NPDES Field Studies Report – SD026

Prepared for
Cliffs Erie L.L.C. and
PolyMet Mining Inc.

September 2011

NPDES Field Studies Report – SD026

Prepared for
Cliffs Erie L.L.C. and
PolyMet Mining Inc.

September 2011

BARR

332 West Superior Street
Duluth, MN 55802
Phone: (218) 727-5218
Fax: (218) 727-6450

NPDES Field Studies Report – SD026

September 2011

Table of Contents

List of Tables

List of Figures

List of Appendices

1.0 Introduction

1.1 Background

The former LTV Steel Mining Company (LTVSMC) tailings basin is located in two local watersheds and is administered by two separate NPDES Permits. The general site layout is shown on Figure 1-1. Surface seepage emanating from the tailings basin and flowing south (via surface discharge station SD026 toward Second Creek, which flows into the Partridge River) is covered under Minnesota Pollution Control Agency (MPCA) NPDES Permit MN0042536. The Permit is currently held by Cliffs Erie L.L.C. (CE). However, PolyMet Mining Inc (PolyMet) is collaborating with CE on the reissuance of the Permit. A key aspect of the Permit renewal process will be the implementation of corrective actions defined in the April 6, 2010 Consent Decree between MPCA and CE. The work required under the Consent Decree is designed to address selected chemical parameters that have had elevated concentrations in the surface seepage (SD026). A one-year program of field study investigations (ending on June 16, 2011) was conducted at the site, following the scope of work described in the May 6, 2010 *NPDES Field Studies Plan – SD026* (approved by the MPCA on June 16, 2010). This Field Studies Report provides a summary of the results from the individual field studies that were conducted for SD026 under the Consent Decree.

In addition to this Field Studies Plan, the Consent Decree required the preparation of a Short Term Mitigation Evaluation Plan for SD026. The objectives of the Short Term Mitigation Evaluation Plan are to investigate existing methods and technologies to partially or completely mitigate the elevated sulfate and parameters of concern. The Short Term Mitigation Evaluation Plan is intended to address and mitigate the existing elevated concentrations of sulfate and the parameters of concern in SD026 to the extent feasible and practical during the period that field studies are being conducted to determine an appropriate long-term mitigation strategy.

As part of Short Term Mitigation under the Consent Decree, a seepage collection and pumpback system was constructed and was placed into operation during the summer of 2011 following completion of the field studies summarized in this document. Seepage from the tailings basin that formerly flowed to SD026 is currently being collected upstream of SD026 and pumped to the tailings basin.

For the purposes of this document, ‘parameters of concern’ are total dissolved solids, bicarbonates, total hardness (Ca + Mg as $CaCO_3$) and specific conductivity in SD026 of NPDES/SDS permit MN0042536.

1.2 Overall Objectives

The purpose of the Field Studies for SD026 was to develop an understanding of the potential impacts of the elevated concentrations of sulfate and parameters of concern and to collect adequate data to support either the development of recommendations for long-term mitigation alternatives or the development of site specific standards. The Field Studies collected data to assess:

- The impact of the elevated sulfate in SD026 on receiving waters supporting the production of wild rice
- The impact of the elevated sulfate in SD026 on methyl mercury concentrations in receiving waters
- The impact of elevated parameters of concern in SD026 on the water quality and aquatic life (fish and macroinvertebrates) of receiving waters

2.0 Historical Data Compilation

2.1 Objectives

The primary objective of the historical data compilation was to: identify, compile, and review readily available information regarding the SD026 site setting, water quality, hydrology, and hydrogeology. This activity was substantially completed in support of determining the detailed scope of the individual studies described in the *NPDES Field Studies Plan – SD026*. This review of available information allowed for a more complete understanding of the site prior to designing the field studies.

2.2 Scope / Sources of Information

The following general sources of information were compiled and reviewed. Specific sources of information reviewed for the individual studies were described in detail in the *NPDES Field Studies Plan – SD026*:

- Permit monitoring data (water quality and flow)
- Other relevant data from field studies at the tailings basin (seepage computations,)
- Data from completed and ongoing studies related to the environmental review for PolyMet's NorthMet Project
- Published reports and maps regarding local geology, hydrogeology, and water quality

3.0 Stream Investigation

3.1 Background

A one year field study (July 2010 to June 2011) was conducted to characterize and assess the water quality and biological condition of streams directly adjacent and downstream of outfall SD026.

