TOXICITY IDENTIFICATION EVALUATION OF EFFLUENT FROM A MINE

James R. Elphick, B.Sc.
Howard C. Bailey, Ph.D.

EVS Environment Consultants
195 Pemberton Avenue
North Vancouver, BC
V7P 2R4

F. Marlin Murphy

Homestake Canada
1100 - 1055 West Georgia
Vancouver, BC
V6E 3P3

Abstract

The toxicity of discharges from mining operations; continue to be of concern to the regulatory community and mine operators. Toxicity in discharges may be caused by a variety of factors, including metals, ammonia, pH, process chemicals and total dissolved solids. In this study, a toxicity identification evaluation (TIE) was performed on samples of a discharge from a gold and silver mine which exhibited toxicity in 7-day partial lifecycle tests using the freshwater cladoceran Ceriodaphnia dubia. The results indicated that an organic anion was responsible for toxicity and that phosphorus concentrations in the treatments were correlated with toxicity. Collectively, the data suggested that a phosphine-based collector (Aerophine 34ISA Promoter) used in the metals flotation process was the most likely cause of the observed toxicity. Consequently, the chemical was evaluated for toxicity and its response to the TIE procedures which were effective at reducing toxicity in the discharge sample. These results, and those of a confirmatory spiking study, consistently suggested that Aerophine was the cause toxicity. Efforts at the mine to reduce the residual concentration of this process chemical resulted in reduced toxicity of the discharge.

Introduction

The Eskay Creek mine is a gold and silver mine operated by Homestake Canada and is located on a subalpine upland on the eastern flank of the southern Boundary Ranges of the Coast Mountains in northwest British Columbia, Canada. The mine is authorized to discharge wastewater via two main discharges subject to conditions stipulated by the British Columbia Ministry of Environment Lands and Parks. Waste rock and mill tailings are deposited into Albino Lake which feeds into an outflow creek and then into Tom MacKay Creek. Treated water from the underground operation, treated mill effluent, and surface run-off from the mine-site are
The D-7 discharge stream flows downhill for approximately 500 m before entering Ketchum Creek, a tributary of the Unuk River.

Samples collected from the D-7 discharge exhibited intermittent toxicity to the cladoceran *Ceriodaphnia dubia* in 7-day chronic toxicity tests. Before any meaningful ecological risk assessment or toxicity control strategies could be initiated, it was first necessary to identify the cause of the observed toxicity. Potential sources of toxicity in the samples include total dissolved solids, metals and chemicals used in the metals recovery process.

Identification of causes of toxicity is usually undertaken using Toxicity Identification Evaluation (TIE) procedures (U.S. EPA, 1991). These methods involve physical and chemical manipulation of samples followed by toxicity tests using the treated and untreated samples. By comparing the results of the manipulated sample to the untreated sample with respect to changes in toxicity, information regarding the cause of toxicity can be obtained. For example, toxicity due to metals such as copper, cadmium or zinc is identified by a reduction in toxicity as a result of addition of a metal chelating agent (EDTA). Organic contaminant-driven toxicity is indicated by a reduction in toxicity following solid phase extraction of organics from solution using an organic substrate. In general, TIEs follow a step-wise process in which the results from previous studies are used to direct additional tests and evidence from a number of tests are used to identify and confirm the cause of toxicity. The use of multiple lines of evidence reduces the risk of inaccurately identifying the cause of toxicity.

**Methods**

Grab samples were collected from D-7 on August 12 and December 7, 1998, and shipped in 20-L polyethylene containers to the EVS laboratory where they were stored in the dark at 4°C. Studies conducted to determine the chronic toxicity of the samples to *Ceriodaphnia dubia* were initiated within 72 hours of sampling. Samples were tested over 7 days using procedures based on methods presented by Environment Canada (1992). *C. dubia* neonates were obtained from in-house cultures and were <24-hr old and born within 8 hr of one another. Individual neonates were exposed in 30-mL plastic medicine cups containing 20 mL of test solution. Ten replicates were used for each concentration and control. Water for control treatments and dilution was dilute mineral water (hardness 80 - 100 mg/L, as CaCO₃). The tests were performed at 25±1°C under a 16:8 hr light:dark photoperiod and fresh test solutions, supplemented with food, were prepared daily. Dissolved oxygen, pH and temperature were measured daily on the freshly prepared solutions and the 24-hr old solutions in which the test organisms had been exposed.
TIE procedures

The TIE was performed with both samples collected from D-7. TIE manipulations were performed on undiluted effluent for the August sample and on a 50% dilution of the sample collected in December; the latter sample was diluted with control water due to its higher toxicity. Procedures generally followed those outlined by the U.S. EPA (1991, 1993). In all cases, an untreated sample was tested concurrently with the TIE procedures to determine whether toxicity in the sample had dissipated and to provide a baseline against which to measure the effectiveness of the treatment at reducing toxicity. In addition, dilution water controls and treatment control blanks (i.e., TIE treatments performed on control water) were tested to monitor culture health and to confirm that the treatments did not contribute artifactual toxicity.

