Date: Thu, Apr 10, 2014 at 4:15 PM
Subject: RE: Workplan for Toxicological Testing of Brine Shrimp and Brine Flies from Great Salt Lake

Doug,

Thank you for inviting us to comment on the *Workplan for Great Salt Lake Toxicity Tests*. Dan Wall and I have combined our comments below. Overall, we agree that this workplan is a great start to understanding the effects of metals introduced into the waters of the Gilbert Bay area on aquatic organisms and wildlife.

Specific comments on the workplan:

Page 1, paragraph 1. Please confirm that there are threatened and endangered species on the GSL.
Page 2, paragraph 5. Will efforts be made to simplify the hatching and rearing process (acclimation to higher salinity)? What are the implications of hatching at a higher salinity? It would be nice if these methods were established for broader application and a simplification of this portion of it would be useful.

General comments/considerations for this or future tox testing:

Effects of mixtures – testing one chemical at a time does not address additive or synergistic toxicity that is possible when organisms are exposed to multiple metals.

The choice of parameters for acute testing (salinity, temperature, pH) are based on averages of conditions in GSL for a 20 year period during the time that brine shrimp are present (April-October). This is a good starting point, however toxicity of a particular metal dose may be affected when changes in these parameters and/or hardness occur resulting in increased or decreased effects from metal exposure. Consider a multifactorial design to address possible changes in metal toxicity under different water parameter scenarios.

According to the Gary Belovsky, et al paper "The Great Salt Lake Ecosystem (Utah, USA): long term data and a structural equation approach", the phytoplankton-based food web (that includes brine shrimp) is limited by phytoplankton production. Will there be considerations of phytoplankton toxicity from metal exposure in the derivation of the numeric water quality criteria for the metals of interest?

Has the most sensitive stage in the lifecycle for each of the test species been properly identified for acute testing of each metal?

Again, thanks for the opportunity to review and comment on the workplan.

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EPA comments on the proposed "Work Plan for Great Salt Lake Toxicity Tests"
4/15/14

General Comments

- It is not clear who will be doing what where. Are both labs conducting the range finding and acute toxicity tests for both species? If NCSU will be doing some of the brine fly tests, how will the flies be delivered to NCSU? It would be useful for the work plan to define the roles and responsibilities.
- What are the anticipated products/report(s) and how data will be provided to UDEQ. We expect that these reports will be necessary to support future water quality criteria proposals.
- Are there any additional scenarios that should be recognized in the work plan that might alter the expected products? For example, if the results of the range finding test suggest that one species is consistently more sensitive than the other, will acute testing continue with both species?

Source animals and rearing conditions

- What are your rearing criteria for the test organisms? How will you ensure that tests will only be initiated with test organisms in good health?

Test Solutions

- We recommend making a super stock for each pollutant to reduce potential exposure variability.

Range Finding Tests

- For the mercury range finding test, UDEQ should consider a dilution series of 0, 0.1, 1, 10, 100, and 1,000 µg/L given the very low concentrations that are typically observed in surface waters and the maximum concentration is still much greater than an ecologically relevant concentration that would be considered protective of aquatic birds.

Acute Toxicity Tests

- Additional details are needed on how the daily water renewals will be conducted. Will you be moving the test organisms or removing the water?
- The brine fly test procedures state that pH, DO and conductivity will be checked daily. We expect that these parameters will also be measured in the brine fly tests. We suggest you consider adding ammonia to your routine water chemistries since pH drift in static-renewal tests can lead to artificial ammonia toxicity. Alternatively, if the expected ammonia concentrations can be determined from the historic rearing data, it would be useful to discuss these data to determine if ammonia analyses would be needed to interpret test results.

- We recommend that you expand the test protocol table to include additional information that is found in the text. See an example of the information needed below. Shaded cells represent areas of uncertainty.

