

Comments and Responses for the draft Work Plan For Great Salt Lake Toxicity Tests, April 2014  
 May 3, 2016

No.	Topic	Comment	Response
1	General	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.	For this project, the UND has primary responsibility for developing the culturing methods for the brine flies and brine shrimp. The NCSU laboratory has primary responsibility for conducting the toxicity tests for both species. Using the methods developed by UND, NCSU will culture brine shrimp from cysts and brine fly larvae (2 <sup>nd</sup> instar) that were shipped from UND to NCSU. Text was added to the Work Plan that identifies the primary responsibilities for the two laboratories.
2	General	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.	The reports are the Work Plan and a results report. The Work Plan has been revised in several places to add sufficient details so that the experiments can be reviewed and duplicated. After implementing the Work Plan, the results of the acute testing (LC50s) for arsenic, copper, and lead will be reported. All raw analytical and toxicity test data will also be submitted to UDEQ.
3	General	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?	As expected, there have been many methodological challenges encountered in developing these new methods and additional unanticipated issues are possible. The Work Plan was revised to note that if the range finding results indicate that acute toxicity exceeds the solubility, acute testing will halted and the pollutant will proceed to chronic testing. With regards to species sensitivities, both species will be tested for this round. One of the goals of this study for brine flies is to determine both the absolute and relative sensitivity relative to brine shrimp because little data are available for brine flies.
4	Analytical	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	The methodology was revised as suggested to collect and measure initial exposure and final exposure filtered (0.45 µm) water chemistry every day. Exposure concentrations will be based on the geometric mean of pre- and post-exposure concentrations. These procedures may be modified if the data support that exposure concentrations are stable or if a different sampling procedure such as unfiltered more accurately represent the organism responses. In addition to the filtered concentrations (0.45 µm), total metals concentrations will

		<p>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.</p>	<p>initially be measured in at least one sample from each dosing concentration.</p>
5	Analytical	<p>Here we provide a visual [See Table in Complete Comments from EPA] 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. 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.</p>	<p>As discussed in response to comment 4, the analytical measurements were increased.</p>
6	Methods	<p>Additional details are needed on how the daily water renewals will be conducted. Will you be moving the test organisms or removing the water?</p>	<p>The Work Plan was revised to clarify that the water will be replaced to reduce the potential for injuring the test organisms. The analytical monitoring for pH, DO and conductivity will be the same for brine flies and brine shrimp. In developing the test conditions at the Belovsky</p>

		<p>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.</p>	<p>laboratory, pH did not drift with the 24-hr water changes. Thank you for the information regarding ammonia toxicity. We agree that measuring the ammonia concentrations during the testing could provide useful data. However, the Work Plan does not include ammonia monitoring based on the following reasons:  The potential sources of ammonia in conjunction with the daily water changes suggest toxic increases in ammonia are unlikely.  The pH has remained stable during the acute test durations.  Based on unpublished testing conducted in Dr. Belovsky's laboratory (<a href="http://science.nd.edu/undergraduate/minors/sustainability/capstone-projects/2015/davila/">http://science.nd.edu/undergraduate/minors/sustainability/capstone-projects/2015/davila/</a>), brine shrimp do not appear to be particularly sensitive to ammonia.</p>
7	General	<p>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).</p>	<p>Agree. References, when available were added to the Work Plan including the suggested U.S. EPA method.</p>
8	General	<p>Are you confident that the analytical methods will have the precision (at both low and high concentrations) necessary to calculate good LC50s?</p>	<p>The Work Plan was revised to identify the William Johnson's analytical laboratory at the University of Utah for the analytical work. Johnson's laboratory has extensive experience with analyzing native Great Salt Lake waters. His laboratory was also successful in accurately measuring these same metals that were spiked (unknown to laboratory) into an artificial hypersaline matrix similar to the toxicity test media. Accurately analyzing the hypersaline matrices of Great Salt Lake continues to be challenging and UDEQ is confident that this laboratory is capable of addressing these challenges. I</p>
9	General	<p>Page 1, paragraph 1. Please confirm that there are threatened and endangered species on the GSL.</p>	<p>Documentation of all of the species present at Great Salt Lake are beyond the scope of the toxicity testing. However, in March, 2015 U.S. EPA and UDWQ hosted an aquatic life use workshop. The data collected during the workshop will be summarized in a report to be released later in 2016. No changes were made in response to this</p>