Solutions were tested in the same manner as in the original toxicity tests, except that only five replicates were used in each treatment. Depending on the treatment, sample manipulations were either performed daily or enough treated solution was prepared at the start of the test to last the entire 7 day test duration. In the latter case, the treated and untreated solutions were stored in plastic bottles at 4°C in the dark.

Phase I TIE procedures

Phase I treatments are designed to determine the class of contaminant that is responsible for toxicity in a sample (U.S. EPA, 1991).

*EDTA chelation* - EDTA treatment is used to identify toxicity caused by divalent cations (e.g., zinc, copper, cadmium). Aliquots of the August D-7 sample were treated with 4 and 8 mg/L EDTA and tested for toxicity.

*Filtration* - Filtration removes toxicants associated with the particulate phase of the sample. The sample collected in August was filtered under vacuum through a 0.45μm filter and the filtrate evaluated for toxicity.

*Solid Phase Extraction: Anion Exchange* - Anion exchange columns remove anions from solution by exchanging them for a counter-ion from the column substrate. The August and December sample were extracted through anion exchange columns and the filtrates evaluated for toxicity. The columns used in this study are generally effective at removing large organic and some inorganic anions, but are not efficient at removing the major anions which contribute to total dissolved solids (TDS) (e.g., bicarbonate, sulfate, chloride).
Since there was concern over potential artifactual toxicity resulting from contaminants leaching from the column, the anion exchange filtrate from the August sample was mixed 50:50 with untreated sample. Our reasoning was that if the anion column removed the toxic component from D-I, then diluting D-7 with the treated sample should reduce toxicity: Similarly, if the column added artifactual toxicity, such toxicity would be diluted by the addition of D-7. This approach assumed that the toxicants present in D-7 and in leachate from the column were not additive. The sample collected in December was diluted by 50% with control water after anion exchange treatment.

**Solid Phase Extraction (SPE): C8 and C18** - C8 and C18 columns are used to remove non-polar and moderately polar organic contaminants from solution; these chemicals can usually be recovered in a solvent elution of the column. Reduced toxicity following SPE with C8 and C18 substrates indicates an organic contaminant is responsible for toxicity; toxicity in the solvent elution from the column added back into control water provides confirmation of this. The August sample was extracted through a CS SPE column and the filtrate and solvent elution were tested for toxicity. The solvent elution was added back into control water at 4 times the concentration relative to the sample to account for any loss in the extraction and recovery steps. This sample was also extracted through a C18 SPE column and the column filtrate tested for toxicity.

The binding affinity of organics to the column substrate can often be affected by pH, particularly for chemicals which are ionized over a particular range of pH. Since the results from anion exchange tests implicated an anionic toxicant, aliquots of the December sample were adjusted to pH 5 and 9 and then extracted through separate CIS columns and tested for toxicity.

**pH adjustment** - Adjustment of pH can result in a reduction of toxicity because of enhanced precipitation, volatilization or hydrolysis of contaminants associated with changes in pH. The August sample was adjusted to pH 9 for two hours and the solution subsequently filtered under vacuum through a 0.45 um filter to remove precipitate from solution. This treatment would reduce toxicity caused by bicarbonate and most metals because these chemicals precipitate under alkaline conditions.

**Phase III TIE procedures**
Phase III procedures are designed to confirm the cause of toxicity which has tentatively been identified by the Phase I procedures (U.S. EPA, 1993).
Synthetic effluent approach - Since TDS was high in these samples, we undertook a test to determine the extent to which the concentration of major ions was responsible for the observed toxicity. Synthetic effluent was prepared by addition of magnesium, sodium, calcium and potassium salts (sulfates, chlorides and carbonates) to pre-aerated bottled mineral water and tested for toxicity with *C. dubia*. The resulting mixture was comparable to D-7 with respect to the concentration of the major ions contributing to TDS (i.e., Mg$^{2+}$, Ca$^{2+}$, Na$^+$, K$^+$, SO$_4^{2-}$, HCO$_3^-$, Cl$^-$).