	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	50 ml	250 ml
Test Solution Volume	30 ml	150 ml
Renewal of Test Solution	daily	daily
Age of Test Organism	48, 96 or 120 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	more details needed	more details needed
Dilution Water	DI water (15L) + Instant Ocean (~800 ml) and Morton Solar Salt Water Softening Crystals (~1200 ml)	DI water (15L) + Instant Ocean (~800 ml) and Morton Solar Salt Water Softening Crystals (~1200 ml)
Endpoint	mortality (LC50) and immobility (EC50)	mortality (LC50) and immobility (EC50)
Test Acceptability	≥ 90% control survivorship	≥ 90% control survivorship

- Please provide additional information to support the proposed acute test feeding/starvation regime. The work plan says that the test organisms will be starved for 24 hours prior to the test and will not be fed at any point during the 96hr exposure. It is our understanding that control survival may be compromised if test organisms are not feed for 120 hrs (24hr starvation period + 96 hr exposure). It is not typical to starve invertebrate test organisms prior to initiating the acute toxicity tests. Furthermore, it would be acceptable to feed the test organisms for the last 2 hours of the 48 hour exposure, prior to test solution renewal, if starvation compromises control survival for a 96 hr test. It is possible that starvation experiments have been conducted to support the proposed approach. If these experiments have been conducted, please discuss the results of those experiments in the background information.
- What will be the age of the brine shrimp nauplii when initiating tests?

Analytical Chemistry

- We are concerned that the work plan proposes an insufficient number of final water chemistry samples. To be able to calculate defensible LC50s, it is critical to have an understanding of exposure throughout the test since changes in the dissolved fraction can occur over 24 hours due to organism excretion, sorption to the test organism and/or sorption to the test chambers. It is our understanding that the results of these toxicity tests will be used for water quality criteria recommendations for the hypersaline portions of the lake. It is possible that the proposed criteria will be derived from the sensitivity of only two species, compared to the 8 diverse families that are typically used for criteria development, which is why we are encouraging an analytical chemistry approach that will result in the highest quality data possible.
- Here we provide a visual of the analytical chemistry associated with a hypothetical side-by-side 96-hr toxicity test using two species, 5 exposure concentrations plus a control, assuming a 3 concentration overlap, and pooled replicates.

Time of test	**0**	**24**		**48**		**72**		**96**
Exposure solutions	Initial	Initial	Final	Initial	Final	Initial	Final	Final
Control –both	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 Fly	1	1	1	1	1	1	1	1
Brine Fly	1	1	1	1	1	1	1	1
Duplicate	1	1	1	1	1	1	1	1

Total	88

Stability of exposure solutions can be examined after conducting the first round of tests with each parameter to determine the necessary water chemistry analyses for future toxicity tests. If the lab can validate that the final 96 hr average concentrations are within 95% of the nominal concentrations for each pollutant, it is possible that the total number of chemical analyses may be reduced.

- Please provide references for all methods cited in the work plan including analytical chemistry and modified toxicity test methods. Consider reviewing and a citing the EPA acute toxicity test methods (EPA-821-R-02-012).
- Are you confident that the analytical methods will have the precision (at both low and high concentrations) necessary to calculate good LC50s?

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Chris Kaiser
Acting Manager - Environment

10 April 2014

Sent via email and US Mail

Jodi Gardberg
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Dear Ms. Gardberg,

Thank you for the opportunity to comment on the Workplan for Toxicological Testing of Brine Shrimp and Brine Flies from Great Salt Lake. Kennecott supports the State in understanding the ecology of Great Salt Lake.