According to Minnesota State Water Rules (Chapter 7050), Second Creek is an unlisted water and is designated for the protection of aquatic life (Class 2B) as well as other use protections. In general, water quality standards for the protection of aquatic life, which are based upon toxicity tests with very sensitive aquatic organisms (e.g., zooplankton), serve as a conservative means to assess whether a given discharge could possibly have an effect on aquatic life. Therefore, if a given water quality standard is met in the discharge, it can be concluded with confidence that aquatic life is protected.

In addition to water quality standards, regulatory agencies may include Whole Effluent Toxicity (WET) testing requirements in permits to determine whether constituents in a discharge have additive toxicological effects, or if constituents lacking applicable water quality criterion (with respect to aquatic life, e.g., total dissolved solids or sulfate) may be toxic. WET testing was included in this study to follow this regulatory construct and to evaluate whether the groups of constituents originating from SD026 have toxic properties at the concentrations observed.

Biological monitoring can be requested by regulatory agencies to further investigate effects from discharge waters. Biological monitoring is important because it highlights the true in-stream effect of a given discharge. Biological monitoring also separates the "chemical" effect from the "habitat" effect. For example, if water quality standards are not met or if WET testing results show some perceptible difference from background, biological monitoring will provide an indication of whether these indicators really result in impacts to the biological communities downstream of the discharge. For this study, aquatic invertebrates were assessed to determine the effect of discharges from SD026. A habitat evaluation was also conducted as part of this study to quantify the difference in habitat quality between the downstream sites and the control site.

The goal of this investigation was to determine whether the biota downstream of outfall SD026 are "ecologically" better or worse than can be reasonably expected given the available habitat and compared to a control stream that is not affected chemically by mining operations.

The overall composition and evaluation of biological communities including fish and macroinvertebrates, can provide valuable information about a site and allow investigators to draw conclusions about the system even without the availability of extrinsic abiotic information. Water chemistry and WET testing results should be viewed as indicators of potential effect, while the invertebrates provide an actual measurement of effect.

Fish also serve as good indicators of ecological health because the taxonomy of fishes is well established; extensive information is available on distributions and life histories of most North American species. Fish populations represent a broad spectrum of community tolerances and respond predictably to changes in abiotic factors such as habitat and water quality. The general public can easily relate to statements about the condition of a particular species or the fish community on the whole. Certain key indicators of severely degraded water quality conditions include measures such as the proportion of fish sampled that have deformities (e.g. eroded fins, lesions or tumors). The species composition in a particular habitat is also indicative of overall water quality conditions. For example, a high proportion of highly tolerant species or omnivorous species, especially in comparison to a reference condition site with minimal disturbance, would suggest poor water quality conditions. By comparison, sites with good water quality conditions and high overall ecological integrity, would contain top carnivorous species (e.g. northern pike, burbot), or a relatively high abundance of insectivorous fish such as perch or minnow species.

Study results provide the initial data for the assessment of the potential effects from SD026 on aquatic life (in a laboratory setting and in the field).

3.2 Objective

The objective of the Stream Investigation Plan was to determine whether there is an effect from the existing tailings basin seepage on aquatic life (fish and macroinvertebrates) in Second Creek.

3.3 Scope and Methods

The detailed scope of the Stream Investigation Plan was defined following the review of historical data. The scope of the work consisted of the following activities:

- **Literature review** on the relationship between dissolved solids/conductivity and aquatic life metrics (survival, growth, reproduction, abundance, diversity). A preliminary review has been completed and is summarized in Section 3.4 below.
- **Review data** available for Second Creek that has been generated by other proposed mining operations.
- **Aquatic life** (fish and macroinvertebrate) monitoring and WET testing just downstream of SD026 (in Second Creek) and at a control site.

- **Data analysis** to evaluate the relationship between dissolved constituents and aquatic life. The analysis also includes comparison of the number, relative abundance, and diversity of species in Second Creek (just downstream of SD026) to the control site.
- **Summary report** that provides an evaluation of any impacts to aquatic life associated with the seepage.