Spiking study approach - Spiking studies are used in TIEs to confirm or rule out tentatively identified contaminants as being the actual cause of toxicity in samples (U.S. EPA, 1993). This procedure involves increasing the concentration of the suspected contaminant by addition of the chemical and testing the spiked and unspiked samples concurrently for toxicity. A proportional increase of toxicity in the spiked sample relative to the untreated sample in response to an increase in chemical concentration indicates that the chemical tested is indeed the chemical responsible for toxicity. If tide toxicity is increased by less than the proportional increase in chemical concentration, the chemical tested is probably not responsible for all or any of the toxicity in the sample.

The alkalinity in both samples was higher than that previously reported to exhibit chronic toxicity to *C. dubia* (Cowgill and Milazzo, 1991). Thus, in order to confirm whether HCO$_3^-$ was responsible for toxicity in effluent from D-7, the sample was spiked with NaHCO$_3$ to approximately double the concentration of HCO$_3^-$ and the spiked and unspiked samples were tested for toxicity.

Because the Phase I results implicated an anionic organic contaminant as the cause of toxicity, the process chemicals used at the mine were evaluated and a chemical used in the flotation process, Aerophine, was consistent with this description. Therefore, Aerophine was tested for chronic toxicity to determine whether it might be responsible for toxicity. This chemical was selected over another anionic process chemical used at the mine (potassium amyl xanthate) because the Phase I results also indicated a strong relationship between phosphorus and toxicity, and Aerophine is a phosphorus-based chemical.

To confirm that Aerophine was responsible for toxicity in the sample, this process chemical was spiked into the sample to double its concentration and the spiked and unspiked samples were tested for toxicity with *C. dubia*. The concentration of Aerophine in the spiked and unspiked solutions were determined analytically by measuring phosphorus and converting on the basis of relative molecular weights of phosphorus and Aerophine.
**TIE fingerprint approach** - Aerophine was also tested using TIE procedures to determine if it exhibited a similar response to the procedures as the sample did. The chemical was spiked into laboratory dilution water and into the sample of D-7 collected in December at a nominal 25 mg/L. The spiked samples were then extracted through CIS and anion exchange SPE columns and the resulting solutions were analyzed to determine the effectiveness of each procedure at removing the chemical.

**Correlation approach** - Toxicity values in five samples collected from D-7 over an 18-month period, including the two samples tested in this study, were compared to analytical data for the concentration of phosphorus. If Aerophine was responsible for toxicity on an on-going basis, a strong correlation between phosphorus and toxicity would be expected.

**Results**

**Toxicity tests**
The samples collected in August and December both caused a reduction in reproductive output. Respective toxicity values were 47.8 and 27.2% for IC25 and 69.8 and 35.9% for IC50 estimates. Thus, the December sample exhibited approximately twice the toxicity compared with the August sample.

**Phase 1 Toxicity Identification Evaluation**
The results of the Phase 1 TIE treatments performed on the sample collected from D-7 in August, 1998, are summarized in Table I. Treatment of the sample with EDTA had no effect on toxicity, suggesting that divalent cationic metals were not responsible for toxicity. Filtration of the sample did not have an appreciable effect on toxicity, indicating that the contaminant was not associated with the particulate phase. Solid phase extraction using a C8 column reduced toxicity marginally; however, there was no toxicity associated with the solvent eluate add-back from this column and extraction of the sample with a C18 column had no effect on toxicity. The overall lack of effect of solid phase extraction using organic substrates suggested that the toxicant in the sample was not a non-polar organic contaminant.

Conversely, anion exchange treatment reduced the toxicity of the August sample in two separate tests, implicating an anionic component of the sample as the cause of toxicity. Anions which might have been present in the samples included anionic contributors to TDS (e.g., SO₄²⁻, HCO₃⁻, Cl⁻), anionic metals (e.g., selenate, antimonate, arsenate) and anionic process chemicals. Metallocyanide complexes are generally anionic and often
associated with gold mine operations; however, these chemicals were not expected to be present in these samples because this mine does not use cyanide in its processes.

Adjustment of the sample to pH 9 reduced HCO₃⁻ due to precipitation of calcium carbonate, but had no effect on toxicity suggesting that bicarbonate in the sample was not a major contributor to toxicity.

Results for the sample collected in December, 1998, are presented in Table 2. Anion exchange treatment removed toxicity from this sample, suggesting that toxicity was caused by the same contaminant as in the previous sample. Toxicity was also removed by extraction through a CIS column at pH 5 and reduced by this treatment at pH 9. This is consistent with toxicity caused by an anionic organic contaminant; in particular, lowering the pH shifts the anion into its non-dissociated form (i.e., non-charged), making the contaminant more likely to adsorb to the organic substrate of the column. These results suggest that an organic contaminant with an anionic group was responsible for toxicity in these samples. The most likely source of this type of chemical would be a chemical used in the extraction processes. For instance, anionic surfactant-like chemicals are used as "collectors" to enhance removal of metals from the mineral surface. This hypothesis was supported by a strong relationship between toxicity and a general analytical measure of anionic surfactants (MBAS) (R² = 0.95; n=4) for these TIE treatments. This relationship was also strong between phosphorus and toxicity (R² = 0.95; n=4). These data suggested that a phosphorus-based surfactant may have been responsible for toxicity. Aerophine is a process chemical used at the site which fits this description.

