

Use of 28 Day Hyalella Toxicity Test vs a 42 Day Test

Mercury Bioaccumulation Toxicity Test

I've discussed the issue of a 28 day vs 42 day test with Joe Goulet, one of EPA's eco-risk assessors who works on the Black Butte Mercury Mine investigation. As the group discussed on the May 8 call, the 42 day test can provide information on reproductivity which won't be directly provided by the 28 day test. However, longer test period used for this test offer greater opportunities for random physical, chemical and biological factors to affect test results. Accordingly, the results from the 42 day test may be expected to be more variable (larger coefficients of variation than results from the shorter test). Also, as noted in EPA's comments, the 42 day test will cost more. The 28 day test will be less expensive and provide most of the information the 42 day test will. Indeed, some studies have shown a significant correlation between reproduction from Day 28 to Day 42 and length of Hyalella on Day 28. That is, smaller amphipods (<3.5 mm) tend to have lower reproduction and larger amphipods (>4.3 mm) tend to have larger reproduction.

In regards to the bioaccumulation toxicity test, I had asked Burt Shepard if there was a good bioaccumulation toxicity test for mercury in sediment. He recommended the use of a 28 day test using oligochaete *Lumbriculus variegatus* which would measure the uptake of contaminants from sediments. However, during my discussions with Joe Goulet it became apparent, I should have asked additional questions of Burt, such as how useful the data from such a test would be in the investigation and cleanup of the site given site specific conditions. Joe's opinion, in consultation with Chris Eckley, is that such testing would not provide much additional useful information regarding the bioaccumulation of mercury from the river sediment.

There are several factors that entered into this revision of EPA's position. The Kuskokwim River is orders of magnitude greater than Red Devil Creek and the river flows through a large mineralized area thus making a determination between Red Devil Creek sediment versus the upstream watershed as the source of mercury in the river sediment difficult. Also, the general area where Red Devil Creek enters the Kuskokwim River is an area of relative high flow, not necessarily an area conducive to methylation of mercury. Based on this information EPA recommends that the bioaccumulation toxicity test not be performed. Instead we recommend that any funds allocated for this study would be better spent collecting more sediment samples for direct measurement of the metals concentration in the sediment and the potential for methylation of mercury in these sediment samples.

The form of Hg that accumulates in biota is MeHg. Unlike inorganic Hg which almost always has an oxidation state of 2+ and can be tightly bound to solid phases; MeHg has a +1 oxidation state and is much more uniform in its availability. From EPA's perspective knowing how much MeHg is present will provide an adequate estimate of the potential for bioaccumulation without performing the tox tests.

Also, Hg methylation is very spatially and temporally variable. We anticipate that a toxicity test would be spatially limited due to costs/logistics and therefore may not be representative of the breadth of Hg methylation dynamics at the site.

Another option, if practical, would be to collect additional fish that have a limited home range, like sculpin, for direct measurement of mercury.

If we need to discuss this further let me know and we can arrange a mutually agreeable time to do so.

BLM Response: Based on the additional comments provided by the EPA in this document and agreement reached during the May 28, 2015 comment resolution call, the BLM will not collect samples for bioaccumulation testing. Instead, selected sediment samples will be analyzed directly for methylmercury content to provide information on the potential for bioaccumulation of mercury for sediment. Based on a call between Chris Eckley (EPA) and Mark Longtine (E&E) on June 1, 2015 to further discuss Kuskokwim River sediment sampling and analysis, it was agreed that analysis for methylmercury would be added to a total of fourteen (14) of the previously planned samples (extending from the area near the mine site to locations as far downriver as KR104 and KR105 as shown in Figure 2-4 of the draft Field Sampling Plan). Chris Eckley added that, although he is interested in methylmercury sediment concentrations at locations further downriver, he recognizes that gathering such information is not part of the current scope.

The final Kuskokwim River sediment sampling approach is as follows:

- Twelve (12) samples will be collected from the area upriver of, in the vicinity of, and downriver of Red Devil Creek delta. These samples are planned for locations KR082 through KR093 (per draft Work Plan). All twelve (12) samples will be analyzed for total target analyte list (TAL) metals, total organic carbon (TOC), and grain size distribution. Eleven (11) of these samples will be analyzed for toxicity using a *Hyallolella azteca* 28-day test rather than a *Hyallolella azteca* 42-day test was proposed in the draft Work Plan. Six (6) of the samples also will be analyzed for methylmercury and mercury selective sequential extraction (SSE).
- Twelve (12) samples will be collected from locations cross-river and downriver from the areas of documented elevated concentrations of antimony, arsenic, and mercury. These samples are planned for locations KR094 through KR105 (per draft Work Plan). Each of these samples will be analyzed for total TAL metals, TOC, and grain size distribution. In addition, eight (8) of these samples also will be analyzed for methylmercury and one of the samples will be analyzed for toxicity using a *Hyallolella azteca* 28-day test.
- Four (4) samples will be collected along two previously defined transects across the river. Two (2) samples will be collected from one transect located upstream of Red Devil Creek and two (2) samples will be collected from one transect located a short distance downstream of Red Devil Creek. Along each transect, one sample will be collected from shallow water near the shore (approximately 10-20 feet from the bank) opposite the mine site, and a second sample will be collected approximately 50 feet from that same shore. All four (4) samples will be analyzed for TAL metals only.
- The water column at all sampling locations will be analyzed in the field for turbidity.