature were similar among treatments.

Table 3. Physical and Chemical Data Measured during the Toxicity Test

Treatment (Nominal Conc., µg Cu/L)	pH		Dissolved Oxygen (mg/L)		Conductivity (mS/cm)		Temperature (°C)	
	Low	High	Low	High	Low	High	Low	High
0 (Control)	7.9	8.5	4.8	5.2	173,000	176,400	18	21
288	7.8	8.4	4.8	5.2	175,400	191,600	18	20
412	7.8	8.4	4.8	5.2	175,800	185,200	18	20
588	7.8	8.4	4.9	5.2	175,000	192,200	18	20
840	7.8	8.4	4.9	5.2	173,000	190,000	18	20
1,200	7.8	8.4	4.8	5.2	173,600	190,800	18	20

Analytical Confirmation

Samples were collected for total and dissolved copper analyses from new and old test solutions as outlined in the Methods section. Average concentrations for all treatments are presented in Table 4.

Table 4. Measured Copper Concentrations

Nominal Conc. (µg Cu/L)	Avg Total Rec. (µg Cu /L)	% of Nominal	Avg. Dissolved (µg Cu/L)	% of Nominal	Diss. / Tot. (%)[a]
0 (control)	21 U	--	21 U	--	--
288	202	70	147	51	59
412	311	75	201	49	48
588	449	76	205	35	36
840	698	83	285	34	35
1,200	913	76	331	28	25

[a] Initial samples, except 412 µg/L, which is calculated from the renewal sample
Note: Refer to Appendix B for a summary of analytical results
U = less than the MRL / MDL (which is reported)

In comparing total recoverable versus dissolved copper concentrations, dissolved copper concentrations were on average 25-59% of the total recoverable copper concentrations and 28-51% of nominal concentrations (Table 4 and Appendix B). On the other hand, total recoverable copper concentrations ranged from 70 to 83% of nominal concentrations. Dissolved copper concentrations were low compared to nominal and measured total recoverable copper concentrations, and therefore, endpoints calculated using dissolved copper values should be interpreted with caution as they may under-represent actual exposure values.

Organism Response

The definitive test was initiated December 18, 2017 at 1445 hours and was terminated on December 22, 2017 at 1500 hours. *A. franciscana* survival at 48 h and 96 h is shown in Table 5.

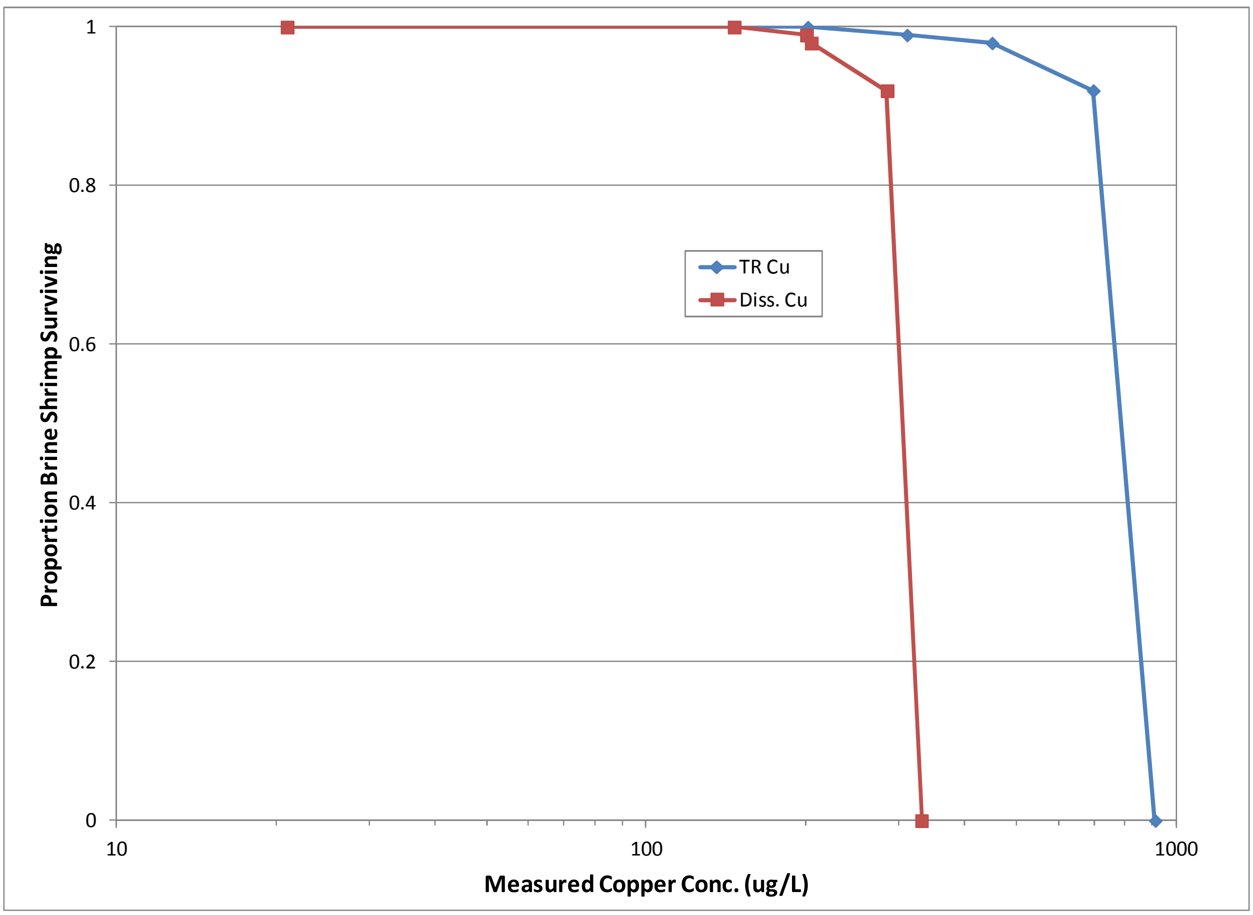
Table 5. Survival (%) of *Artemia franciscana*

Nominal Conc. (µg Cu/L)	% Survival		
	0 h	48 h	96 h
0 (control)	100	100	100
288	100	100	100
412	100	99.0	99.0
588	100	99.0	98.0
840	100	97.0	92.0
1,200	100	34.0	0.0

Note: See Appendix C for a copy of raw data

A graphical representation of the 96-hour survival data, based on measured copper concentrations (log scale) is shown in Figure 1.

Figure 1. Proportion of *Artemia franciscana* Surviving at 96 hours versus Measured Copper Concentrations.



Dissolved copper concentrations were included in Figure 1 for comparison, even though dissolved copper concentrations may not provide a good estimate of actual exposure concentrations.

The calculated 96 hour LC_{50} values for *A. franciscana* are provided below for total recoverable and dissolved copper (Table 6). As mentioned above, the median lethal concentration for dissolved copper should be used with caution due to the low recoveries compared to nominal and total recoverable copper values.

Table 6. 96 hour Median Lethal Copper Concentrations (µg/L)

Endpoint	Total Rec. (µg Cu/L)	Dissolved (µg Cu/L)
LC_{50}	766.8	299.6
95% C.I.	748.9 – 785.1	295.3 – 303.9
Method	Spearman-Karber	Spearman-Karber

Reference Toxicant Studies

Three additional studies were initiated with *A. franciscana* using arsenic for the purpose of reference toxicant tests. The average results of these reference toxicant studies (mean ±2 standard deviations) are presented below (Table 7).

Table 7. Reference Toxicant Test Results for *A. franciscana*

96-h LC_{50} (mg As/L)	TRE Historical 95% Control Limits (mg As/L)	
	Low	High
70.57	53.97	87.16

Note: Values are expressed as mg/L of nominal arsenic.

References

ASTM. 2012. Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians. E 729-96. Annual Book of ASTM Standards, Volume 11.05, Section 11, Water and Environmental Technology.

Belovsky, G. Work Plan for Great Salt Lake Toxicity Tests, Version 8. October 23, 2016. Environmental Research Center and Department of Biological Sciences. University of Notre Dame.

CETIS. 2014. Comprehensive Environmental Toxicity Information System. User Guide (version 1.8.7). Tidepool Scientific, LLC. McKinleyville, CA.

STATEMENT OF PROCEDURAL COMPLIANCE

I certify that this document and all attachments were prepared under my direction or supervision in accordance with a system designed 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, accurate and complete.

[signature] February 25, 2019

Rami B. Naddy, Ph.D.
Study Director

Date

STATEMENT OF QUALITY ASSURANCE

The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data generated at TRE.

[signature] 02/25/2019

Quality Assurance Unit

Date

APPENDIX A

WORK PLAN

<u>Work Plan For Great Salt Lake Toxicity Tests, Version 8</u>
<u>October 23, 2016</u>

Gary Belovsky
Environmental Research Center & Department of Biological Sciences
University of Notre Dame

<u>Introduction</u>:

Great Salt Lake (GSL) is a unique ecosystem, the fourth largest (largest in the western hemisphere) hypersaline lake in the world[16]. Invertebrate life in the GSL is relatively species poor due to the high salinity of the lake and is dominated by brine shrimp (*Artemia franciscana*) and brine fly larvae. Two or more species of brine flies occur in the GSL with *E. cinerea* the most abundant by several orders of magnitude. Thus, *Artemia franciscana* and *Ephydra cinerea* are the dominant grazers in the GSL food web in Gilbert Bay (South Arm) of Great Salt Lake, and are the focus of this study. These invertebrates are very abundant and are the main source of food for many resident and migrating water birds, which have important ecological and conservation value. Some of these water bird species are threatened or endangered or have other legal protections.

The purpose of this project is to determine toxic levels of copper, arsenic and lead, to brine shrimp and brine fly larvae so that water quality criteria (WQC) can be developed for GSL as described in the State of Utah Division of Water Quality (UDWQ) Great Salt Lake Strategy[22]. These potential pollutants were identified as the highest priorities by UDWQ after public feedback and comments.

We will conduct acute toxicity tests of the above substances for brine shrimp and brine fly larvae (*E. cinerea*) and develop a plan of work to assess chronic toxicity of these trace elements. *E. cinerea* has been chosen as a test species because of its local abundance end ecological dominance and it has been successfully cultured in the laboratory. American Society for Testing and Materials (ASTM) has not sanctioned standard methods for toxicity testing with either of these species. However, other ASTM-approved methods for invertebrates are established and will be used as a guide for the conduct of the toxicity testing proposed here.

In this work plan we present the protocols that lead to uniform rearing of the brine shrimp and brine fly larvae, as well as production of control and test (pollutant) solutions. We then focus on range-finding and acute testing, which must be completed before proceeding with the ecologically more relevant chronic testing (survival and reproduction with life-time exposure to the pollutant), which will be detailed in a future work plan. <u>Range-finding</u> establishes the range of concentrations for each pollutant that produces short term (96 hour) toxicity (assessed by mortality and growth). Once the range for each pollutant has been established, <u>acute tests</u> will be conducted to establish the concentration of each pollutant that will produce 50% mortality over 96 hours of exposure (LC50). For this project, the Belovsky laboratory has primary responsibility for developing the culturing methods for the brine flies and brine shrimp and will provide brine flies to the bioassay laboratory. The bioassay laboratory has primary responsibility for conducting the toxicity tests for both species –*Artemia franciscana* cultured at the bioassay laboratory per protocols provided by the Belovsky lab, and *Ephydra cinerea* which will be provided by the Belovsky lab.

Source animals and rearing conditions:

Given the uniqueness of Gilbert Bay, Great Salt Lake compared to other aquatic environments for which ecotoxicology studies have been developed, it is critical that our toxicology studies provide results that are applicable to the lake's environment. The environmental conditions found in the Great Salt Lake in April – October (the time when brine shrimp are present) over a 20 year period (1994 – 2013[16, 17]) are summarized in the table below.

Consistency of *Artemia* will be ensured by using a single batch of brine shrimp cysts (resting eggs) that have been commercially harvested from GSL. Brine fly larvae will be obtained from a colony maintained at UND, where GSL was the source of individuals starting the colony and the colony has been in existence for approximately two years (6 – 8 generations). The 3rd instar were selected because this is the longest and final larval stage when the most growth and development occurs[20]. This stage can be easily collected without damaging them from the rugose surface of bioherms on which they are raised in the laboratory colonies. Furthermore, this life stage is long enough to support shipping the larvae and robust enough to experience <10% mortality in transit.

Both species will be reared in environmental chambers that maintain temperature ($\pm$ 1^{o}C) and a light:dark cycle (16:8, ~summer day using full spectrum lighting) using the following protocols:

> **Brine shrimp** will be hatched in 10 gallon aquaria at a salinity of 45 ppt, the optimum for hatching and hatchling survival. Nauplii will be used in the bioassays as it is thought that they are most susceptible due to their small size and less developed exoskeleton. Twenty-four hours after hatching, the nauplii will be transferred to artificial GSL water (see below). Over the initial 2 days post hatch, individuals are fed ad libidum a high quality phytoplankton (*Dunaliella* sp.: 40 µg Chl_a/L/2 days) maintained in culture.
>
> **Brine fly larvae** will be reared in plastic containers (60 cm X 60 cm X 25 cm) that contain 12 cm of water that is maintained at average lake conditions specified above at the University of Notre Dame. Gravel and GSL bioherm (approximately 30 cm X 15 cm X 15 cm) serve as a substrate for larvae and pupae, and an above water platform is provided to emerging adults for resting and mating. Larvae will be fed ad libidum (pupae and adults do not feed) a high quality food (*Dunaliella* sp.: 40 µg chl_a/L/2 days) from a colony established from the GSL. Notre Dame personnel ship late 2nd or 3rd instar larvae based on size (FedEx overnight) with a resin fiber pad (3M ™ Scotch Brite #86) for attachment and food (*Dunaliella* sp.) in a plastic bag with head space and bags in a cooler with ice. Larvae will then be acclimated in artificial GSL water (see below) for a minimum of four days prior to the beginning of each test.

Dilution Water:

Artificial reconstituted GSL water (rGSL) (Table 1, salinity = 120 ppt mass/volume) will be made to duplicate Gilbert Bay water as close as practical. This specific artificial reconstituted water was selected based on considerations of the data quality objectives (see Appendix 1).

The rGSL attempted to match the average concentrations of salts that are shown in Table 2 based on Utah Geological Survey measurements from Gilbert Bay[18] (Table 2). Initially, no attempt will be made to mimic the dissolved organic content (DOC) of Gilbert Bay. DOC in Gilbert Bay water has been reported to reduce the toxicity of copper and other metals compared to artificial Great Salt Lake water[3] (Brix et al 2006). Using Gilbert Bay water instead of artificial water would duplicate the DOC concentrations at the time the water was collected but the representativeness over time is unknown. For instance, reported DOC concentrations include 7 mg/L to 42 mg/L[3,2]. Amending the rGSL with DOC was considered but was primarily rejected by UDWQ because of the lack of data to support determining an appropriate target DOC concentration.

For the acute tests, rGSL will be made using reagent grade Crystal Sea™ Bioassay Laboratory Formula Marinemix® (Marine Enterprises, Baltimore, Maryland). Marinemix is approved by EPA for toxicity testing[19]and American Chemical Society (ACS) grade salts to deionized water in 20L Nalgene® carboys. Facility Deionized filtered water will be used. pH will be maintained at 7.9 ± 0.2 via the addition of 0.1N nitric acid or 0.1N sodium hydroxide as necessary. Filtered triplicate samples will be collected from each batch to verify that the salt concentrations and pH are within acceptable ranges.