			comment.
10	General	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 purpose of the toxicity testing is to support the future development of water quality criteria for Great Salt Lake. In accordance with U.S. EPA's 1985 <i>Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses</i> , numeric criteria are based on testing with single toxicants. Exceptions are toxicants that are structurally similar such as PCBs. No changes were made in response to this comment.
11	Feeding	In the Table that summarizes the test protocol, please define the feeding protocol. 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.	The Work Plan was revised to include the feeding protocols. As discussed in the Work Plan, Dr. Belovsky's laboratory demonstrated that minimal feeding of both nauplii and brine fly larvae is necessary to achieve control survivals of at least 90%. The methods for ensuring that the amounts of food are controlled are presented in the Work Plan.
12	Test Organisms	We appreciate you citing papers Kennecott has produced/published (attached) but left	Thank you for the information. No modifications were made to the Work Plan because multi-generational testing is not within the scope

		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 than the parental generation (arsenic). This was not mentioned in the draft work plan.	of this project but may be the subject of future testing.
13	Test Organisms	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.	The Work Plan was clarified that brine shrimp cysts are hatched at a salinity of 45ppt and the nauplii are transferred to water with a salinity of 120 ppt (test salinity) directly in 24 hours. Acclimation was unnecessary. The initial lower salinity for hatching markedly increases hatching success.
14	Test Organisms	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?	The methods proposed are adapted from the U.S. EPA's 1985 <i>Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses</i> . Based on the work of Belovsky et al., UDEQ anticipates that the U.S. EPA methods will have to be modified with regards to the minimum number of taxonomic families required because Gilbert Bay doesn't exhibit the diversity of most aquatic ecosystems. UDEQ also anticipates that toxicity testing of phytoplankton will be necessary in the future to support the derivation of numeric criteria. No changes were made in response to this comment.
15	Test Organisms	Has the most sensitive stage in the lifecycle for each of the test species been properly identified for acute testing of each metal?	Different life stages were not tested for brine shrimp nor brine flies. However, brine shrimp nauplii are assumed to be more sensitive than juveniles or adult life stages because of their small size and limited exoskeleton development. The 3 <sup>rd</sup> instar of brine fly larvae are the youngest that can be tested for the practical reasons explained in the Work Plan. These details were added to the Work Plan.
16	Test Pollutants	Ammonia should not be tested because: <ul style="list-style-type: none"> <li>Existing data on concentrations of ammonia in Gilbert Bay and the toxicity of ammonia to brine shrimp</li> </ul>	In response to the comment, UDWQ investigated the discrepancies regarding reported ammonia concentrations for Gilbert Bay. Due to laboratory methodological errors, the UDWQ data from 2011 and 2012 used for the pollutant prioritization is rejected and not usable. Other