3.3.1 Study Sites

A reconnaissance visit to potential stream sites was conducted during the week of April 26, 2010 to identify sites suitable for both fish and macroinvertebrate sampling. Following MPCA Reconnaissance Procedures (Standard Operating Procedures; http://www.pca.state.mn.us/water/biomonitoring/bio-streams-fish.html; accessed on May 4, 2010), stream reaches were evaluated for such characteristics as substrate, morphology, and habitat so that selected reaches would have the potential to support macroinvertebrates and fish. The reconnaissance area encompassed Second Creek from SD026 downstream to County Road 666. County Road 666 is considered the approximate extent of the Cliffs Erie / PolyMet property. The portion of Second Creek downstream of County Road 666 flows through Mesabi Nugget's property and would not be considered pertinent sampling locations to this repermitting effort. Stream reaches included in the Stream Investigation are identified in Figure 3-1.

In Second Creek, between SD026 and County Road 666, one sampling location for macroinvertebrates was identified. This sampling location is just downstream of SD026 (within 0.2 miles downstream of SD026).

The site reconnaissance visit determined that the stream reach within 0.25 miles downstream of SD026 did not have fish habitat. Therefore, no fish sampling was proposed for the stream reach immediately downstream of SD026. In addition, the portion of Second Creek from about 0.25 miles downstream of SD026 to County Road 666 is characterized by open water wetland and numerous beaver ponds. Therefore, no fish sampling was proposed for this upper portion of Second Creek (i.e., no sampling from SD026 to County Road 666).

A control stream was also identified: Bear Creek. The specific stream reach that was determined to be suitable for macroinvertebrate sampling for this study is upstream of monitoring site SW003 (alternatively known as site PM20). The control reach is approximately 0.1 miles to the west of the intersection of County Road 969 (Forrest Road) and County Road 960 (Hayland Road); approximately 2.4 miles north of the intersection of Bear Creek with State Highway 21 (Figure 3-1).

Macroinvertebrate community sampling was conducted at two separate time periods: spring (early June 2011) and summer (late August 2010). Water chemistry data was collected at site SD026 and Bear Creek at the same time that macroinvertebrate sampling was conducted.

Bear Creek served as the control stream for the stream investigations conducted for SD026, SD033, and the Tailings Basin. Macroinvertebrate and fish sampling were conducted in Bear Creek. Because no fish habitat was identified for the upper portion of Second Creek, including the stream reach within 0.25 miles downstream of SD026, no fish sampling was conducted. Therefore, only the water chemistry data and macroinvertebrate data from Bear Creek are included in this report when comparing data from SD026 to the control stream.

3.3.2 Physical Habitat Assessment

In Bear Creek, the monitoring site was composed of a stream reach that was 150 meters in length. However, in Second Creek the stream length for sampling was limited to 70 meters because of a beaver dam upstream and a culvert downstream of the selected stream reach. The respective mid-point, upstream and downstream ends of the reach were marked with surveyor tape and coordinates (NAD 83, Zone 15) were collected using a Global Positioning System (GPS) with submeter accuracy to provide consistency for future sampling efforts.

A physical habitat assessment was completed at the monitoring sites in July 2010 utilizing the MPCA *Physical Habitat and Water Chemistry Assessment Protocol for Wadeable Stream Monitoring Sites* (Appendix 3A).

During the macroinvertebrate survey in June 2011, a physical habitat evaluation was completed for the stream monitoring sites to assess differences and/or similarities between sites using the *MPCA Stream Habitat Assessment Worksheet*, revised 03-07 (Appendix 3-B). Scores for the worksheet are based on a scale from -5 to 100, with higher numbers representing better quality habitat. This field worksheet provided information about the substrates, channel characteristics, riparian characteristics, and general area information.

The streambed gradient for each monitoring site was determined by reviewing ten-foot topographic contours using the digital raster graphic (DRG) developed by the United States Geologic Survey (USGS), which were overlain on the 2010 Farm Services Association (FSA) aerial imagery using ArcMap 9.3. Sinuosity was determined using the 2010 FSA imagery in ArcMap 9.3. The results were used in the MPCA's worksheets to assess the similarities and differences between the physical habitats of the sites. Stream flow was measured at each site using a Marsh McBirney Flo-Mate 2000 flowmeter.