Recipe	**g/L**
MarineMix	50.95
KCl	2.99
$MgSO_4$	6.19
NaCl	65.77

Table 1. Reconstituted Great Salt Lake water used for acute toxicity testing.

Constituent	**Great Salt Lake Average**	**rGSL**	**% match**
Na	42.0	41.53	98.54
Mg	4.4	3.19	100.77
Cl	74.0	69.33	100.48
K	2.8	2.14	100.40
Ca	0.3	0.62	99.73
SO_4	9.3	8.85	99.95
HCO_3	0.4	0.22	100.14
CO3	No data	0.02	No data

Table 2. Reconstituted Great Salt Lake (rGSL) water nominal concentrations compared to Utah Geological Survey average Great Salt Lake (Gilbert Bay) Concentrations from 1966-2013[18] .

Prior to initiating testing using organisms, the rGSL will be characterized for trace metals and metalloids and the analytical results from the copper range finding test (Appendix 2) will be confirmed. To characterize the rGSL, the concentrations of antimony, arsenic, beryllium, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, thallium and zinc will be measured. To confirm the analytical results from the copper range finding test, a sample of the rGSL will be spiked with 450 ± 50 µg/l of copper as $CuCl_2$. The salinity of the rGSL may need adjustment to achieve the target salinity of 120 ppt after spiking with $CuCl_2$. The copper-spiked rGSL will be stirred to mix and both filtered (0.45µm) and unfiltered samples collected and analyzed. The measured concentrations in the filtered samples should be at least 70% of nominal. If measured concentrations are less than 70% of nominal, additional experiments will be pursued to determine the fate of the copper spikes.

Test solutions (treatments):

The pollutants chosen for these studies were selected by UDWQ after soliciting public comment. American Chemical Society (ACS) reagent grade chemicals will be employed. To maximize comparability of test results with published ecotoxicology studies[2-14] that have been conducted with other aquatic invertebrates, the following chemicals will be used to produce test solutions:

- As (arsenic) as sodium arsenate;
- Cu (copper) as copper chloride;
- Pb (lead) as lead nitrate.

Stock solutions of each trace metal will be made fresh for each of three rounds of acute toxicity testing as recommended by US EPA. Solutions will be made in Teflon sample bottles and solution concentrations will be verified at the beginning and end of each exposure 24-hour day of the bioassay (see details below).

Range finding tests:

For each pollutant to be tested, a preliminary range finding test will be conducted to establish concentrations to be used in the acute tests so that well resolved concentration-response (mortality or immobility) curves result. Initial range finding will be conducted with 10-fold increases in dissolved concentrations (e.g., 0 (controls), 10, 100, 1,000, 10,000, 100,000 µg of the pollutant/L) to ensure that we can develop well-resolved concentration response curves. Exposure to each concentration will be tested in triplicate for each test species over a 96 hour period on a static renewal basis (100% water changes occurring at 24, 48 and 72 hours), as follows:

Brine shrimp – 48 hour old nauplii will be used for all acute tests (including preliminary range finding tests). Test chambers will be 250 mL HDPE beakers containing 150 mL of test solution. Nauplii will be hatched in Marine Mix formula at 45ppt and then transferred to MarineMix at 120 ppt for tests. The shrimp nauplii will be fed *Dunalliela* from culture at a rate of 190 µg Chl_a/L/day. These feeding rations were determined to be the minimum rations required to achieve ≥ 90% control survivorship in feeding trials were conducted at UND. The feeding trials were conducted using 5 replicate treatments using the same rGSL, temperature, number of nauplii and container size as the acute tests. The initial survival was <90% and the food was increased by 50% until >90% survival was observed. Food was decreased from this rate to the midpoint of the next lowest feeding rate and the trials repeated until the minimum amount of food resulting in ≥ 90% survival was determined (190 µg Chl_a/L/day).

For the acute tests, feeding rates will be measured by taking at least three (3) Chl_a measurements of the feeding solution with the fluorometer (equivalent FSUs, fluorescent signal units, for the necessary µg Chl_a feeding level) will be made after mixing vigorously between measurements. The test conditions are summarized in Table 3.

Brine fly larvae – 3rd instar larvae will used in all acute and range finding tests. Ten individuals per replicate will be held in a 250 ml HDPE beaker containing 150 ml of test solution. The larvae will be fed *Dunalliela* from culture at the minimum necessary to achieve rate ≥ 90% control survivorship as determined at UND using the same feeding trial methods as for nauplii. This food level is attained by vacuum filtering at ~1 atmosphere 150 mL of 490 µg Chl_a/L through a glass microfibre filter (Whatman Catalog # 1825 024) 50 mL at a time. The FSU of the filtrate will be measured and recorded to verify that the phytoplankton were captured on the filter. The filter paper will be placed phytoplankton-side up in the bottom of the empty beaker. A piece of a resin fiber pad (3M ™ Scotch Brite #86) weighted with a paperclip will cover the top of the filter and provide a substrate for the larvae. The larvae are then added to the beaker.

For brine shrimp and brine fly larvae, beakers will be acid washed (5% HNO_3), rinsed several times with deionized water and dried under a laminar flow hood prior to use. Each beaker will be covered with parafilm® to reduce evaporative water loss and associated changes in salinity and pollutant concentration. The resulting headspace in each beaker will provide ample gas exchange (O_2 and CO_2) on a 24 hour basis for the animals between daily treatment solution changes. Individuals will be randomly assigned to treatment groups, and the placement of beakers in the incubators will be randomized as well. pH, DO, and conductivity in each beaker will be checked daily, as well as in a "monitoring replicate" (no test organisms present) for each concentration.

Dead and immobile individuals will be recorded daily. Dead individuals are those that are immobile and unresponsive to stimuli (touched with pipette), while immobile individuals are not observed to move until touched with the pipette. Dead individuals are removed by pipette daily at the time of treatment solution change. After the sampling and methods for measuring concentrations in the test solutions are verified to be accurate, analytical chemistry of the treatment solutions will not be conducted in the range testing work, because it is only necessary to establish which treatment solutions produce mortality or immobility and to reduce analytical costs.

Acute Toxicity Assays:

Concentration ranges for acute assay treatment solutions – Based on range finding tests, we will run appropriate dilution series such that we can make robust statistical estimates of LC50 concentrations for each species. Six test concentrations (including controls) will be used for each species. If it is determined that the concentrations required to elicit mortality exceed the solubility limit of the metal under our test conditions, acute testing will be halted and the pollutant will proceed to chronic testing.

Acute assay protocols – Acute assay protocols are summarized in Table 5. Assays will be executed as described in range finding tests (see above) with the exception that 5 replicates will be used per test concentration (as opposed to 3 for the range finding tests). Fresh test solutions will be made daily and verified (see below). Tests will be run on a static renewal basis with a 100% water change and the removal of dead test organisms occurring on a daily basis. At the termination of each test, surviving organisms will be counted, and dried on pre-weighed filter paper for analysis of growth differences between treatments. Mortality data will be analyzed via probit analysis.

Analytical chemistry. Test exposure concentrations will be verified daily at the beginning and end of each 24 hour exposure period to ensure that we are accurately characterizing exposure conditions. When possible and supported by the range finding results, brine flies and brine shrimp will be tested simultaneously to reduce analytical costs. Table 4 illustrates the number of analytical samples (88) needed assuming that both brine shrimp and brine flies are tested simultaneously with 5 exposure concentrations plus the control. Three of these exposure concentrations are assumed to be identical for both brine flies and brine shrimp and 2 of the exposure concentrations are unique to both brine flies and brine shrimp.

Note that the addition of *Dunaliella* as a food source for *Artemia* nauplii and brine fly larvae will require that post exposure water samples are filtered to remove particulates (*Dunaliella).* Because the addition of live cells (*Dunaliella*) as a food source is likely to decrease the dissolved concentrations of the test chemicals, all statistical analyses will be based on the geometric mean of the initial (pre-exposure) and final (post-exposure) dissolved concentrations. Treatment solution samples will be filtered through acid washed (5% HNO_3) 0.45 µm syringe filters that have had 3 volumes of sample water passed through them prior to retaining the sample in the appropriate acid washed (5% HNO_3) sample tubes (500 ml). Comparisons of pre-exposure test solutions will be made between filtered and unfiltered samples to describe the relationship between total and dissolved metals under our test conditions. Samples will be preserved and kept at 4^oC in the dark in 15mL conical tubes for As, Cu and Pb samples which will be stabilized with Omnitrace nitric acid or as instructed by the analytical laboratory

Acute assay data analyses – Two toxic endpoints will be recorded - mortality and immobility. Records of daily deaths and immobility will be recorded, but analyses will be conducted on overall mortality and immobility over the entire 96 hour assay period for a given pollutant concentration. Measures of mortality and immobility for a given pollutant concentration will be presented relative to the respective values observed in the simultaneous controls (no pollutant). For an assay to be considered successful, $\geq$ 90% of individuals in the control must survive.

With the above measures, the concentration-response (mortality or immobility) curves will be developed for a given pollutant and organism. These curves will be calculated via standard analytical procedures with diagnostic checks for homogeneity of variances using standard statistical packages . With the concentration-response curves, a number of toxicity effects for a pollutant can be estimated:

1) LC50 and EC50 is computed as the concentration eliciting 50% mortality (LC50) and 50% immobility (EC50) relative to the organism's control values.

2) Lowest concentration (LOEC) affecting mortality and immobility is defined as the first test concentration to produce a statistically significant increase in mortality or immobility relative to control values.

3) No effect concentrations (NOEC) is the next lowest concentration tested relative to the LOEC.

Data archiving -- all water chemistry, QA/QC data, and toxicity (mortality and immobility) data will be provided to UDWQ and made available to any interested parties

	Brine shrimp	Brine Flies
Test Type	Static renewal	Static renewal
Test Duration	96-hr	96-hr
Temperature	20 +/- 1 °C	20 +/- 1 °C
pH	7.9 +/-0.1; adjusted with 0.1N nitric acid and/or 0.1N sodium hydroxide as needed	7.9 +/-0.1; adjusted with 0.1N nitric acid and/or 0.1N sodium hydroxide as needed
Salinity	120 ppt	120 ppt
Photoperiod	16 hr light/8 hr dark	16 hr light/8 hr dark
Test Concentration or Dilution Series	5 exposures + control (40% dilution series TBD by range finder test)	5 exposures + control (40% dilution series TBD by range finder test)
Test Chamber Size	250 ml	250 ml
Test Solution Volume	150 ml	150 ml
Renewal of Test Solution	daily	daily
Age of Test Organism	48 hr nauplii	3rd instar
Number of Organisms per Test Chamber	20	10
Number of Replicates per Concentration: range finder (acute test)	3(5)	3(5)
Number of Organisms per Concentration: range finder (acute test)	60(100)	30(50)
Feeding	Daily (see text)	Daily (see text)
Endpoint	mortality (LC50) and immobility (EC50)	mortality (LC50) and immobility (EC50)
Test Acceptability	≥ 90% control survivorship	≥ 90% control survivorship

Table 3. Summary of acute testing methods.

Time (hours)	0	24		48		72		96
Exposure Solutions	initial	initial	final	initial	final	initial	final	final
Control	1	1	2	1	2	1	2	2
Brine Shrimp	1	1	1	1	1	1	1	1
Brine Shrimp	1	1	1	1	1	1	1	1
Both	1	1	2	1	2	1	2	2
Both	1	1	2	1	2	1	2	2
Both	1	1	2	1	2	1	2	2
Brine Flies	1	1	1	1	1	1	1	1
Brine Flies	1	1	1	1	1	1	1	1
Duplicate	1	1	1	1	1	1	1	1

Table 4. Minimum number of analytical samples for each toxicant tested (see text for details

Chronic Toxicity Test Plan of Work:

While conducting the acute testing portion of this project, we will develop a plan of work for chronic testing of the priority pollutants described above.

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Appendix 1

Bioassay Water For Great Salt Lake Bioassays

State of Utah
GARY R. HERBERT
Governor

Department of
Environmental Quality

Alan Matheson
Executive Director

M E M O R A N D U M

TO: Great Salt Lake Bioassay Team

FROM: Chris Bittner

DATE: April 23, 2015 (edited for inclusion in Work Plan 5/4/16)

PROJECT: Great Salt Lake Toxicity Bioassays for Brine Shrimp and Brine Flies

SUBJECT: Bioassay water for Great Salt Lake Toxicity Bioassays

Conclusions

After evaluating how well each media met the data quality objectives, none of the media can currently be concluded to be superior. The DWQ Round Robin medium was scored the highest but has not been tested with the test organisms and concerns remain regarding the long-term stability.

Data Quality Objectives

1. USEPA approval is required because the test results are intended to ultimately support the development and promulgation of numeric criteria. USEPA approval is not independent of the other data quality objectives (DQOs) because if the other DQOs are met, USEPA would likely approve the results for criteria development. However, the salinity of Great Salt Lake (GSL) is not specifically addressed by USEPA Guidance or Rules and unavoidable deviations from the existing guidance and rules are anticipated. These deviations must ultimately be acceptable to USEPA for criteria development.

2. The test medium must support the test organisms which at minimum are anticipated to include brine shrimp, brine flies, and green algae. If the test medium doesn't support the test organisms as defined by acceptable survival, growth, and reproduction in the negative controls, the results won't support the development of numeric criteria.

3. The test medium must have minimal potential confounders that either increase or decrease toxicity. The goal of having no potentially confounding issues is the ideal and meeting this

DQO is anticipated to limited to accepting the least amount of potential confounders. Confounders include factors such as ion balance, pH, presence of pollutants to be tested, dissolved organic carbon and hardness. Some of these factors are discussed in the context of DQO 5 because ideally, the medium mimics GSL's concentrations of these modifiers of toxicity.

4. The medium must be able to be replicated over time. Considerations for this DQO include that the source of the materials used for the medium should be stable over time and have documented quality control to ensure that any deviations can be identified and addressed if necessary. Based on the resources that are anticipated to be available for conducting bioassays and the number of existing pollutants, numeric criteria development for GSL is a long term project (e.g., 20+ years). Tests conducted 20 years should give the same results.

5. The DQO that the medium should be representative of Great Salt Lake is related to DQO 3 for minimal confounders affecting toxicity. The representativeness DQO is specifically specified because in a regulatory context, if bias is present, bias that overestimates toxicity is much more acceptable than bias that may underestimate toxicity. USEPA recommendations for toxicity testing media are intended to avoid underestimating toxicity for waters across the nation at the expense of potentially overestimating toxicity. To address this potential overestimation, effluent limits or potentially criteria can be modified on a site-specific basis using the Water Effects Ratio (WER). A WER is the ratio of toxicity between conducting bioassays using USEPA standard laboratory bioassay water and site water for dilution. The results may be the national criteria are either over- or under protective for the specific site. The GSL criteria are intended to be site-specific, so the bioassay medium should accurately reflect the toxicity, or lack thereof, of GSL waters obviating the need for WERs.

6. The medium should be stable over time. At minimum, the media must be stable for the test duration and ideally, the media would be stable over longer time frames. The stability of the medium may also affect the reproducibility of the toxicity testing.