We have the following concerns:

1. Kennecott is concerned the methods do not provide sufficient detail to determine whether or not the ionic composition of the artificial GSL water to be used will actually match up with real GSL water. Table 1 below is from the Brix et al publication where they examined toxicity of Cu, Cd and Zn on hatching success of brine shrimp. Instant ocean type water resulted in lower toxicity values (less toxic) than GSL water, especially for copper.
2. Kennecott cannot find any mention of adding or measuring dissolved organic carbon. We feel it if very important that DEQ mimic the GSL – especially for copper. Kennecott measures of dissolved organic carbon indicated a value of 0.6 ppm.
3. It will be important that the artificial GSL water is monitored for As, Cd, Cu, and Zn before the tests are performed.
4. Has the Buchwalter Lab ever performed a chronic toxicity test with Brine shrimp? We feel there needs to be a demonstration that suitable survival and reproduction can be obtained under test conditions (without a toxicant) before the first test with one of the metals is performed. A demonstration that the control organisms are healthy is a key prerequisite.
5. We did not see any evidence of cited literature for any chronic toxicity test performed with brine flies. We are concerned there is no precedent for handling and growing healthy brine flies for both the test conditions and control.
6. We appreciate you citing papers Kennecott has produced/published (attached) but left out a lot of the details on how to manage the organisms to achieve successful reproduction. Also we observed that the second generation was less sensitive that the parental generation (arsenic). This was not mentioned in the draft work plan.

Rio Tinto

Please let us know if you have any questions or concerns.

Regards,

Chris Kaiser
Principal Advisor
Environmental Manager (Acting)

Table 1. Ionic Composition of Dilution Waters. All units in (mM) except pH which is in standard units.

Parameter	Bagshaw et al. Media	Synthetic Seawater	Great Salt Lake Water
Ca^{2+}	1.4	10.3	1.6
Mg^{2+}	48.1	49.2	40.9
Na^{+}	423	485	492
K^{+}	7.2	8.3	15.6
Cl^{-}	478	410	413
SO_4^{2-}	25.5	21.7	19.1
CO_3^{2-}	0.023	0.214	0.089
HCO_3^{-}	0.470	2.171	0.905
pH	7.8	8.1	8.1
DOC	<0.04	<0.04	0.63

Salinities Morton's Water Salt Instant Ocean Hydrometer
(1.5%) 15 ppt 85 ml/15 L 55 ml/15 L 1.009
(2.5%) 25ppt 190 ml/15 L 125 ml/ 15 L 1.016
(3.0%) 30 ppt 270 ml/15 L 170 ml/15 L 1.02
(4.5%) 45ppt 400 ml/15 L 250 ml/15 L 1.03
(6.0%) 60 ppt 540 ml/15 L 350 ml/15 L 1.04
(9.0%) 90 ppt 810 ml/15 L 510 ml/15 L 1.06
(12.0%) 120 ppt 1200 ml/15 L 800 ml/15 L 1.082
(15.0%) 150 ppt 1500 ml/15 L 980 ml/15 L 1.104

Brix et al 2004 GSL Se site Specific.pdf

Arsenic chronic brine shrimp publication.pdf

Comments of

Great Salt Lake Brine Shrimp Cooperative, Inc.

On

" DRAFT WORKPLAN FOR TOXICOLOGICAL TESTING OF BRINE SHRIMP AND BRINE FLIES FROM GREAT SALT LAKE'S GILBERT BAY"

April 10, 2014

Submitted by Thomas Bosteels

To Utah Division of Water Quality, 195 North 1950 West, Salt Lake City, Utah 84114-4870

Attention Jodi Gardberg

Summary Statement:

There is no credible scientific information indicating observed or potential adverse impacts of ammonia on Great Salt Lake (GSL) *Artemia*, nor is there any other compelling reason to study ammonia toxicity on *Artemia* from GSL.

Comments:

My greatest concern regarding the proposed study, pertains to the assertion that ammonia be added to the toxicity testing protocol.

This proposed addition is surprising, given the precedent already established through previous meetings and discussion groups in which it was recognized that there are other, far more critical pollutants of concern than ammonia in GSL. Detailed data was provided to the Division of Water Quality (DWQ) regarding observed ammonia levels in the mixolimnion (upper mixed layers--UML) of Gilbert Bay. Values observed during the 2012-2013 sampling season by the research team of Great Salt Lake Brine Shrimp Cooperative, Inc. (GSLBSC) are shown in Table 1.0 below.