3.3.3 Water Chemistry

Field measurements for water chemistry parameters were collected at SD026 and Bear Creek in July 2010, September 2010, October 2010 and June 2011. The parameters, measured using a YSI multiprobe unit, included dissolved oxygen (DO), temperature, pH, oxidation reduction potential (ORP), specific conductance and turbidity. The protocols for the water chemistry assessment presented in the MPCA document *Physical Habitat and Water Chemistry Assessment Protocol for Wadeable Stream Monitoring Sites* (see Appendix 3-A) were used as a guide for chemical measurement and sampling.

Water samples collected in the field were also processed in the laboratory to measure a suite of physico-chemical variables as well as concentrations of 23 metals including known toxicants. All measured field and laboratory parameters have been summarized in Table 3-1.

Data Analysis

All water chemistry parameters (except pH) and metal concentration values were $\log_{10}$ (Y+1) transformed to improve homogeneity of variances and normality of the data. A spearman rank correlation matrix was used to identify redundancy among the set of variables. In the case where two variables were significantly correlated, only one of the two variables was chosen for further analysis (e.g. total suspended solids and total dissolved solids; Nitrate+Nitrite and Nitrogen (total kjeldahl)).

To determine if the sites, Second Creek (SD026) and Bear Creek, were significantly different based on water chemistry parameters, a randomized block Analysis of Variance (ANOVA) (blocking factor: season) was conducted for each of the measured parameters across sampling periods.

Water chemistry parameter and concentration values from all biological sampling events were combined (July 26, 2010; September 15-17, 2010; October 26, 2010; June 2011), and the average values were compared to the Minnesota Water Quality Standards criteria for each individual parameter value or concentration (including metal concentrations).

Finally, as a further step in determining the overall surface water quality, a water quality index classification system (developed by Prati, et al. 1971) was used to categorize the sites into one of five different water quality classes, each of which corresponds to an “implicit index of pollution” (IIP), ranging from 1-8. The five classes correspond to conditions of ‘excellent’ (index value = 1), ‘acceptable’ (index value = 2), ‘slightly polluted’ (index value = 4), ‘polluted’ (index value = 8) and ‘heavily polluted’ (index value > 8) (terminology as prescribed by Prati, et al. 1971). The parameters evaluated were – dissolved oxygen, pH, 5-day biological oxygen demand (B.O.D.), chemical oxygen demand (C.O.D.), total suspended solids, ammonia, chlorides, iron and manganese. Parameter values were averaged across the four sampling periods. For each parameter, an explicit mathematical function was used to determine the value of each IIP and its corresponding classification.

3.3.4 Whole Effluent Toxicity (WET) Testing

WET testing is a commonly used technique to determine whether constituents in a discharge have additive toxicological effects, or if constituents lacking applicable water quality criterion (with respect to aquatic life, e.g., bicarbonate) may be toxic. This test is conducted in a controlled laboratory environment whereby test species are exposed to a range of effluent and receiving water mixtures. The test is typically conducted in a 125 milliliter cup and the effluent/receiving water mixtures are replaced daily during the test. The test species can vary, but for the purposes of this study the test species used was *Ceriodaphnia dubia* because it is commonly used and is regarded as one of the most sensitive test species. The test was conducted for seven days (a chronic test), and the testing endpoint was survival and reproduction.

WET testing with *C. dubia* is an indicator of the potential for a particular discharge to cause adverse effects to downstream biota. It is important to understand that WET testing is a "potential" indicator because of the sensitivity of the test and because the test results must be interpreted properly with respect to the severity of the test results. For example, mortality is a strong indicator of a potential effect. If there is mortality associated with a test solution that is only the discharge being evaluated, there is a potential to affect downstream aquatic life on some level, although there remains some uncertainty given the sensitivity of the test. However, if the effluent causes mortality with a highly diluted (e.g., 12 percent discharge and 82 percent receiving water) test solution, it can be interpreted that the discharge has a much greater potential to affect downstream aquatic life.

Reproduction is a more sensitive indicator since reproduction is much more easily disturbed by discharges that in some cases are not toxic but simply have a chemical composition that *C. dubia* are not accustomed to. The results of the WET tests discussed below must be interpreted with respect to the gradient of results that WET tests can provide.

WET testing was required for two discharge locations; SD033 (Area 5) and SD026. For efficiency and convenience, the water sampling and WET testing for SD026 and SD033 were conducted simultaneously and laboratory reports include the results from both SD026 and SD033.