7. The medium should be able to be replicated by any qualified laboratory. This DQO is similar to the other precision-related DQOs such as replication over time. The media composition must be sufficiently documented and the materials readily available to any qualified laboratory to meet this DQO.

8. Cost and convenience are the least important consideration but resource constraints are still an influential factor. When the scores for each DQO were summed, the cost and convenience score was not included.

Evaluation of Media

Several potential media were investigated:

- GSL Water (Brix et al., 2006)
- Barnes and Wurtsbaugh (2015)
- Belovsky
- DWQ Marinemix
- DWQ Round Robin

The advantages and disadvantages of each approach relative to the data quality objectives (DQOs) are summarized in the following text. Media that met, or were anticipated to potentially meet the minimum requirements are qualitatively scored on a scale of 1 to 10 for each DQO. Scores are summarized in Table 5.

Brix et al. 2006

Brix et al. (2006) used GSL water, artificial seawater, and the Bagshaw et al. (1986) media for conducting their bioassays. Although not documented in the paper, Bill Adams' (coauthor with Brix) recollection was that the GSL water was not filtered. When contacted, Mr. Adams opined that because of the quantities of water needed, filtering through a 0.45 µm filter wasn't a practical option. While a larger filter may not clog as fast, decanting may be just as efficient as filtering. Brix et al. (2006) diluted the GSL water with deionized water to the salinity of seawater. Brix et al. (2006) concluded that the toxicity of copper in GSL water was much less compared to artificial seawater or the media of Bagshaw et al. (1986). Neither the artificial seawater medium nor the Bagshaw medium were considered further because they do not appear representative of Gilbert Bay water with regards to toxicity.

The use of GSL water might be approvable by USEPA. At minimum, this will require addressing the existing contaminant concentrations assuming they are low enough to not significantly affect the test results. A score of 7 is assigned for USEPA approval because using GSL water would require a deviation from existing USEPA protocols.

GSL water is anticipated to support the test organisms under current lake conditions and is scored a 10 for this DQO.

GSL water has many potential confounders that could affect the toxicity results. Existing pollutant concentrations (further discussed in the Marinemix section) for some metals are known but data for the majority of organic priority pollutants are unavailable. GSL water may also have unidentified pathogens or introduce other undesirable organisms into the bioassays. The concentration of dissolved organic carbon, sulfate, and hardness are expected to decrease the toxicity of metals. However, these affects are reflective of actual site conditions and are not considered undesirable for criteria development specific and limited to GSL. GSL water is scored a 5 for this DQO because of existing pollutant concentrations.

The consistency of GSL water over time is uncertain. Lake salinity is known to fluctuate over time (see Figure 1) and a protocol to address these potential fluctuations would need to be developed if salinity is determined to significantly affect toxicity. For instance, the test protocol may require salinities of 11 to 13%. If GSL water was higher, deionized water could be used to lower the salinity to the target range. UGS reports that the major ion ratios have remained similar (Gwynn, 1998). However, future changes are possible because of for instance, changes by the mineral extraction industries that selectively remove some of the salts and are required to return the remainder to the Lake. At GSL salinities lower than 11%, additional salts would have to be added. Depending on the quantity of salt required, the ion ratios of GSL water may not be achievable because maintaining the ion ratios may result in precipitation. This is judged to have a small potential because much higher salinities exist in GSL. Pollutant concentrations in GSL may increase over time resulting in the water being unacceptable for criteria development at some time in the future. Other factors affecting toxicity such as pH, dissolved organic carbon, and sulfate would have to be tracked and their impacts on toxicity accounted for. GSL water is scored a 5 because of the identified uncertainties.

GSL water is representative of current conditions in GSL and is scored a 10. GSL water has several physiochemical parameters that are known to decrease toxicity and accurately accounting for these effects is important. GSL water is scored a 10 because the potential changes over time were addressed by the preceding DQO.

GSL water is anticipated to be stable relative to the concentrations of ions. The potential for biologically mediated changes are unknown. Based on the difficulty in obtaining and shipping the water, GSL water would have to be stable over time to allow large quantities to be collected and stored. Protocols would need to be established that verify the stability of GSL water over time. GSL water is scored an 8 for this DQO.

GSL water can be replicated by any laboratory provided that protocols are developed for collecting and storing the water. The protocols should also establish the tolerance ranges for key parameters. GSL water is scored a 10 for this DQO.

GSL water is anticipated to be costly because of the logistical issues of collection presumably by boat from the more remote areas of the Lake and the cost of shipping large quantities of water. Storage of the water may be inconvenient and the collection of additional water may be restricted by factors such as weather and/or season. GSL water is scored a 4 for this DQO.

Barnes and Wurtsbaugh, 2015

Barnes and Wurtsbaugh (2015) prepared a medium with salinity concentrations ranging from 10 g/l to 275 g/l. The media were prepared using equal parts deionized and GSL water and an inorganic salt mix consisting of 84% Instant Ocean, 14% NaCl, and 3% K_2SO_4. Final salinities and major ion concentrations were measured.

The Barnes and Wurtsbaugh (2015) medium would require similar analyses as the GSL water because it includes GSL water to meet USEPA approval. In addition, Instant Ocean is not a currently approved artificial salt mix for conducting bioassays. Presumably, USEPA could approve the use of this mix provided that protocols were developed to document the contents of the final solutions. This media is scored a 7.

The Barnes and Wurtsbaugh (2015) medium was used successfully to conduct experiments on brine shrimp. The suitability of this media for culturing algae or brine flies is unknown resulting in a score of 8.

Because the Barnes and Wurtsbaugh (2015) medium contains GSL water, the same concerns regarding confounders applies but to a lesser degree because only ½ of the liquid portion of the media is GSL water. The Instant Ocean has trace concentrations of the same inorganic substances that will be tested for the bioassays. Figures 2 through 8 compare the concentrations of metals in Instant Ocean at 120 ppt versus GSL water when data were available for both media. Note that none of the media considered is either 100% Instant Ocean or 100% Marinemix. With the exception of the DWQ Marinemix medium, this assumption is not anticipated to significantly overestimate the metals concentrations because metals were not measured in all materials by Barnes and Wurtsbaugh (2015) or Belovsky.

The Instant Ocean concentrations were scaled up from the data of Hovanec and Coshland (2002). Atkinson and Bingman (1999), the only source for Instant Ocean trace metals that was peer reviewed, reported much higher trace metal concentrations than Hovanec and Coshland (2002) whose concentrations are corroborated by the data reported by Marulla and O'Toole (2005). As noted by Hovanec and Coshland (2002), the ICP/MS analytical method that they employed is considered more reliable than the ICP used by Atkinson and Bingman (1999). The comparisons were subsequently based on the data from Hovanec and Coshland (2002). The GSL data were reported by Adams et al., (2015) or were based on 2 years of recent sampling by DWQ. The GSL data were not normalized to 120 ppt salinity. The figures also include the same data for Crystal Sea Bioassay Laboratory Formula Marinemix which was provided by the manufacturer.

The following observations are based on a qualitative analysis of the comparisons shown on Figures 2 through 8. Arsenic concentrations are higher in the lake than in the Marinemix (Figure 2). No data were found for the Instant Ocean arsenic concentrations. Cadmium concentrations in Instant Ocean are higher than Marinemix or GSL (Figure 3). Copper concentrations are similar between Marinemix, Instant Ocean (not detected), and GSL (Figure 4). Lead (Figure 5) and zinc (Figure 9) concentrations in Marinemix are higher than Instant Ocean or GSL. Mercury concentrations are similar between Marinemix and GSL but no data were available for Instant Ocean (Figure 6). Nickel concentrations in Instant Ocean are higher than Marinemix and GSL (Figure7). Selenium concentrations are higher in Marinemix than GSL but no data were available for Instant Ocean (Figure 8). The media mixtures using Instant Ocean and Marinemix were was assigned a score of 7.

The Barnes and Wurtsbaugh (2015) media can be replicated over time. Although the medium would have some of the same potential deficiencies as were discussed for GSL water, the salinity can be increased with the salt mixture which would negate the impacts of varying salinity in the lake. However if other parameters in the GSL water changed significantly, the media may not easily replicated over time. The media was assigned a score of 7.

The Barnes and Wurtsbaugh (2015) media is reasonably similar to GSL water with respect to major ion concentrations (Table 2). However, the media has about half Ca and Mg as GSL water and higher concentrations of Cl and SO_4. The dissolved organic carbon concentration is not reported but is likely lower than the 7 to 42 mg/l reported by Brix et al. (2006) and Wurtsbaugh and Jones (2012), respectively. This media was scored 6 primarily due to the uncertainties regarding organic carbon.

The Barnes and Wurtsbaugh (2015) medium is presumed to be stable based on the duration of the microcosm experiments conducted. The medium was assigned a score of 10. The medium could also be replicated by a qualified laboratory and was assigned a score of 10. For cost and convenience, the medium was assigned a score of 6 because compared to 100% GSL water, half as much water is needed.

Table 2 excerpted from Barnes and Wurtsbaugh (2015)

Table 1. Ionic weight proportions of the Gunnison Bay (328 g L^{-1}) and Gilbert Bay (110 g L^{-1}) of the Great Salt Lake (from Sturm 1980) and measured ionic composition of water from six of the salinity treatments in the microcosm experiment.

	Great Salt Lake		Microcosm Experiment (Nominal Salinities)					
Major Ions	Gunnison Bay (328 g L^{-1})	Gilbert Bay (110 g L^{-1})	10 g L^{-1}	50 g L^{-1}	100 g L^{-1}	150 g L^{-1}	200 g L^{-1}	250 g L^{-1}
Na^+	.320	.313	.350	.330	.307	.314	.313	.320
K^+	.026	.027	.017	.025	.024	.025	.024	.025
Mg^{+2}	.032	.035	.037	.020	.017	.016	.016	.016
Ca^{+2}	.001	.002	.004	.001	.001	.001	.001	.001
Cl^-	.554	.551	.502	.538	.571	.562	.563	.562
SO_4^{-2}	.067	.073	.090	.086	.080	.082	.083	.076

Belovsky

Belovsky has successfully used a 60:40 mix by volume of Morton Solar Salt and Instant Ocean to lab to culture brine shrimp and brine flies for the proposed bioassays. The Morton Solar Salt is commercial water softening salt extracted from GSL and the ion concentrations are as reported by the manufacturer. Both Belovsky and Wurtsbaugh use Instant Ocean in their media combined with either GSL water or salt extracted from GSL water and the following includes a specific discussion only where the scores or rationale differ.

Belovsky's medium has been successfully used to culture GSL algae, brine shrimp and brine flies and was assigned a score of 10. Belovsky's medium was assigned a score of 6 for potential confounders. No data for trace metals were available for the Morton Solar Salt and the score could increase or decrease if this data were obtained.

A comparison of the major ions in Belovsky's medium to GSL water is shown in Figure 10 for assessing the representativeness compared to GSL water. Belovsky's media has more NaCl and less K, SO_4, Ca, and Mg than GSL water. These concentrations were estimated assuming that 60:40 ratio by volume was equivalent to 60:40 by weight and the scores could change based on actual analytical or more refined nominal estimates.

Belovsky's medium is the least expensive medium considered and uses salts that are easily obtained and was scored 10 for cost and convenience.

DWQ Marinemix

A DWQ proposed medium consisting of Marinemix, NaCl and KCl in ratios of 84:13:2, respectively was attempted. This medium may have matched GSL water better than Barnes and Wurtsbaugh (2015) or Belovksy but when the medium was attempted, a precipitate formed and preliminary testing indicated lower survival of brine flies and brine shrimp, potentially in part due to reduced algal production. Jim from EPA unsuccessfully attempted to permanently dissolve the precipitate by reducing the pH. After consulting with the Marinemix manufacturer, Belvosky reported that at salinities above approximately 5%, calcium would precipitate. This medium was abandoned because of the precipitate. The precipitation also suggests that the Marinemix (USPEPA approved) could not simply be substituted for Instant Ocean in Barnes and Wurtsbaugh's (2015) or Belovsky's media recipes.

DWQ Round Robin

DWQ initiated a laboratory round robin study to verify the analytical methods commonly used for analyzing the GSL samples. The initial matrix was formulated using reagent grade salts only and precipitates formed at salinities well below 12%. Different chemical forms of the salts were attempted and the medium shown in Table 3 did not exhibit a precipitate when anhydrous salts were used. Precipitates did form at salinities above 13% and the SO_4 was reduced by 50% as shown in Table 4 to prevent precipitation up to a salinity of 20%. The medium described in Table 4 was not considered further because the major ion concentrations deviate substantially from GSL. With salts of sufficient quality (e.g., laboratory grade), the medium in Table 3 would be approvable by USEPA. The primary unknown with this media is if the test organisms would tolerate it and if potential nutrient deficiencies exist. Because this is unknown, a score of 3 was assigned which could change if the organism were tested.

No potential confounders are identified and the medium was scored a 10. The medium would be replicable over time. The media is representative of GSL for the major ions but is lacking dissolved organic carbon, so was scored a 7. The potential addition of organic carbon could potentially change this score. The medium was stable over a couple of weeks but longer-term stability was not tested, so a score of 9 was assigned. The media can be replicated by any qualified laboratory and was scored a 10.

The salts are available from laboratory suppliers but the costs will be higher than e.g., Instant Ocean. Large term storage may require conditions to preserve the anhydrous condition of the salts resulting in a score of 6.

