Toxicity Identification Evaluation of Mill Water Sample

Draft Report

Report date:
April 8, 2011

Submitted to:

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1.0 INTRODUCTION

Toxicity tests conducted on two mixtures of Mine Water and Mill Water produced to reflect future anticipated conditions at the Canadian Zinc operation indicated that reproduction of the freshwater cladoceran, *Ceriodaphnia dubia*, was adversely affected by the mixtures. Conversely, rainbow trout and duckweed were not adversely affected in acute and chronic tests, respectively, and only a marginal adverse effect was apparent in one of the mixtures using acute tests with *Daphnia magna*. The results of these tests are provided in a separate test report.

Adverse effects on reproduction of *Ceriodaphnia* appeared to be derived from the Mill Water, since the degree of toxicity observed was related to the proportion of Mill Water in the samples, and the Mine Water tested alone exhibited no adverse effect on *Daphnia*. Consequently, efforts were undertaken to establish the cause of toxicity to *Ceriodaphnia* in the Mill Water using a Toxicity Identification Evaluation. This process involves conducting a series of physico-chemical manipulations on the sample, following by toxicity tests on the treated and untreated samples. Alterations in the degree of toxicity present as a result of the treatments provides an indication of the characteristics of the contaminant that is responsible for toxicity in the sample. The actual identity of the toxicant can then be established through a series of follow-up procedures.

2.0 METHODS

The following treatments were conducted:

EDTA treatment – Chelation of the sample with EDTA was used to identify whether divalent metals, such as copper, cadmium and zinc, were responsible for toxicity. This chemical binds to divalent metals and reduces their bioavailability and, therefore, toxicity. Treatments were conducted at 5 mg/L EDTA.

C18 solid phase extraction – Treatment of the sample through a C18 substrate was utilized to identify whether toxicity was caused by a non-polar organic contaminant. This material binds and removes these materials and, therefore, if toxicity is reduced following treatment with C18, this indicates that organic contaminants are responsible for toxicity.

Anion Exchange – Extraction of the sample through a strong anion exchange column was used to establish whether strong anions were responsible for toxicity. This treatment is similar to the

C18 treatment, except that the substrate contains positively charged amine groups which have an affinity for anions in the sample. Anions that would be expected to be removed include anionic surfactants, but not major anions such as sulphate, carbonate or chloride.

Filtration – Filtration of the sample through a 0.45 µm filter was conducted to remove particulate-bound contaminants.

pH adjustment – Adjustment of the pH of a sample can alter the characteristics of the toxicant, resulting in an alteration in toxicity, or a change in the effectiveness of other TIE procedures. In this case, adjustment of the pH of the sample to 5 and 9 was used in conjunction with C18 and anion exchange in an attempt to establish whether the toxicant exhibited a higher affinity for these materials under different pH conditions. In addition, the sample was filtered after adjustment to pH 10, which would be expected to remove metals, such as zinc.

In order to evaluate the potential contribution to toxicity of a flocculent (Magnafloc 10) that was used in preparation of the sample, a sample of this chemical was obtained from SGS and evaluated for toxicity using *Ceriodaphnia*.

Test procedures used here were consistent with those typically applied for chronic tests using this species, with the exception that the degree of replication was reduced from 10 to 5. This stream-lining of the procedure is appropriate in Toxicity Identification Evaluations, where the purpose is to look for substantial changes in effect as a result of the treatments.

3.0 RESULTS

Initial treatments were conducted on the Mill Water sample diluted to 10%. None of the treatments (filtration, C18 extraction, anion exchange and EDTA) had an appreciable effect on toxicity to *Ceriodaphnia*, indicating that toxicity did not appear to be caused by particulate-bound contaminants, non-polar organic contaminants, strong anions, or divalent metals (Table 1).

These treatments were repeated using a sample diluted to 5% in case there had been too much toxicity present in the 10% sample for the treatments to be effective; however, the results of these treatments were not useful because the reproduction in the untreated sample diluted to 5% (21.2 ± 1.8 offspring per adult) were not significantly lower than the control (22.8 ± 2.9 offspring per adult). Thus, since the 5% sample did not exhibit toxicity, no information with respect to the cause of toxicity could be obtained from these treatments.

Adjusting the pH of the sample to 5 or 9 prior to treatment using C18 and anion exchange did not improve the effectiveness of these treatments at reducing toxicity in the 10% sample. Results of these treatments are also shown in Table 1. These results did not provide further indication as to the cause of toxicity, but are consistent with the initial findings described above.