Water was collected from SD026, SD033, and the control stream (Bear Creek) for WET testing on July 26, 2010, October 26, 2010, and June 2, 2011. For each WET test event, water was collected from SD026 and from a water body that is either unaffected by mining activity, can be considered as background, or the water body was downstream of the mining-affected outfall and hence consisted of a mixture of mining and background waters. For all WET tests, the background (control) water was obtained from Bear Creek. For WET tests for site SD026, water was also collected from the Partridge River (just upstream of the confluence of Second Creek with the Partridge River) (i.e., a receiving water) and used as dilution water for the October 2010 and June 2011 WET tests, respectively.

For the October 2010 and June 2011 WET tests, water samples downstream of the respective discharge locations were also collected. Samples for WET testing and water chemistry were collected from Second Creek (Site PM17, downstream of SD026).

Mixtures of permitted discharge waters (SD026) and background waters were prepared in the WET testing laboratory to evaluate whether there were biologically perceptible differences between the mining-related water and the background (Bear Creek) and receiving water (Partridge River for SD026). The degree of difference can be determined using two statistics: (1) the NOEC (no observed effect concentration) is used for mortality to determine the concentration of effluent-receiving water mixtures which cause no mortality effects, and (2) the IC25 (concentration at which there is a 25 percent decrease in young production) which is based upon reproduction and is a more sensitive indicator. If the NOEC is > (greater than) 100 percent, then there is no statistically significant difference between the permitted discharge waters and the background or receiving water. If the IC25 is > 100 percent, this also means that there is no statistically significant difference between the receiving water and the effluent with respect to reproductive capacity. If the NOEC or the IC25 are less than 100 percent, then it can be concluded that the biological properties of the discharges are different from the receiving water.

Results of data collected and analysis performed are provided in this report. WET testing and chemical data for SD026 are provided in this report. However, in order to have a large enough data set that could be statistically analyzed (e.g., the number of response variables-survival and reproduction, had to be large enough to provide enough degrees of freedom), data were combined for outfalls SD033 and SD026; all background waters and all downstream waters. Using the entire data set, multivariate logistic regression, which is similar to linear regression but the curve has an S-shape, was used to identify those chemical constituents that appear to have the most influence on the WET testing results. Once the best logistic regression model was built, it was used to determine the importance of the monitored constituents on the WET testing results.

3.3.5 Macroinvertebrates

Biological monitoring required an assessment of the status of the biota in terms of the physical, chemical and biological conditions of the water body. Biological monitoring in Bear Creek and Second Creek assessed macroinvertebrate communities. The physical components of the streams were measured utilizing stream geomorphology concepts and data, while parameter values and chemical concentrations were obtained from the analysis of water samples that were collected in July 2010, September 2010, October 2011 (for WET test purposes) and June 2011 (field and laboratory analysis).

The MPCA Standard Operating Procedures (SOPs) were followed for this study.

Macroinvertebrate Sampling

Aquatic macroinvertebrates were sampled using the MPCA multi-habitat sampling procedures (MPCA protocol EMAP-SOP4 (Appendix 3-C)). For each site, the relative proportion of available habitat was identified and the various habitats of Second Creek were sampled according to their relative proportion to obtain similar samples of macroinvertebrates. A total of 20 samples were collected at each site. All macroinvertebrates were collected using D-frame dip nets.

The debris (large twigs, leaves, plants, rocks, etc.) was washed with stream water, visually inspected and discarded. Collected macroinvertebrates were composited in a sieve bucket, transferred into 500-ml plastic bottles, and preserved in 85 percent reagent alcohol. All containers were labeled (inside and outside) with information including site identification, habitat type and collection date.

Macroinvertebrates were sorted using the MPCA *Invertebrate Identification and Enumeration* (SOP BMIP03; Appendix 3-D) procedures as a reference. Macroinvertebrates were identified by Dr. Dean Hansen, and the MPCA procedures were provided to Dr. Hansen. Macroinvertebrates were identified to the genus level if at all possible for all organisms. Large macroinvertebrates were picked and identified for the entire sample.