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Table 3 DWQ Round Robin Media Matrix at 13% Salinity										
Salt	**Added (g/l)**	**[Na+]**	**[Mg++]**	**[K+]**	**[Ca++]**	**[H+]**	**[Cl-]**	**[SO4=]**	**[CO3=]**	**[OH-]**
CaCl2+2H2O	1.1				0.299944		0.530661			
MgCl2 (anhy)	8.1		2.06775				6.032335			
NaCl	106.56	41.91779					64.64221			
KCl	5.38			2.821532			2.558468			
CaSO4										
MgSO4 (anhy)	11.6		2.342456					9.258026		
Na2SO4										
K2SO4										
NaHSO4										
CaCO3										
MgCO3										
Na2CO3										
K2CO3										
NaHCO3	0.65	0.177883				0.007737			0.464379	
Ca(OH)2										
Mg(OH)2										
NaOH										
KOH										
Mass Fraction		0.317328	0.033245	0.021269	0.002261		0.556049	0.069789		
Target Conc. (g/l)	133.39	42	4.4	2.8	0.3	1E-08	74	9.3	0.43	0.000017
% of Target		**100.2**	**100.**	**100.8**	**100.0**		**99.7**	**99.5**	**108**	

Table 5
Qualitative Scores for Bioassay Media Considered

Data Quality Objective	**GSL Water**	**Barnes and Wurtsbaugh (2015)**	**Belovsky**	**DWQ Round Robin**
USEPA Approval	7	7	7	10
Media supports test organisms	10	8	10	3
Media has minimal potential confounders affecting the toxicity	5	7	6	10
Media can be replicated over time	5	7	7	10
Media is representative of Great Salt Lake	10	6	6	7
Media is stable over test duration	8	10	10	9
Media can be replicated at any laboratory	10	10	10	10
Low Cost and Convenient	4	6	8	6
SUM without cost score	55	55	56	59

References

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Figures

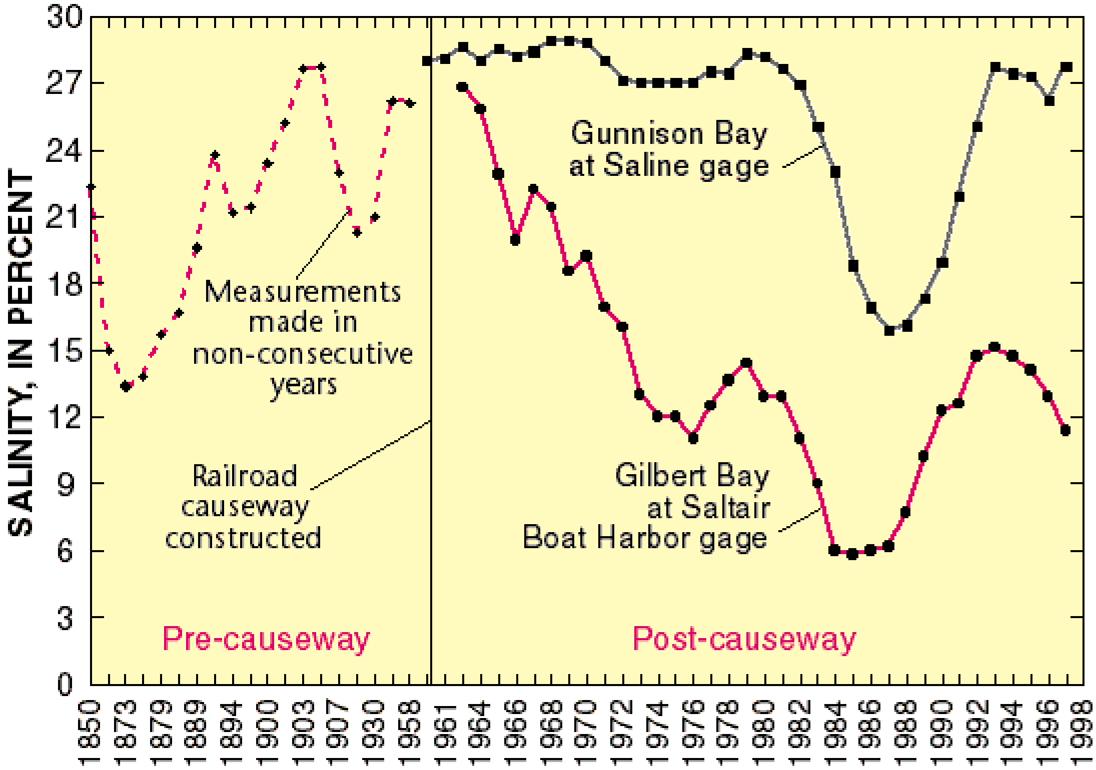


Figure 1. Great Salt Lake Salinity over time from USGS (http://ut.water.usgs.gov/greatsaltlake/salinity/)

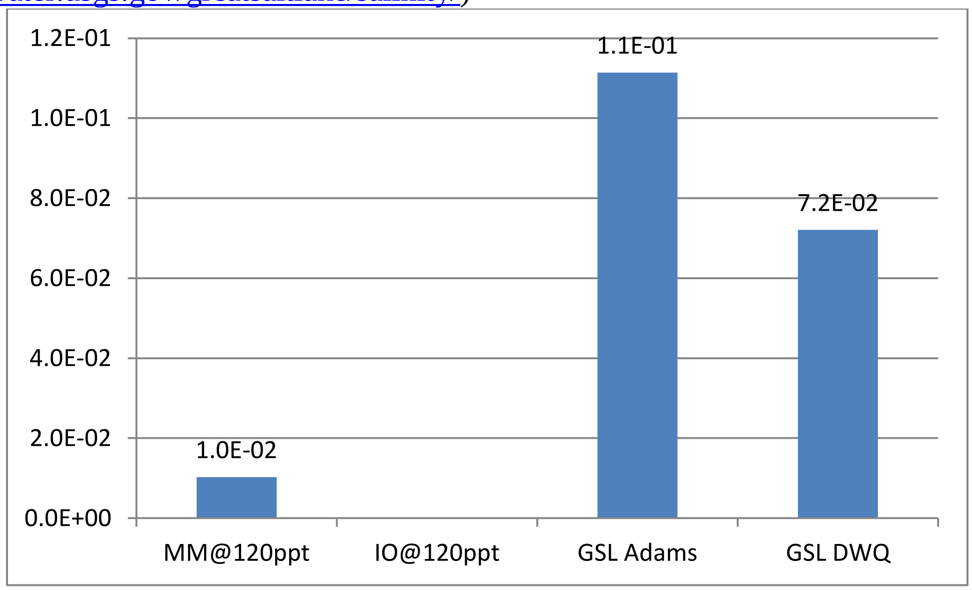


Figure 2. Comparison of mean arsenic concentrations. MM=Marinemix, IO=Instant Ocean, GSL Adams=Great Salt Lake Adams et al.,2015, GSL DWQ= Great Salt Lake based on 2 years of sampling by the Utah Division of Water Quality. No data available for Instant Ocean (IO).

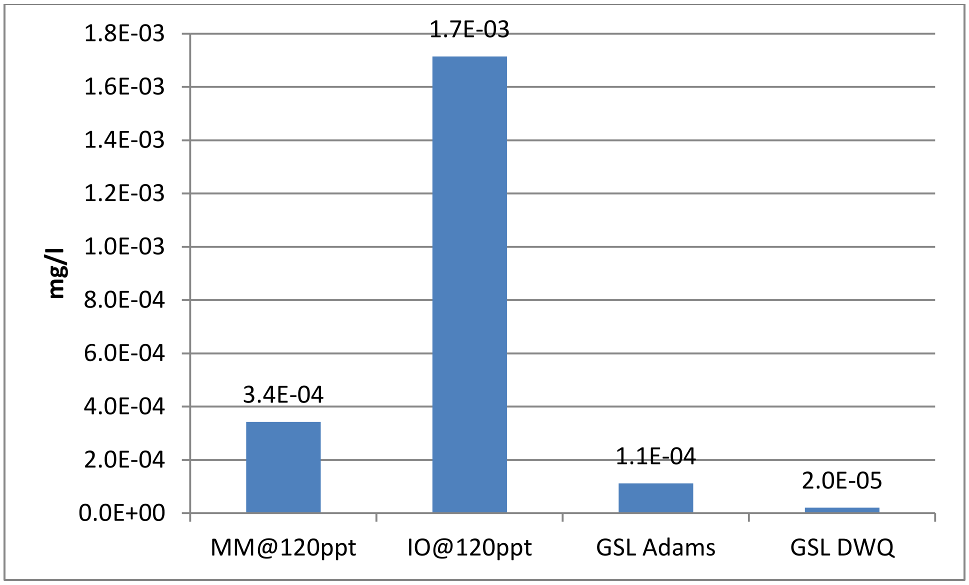


Figure 3. Comparison of mean cadmium concentrations. MM=Marinemix, IO=Instant Ocean, GSL Adams=Great Salt Lake Adams et al.,2015, GSL DWQ= Great Salt Lake based on 2 years of sampling by the Utah Division of Water Quality

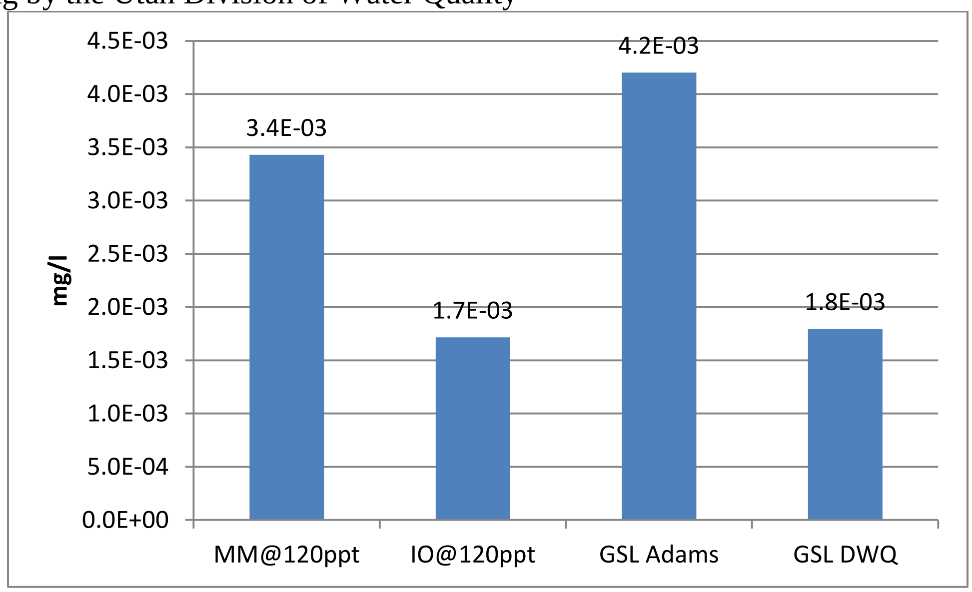


Figure 4. Comparison of mean copper concentrations. MM=Marinemix, IO=Instant Ocean, GSL Adams=Great Salt Lake Adams et al.,2015, GSL DWQ= Great Salt Lake based on 2 years of sampling by the Utah Division of Water Quality. Copper was nondetect for Instant Ocean.

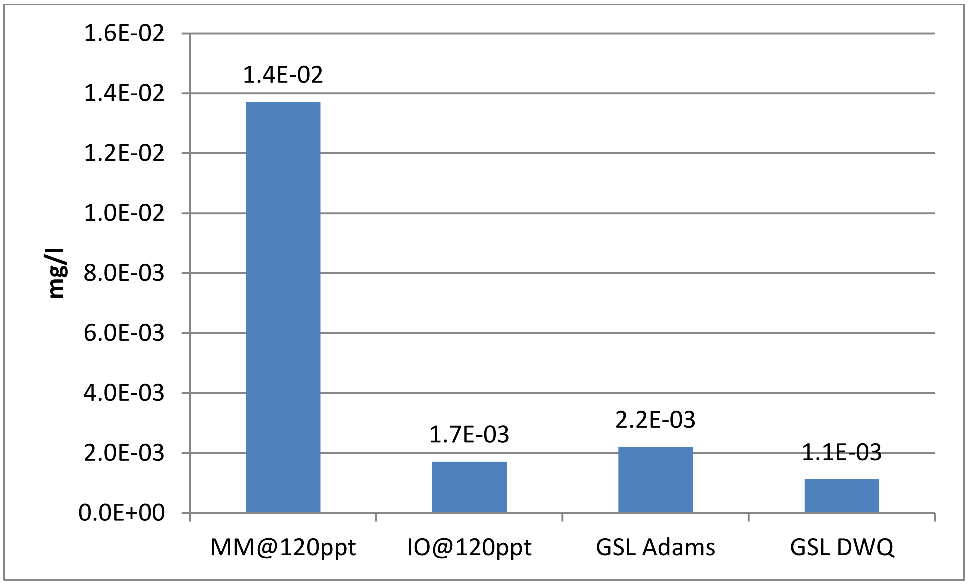


Figure 5. Comparison of mean lead concentrations. MM=Marinemix, IO=Instant Ocean, GSL Adams=Great Salt Lake Adams et al.,2015, GSL DWQ= Great Salt Lake based on 2 years of sampling by the Utah Division of Water Quality. Lead was nondetect for Instant Ocean.

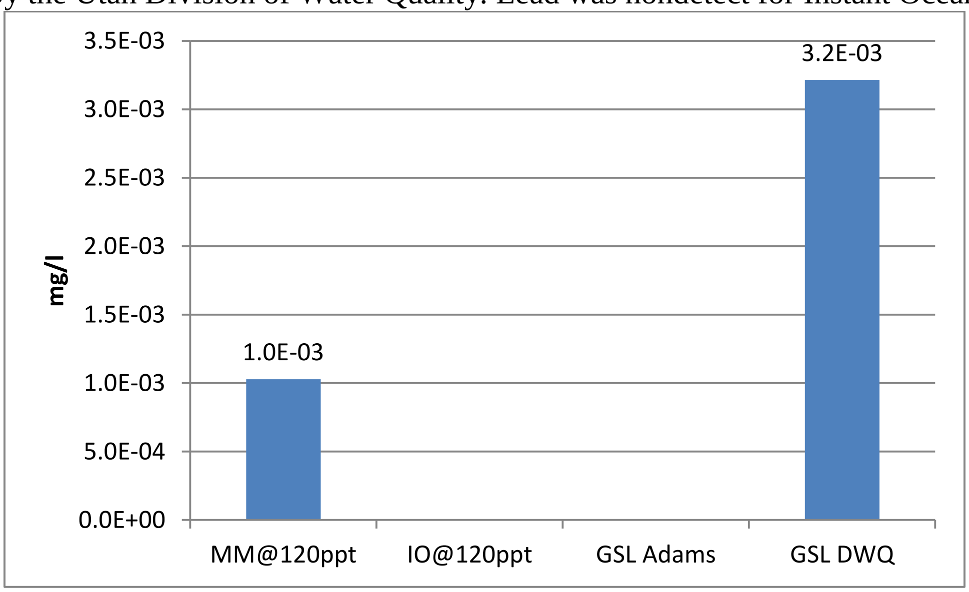


Figure 6. Comparison of mean lead concentrations. MM=Marinemix, IO=Instant Ocean, GSL Adams=Great Salt Lake Adams et al.,2015, GSL DWQ= Great Salt Lake based on 2 years of sampling by the Utah Division of Water Quality. No data were available for Instant Ocean or GSL Adams.