Table 1.0. Ammonia levels in GSL mixolimnion composite samples taken from shallow (1m), Medium (1-3M), and Deep Sites (1,3,5M). The maximum concentration observed was 0.348 mg/L for medium depth composite sample in June of 2013.

	Values in mg/L Ammonia In the GSL South Arm Oxic Stratum			
	composite sample (1m)	composite sample (1 and 3 m)	composite sample (1,3 and 5m)	lake average
	shallow sites	medium depth sites	deep sites	all
	(3 shallow sites)	(3 medium depth sites)	(3 deep sites)	sites
Jun-12	0.321	0.228	0.256	0.268
Jul-12	0.170	0.154	0.189	0.171
Aug-12	0.103	0.105	0.098	0.102
Sep-12	0.019	0.039	0.049	0.036
Oct-12	0.049	0.051	0.038	0.046
Nov-12	0.041	0.041	0.058	0.046
Dec-12	0.200	0.149	0.195	0.181
Jan-13	0.081	0.088	questionable value	0.085
Feb-13	0.017	0.011	0.025	0.018
Mar-13	0.042	0.031	0.033	0.035
Apr-13	0.038	0.029	missing value	0.033
May-13	0.138	0.158	0.168	0.155
Jun-13	0.144	0.178	0.225	0.182
Jul-13	0.036	0.044	0.093	0.057
Aug-13	0.052	0.032	0.046	0.043
Sep-13	0.011	0.032	0.021	0.021
Oct-13	0.048	0.072	0.082	0.067

GSLBSC has no concerns regarding the lethal toxicity of ammonia to brine shrimp at these concentrations. Although DWQ reported slightly higher concentrations as compared to GSLBSC studies (and the reason for those difference remain un-answered), the concentrations reported by both DWQ and GSLBSC are below the chronic benchmarks for fresh water and marine environments (as reported by DWQ in "Review draft prioritization of Brine Shrimp and Brine Fly Bioassay Test Pollutants for Gilbert Bay, Great Salt Lake, Utah July 22, 2013"). Furthermore, the concentrations are several orders of magnitude below toxicity levels for *Artemia* reported in the literature, as further demonstrated below.

Whereas, there are occasional temporal spikes in the concentration of ammonia, these transient increases remain below chronic benchmarks and are several orders of magnitude below toxic levels reported in the literature. In addition, they coincide with significant population fluctuations of *Artemia*, pointing to a conclusion that they may have more to do with the fecal production by *Artemia* or decomposition of *Artemia* than any influence from tributary or other external sources. Moffett and Fisher (2011) have demonstrated that *Artemia* reared in a closed system can contribute 46% of the total ammonia in the culture system. Hernandorena and Kaushik (1981) found that *Artemia* meta-nauplii and adults have an approximate per capita production of ammonia of 26 ug/day. These and other studies (Post, 1977) suggest that ammonia concentrations in GSL may be a function of input sources, internal cycling and also of production by the *Artemia*. Although there are some small temporal increases in ammonia in the mixolimnion of Gilbert Bay, there is no evidence, whatsoever, of any adverse impact on *Artemia* by ammonia in the GSL ecosystem.

Finally, DWQ previously suggested that somewhat higher levels of ammonia detected in the deep brine stratum should also be considered and weighed into the decision for priority pollutants because of potential mixing events between both the deep brine layer and the oxic stratum suggested ("Review draft prioritization of Brine Shrimp and Brine Fly Bioassay Test Pollutants for Gilbert Bay, Great Salt Lake, Utah July 22, 2013"). GSLBC previously commented that since brine shrimp and brine flies are not typically exposed to the deep brine stratum, ammonia concentrations in the deep brine stratum need not be considered. Clearly, the concentration of pollutants in the oxic stratum already accounts for any

contribution of the pollutant from the deep brine stratum to the oxic stratum and, consequently, to the exposure of the brine shrimp and brine flies to that source of pollutant. Finally, even if there were direct exposure of brine shrimp to the deep brine stratum, the ammonia results reported for the deep brine stratum remain several orders of magnitude below toxic values reported in the literature.