Measures of Biological Diversity – Macroinvertebrate Community

Biological monitoring can be used to evaluate the relative condition of biological communities in streams. This monitoring is usually conducted in association with physical and chemical monitoring at the site to assess all aspects of the stream reach. Several metrics can be used to evaluate and compare the biological communities of streams.

Abundance

Abundance (n) for a site was determined as the total number of organisms collected in the sampling effort. Samples were subsampled to a minimum of 300 organisms as per MPCA's general guidelines for aquatic invertebrate monitoring in streams (http://www.pca.state.mn.us/index.php/water/water-monitoring-and-reporting/biological-monitoring/stream-monitoring/stream-monitoring-aquatic-invertebrates.html?menuid=&redirect=1#sops; Date Accessed: August 29, 2011).

Richness

For the macroinvertebrate data, the number of families and genera was used to determine richness.

Shannon-Wiener Diversity Index

The Shannon-Wiener Diversity Index (H´) was used in conjunction with abundance and richness to detect environmental disturbances that may cause a decrease in diversity. H´ is calculated as:

$$H' = -\sum_{i=1}^{s} (n_i/n)\ln_2(n_i/n),$$

where n is the total number of individuals of all taxa, n_i is the number of individuals in the i^{th} taxon, and s is the total number of taxa in the community. The values of n and s were used as previously indicated for abundance and richness.

Evenness

Evenness was calculated to determine how equally abundant the species are among the families. Evenness (E) was calculated as:

$$E = H'/\ln s$$

where H´ is the calculated Shannon-Wiener Diversity Index and "ln s" is the natural logarithm (ln) of the total number of taxa in a community (s). High evenness occurs when species are equal or nearly equal in abundance and it is usually equated with high diversity. The maximum diversity would be possible if all species were equally abundant. By contrast, low evenness occurs when one or more species dominate the community which indicates low diversity.

Hilsenhoff Biotic Index (HBI) for Macroinvertebrates

The 2010 and 2011 macroinvertebrate data were evaluated using the Hilsenhoff Biotic Index (HBI). The Hilsenhoff Biotic Index (HBI) provides a method to assess water quality based on taxa pollution-tolerance (Hilsenhoff 1987). The HBI was developed from research on more than 1,000 small streams in Wisconsin (Hilsenhoff, 1982 and 1987). Small streams typically have a naturally low biological diversity, which is unrelated to their water quality. Small low-gradient streams in northeast Minnesota are also generally naturally low in DO without the introduction of nutrient or organic pollutants. Other water quality indices attribute biological diversity to stream condition and water quality. However, research indicates the HBI does an excellent job of ranking small streams in this region according to their stream condition.

The HBI was developed using macroinvertebrate populations in streams with a range of organic and nutrient levels, and therefore DO levels. The HBI is typically used to measure biodiversity in streams that may be affected by nutrient or organic pollution that causes excessive plant growth which reduces the DO and may affect the growth of other aquatic biota (e.g. macroinvertebrates). In general, species resident in streams with high organic levels and low DO levels were assigned high tolerance values and those species absent from these types of streams were given lower tolerance values. Using the tolerance values developed by Hilsenhoff and the U.S. Environmental Protection Agency (EPA) (*Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers*, July 1999), every species or genus identified at the monitoring sites has been assigned an index value from 0-10, with 0 assigned to the most intolerant species and 10 assigned to the most tolerant species. Species with tolerance values that are less than or equal to 3 are considered to be sensitive (intolerant) and species with values greater than or equal to 7 are considered to be tolerant.

When evaluating water quality conditions at a site, only those taxa with assigned tolerance values are included in the analysis. The HBI is an average of tolerance values for all individuals collected from a site. The calculations result in a HBI value that is a tolerance score for the sample weighted by the number of individuals in each contributing taxon. The calculated HBI scores can range from 0 to 10.

An HBI score at the high end of the scale indicates the macroinvertebrate community is dominated by pollution-tolerant taxa and that the site has some amount of pollution or that conditions are stressing the resident populations. A score at the low end of the scale indicates the macroinvertebrate community is dominated by organisms intolerant of pollution or stressor conditions (i.e., sensitive taxa) and implies that the water quality is good.

It is noted that the stream evaluations based on the HBI may underestimate the biologic integrity of the streams discussed in this report. The HBI is generally a measure of organic or nutrient pollution which affects organisms resulting from low DO or fluctuating DO levels. The study streams may have naturally low DO levels since they generally flow through wetland complexes and may not have any relationship to “organic pollution”. However, even with these limitations, the HBI values are presented as a method for comparing the streams included in this study.