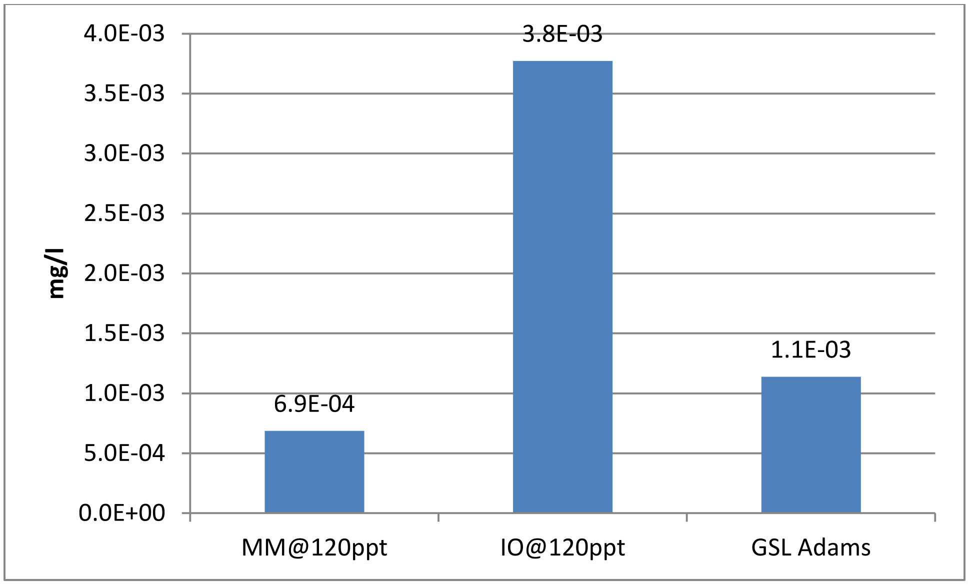


Figure 7. Comparison of mean nickel concentrations. MM=Marinemix, IO=Instant Ocean, GSL Adams=Great Salt Lake Adams et al.,2015, GSL DWQ= Great Salt Lake based on 2 years of sampling by the Utah Division of Water Quality

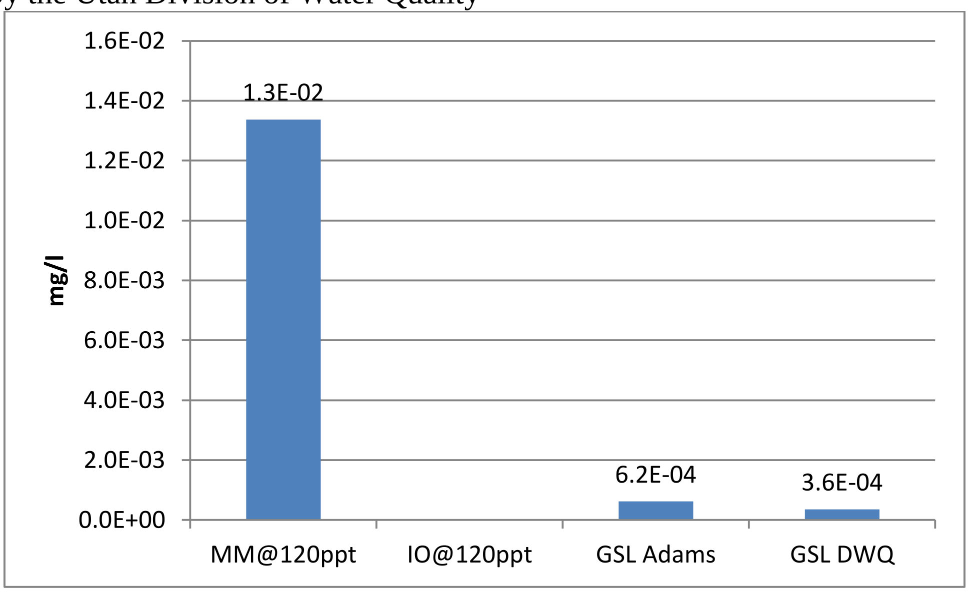


Figure 8. Comparison of mean selenium concentrations. MM=Marinemix, IO=Instant Ocean, GSL Adams=Great Salt Lake Adams et al.,2015, GSL DWQ= Great Salt Lake based on 2 years of sampling by the Utah Division of Water Quality. No data were available for Instant Ocean.

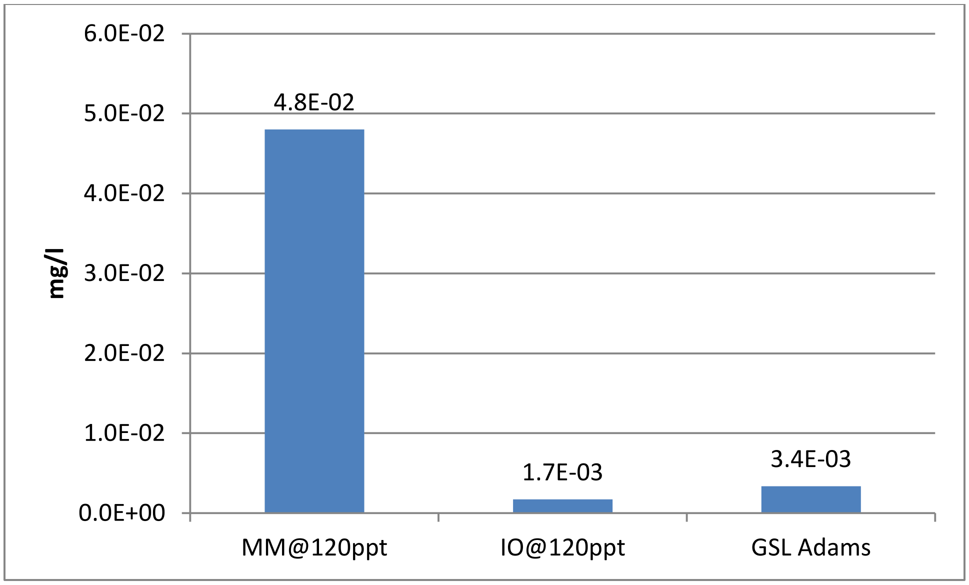


Figure 9. Comparison of mean zinc concentrations. MM=Marinemix, IO=Instant Ocean, GSL Adams=Great Salt Lake Adams et al.,2015, GSL DWQ= Great Salt Lake based on 2 years of sampling by the Utah Division of Water Quality. Zinc was nondetect for Instant Ocean.

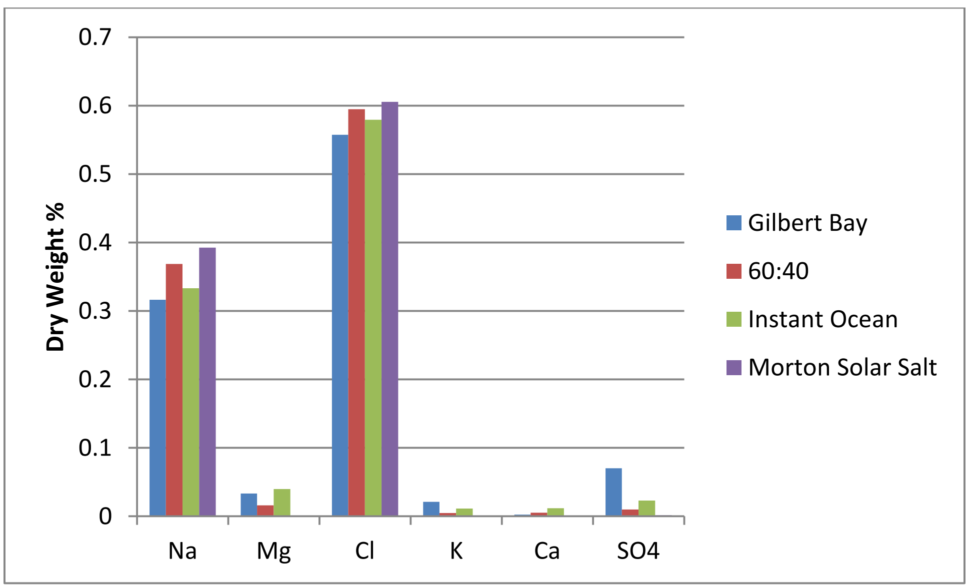


Figure 10. Comparison of major ions between Great Salt Lake (Gilbert Bay), a 60:40 Morton Solar Salt : Instant Ocean assuming volume is accurate surrogate for mass, Instant Ocean and Morton Solar Salt

APPENDIX 2

Results from Initial Range Finding for Copper and Brine Shrimp

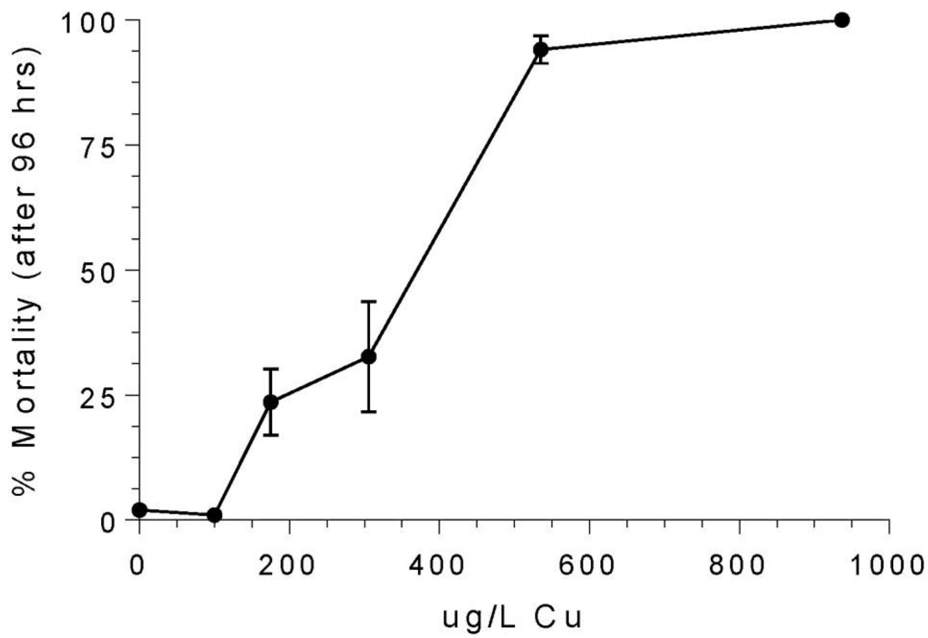
The pre-test water samples were collected after the water had been renewed, but prior to the addition of algae and brine shrimp each day as to hopefully ensure the correct copper concentrations were in solution.

The post-test water samples were collected each following day, prior to water renewal, as why those samples start at 24 hours. These samples were filtered in order to remove the algae.

The blanks were one replicate that was treated the same as all other replicates (water renewal each day and algae present), but it did not have brine shrimp in solution. Water samples were taken from these replicates after each 24 hours (post-test) to monitor the copper concentrations without brine shrimp in solution.

Ultra-pure nitric acid prior to shipment to the lab and were clear at the time of shipping.

96-Hr Acute Copper Assay Results

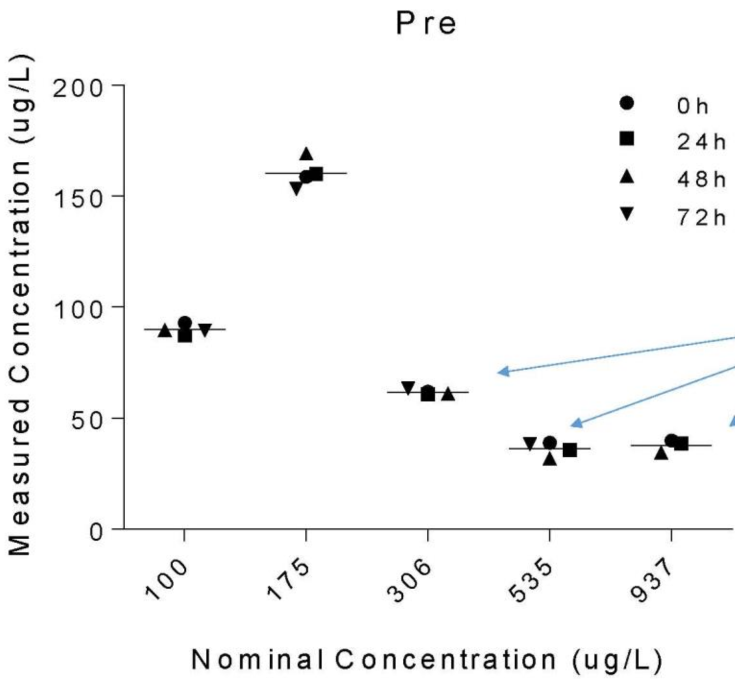


<u>Observations:</u>

- Mortality increased with increased nominal concentrations of copper

- However, measured copper concentrations are not in agreement with nominal values…..see slides 2-5

Measured Copper Concentrations **Pre-test** for Each Day



*All samples were filtered via 0.2um syringe filters

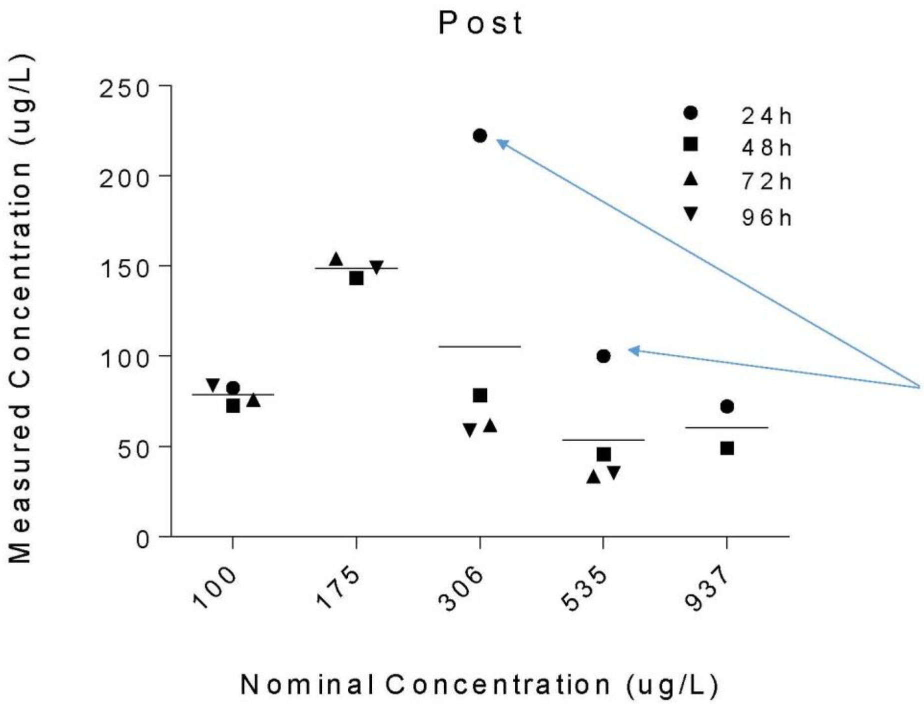
Observations:

- Background copper in control solutions varied from 5.3-9.6 ug/L

- Measured concentrations of copper at first 2 concentrations were acceptable

- The three highest concentrations had lower measured concentrations than expected
<u>Possible Explanation:</u> Precipitation and removal via filtration

Measured Copper Concentrations **Post-test** for Each Day



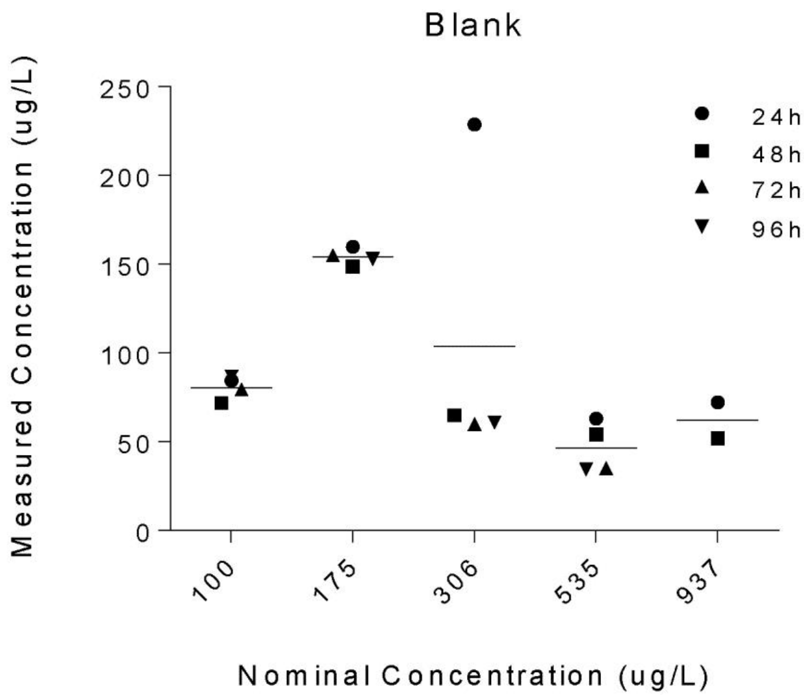
Observations:

- Similar trends in measured copper across the range of concentrations

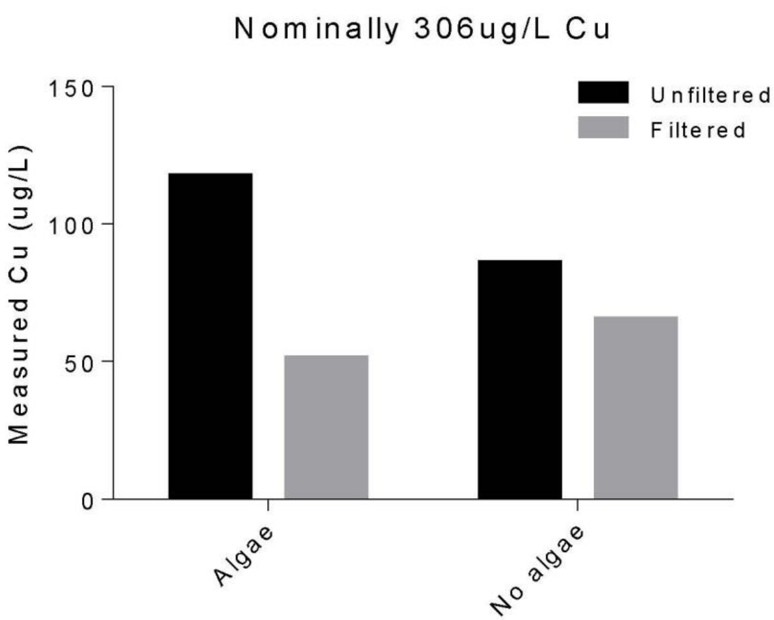
- Reduction from pre-test concentrations, especially for 175 ug/L
Possible explanation: Accumulation in algae (see slide 5)

- Higher concentration than pre test for certain measurements

Measured Copper Concentrations <u>**Post-test (Blanks)**</u> for Each Day



Filtered vs. Unfiltered Samples

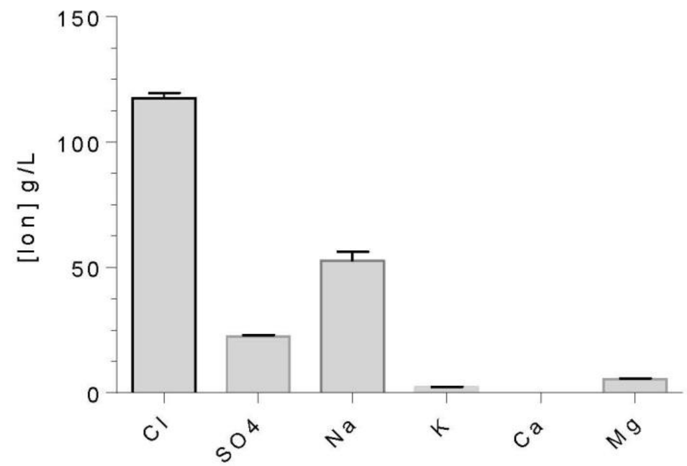


Observations:

- Filtering the sample via .2um has an effect on measured concentrations
- Copper appears to be associated with algae, either apically bound or accumulated
- Measured copper is lower than target concentration

Major Ion Concentrations

Ion	Average in lake (g/L)	Average measured (g/L)	Difference
Cl	74	116.5	42.5
SO4	9.3	22.8	13.5
Na	42	55.8	13.8
K	2.8	2.3	-0.5
Ca	0.3	0.34	0.04
Mg	4.4	5.3	0.9



A run of background metals will be important moving forward. These initial samples were with a lower grade NaCl than what we are using now

APPENDIX B

ANALYTICAL CHEMISTRY INFORMATION

KSW 2/25/18

Long list of metals - pre-definitive studies
RW#: 13060
date: Oct. 2017

	ug/L TR	ug/L Diss.
Antimony	1.0 U	1.0 U
Arsenic	0.50 U	0.50 U
Beryllium	0.020 U	0.020 U
Cadmium	0.020 U	0.020 U
Chromium	1.82	0.65
Copper	0.29	0.35
Lead	0.271	0.304
Mercury	0.20 U	0.20 U
Nickel	6.88	4.29
Selenium	1.0 U	1.0 U
Silver	0.020 U	0.020 U
Thallium	0.053	0.160
Zinc	2.00	1.07

Long list of metals for arsenic definitive study
RW#: 13090
date: Nov. 2017

	ug/L TR	ug/L Diss.
Antimony	2.0 U	2.0 U
Arsenic	0.50 U	0.50 U
Beryllium	0.020 U	0.020 U
Cadmium	0.020 U	0.020 U
Chromium	0.44	0.47
Copper	0.21	0.36
Lead	0.043	0.150
Mercury	0.20 U	0.20 U
Nickel	14.6	14.1
Selenium	1.0 U	1.1
Silver	0.020 U	0.020 U
Thallium	0.020 U	0.038
Zinc	2.74	7.63

Long list of metals for lead definitive study
RW#: 13175
date: Feb. 2018

No Food	SP1.0hN-T/D(NF) ug/L TR	ug/L Diss.
Antimony	1.0 U	1.0 U
Arsenic	0.50 U	0.50 U
Beryllium	0.020 U	0.020 U
Cadmium	0.020 U	0.020 U
Chromium	0.56	0.25
Copper	0.41	0.28
Lead	0.077	0.032
Mercury	0.20 U	0.20 U
Nickel	22.0	22.0
Selenium	1.0 U	1.0 U
Silver	0.044	0.020 U
Thallium	0.102	0.022
Zinc	9.68	6.21

Food	SP1.0hN-T/D(F) ug/L TR
Antimony	1.0 U
Arsenic	0.50 U
Beryllium	0.020 U
Cadmium	0.026
Chromium	37.2
Copper	4.93
Lead	0.898
Mercury	--- *
Nickel	23.4
Selenium	--- *
Silver	0.208
Thallium	0.465
Zinc	52.6

U = < MRL / MDL

*-insufficient volume for Hg and Se

[handwritten initials] 7/16/14

GSL - Definitive Brine Shrimp study with Copper (as Copper Chloride)
filename: analytical results_Cu_BS.xlsx

Sample ID	Nominal conc. (ug/L)	New T1 Initial Total	New D1 Initial Diss	Old T2 Day 2 Total - old	Old D2 Day 2 Diss - old	New T3 Day 2 Total - new	New D3 Day 2 Diss - new	Old T4 Day 4 Total - old	Old D4 Day 4 Diss - old	AVG Total	AVG Diss	Total % of nominal	Diss % of nominal	D1/T1	Dissolved D2 / D1
SC1	-	21 U	21 U	21 U	21 U	21 U	21 U	21 U	21 U	21.0	21.0	---	---		
SC2	288	200	118	207	130	197	172	203	166	201.8	146.5	70.1%	50.9%	59.0%	110%
SC3	412	275			120	422	204	235	278	310.7	200.7	75.4%	48.7%	0.0%	#DIV/0!
SC4	588	399	143	400	174	617	232	381	270	449.3	204.8	76.4%	34.8%	35.8%	122%
SC5	840	670	234	635	322	906	236	580	347	697.8	284.8	83.1%	33.9%	34.9%	138%
SC6	1,200	936	236	912	394		201	892	492	913.3	330.8	76.1%	27.6%	25.2%	167%
											Average	76.2%	39.2%	31.0%	#DIV/0!

Note: Calculations (e.g., % nominal) may appear slightly different due to rounding differences.
U = < MRL / MDL

suspect values removed from average determination

D3/T3	D4/D3
87.3%	96.5%
48.3%	136.3%
37.6%	116.4%
26.0%	147.0%
#DIV/0!	244.8%

NZW 2/14/18

GSL - Definitive Brine Shrimp study with Copper (as Copper Chloride)
filename: analytical results_Cu_BS.xlsx

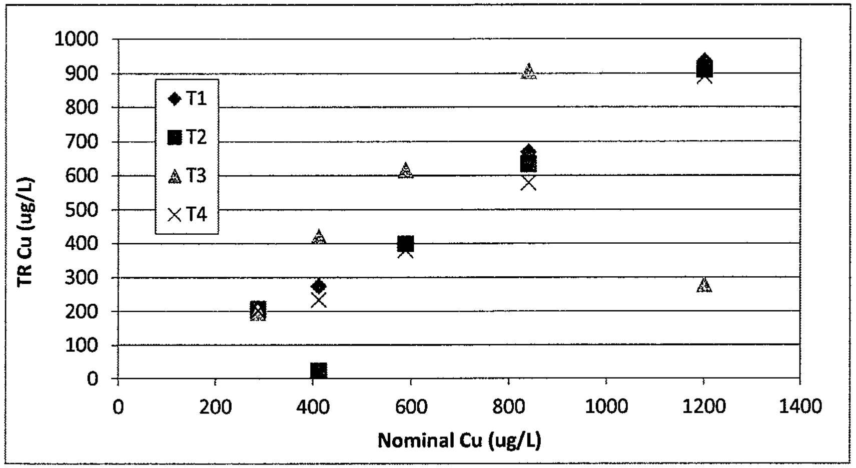
Sample ID	Nominal conc. (ug/L)	New T1 Initial Total	New D1 Initial Diss	Old T2 Day 2 Total - old	Old D2 Day 2 Diss - old	New T3 Day 2 Total - new	New D3 Day 2 Diss - new	Old T4 Day 4 Total - old	Old D4 Day 4 Diss - old	AVG Total	AVG Diss	Total % of nominal	Diss % of nominal	D1/T1	Dissolved D2 / D1
SC1	-	21 U	21 U	21 U	21 U	21 U	21 U	21 U	21 U	21.0	21.0	---	---		
SC2	288	200	118	207	130	197	172	203	166	202	147	70%	51%	59%	110%
SC3	412	275	<u>80</u>	<u>25.3</u>	120	422	204	235	278	239	171	58%	41%	29%	150%
SC4	588	399	143	400	174	617	232	381	270	449	205	76%	35%	36%	122%
SC5	840	670	234	635	322	906	236	580	347	698	285	83%	34%	35%	138%
SC6	1,200	936	236	912	394	<u>280</u>	201	892	492	755	331	63%	28%	25%	167%
											Average	70.1%	37.7%	36.8%	137.3%

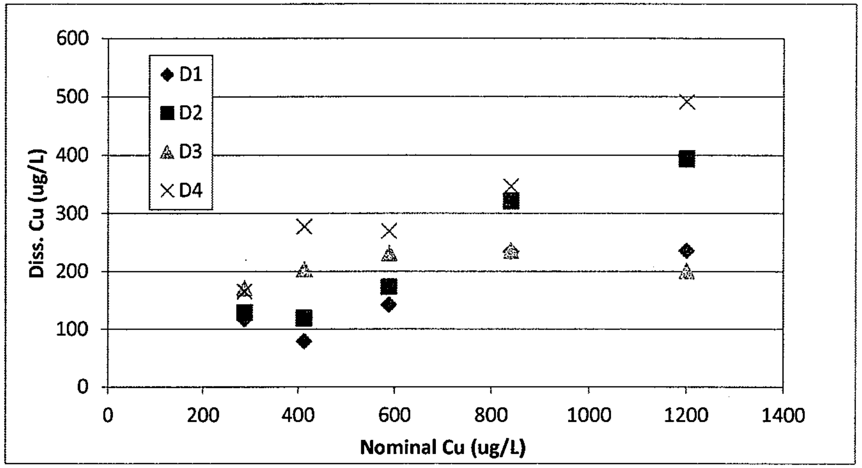
Note: Calculations (e.g., % nominal) may appear slightly different due to rounding differences.
U = < MRL / MDL

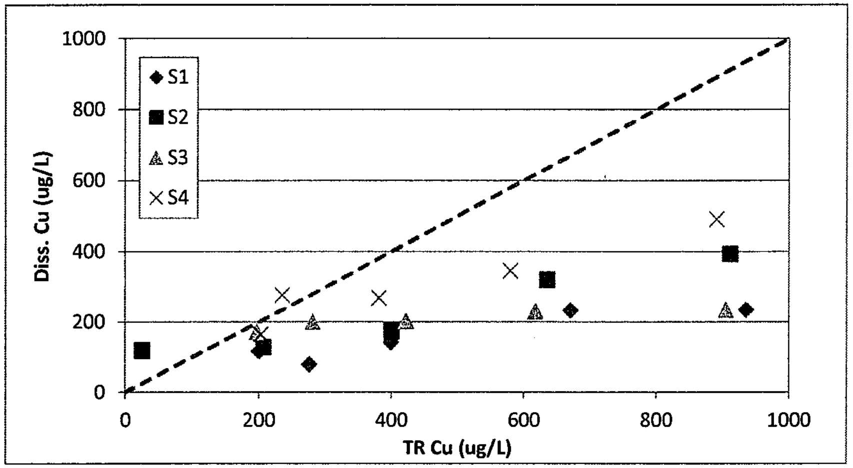
<u>underlined</u> values seem to be suspect

Analyzed for BS Cu

Nominal Cu	T1	T2	T3	T4	D1	D2	D3	D4	0	0
	Total Recoverable Cu (ug/L)				Dissolved Cu (ug/L)					
288	200	207	197	203	118	130	172	166	1000	1000
412	275	25.3	422	235	80	120	204	278		
588	399	400	617	381	143	174	232	270		
840	670	635	906	580	234	322	236	347		
1200	936	912	280	892	236	394	201	492		







APPENDIX C

BIOLOGICAL TEST DATA

QA: DAP 2/25/18

TOXICITY DATA PACKAGE COVER SHEET

Test Type: Acute

Project Number: 17001-474-~~025~~ 014 ①

Test Substance: Cu

Species: *Artemia franciscana*

Dilution Water Type: 120 ppt rGSL

Organism Lot or Batch Number: 121617

Concurrent Control Water Type: N/A

Age: 48hr (48 hr)

Supplier: TRE

Date and Time Test Began: 12/18/17 @ 1445

Date and Time Test Ended: 12/22/17 @ 1500

Protocol Number:

Investigator(s): DM/[illegible]/DAP/NZN

Background Information

Type of Test: Static - Renewal

pH control?: Yes **No**

If yes, give % CO_2: N/A

Test Temperature: 20 ± 1 °C

Env. Chmbr/**Bath #**: 1

Test Chmbrs: 384 ml cups

Photoperiod: 16 h light : 8 h dark

Light Intensity: 50 -100 ft.-c.

Test Solution Vol.: 150 ml

Number of Replicates per Treatment: 5

Length of Test: 96 h

Number of Organisms per Replicate: 20

Type of Food and Quantity per Chamber: see food sheet

Feeding Frequency: Day 0 and 2

Test Substance Characterization Parameters and Frequency:

Hardness: Initiation — Alkalinity: Initiation — NH_3: Initiation — TRC: Initiation

pH: Daily* — Conductivity: Daily* & Termination — Salinity: Initiation

Test Concentrations (Mass:Volume): 0 (Control), 288, 412, 588, 840, and 1,200 ug/L Cu (nominal)

Agency Summary Sheet(s)?: NA

Reference Toxicant Data: Test Dates: 2/5/18 to 2/28/18		LC_{50}: 70.57
Hist. 95% Control Limits: 53.97 to 87.16	Method for Determining Ref. Tox. Value: Probit	

Special Procedures and Considerations:

From 101117 culture

* Measure chemistries at test termination or when 100% mortality is observed in a treatment.