Phase 3 Toxicity Identification Evaluation
The synthetic effluent was comparable to D-7 with respect to the concentration of major ions contributing to TDS; however, it did not cause adverse effects on reproduction (IC25 was > 100%). These data indicate that the composition of major ions in the sample was not responsible for toxicity. Further evidence that TDS was not responsible for toxicity comes from the fact that the toxicity of the sample dissipated over time; in general, the concentration of major ions did not change significantly over time. Alkalinity (a measure of HCO₃⁻) did decrease in the August sample as the toxicity dissipated; however, it is unlikely that the small reduction in alkalinity (approximately 15%) could have accounted for the large change in toxicity (more than a factor of two). Furthermore, spiking NaHCO₃ into the sample did not increase toxicity appreciably, despite increasing alkalinity to more than 50% higher than in the original sample.

The chronic toxicity test using Aerophine added into control water resulted in an IC25 estimate for Aerophine of 13.3 mg/L (Table 3). In the Aerophine spiking study using the December 1998 sample, an increase in the
concentration of Aerophine by a factor of 2.2 and resulted in a reduction in the IC25 and IC50 estimates by 1.9 and 2.5 times, respectively. The general agreement between the relative increase in toxicity and concentration of Aerophine indicates that Aerophine was the cause of toxicity in the sample. When the IC25 and IC50s of the spiked and unspiked sample are calculated on the basis of Aerophine concentration, the values are very similar. These data are also shown in Table 3.

The chemical concentration of Aerophine was reduced by more than 80% following anion exchange and by 56% with C18 solid phase extraction. These results are consistent with those shown by the sample with respect to reduced toxicity following treatment. Thus, the fingerprint of Aerophine with respect to the TIE treatments which were effective at altering the toxicity of the sample matched that of the sample.

When comparing the toxicity of the December sample when originally tested and when tested as the untreated sample in the spiking study, the change in Aerophine concentration (decreased by a factor of 1.8), reflected a comparable change in toxicity (IC50 increased by a factor of 2.0). This information provides further evidence that Aerophine was the cause of toxicity in the sample.

Toxicity in six samples collected from D-7 shows a strong correlation with phosphorus (R² = 0.93) (Figure 1). This provides additional evidence that Aerophine was the cause of toxicity in the samples presented in this study, and has contributed to toxicity in previous samples.

Discussion

TIE procedures were applied in this study to determine which contaminants might have been responsible for toxicity. Once tentatively identified, additional procedures were applied to either rule out or confirm whether suspected toxicants were the actual cause of toxicity. Divalent metal cations, bicarbonate and TDS were eliminated from consideration and the results indicated that an organic anion was the cause of toxicity. Consequently, a candidate anionic process chemical, Aerophine, was tested using selected TIE procedures to evaluate whether it exhibited a similar "fingerprint" with respect to the effectiveness of different TIE treatments which were previously shown to remove toxicity in the sample. These data suggested that Aerophine was indeed responsible for toxicity, and a follow-up spiking study confirmed that it was the cause of toxicity (i.e., the increase in the concentration of Aerophine resulted in a proportional increase in toxicity). These data were further supported by a strong correlation between toxicity and analytical measures for Aerophine.
The toxicity of Aerophine in lab dilution water was lower than that exhibited in the sample spiking study. This difference is certainly within the range of intra-laboratory variability; however, it is also possible that the higher toxicity of Aerophine in the sample matrix resulted from metals in the sample that are not present in the dilution water. Process chemicals such as Aerophine form complexes with heavy metals which render the metals more hydrophobic and, therefore, more bioavailable (Block, 1991). Presence of these hydrophobic metal complexes has been shown to enhance the uptake rate of metals in fish and daphnids (Block and Glynn, 1992; Tjalve and Gottofrey, 1991; Poldoski, 1979). Greater than additive toxicity between metals and anionic surfactants has also been demonstrated for surfactants found in detergents (Calamari and Marchetti, 1973).

The formation of hydrophobic complexes may also explain why the efficiency of the C8 and CIS extractions were not consistent between the first and second sample. Removal of contaminants by these substrates is generally better for more hydrophobic chemicals; therefore, the relative proportion of the chemical which is in the hydrophobic metal complex versus the anionic form will affect its affinity for the C8 and CIS substrates.