Investigations of LC50 values for ammonia and ammonium using either nauplii or adult *Artemia* defined LC50 values that are on the order of a thousand times higher than the highest values we observed. Ostrensky et al., (1992) found that the LC50 values for nauplii, when exposed to ammonium, were: 650 mg/L (24 hour) and 399 mg/L (96 hour). For the adults the values were higher: 1290 mg/L (24 hour) and 600 mg/L (96 hour). Similarly Chen, Chen and Liau (1989) observed a Total Ammonia-Nitrogen (TAN) LC50 of 839 mg/L (24 hour) for nauplii *Artemia*. Landau and Sanchez (1991) found that there were protective factors at a pH of 8.5 (close to GSL pH) compared to those at a pH of 6.5 when exposing *Artemia* to ammonium. In their study Landau and Sanchez (1991) defined Ammonia-N and Ammonium-N LD50 values of: 730 mg/L at 6.5 pH and 990 mg/L at 8.5 pH. The amount of unionized ammonia at each pH was: 1.05 mg/L at pH 6.5 and 125.04 mg/L at pH of 8.5. These authors further opine that at higher salinities the adverse effects of ammonium would be diminished further because the high concentration of Na^+ in the surrounding water would reduce the competitive uptake of NH_4^+ versus Na^+. In a study of Leachate water from landfills, Svensson et al. (2005) observed an immobility percentage of only 20% among exposed *Artemia* nauplii (instar 2-3 stages) to 800 mg/L ammonium. The calculated concentration of ammonia in the solution was around 8-10 mg/L, but was not the primary nutrient of concern in the mixture.

These toxicity studies collectively reveal a toxic threshold far in excess of ammonia concentrations in the mixolimnion of Gilbert Bay and, therefore, there is no compelling reason to be concerned that GSL *Artemia* are currently being, or in some foreseeable time in the future would be, adversely impacted by ammonia in Gilbert Bay. Given the levels of ammonia measured In Great Salt Lake by GSLBSC Researchers, there is simply no evidence of an existing or looming potential adverse impact.

The nutrient dynamics within the open waters of the GSL, and in particular Gilbert Bay, exhibit a temporal and spatial variability. Studies conducted by Stephens and Gillespie (1976) and Wurstbaugh (1988) have indicated that Gilbert Bay is nitrogen limited. Other investigations by Wurtsbaugh, Naftz and Brandt (2008) have shown that, whereas, the input sources such as Bear River and Farmington Bay are important contributors of nutrients to support primary productivity in Gilbert Bay, these nitrogen inputs are short lived as there is rapid uptake by the phytoplankton and benthic algae. The available nitrogen is systematically incorporated into biological molecules as the nitrogen ascends through the trophic structure of the GSL. Although these authors suggest that nitrogen loading into Gilbert Bay, (i.e., >2g N/m^2) is sufficient to cause eutrophic conditions, such conditions do not occur in any meaningful manner when *Artemia* are present to graze on the algal blooms (Wurtsbaugh, Naftz and Brandt, 2009). Wurtsbaugh (2008) further states that under the current loading conditions of nitrogen into Gilbert Bay the brine shrimp population will derive a benefit rather than harm. Overall, the impression of nitrogen input into the GSL is that it is one of the limiting factors controlling primary production and, therefore, the food base for the *Artemia* population.

Toxicity studies should focus on those persistent toxins that have been demonstrated to be present in GSL at levels of concern for the health and integrity of the ecosystem. There is simply no evidence to suggest that ammonia in GSL is causing harm to *Artemia* or that it will cause harm in the near future. Therefore, ammonia should be excluded from the pending toxicological testing of brine shrimp from Great Salt Lakes Gilbert Bay.

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