Study Director Initials: NZN Date: 12/18/17

① NZN 12/18/17 E

TEST SUBSTANCE USAGE LOG

Project Number: 17001-474-~~025~~ 014

QA: 2/25/19

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number	TAE# C04-008 (Copper Chemical)			
Test Substance Stock Solution Preparation Date	12/18/17			
Sample Type	N/A			
Date Test Substance Received	N/A			
Dilution Water Number RW# or TRE#, circle one	13121			
Concurrent Control Water RW#	NA			
Date(s) Used	12/18/17			

Preparation of Test Solutions

Test Substance Conc. (mg/L As Nominal)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)		Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)
0				see Cu spiking sheet			
288				"			
412				"			
588				"			
840				"			
1200				"			
Total	0	0	0				
Initials / Date	Am 12/18/17						
Initials / Date							
Initials / Date							
Initials / Date							
Initials / Date							
Initials / Date							
Initials / Date							
Initials / Date							

DLSN 12/18/17 E

QA Form No. 052
Revision 3
Effective 02/14

ACUTE BIOLOGICAL DATA

QA: [initials] 3/25/19

Project Number:	17001-474-~~025~~ 014 (1)
Test Species:	*Artemia franciscana*

~~mg/L~~ µg/L (1) Conc.	Test Replicate	Number of Surviving Organisms: 0 Hours	24 Hours (1)	48 Hours	72 Hours	96 Hours	Remarks
0	A	~~20~~ 22		22		22	100%
	B	20		20		20	
	C	20		20		20	
	D	20		20		20	
	E	20		20		20	
288	A	20		20		20	100%
	B	20		20		20	
	C	~~20~~ 21 (1)		21		21	
	D	20		20		20	
	E	20		20		20	
412	A	20		(2) ~~19~~ 20		20	99%
	B	20		(2) ~~20~~ 19		19	
	C	20		20		20	
	D	20		20		20	
	E	20		20		20	
588	A	~~20~~ 21 (1)		21		21	98%
	B	20		19		18	
	C	20		20		20	
	D	~~20~~ 21 (1)		21		21	
	E	20		20		20	
840	A	20		20		19	Smaller organisms 92%
	B	20		18		16	
	C	20		19		19	
	D	20		20		19	
	E	20		20		19	
1200	A	20		10		0	0%
	B	20		4		0	
	C	20		5		0	
	D	20		6		0	
	E	20		9		0	
	Date:	12/18/17		12/20/17		12/22/17	
	Time:	1484S (1)		1505		1500	
	Initials:	[initials] / [initials]		DAP/[initials]		[initials] / [initials]	

[initials] DW3N 12/18/17E DW3N 12/20/17E

ACUTE CHEMICAL DATA

QA: DEP 3/25/19

Project Number: 17001-474-~~025~~ 014 ①
Test Species: *Artemia franciscana*

① ~~(mg/L)~~ µ	NEW Day 0	NEW Day 1	NEW Day 2	NEW Day 3	OLD Day 1	OLD Day 2	OLD Day 3	OLD Day 4	Meter # (All Conc.) Day 0	Day 1	Day 2	Day 3	Day 4
Conc.: 0					Rep. A	Rep. B	Rep.	Rep. D					
pH	8.5		8.4		8.0	7.9	8.0	7.9	FM24	FM24	FM26	FM26	FM24
D.O. (mg/L)	*5.0		4.8*		*5.2	5.1*	5.1*	5.1*	LDO	LDO	LDO	LDO	LDO
Temp. (°C)	20	20①	20		21	19	18^	19	L12	L12	L13	L13	L12
Cond. (µS/cm)	□88200		87200□			~~87300~~ ①		139300□	15		15		15
Hard. (mg/L)	(176400)+		(174400)+					86,500□	Titration				
Alk. (mg/L)								(173000)+	Titration				
TRC (mg/L)													
NH3 (mg/L)													
Salinity (ppt)	118		115			117		118	1				
Conc.: 288													
pH	8.4		8.4		8.1	7.8	8.0	7.9					
D.O. (mg/L)	*4.9		4.8*		*5.2	5.1*	5.1*	5.1*					
Temp. (°C)	20	20①	20		20	19	18^	19					
Cond. (µS/cm)	□95800		87700□			~~87100~~ ①□		142000					
Hard. (mg/L)	(191600)+		(175400)+					89,300□					
Alk. (mg/L)								(178600)+					
TRC (mg/L)													
NH_3 (mg/L)									ppt=118		120		
Conc.: 412													
pH	8.4		8.4		8.1	7.8	8.0	7.9					
D.O. (mg/L)	*4.9		4.8*		*5.2	5.1*	5.1*	5.1*					
Temp. (°C)	20	20①	20		20	20	18^	19	87,900□				
Cond. (µS/cm)	□97600		88100□			~~88100~~ ①□		141400	ppt=120		120		
Conc.: 588	(185200)+		(176200)+					(175800)+					
pH	8.4		8.3		8.1	7.8	8.0	7.9				(192200)+	
D.O. (mg/L)	*4.9		4.9		*5.2	5.2*	5.1*	5.1*				(175,000)+	
Temp. (°C)	20	20①	20		20	20	18^	19	89,300□			(178,600)+	
Cond. (µS/cm)	□~~86200~~ 86100		87500□			~~87500~~ ①□		141200	ppt=120		120		
Date:	12/18/17		12/20/17		12/19/17	12/20/17	12/21/17	12/22/17					
Time:	1645		1445		1350	1435	1150	1610					
Initials:	DM		AB		DM	AB	AB	NZW					

Note: Hardness, alkalinity, TRC, and NH3 data appearing on this page have been transcribed from the wet chemistry log, QA Form No. 084

*D.O. readings read @ 70 ppt
□ 1:1 dilution w/ MQ
^ checked all ...

① NZW 12/18/17 E
② AB 12/20/17 ewp

+ 2x the diluted amt for cond.

① **ACUTE CHEMICAL DATA**

QA: ADU 3/25/19

Project Number:	17001-474-025 014
Test Species:	*Artemia franciscana*

① mg/L	NEW				OLD				Meter # (All Conc.)				
	Day 0	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 4	Day 0	Day 1	Day 2	Day 3	Day 4
Conc.:					Rep. A	Rep.	Rep.	Rep. D					
pH													
D.O. (mg/L)													
Temp. (°C)													
Cond. (µS/cm)													
Conc.:													
pH													
D.O. (mg/L)													
Temp. (°C)													
Cond. (µS/cm)													
Conc.:													
pH													
D.O. (mg/L)													
Temp. (°C)													
Cond. (µS/cm)													
Conc.: 840													
pH	8.4		8.3		8.1	7.8	8.0	8.0					
D.O. (mg/L)	*4.9		4.9*		*5.1	5.2*	5.2*	5.2*					
Temp. (°C)	20	~~20~~	20		20	20	18°	20	88,900				
Cond. (µS/cm) □	95000		86500□					142000	ppt=120		120		
Conc.: 1200	(190,000)+		(173,000)+					(177800)+					
pH	8.4		8.3		8.1	7.8	8.0	8.0					
D.O. (mg/L)	*4.9		4.8*		*5.1	5.1*	5.2*	5.2*					
Temp. (°C)	20	~~20~~	20		20	20	18°	19					
Cond. (µS/cm) □	95400		86800□					142800					
Hard. (mg/L)	(190,800)+		(173600)+					88,400					
Alk. (mg/L)								(176,800)+					
TRC (mg/L)													
NH3 (mg/L)													
Salinity (ppt)						122		122	ppt=120		120		
Date:	12/18/17		12/20/17		12/19/17	12/20/17	12/21/17	12/22/17					
Time:	1645		1445		1350	1435	1150	1610					
Initials:	DM		AB		DM	AB	AB	KBN					

Note: Hardness, alkalinity, TRC, and NH3 data appearing on this page have been transcribed from the wet chemistry log, QA Form No. 084

* D.O. readings @ 70 ppt
□ 1:1 dilution w/ MQ
+ 2x the diluted amt for conductivity
① KBN 12/18/17 E
② AB 12/20/17 E

DAILY TOXICITY TEST LOG

QA: [illegible] 2/25/19

Project Number:	17001-474-~~025~~ 014 ①
Test Species:	***Artemia franciscana***

General Comments	Measured salinity of rGSL water: 120 ppt Random Chart ✓ Thermometer #: M-15	Feeding	Initials/Date
Test Day 0	Test Solution Mixed at: 1145 Food Added at: 1145 Test Organisms Added at: 1445 new analytical collected	Fed @1145	[illegible] 12/18/17
Test Day 1	Real Time Temp.= 18 °C Range = 18-20 °C	None	[illegible] 12/19/17
Test Day 2	Real Time Temp.= 18 °C Range = 18-20 °C New + old analytical samples collected	Fed @ see spike sheet	[illegible] 12/20/17
Test Day 3	Real Time Temp.= 17 °C Range = 17-20 °C checked 1200's – 1 alive	None	[illegible] 12/21/17
Test Day 4	Real Time Temp.= 17 °C Range = 17-18 °C old analytical sxs collected placed additional heater in water bath @ ~0820 temp at 19°C at takedown	none	[illegible] 12/22/18
Test Day 5			
Test Day 6			
Test Day 7			
Test Day 8			

① [illegible] 12/18/17 E

17001-474

SIX TREATMENTS FIVE REPLICATES
RANDOM CHAMBER LOCATION "V"

	1	2	3	4	5	6
A	2E	6A	6D	4B	1C	3B
B	1D	3C	6B	5B	4E	2D
C	1B	2B	3A	4D	5D	6C
D	4A	4C	5C	5E	3E	2A
E	1A	1E	3D	5A	2C	6E

1 = Control
2 = 288
3 = 412
4 = 588
5 = 840
6 = 1,200 ug/L

Stocks

1000 mg/L Cu
100,000 mg/L Cu → 400 mg/L

QA: [initials] 2/25/19

Brine Shrimp Acute Studies

DEFINITIVE TEST

Dec 2017

Primary stock @ 365.00 mg/L Cu : 0.97942 g CuCl2.2H2O/L H2O

Volume per treatment (L) 1

Conc. at Test Initiation

0.7 dilution series

Trtmnt	Conc. ug/L	Stock (ml)	Total Vol(L)	
6	1200	3.288	1.00	3.29
5	840	2.301	1.00	2.3
4	588	1.611	1.00	1.61
3	412	1.128	1.00	1.13
2	288	0.789	1.00	0.79
1	0	0.00	1.00	
Total		9.12	6.0	

0.3% % spike of vol

Volume needed:

n = 5 reps
vol 150 ml
750

new analytical
100 ml - TR
80 ml - diss

930 ml

TOTAL VOL
12.0 L
3.43 gallons

Conc. at Test Renewal

Trtmnt	Conc. ug/L	Stock (ml)	Total Vol(L)	
6	1200	3.288	1.00	3.29
5	840	2.301	1.00	2.3
4	588	1.611	1.00	1.61
3	412	1.128	1.00	1.1
2	288	0.789	1.00	.79
1	0	0.00	1.00	
Total		9.12	6.0	

old analytical
100 ml - TR
80 ml - diss

Spiked on 12/18/17 @ 1145 [initials]

Spiked on 12/20/17 from 1125 - 1150 [initials]

Add ~ 500 ml rGSL, add Cu spike, add 73 ml algae bring to vol (exception 288 - added Cu after algae) put in water bath for 3h equilibration period

Calculation of Volume of *D. viridis* Culture Media Required for Feeding

10/11/17 culture

Method Used to Determine Chl a Concn. (ug/L) in Culture

	Enter "Y" or "N"
450 nm (2.5 cm Cuv.)?	N
450 nm (1.0 cm Cuv.)?	Y
Other (enter value):	2000

Enter Chlorophyll. Estimation Source/Concn.

Chl a Concentration in Culture (ug/L):	1506.08
Required Chl a Food Concentration (ug/L):	100
Volume of Test Water/Chamber (ml)	50
Replicates:	1
Treatments:	1

Enter Test Data Here

Intermediate Calculations

Culture Concentration (ug/ml):	1.50608
Food Concentration (ug/ml):	0.1
Total Chl a needed/Chamber (ug):	5

Final Values

Culture Water needed/Chamber (ml):	3.319877
Culture Water needed/Treatment (ml):	3.319877
Culture Water needed/Test (ml):	3.319877
Test Vol./Chamber before Food Addition (ml):	46.68012
Total Vol./Treatment before Food Addition (ml):	46.68012

NOTE: Change values only in cells outlined in GREEN; do not change cells outlined in RED; these are equations

12/16 RJN
3.3 ml of algae in 50 ml grad cylinder w/ rGSL (EWte 13121) to volume

pipet ~200 < 24h old BS nauplii in to 4 beakers 1245

Predicting Chl a from Absorbance at 450 nm

Models Update on:

Predict. Chl a (ug/L) from abs at 450 nm (2.5 cm cuvette):	Chl a = 1092.5x - 96.878
Predict. Chl a (ug/L) from abs at 450 nm (1.0 cm cuvette):	Chl a = 1983.6x - 179.98

	Absorb.	Calc. Chl a Concn. (ug/L)
Enter Absorbance (2.5 cm) here:	0	-96.878
Enter Absorbance (1.0 cm) here:	0.85	1506.08

From 10/11/17 culture

5/22/2017

	Slope	Y-intercept
	1092.5	-96.878
	1983.6	-179.98

Calculation of Volume of *D. viridis* Culture Media Required for Feeding

Method Used to Determine Chl a Concn. (ug/L) in Culture	Enter "Y" or "N"
450 nm (2.5 cm Cuv.)?	N
450 nm (1.0 cm Cuv.)?	Y
Other (enter value):	2000

Enter Chlorophyll. Estimation Source/Concn.

Chl a Concentration in Culture (ug/L):	1567.572
Required Chl a Food Concentration (ug/L):	145
Volume of Test Water/Chamber (ml)	150
Replicates:	1
Treatments:	1

Enter Test Data Here

Intermediate Calculations

Culture Concentration (ug/ml):	1.567572
Food Concentration (ug/ml):	0.145
Total Chl a needed/Chamber (ug):	21.75

Final Values

Culture Water needed/Chamber (ml):	13.87496
Culture Water needed/Treatment (ml):	13.87496
Culture Water needed/Test (ml):	13.87496
Test Vol./Chamber before Food Addition (ml):	136.125
Total Vol./Treatment before Food Addition (ml):	136.125

NOTE: Change values only in cells outlined in GREEN; do not change cells outlined in RED; these are equations

Day 0 + 2 feedings

Calculation of Volume of *D. viridis* Culture Media Required for Feeding

Method Used to Determine Chl a Concn. (ug/L) in Culture	Enter "Y" or "N"
450 nm (2.5 cm Cuv.)?	N
450 nm (1.0 cm Cuv.)?	Y
Other (enter value):	2000

Enter Chlorophyll. Estimation Source/Concn.