TIEs for samples from mines are often problematic because of the complex nature of sample matrices resulting from processes associated with mining activities. For instance, a high concentration of TDS can cause acute and chronic toxicity to test animals (Cowgill and Milazzo, 1991; Mount et al., 1998) and is not easily identified with TIE procedures (U.S. EPA, 1991). Toxicity can be attributed to TDS when no TIE procedures are effective at reducing toxicity (U.S. EPA, 1991). However, care must be taken in making this conclusions because toxicity can be contributed by TIE procedures themselves, thus masking a reduction of the actual sample toxicity. In addition, other toxicants present in mine discharges behave in a similar manner to TDS with respect to TIE procedures. For instance, surfactant-like chemicals are not easily identified using TIE methodology (U.S. EPA, 1991) and are used as process chemicals in the mining industry. Finally, we do not know how certain trace metals respond with respect to TIE treatments and these metals are often more concentrated in mining effluent than typically seen in natural environments (for example, thallium and antimony).

The data presented in this study suggest that TIEs must be performed with great care and the results carefully validated prior to finalizing conclusions and implementing any associated toxicity reduction strategies. When performed appropriately, TIEs are a useful component of an Environmental Effects Monitoring (EEM) program because they provide a tool to focus efforts aimed at reducing impacts associated with mining activities (Raggett et al., 2001).
References


U.S. EPA. 1993. Methods for aquatic toxicity identification evaluations. Phase III toxicity confirmation procedures for samples exhibiting acute and chronic toxicity. EPA/600/R-92/081. Duluth, MN, USA,
Table 1. Results of TIE treatments performed on the sample of D-7 collected in August, 1998. Data presented are the average number of young produced per female.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Treatment Control (Blank)</th>
<th>Untreated D-7</th>
<th>Treated D-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filtration</td>
<td>16.0</td>
<td>0</td>
<td>2.4</td>
</tr>
<tr>
<td>Solid Phase Extraction - C8</td>
<td>15.4</td>
<td>0</td>
<td>5.0</td>
</tr>
<tr>
<td>Solvent Add-back (4X)</td>
<td>8.4</td>
<td>0</td>
<td>11.2 *</td>
</tr>
<tr>
<td>Test 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDTA chelation</td>
<td>15.1</td>
<td>8.2</td>
<td>8.6</td>
</tr>
<tr>
<td>Test 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anion Exchange</td>
<td>12.0</td>
<td>1.8</td>
<td>9.6</td>
</tr>
<tr>
<td>Test 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anion Exchange</td>
<td>NT</td>
<td>6.6</td>
<td>15.2</td>
</tr>
<tr>
<td>Solid Phase Extraction - C18</td>
<td>17.8</td>
<td>6.6</td>
<td>4.8</td>
</tr>
<tr>
<td>pH 9 adjustment + filtration</td>
<td>17.0</td>
<td>6.6</td>
<td>6.8</td>
</tr>
</tbody>
</table>

NT  Not tested
* Methanol eluate added to control water

Table 2. Average number of young produced in TIE manipulations and untreated sample of D-7 collected in December, 1998.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>Untreated D-7</th>
<th>Treated D-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anion Exchange</td>
<td>20.0</td>
<td>11.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Solid Phase Extraction - C18; pH 5</td>
<td>20.0</td>
<td>11.0</td>
<td>22.4</td>
</tr>
<tr>
<td>Solid Phase Extraction - C18; pH 9</td>
<td>20.0</td>
<td>11.0</td>
<td>16.0</td>
</tr>
</tbody>
</table>
Table 3. *C. dubia* 7-day chronic toxicity test results for Aerophine, untreated sample D-7 and Aerophinespiked sample D-7. Data are presented as mg/L Aerophine. The similarity in toxicity between the spiked and unspiked samples from D-7 (when expressed in terms of Aerophine) suggests that Aerophine was responsible for toxicity.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Aerophine mg/L</th>
<th>D-7 Untreated Mg/L</th>
<th>D-7 Spiked mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC50</td>
<td>71</td>
<td>&gt; 13.5</td>
<td>&gt; 29.9</td>
</tr>
<tr>
<td>IC25</td>
<td>13.3</td>
<td>7.4</td>
<td>6.6</td>
</tr>
<tr>
<td>IC50</td>
<td>19.4</td>
<td>9.5</td>
<td>11.1</td>
</tr>
</tbody>
</table>

Figure 1. Reproductive effects as a function of phosphorus for six samples collected from discharge D-7.