The results described above are consistent with a number of contaminants, including charged or highly soluble organic contaminants, cations, total dissolved solids, and other chemicals. In order to establish whether one of the process chemicals used in preparation of the samples might have been responsible, the characteristics of the materials were reviewed and Magnafloc 10 was identified as being potentially consistent with the results, and of unknown toxicity to *Ceriodaphnia*. The results of a toxicity test conducted using this chemical are provided in Table 2; in general, this chemical resulted in no adverse effect on reproduction at 1.25 mg/L or less, but reduced reproduction was observed in the 2.5 and 5 mg/L solutions. Since the treatment rate of this material was 14 mg/L in the Mill Water, and most of the material would be expected to be removed during the treatment process, it appears unlikely that this was the cause of toxicity.

Table 1. Results of TIE treatments conducted on 10% Mill Water.

	Survival (%)	Reproduction (offspring per adult)
Control	100	23.6 ± 5.2
Untreated	100	1.2 ± 1.8
Filtered sample	100	1.0 ± 1.4
C18-treated sample	100	0.6 ± 1.3
Anion Exchange-treated sample	100	2.6 ± 1.8
EDTA treated sample	100	0.0 ± 0.0
Control	100	18.0 ± 3.3
Untreated	100	0.0 ± 0.0
pH 5 + anion exchange	100	0.0 ± 0.0
pH 9 + anion exchange	100	0.0 ± 0.0
pH 5 + C18	100	0.0 ± 0.0
pH 9 + C18	100	0.0 ± 0.0
pH 10 + filtration	100	1.4 ± 1.9

Table 2. Results of toxicity test conducted on Magnafloc 10.

Mangafloc 10 (mg/L)	Survival (%)	Reproduction (offspring per adult)
Control	100	21.8 ± 6.6
0.08	100	22.2 ± 10.1
0.16	100	25.0 ± 2.0
0.31	100	20.8 ± 4.2
0.62	100	20.8 ± 6.9
1.25	100	20.2 ± 4.3
2.5	100	14.0 ± 5.7
5.0	100	13.2 ± 2.7

4.0 DISCUSSION

The results of the TIE procedures described here were not conclusive in establishing the cause of toxicity in the Mill Water; however, the results indicate that non-polar organic contaminants, strong anions and divalent metals did not appear to be the primary cause of toxicity in the sample, although it should be noted that these materials may have contributed to toxicity at higher concentrations of sample.

The concentration of sulphate present in the Mill Water would most likely have contributed some portion of the adverse effect observed to *Ceriodaphnia*. For example, Elphick et al. (2011) reported an IC25 value for effects of sulphate for this species of 1212 mg/L sulphate at a hardness of 160 mg/L. Since the Mill Water contained 4500 mg/L sulphate, there was clearly sufficient sulphate present to cause some proportion of the observed effect. Total dissolved solids, in general, which includes sulphate and other major ions, such as calcium, magnesium, sodium, potassium, chloride and carbonate causes effects on this species when elevated as a result of osmotic stress, and so sulphate, or major ions in general, likely explains some of the observed effect. However, the sample diluted to 10% would likely not have contained sufficient major ions to explain the effect observed in the diluted sample.

The Mill Water exhibited toxicity to *Ceriodaphnia* in the sample diluted to 10%, but not when tested at 5%. This result differs somewhat from the initial tests using the Mixtures, in which toxicity was observed in all concentrations tested, as low as 5% sample. Since the Mixtures were comprised of only a portion of Mill Water, the adverse effect observed here with the Mill Water is not consistent with the extent of adverse effect observed in the mixtures. This implies that either: 1) the toxicity of the Mill Water dissipated in between when the original test was conducted and when the TIE treatments were performed; 2) other components of the mixtures (i.e., Mine Water) also contributed to toxicity in the mixtures; or 3) there was some interaction between components in the mixture that exacerbated toxicity. The most likely explanation would be that toxicity dissipated over time in the sample; however, additional investigation would be necessary to fully characterize and identify the cause of toxicity in this sample.

5.0 REFERENCES

Elphick, J.R., Davies, M. Gilron, G., Canaria, E.C., Lo, B. and Bailey, H.C. 2011a. An aquatic toxicological evaluation of sulphate: the case for considering hardness as a modifying factor in setting water quality guidelines. *Environ. Toxicol. Chem.* 30:247-253.