Chl a Concentration in Culture (ug/L):	1567.572
Required Chl a Food Concentration (ug/L):	145
Volume of Test Water/Chamber (ml)	1000
Replicates:	1
Treatments:	1

Enter Test Data Here

Intermediate Calculations

Culture Concentration (ug/ml):	1.567572
Food Concentration (ug/ml):	0.145
Total Chl a needed/Chamber (ug):	145

Final Values

Culture Water needed/Chamber (ml):	92.49976
Culture Water needed/Treatment (ml):	92.49976
Culture Water needed/Test (ml):	92.49976
Test Vol./Chamber before Food Addition (ml):	907.5002
Total Vol./Treatment before Food Addition (ml):	907.5002

NOTE: Change values only in cells outlined in GREEN; do not change cells outlined in RED; these are equations

93 x 6 = 558 ml total algae needed

$\frac{92.5}{1000} = \frac{x}{150}$

x = 13.87 ml

Predicting Chl a from Absorbance at 450 nm

Models Update on:

Predict. Chl a (ug/L) from abs at 450 nm (2.5 cm cuvette):	Chl a = 1092.5x - 96.878
Predict. Chl a (ug/L) from abs at 450 nm (1.0 cm cuvette):	Chl a = 1983.6x - 179.98

	Absorb.	Calc. Chl a Concn. (ug/L)
Enter Absorbance (2.5 cm) here:	0	-96.878
Enter Absorbance (1.0 cm) here:	0.881	1567.5716

From 10/1/17 culture

5/22/2017

	Slope	Y-intercept
	1092.5	-96.878
	1983.6	-179.98

Survival for Copper Brine Shrimp Study - 17001-474-014 Dec. 2017 NW 2/16/19

filename: Definitive study survival.xlsx

QA: NW 3/25/19

		96-h Survival				48-h Survival		
Trt	Rep	# start	# alive	% Survival		# alive	% Survival	
0	a	22	22	100%		22	100%	
	b	20	20	100%		20	100%	
	c	20	20	100%		20	100%	
	d	20	20	100%		20	100%	
	e	20	20	100%	100.0%	20	100%	100.0%
288	a	20	20	100%		20	100%	
	b	20	20	100%		20	100%	
	c	21	21	100%		21	100%	
	d	20	20	100%		20	100%	
	e	20	20	100%	100.0%	20	100%	100.0%
412	a	20	20	100%		20	100%	
	b	20	19	95%		19	95%	
	c	20	20	100%		20	100%	
	d	20	20	100%		20	100%	
	e	20	20	100%	99.0%	20	100%	99.0%
588	a	21	21	100%		21	100%	
	b	20	18	90%		19	95%	
	c	20	20	100%		20	100%	
	d	21	21	100%		21	100%	
	e	20	20	100%	98.0%	20	100%	99.0%
840	a	20	19	95%		20	100%	
	b	20	16	80%		18	90%	
	c	20	19	95%		19	95%	
	d	20	19	95%		20	100%	
	e	20	19	95%	92.0%	20	100%	97.0%
1200	a	20	0	0%		10	50%	
	b	20	0	0%		4	20%	
	c	20	0	0%		5	25%	
	d	20	0	0%		6	30%	
	e	20	0	0%	0.0%	9	45%	34.0%

CETIS Analytical Report

Artemia franciscana ②

TR Cu

Report Date: 16 Feb-19 08:08 (p 1 of 2)
Test Code: 474-014 | 00-6304-4209

~~Mysidopsis~~ 96-h Acute Survival Test — TRE Environmental Strategies

Analysis ID:	05-8097-0352	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	16 Feb-19 8:07	**Analysis:**	Untrimmed Spearman-Kärber	**Official Results:**	Yes
Batch ID:	14-6516-2496	**Test Type:**	Survival (96h)	**Analyst:**	Lab Tech
Start Date:	18 Dec-17 14:45	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	~~Seawater~~ GSL
Ending Date:	22 Dec-17 15:00	**Species:**	~~Mysidopsis bahia~~ Artemia franciscana ②	**Brine:**	Crystal Sea ②
Duration:	4d 0h	**Source:**	In-House Culture	**Age:**	3d
Sample ID:	19-6046-9487	**Code:**	74DA63EF	**Client:**	GSL-ND
Sample Date:	18 Dec-17 10:00	**Material:**	Ambient Sample	**Project:**	Special Studies
Receive Date:	18 Dec-17 11:30	**Source:**			
Sample Age:	5h	**Station:**			

Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0	0.00%	2.885	0.005126	766.8	748.9	785.1

96h Survival Rate Summary — Calculated Variate(A/B)

① C-~~m~~µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
21	Dilution Water	5	1	1	1	0	0	0.0%	0.0%	102	102
201.8		5	1	1	1	0	0	0.0%	0.0%	101	101
310.7		5	0.99	0.95	1	0.01	0.02236	2.26%	1.0%	99	100
449.3		5	0.98	0.9	1	0.02	0.04472	4.56%	2.0%	100	102
697.8		5	0.92	0.8	0.95	0.03	0.06708	7.29%	8.0%	92	100
913.3		5	0	0	0	0	0		100.0%	0	100

96h Survival Rate Detail

① C-~~m~~µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
21	Dilution Water	1	1	1	1	1
201.8		1	1	1	1	1
310.7		1	0.95	1	1	1
449.3		1	0.9	1	1	1
697.8		0.95	0.8	0.95	0.95	0.95
913.3		0	0	0	0	0

PMSD was not appropriate

96h Survival Rate Binomials

① C-~~m~~µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
21	Dilution Water	22/22	20/20	20/20	20/20	20/20
201.8		20/20	20/20	21/21	20/20	20/20
310.7		20/20	19/20	20/20	20/20	20/20
449.3		21/21	18/20	20/20	21/21	20/20
697.8		19/20	16/20	19/20	19/20	19/20
913.3		0/20	0/20	0/20	0/20	0/20

① ABW 2/16/19 E ② ASP 2/25/19 E

CETIS Analytical Report

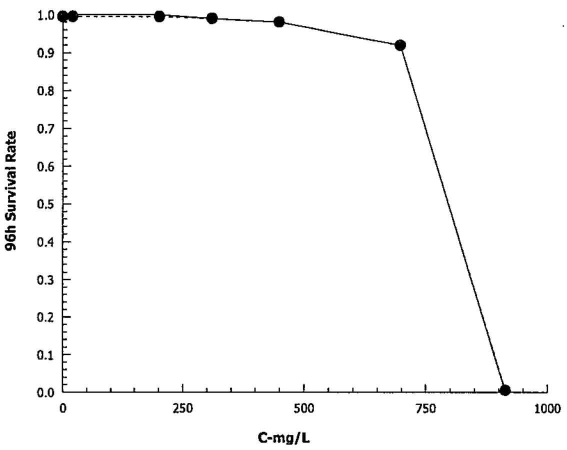
Artemia franciscana

Report Date:	16 Feb-19 08:08 (p 2 of 2)
Test Code:	474-014 | 00-6304-4209

~~Mysidopsis~~ (1) 96-h Acute Survival Test — TRE Environmental Strategies

Analysis ID:	05-8097-0352	Endpoint:	96h Survival Rate	CETIS Version:	CETISv1.8.7
Analyzed:	16 Feb-19 8:07	Analysis:	Untrimmed Spearman-Kärber	Official Results:	Yes

Graphics



(1) DAP 2/25/19 E

CETIS Analytical Report

Artemia franciscana ③

TR Cey

Report Date: 16 Feb-19 08:08 (p 1 of 2)
Test Code: 474-014 | 00-6304-4209

~~Mysidopsis~~ 96-h Acute Survival Test — TRE Environmental Strategies

Analysis ID:	04-3737-7521	Endpoint:	① 48h Survival Rate	CETIS Version:	CETISv1.8.7
Analyzed:	16 Feb-19 8:08	Analysis:	Trimmed Spearman-Kärber	Official Results:	Yes
Batch ID:	14-6516-2496	Test Type:	Survival (96h)	Analyst:	Lab Tech
Start Date:	18 Dec-17 14:45	Protocol:	EPA/821/R-02-012 (2002)	Diluent:	~~Seawater~~ rGSL ②
Ending Date:	22 Dec-17 15:00	Species:	~~Mysidopsis bahia~~ Artemia franciscana ②	Brine:	Crystal Sea
Duration:	4d 0h	Source:	In-House Culture	Age:	3d
Sample ID:	19-6046-9487	Code:	74DA63EF	Client:	GSL-ND
Sample Date:	18 Dec-17 10:00	Material:	Ambient Sample	Project:	Special Studies
Receive Date:	18 Dec-17 11:30	Source:			
Sample Age:	5h	Station:			

Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0	34.00%	2.931	0.006606	853	827.4	879.3

48h Survival Rate Summary

① C-~~mg/L~~ Mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
			Calculated Variate(A/B)								
21	Dilution Water	5	1	1	1	0	0	0.0%	0.0%	102	102
201.8		5	1	1	1	0	0	0.0%	0.0%	101	101
310.7		5	0.99	0.95	1	0.01	0.02236	2.26%	1.0%	99	100
449.3		5	0.99	0.95	1	0.01	0.02236	2.26%	1.0%	101	102
697.8		5	0.97	0.9	1	0.02	0.04472	4.61%	3.0%	97	100
913.3		5	0.34	0.2	0.5	0.05788	0.1294	38.1%	66.0%	34	100

48h Survival Rate Detail

① C-~~mg/L~~ M	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
21	Dilution Water	1	1	1	1	1
201.8		1	1	1	1	1
310.7		1	0.95	1	1	1
449.3		1	0.95	1	1	1
697.8		1	0.9	0.95	1	1
913.3		0.5	0.2	0.25	0.3	0.45

Probit was not appropriate

48h Survival Rate Binomials

① C-~~mg/L~~ M	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
21	Dilution Water	22/22	20/20	20/20	20/20	20/20
201.8		20/20	20/20	21/21	20/20	20/20
310.7		20/20	19/20	20/20	20/20	20/20
449.3		21/21	19/20	20/20	21/21	20/20
697.8		20/20	18/20	19/20	20/20	20/20
913.3		10/20	4/20	5/20	6/20	9/20

①DRW 2/16/19 E ②DRP 2/25/19 E

CETIS Analytical Report

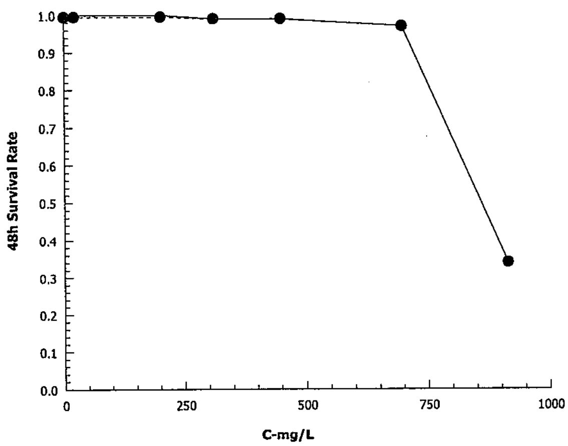
Artemia franciscana

Report Date:	16 Feb-19 08:08 (p 2 of 2)
Test Code:	474-014 | 00-6304-4209

~~Mysidopsis~~ (1) 96-h Acute Survival Test — TRE Environmental Strategies

Analysis ID:	04-3737-7521	Endpoint:	48h Survival Rate	CETIS Version:	CETISv1.8.7
Analyzed:	16 Feb-19 8:08	Analysis:	Trimmed Spearman-Kärber	Official Results:	Yes

Graphics



(1) ADP 2/25/19 E

CETIS Analytical Report

Artemia franciscana ②   Diss Cu

Report Date: 16 Feb-19 08:13 (p 1 of 2)
Test Code: 474-014 | 00-6304-4209

~~Mysidopsis~~ 96-h Acute Survival Test

TRE Environmental Strategies

Analysis ID:	00-4094-5388	Endpoint:	96h Survival Rate	CETIS Version:	CETISv1.8.7
Analyzed:	16 Feb-19 8:13	Analysis:	Untrimmed Spearman-Kärber	Official Results:	Yes
Batch ID:	14-6516-2496	Test Type:	Survival (96h)	Analyst:	Lab Tech
Start Date:	18 Dec-17 14:45	Protocol:	EPA/821/R-02-012 (2002)	Diluent:	~~Seawater~~ GSL ②
Ending Date:	22 Dec-17 15:00	Species:	~~Mysidopsis bahia~~ Artemia franciscana	Brine:	Crystal Sea
Duration:	4d 0h	Source:	In-House Culture ②	Age:	3d
Sample ID:	19-6046-9487	Code:	74DA63EF	Client:	GSL-ND
Sample Date:	18 Dec-17 10:00	Material:	Ambient Sample	Project:	Special Studies
Receive Date:	18 Dec-17 11:30	Source:			
Sample Age:	5h	Station:			

Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0	0.00%	2.477	0.003097	299.6	295.3	303.9

96h Survival Rate Summary

① C-µg/L (~~mg~~)	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
			Calculated Variate(A/B)								
21	Dilution Water	5	1	1	1	0	0	0.0%	0.0%	102	102
146.5		5	1	1	1	0	0	0.0%	0.0%	101	101
200.7		5	0.99	0.95	1	0.01	0.02236	2.26%	1.0%	99	100
204.8		5	0.98	0.9	1	0.02	0.04472	4.56%	2.0%	100	102
284.8		5	0.92	0.8	0.95	0.03	0.06708	7.29%	8.0%	92	100
330.8		5	0	0	0	0	0		100.0%	0	100

96h Survival Rate Detail

① C-µg/L (~~mg~~)	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
21	Dilution Water	1	1	1	1	1
146.5		1	1	1	1	1
200.7		1	0.95	1	1	1
204.8		1	0.9	1	1	1
284.8		0.95	0.8	0.95	0.95	0.95
330.8		0	0	0	0	0

probit not appropriate

96h Survival Rate Binomials

① C-µg/L (~~mg~~)	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
21	Dilution Water	22/22	20/20	20/20	20/20	20/20
146.5		20/20	20/20	21/21	20/20	20/20
200.7		20/20	19/20	20/20	20/20	20/20
204.8		21/21	18/20	20/20	21/21	20/20
284.8		19/20	16/20	19/20	19/20	19/20
330.8		0/20	0/20	0/20	0/20	0/20

① MZW 2/16/19 E   ② MAA 3/25/19 E

CETIS Analytical Report

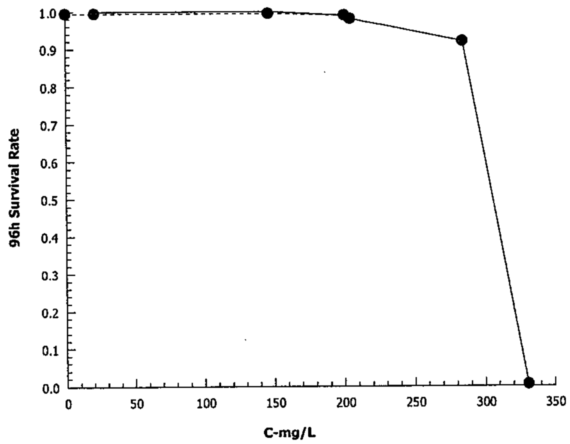
Artemia franciscana

Report Date:	16 Feb-19 08:13 (p 2 of 2)
Test Code:	474-014 | 00-6304-4209

~~Mysidopsis~~ (1) 96-h Acute Survival Test — **TRE Environmental Strategies**

Analysis ID:	00-4094-5388	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	16 Feb-19 8:13	**Analysis:**	Untrimmed Spearman-Kärber	**Official Results:**	Yes

Graphics



(1) ASO 2/25/19