		<p>do not support ammonia being prioritized for testing.</p> <ul style="list-style-type: none"> <li>• Brine shrimp are a likely source for observed ammonia spikes in Gilbert Bay.</li> <li>• Ammonia concentrations observed in the anoxic deep brine layer do not threaten brine shrimp.</li> <li>• Ammonia is a source of nitrogen that is the limiting nutrient in Gilbert Bay.</li> </ul>	<p>data available, including that provided by the Great Salt Lake Brine Shrimp Cooperative indicate lower concentrations than indicated by the erroneous UDWQ 2011-2012 data.</p> <p>The supposition that ammonia is not currently threatening brine shrimp is further supported by unpublished ammonia testing conducted in Dr. Gary Belovsky's laboratory (<a href="http://science.nd.edu/undergraduate/minors/sustainability/capstone-projects/2015/davila/">http://science.nd.edu/undergraduate/minors/sustainability/capstone-projects/2015/davila/</a>). This work was conducted as part of the methods development work summarized in the Work Plan.</p> <p>Ammonia is no longer proposed for this round of toxicity tests primarily because of budgetary constraints and the other pollutants being tested are judged higher priorities. However, ammonia is a U.S. EPA priority pollutant and is known to be toxic to aquatic organisms. As discussed in the Great Salt Lake Water Quality Strategy, the goal is to establish numeric criteria for all U.S. EPA Priority Pollutants including ammonia. Establishing these criteria will require toxicity testing at some time in the future. The Work Plan was revised to not include ammonia.</p>
17	Test Organisms	In the Table that summarizes the test protocol, please define the precise age of the brine shrimp nauplii for testing.	The Work Plan was revised to indicate that nauplii brine shrimp will be no older than 96 hours. The brine fly larvae cannot be aged exactly as they do not hatch out synchronously like the brine shrimp. However, the brine fly larvae will be at the 3 <sup>rd</sup> instar developmental stage for testing.
18	Test Organisms	What are your rearing criteria for the test organisms?	Additional details were added to the Work Plan to document the culturing methods Plan. These methods are documented to achieve <90% survival.
19	Test Organisms	How will you ensure that tests will only be initiated with test organisms in good health?	Currently, we have no specific measure of individual organism health other than only active individuals are used for testing. The test acceptability criteria require at survival of <90% of the control treatment organisms. Future efforts include establishing reference toxicants. No changes were made to the Work Plan in response to this comment.

20	Test Organisms	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.	Very few laboratories have tested brine shrimp under Great Salt Lake conditions and even less have tested brine flies. One of the primary goals of this work is to develop new methods that are specific for Great Salt Lake and repeatable at other laboratories. Dr. Belovsky's laboratory has the most experience in culturing brine shrimp and brine flies. Dr. Belovsky's laboratory empirically determined appropriate test conditions to ensure acceptable control survivals. For the results to be accepted as valid, control survival is mandatory.
21	Test Organisms	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.	Dr. Belovsky's laboratory is the only laboratory that has successfully cultured brine flies in sufficient quantities to support toxicity testing. A major goal of the current work is to develop toxicity test methods for brine flies, one of the keystone species of Great Salt Lake. These methods will be the first step in establishing standard methods for future testing. The test protocols can be modified as more data are available but based on the currently available data, the methods proposed are viable and will achieve the desired goals.
22	Test Pollutants	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.	Mercury was proposed for acute and chronic testing of brine flies and brine shrimp in the draft Work Plan but due to budgetary constraints, mercury will not be tested at the present time. The metals that will be tested were judged a greater threat to Great Salt Lake's uses than mercury, in part because birds are anticipated to be more sensitive to mercury than brine shrimp or brine flies.
23	Test Water	We recommend making a super stock for each pollutant to reduce potential exposure variability.	Agreed. The Work Plan was revised to include super stock solution.
24	Test Water	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	The work plan was revised to include a detailed description of the test water. Many different formulations were considered including using water from Great Salt Lake. The data quality objectives for the test water were:

		<p>match up with real GSL water. In Brix et al. (2006 Effects of Copper, Cadmium, and Zinc on the Hatching Success of Brine Shrimp (publication where they examined toxicity of Cu, Cd and Zn on hatching success of brine shrimp (<i>Artemia franciscana</i>)). Instant ocean type water resulted in lower toxicity values (less toxic) than GSL water, especially for copper.</p>	<ul style="list-style-type: none"> <li>• Acceptable to EPA</li> <li>• Test water supports brine flies and brine shrimp</li> <li>• Test water has minimal toxicity confounders</li> <li>• Test water can be replicated over time</li> <li>• Test water is representative of Great Salt Lake water</li> <li>• Test water is stable over test duration</li> <li>• Test water can be replicated at any laboratory</li> <li>• Cost/convenience.</li> </ul> <p>The Work Plan was revised to include comparisons of major ion concentrations of the test water and average concentrations in Great Salt Lake. The test water is well matched to Gilbert Bay water for the major ions, the test water has been empirically demonstrated to be suitable for brine shrimp and brine flies, and meets EPA quality assurance requirements.</p>
25	Test Water	<p>Kennecott cannot find any mention of adding or measuring dissolved organic carbon. We feel it is very important that DEQ mimic the GSL - especially for copper. Kennecott measures of dissolved organic carbon indicated a value of 0.6 ppm.</p>	<p>As discussed in response to comment 24, artificial Great Salt Lake water was fabricated by mimicking the major ion content of Great Salt Lake, and specifically Gilbert Bay of Great Salt Lake. When the test water was made with the same ion concentrations as measured in the Lake, a precipitate consistently formed. The recipe was iteratively changed until a stable test solution resulted. This match is imperfect. For example, sulfate concentrations in the test water are lower than the average measured in Gilbert Bay to avoid generating a precipitate. To meet the data quality objective of generating data acceptable to U.S. EPA for criteria development requires that the test results not underestimate toxicity. The DOC concentration and type needs to be protective of the range of conditions observed in the Lake. Attempting to mimic the dissolved organic carbon (DOC) content of Great Salt Lake water was considered but rejected for several reasons. Without a more complete understanding of DOC dynamics in the Lake, an appropriate test concentration or composition of DOC could not be determined. No data are available to characterize seasonal or annual fluctuations and very little data on the composition of the DOC (Domagalski et al., 1989. Organic geochemistry and brine composition in Great Salt, Mono, and Walker Lakes. <i>Geochimica et Cosmochimica Acta</i>; Domagalski, J.L. et al.</p>



			<p>1990. Trace metal geochemistry of Walker, Mono, and Great Salt Lakes. Fluid-Mineral Interactions: A Tribute to H.P. Eugster. The Geochemical Society, Special Publications No. 2; Wurtsbaugh, W.A. and E.F. Jones. 2012. The Great Salt Lake's Deep Brine Layer and Its Importance for Mercury Bioaccumulation in Brine Shrimp (<i>Artemia franciscana</i>). Watershed Sciences Faculty Publications. Paper 551. <a href="http://digitalcommons.usu.edu/wats_facpub/551">http://digitalcommons.usu.edu/wats_facpub/551</a>; USGS NWIS database total inorganic and organic carbon); Leenheer et al. 2004. Characterization and origin of polar dissolved organic matter from the Great Salt Lake. Biogeochemistry Vol.69 1:125-141). Any potential future water quality criteria or effluent limits based on these bioassays can be adjusted on a site-specific basis using the U.S. EPA water effects ratio. Specific guidance for evaluating copper is available from <a href="#">EPA-822-R-01-005</a>. No changes were made in response to this comment.</p>
26	Test Water	It will be important that the artificial GSL water is monitored for As, Cd, Cu, and Zn before the tests are performed.	Agreed. The Work Plan was revised to list the target analytes including As, Cd, Cu, and Zn prior to conducting testing.
27	Test Water	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.	The test conditions were based on bioassays for brine shrimp that evaluated the effects of salinity, temperature and pH (Belovsky XXXX). Hardness is known to affect the toxicity of metals as evidenced by U.S. EPA adjustments to water quality criteria based on hardness. The oolitic sands and bioherms at Great Salt Lake indicate that calcium precipitates from solution and the water is at saturation for calcium. The dissolved salts content of the bioassay test water had to be iteratively decreased from the <u>average</u> Gilbert Bay concentrations shown in the Work Plan to avoid creating a precipitate. The calculated average hardness of Gilbert Bay water is over 4,000 mg/L calcium and magnesium. Toxicity testing with varying hardness is not being proposed because the test water has a lower hardness than the Gilbert Bay. Based on the hardness relationships developed by U.S. EPA for water quality criteria, the bioassay test water may overestimate the toxicity and therefore, will be protective. One method of quantifying the potential overestimation that may be pursued in the future would be to conduct additional tests using Gilbert Bay water instead of water

			prepared in the laboratory. Gilbert Bay is anticipated to remain close or at hardness saturation because of continued inputs to the Lake and the existing reservoirs of precipitated calcium and magnesium. No changes were made to the Work Plan in responding to this comment.
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