Other Biotic Measures of Integrity for Macroinvertebrates

There are other metrics or measures of biological communities that are often used to provide some additional understanding of biological communities. The metrics that include composition and habitat include percent Ephemeroptera, Plecoptera, and Tricoptera (% EPT); percent Ephemeroptera, Plecoptera, Tricoptera, and Odonata (% EPTO); and percent insecta versus percent non-insecta.

Composition metrics require identification of key genera and their associated ecological patterns. The presence of a nuisance genus, or notable lack of a preferred genus, relates to stream condition. Composition metrics also provide information on the relative contribution of the genera to the total assemblage. There is a high level of redundancy in the input values used to calculate various composition metrics when the pollution tolerant genera are dominant and there is low diversity, and estimated scores tend to be similar.

Habitat metrics explain the morphological adaptation of genera for feeding and movement in the aquatic habitat. Insects are clinger taxa and require adaptations for attachment in flowing water to maintain position. Typically, with increased pollution, the number of insect taxa decreases. These additional biotic metrics can be used to provide additional understanding of macroinvertebrate populations at each site.

The EPA Biological Indicators of Watershed Health (2007) identifies the benthic macroinvertebrate orders that indicate stream health. In a degraded stream, pollution tolerant organisms (midgeflies, worms, leeches, pouch snails) would dominate the population. In comparison, sites dominated by sensitive (stoneflies, riffle beetles, mayflies) and moderately tolerant (dragonflies, crayfish, scuds, blackflies, caddisflies) orders indicate good stream health.

3.4 Results and Discussion

Results for the stream habitat surveys, surface water samples (chemistry), WET testing and macroinvertebrate sampling are presented and discussed in the following sections.

3.4.1 Physical Habitat

The physical and chemical measurements that were taken in the field during the macroinvertebrate surveys are presented in Table 3-2. The water level was within normal levels in all streams based on observations of vegetation along the bank. The water level was within the banks of all streams when the macroinvertebrate samples were collected.

With regard to precipitation, the following is noted:

- There was 0.24 inches of rainfall in the seven days prior to sampling on September 15 and 17, 2010, with the 0.24 inches occurring on September 11 (precipitation data from state climatologist network, Station: 210390 Babbitt 2SE, http://climate.umn.edu/HIDradius/radius.asp). In addition, during the day on September 16 there was 0.17 inches of rain.

- In the seven days prior to the June 2, 2011 sampling, there was 0.73 inches of rain, occurring on May 28 (0.15 inches), 29 (0.53 inches), and 31 (0.05 inches).

- Recent precipitation data were compared to historic data for evaluating annual and monthly deviations from normal conditions and to determine if the macroinvertebrate sampling and water chemistry were representative of "normal" conditions. Precipitation data were obtained from the Minnesota Climatology Working Group, Wetland Delineation Precipitation Data Retrieval from a Gridded Database (http://climate.umn.edu/wetland/) for St. Louis County, Township 60N, Range 13W, Section 1. Precipitation during the 2 months prior to the 2010 sampling was above normal in July and August. In 2011, the previous 2 months prior to sampling were above the normal range in April and within the normal range in May).

The precipitation data suggests that sampling in September 2010 and June 2011 was conducted during a wet period; however, water levels in the streams were within the banks and do not indicate that sampling was conducted during high flow or flooding conditions. Therefore, the macroinvertebrate sampling is considered to have been completed under relatively normal precipitation conditions.

Reference Stream – Bear Creek

For the stream reach assessed, available habitat types at Bear Creek included undercut banks/overhanging vegetation, woody debris, emergent vegetation and sediment (Table 3-2). The riparian zone was characterized by reed canarygrass, alders and willows. The substrate included muck and detritus. The Qualitative Habitat Evaluation Index (QHEI) for the MPCA worksheet was 44/100. The lower Index score reflects the low diversity of habitat types, substrate and in-stream cover. Discharge (in cubic feet per second, cfs) was higher in 2011 compared to 2010, with a maximum water depth of 1.8 feet. The stream shading was similar in 2010 and 2011 for the reach. The water temperature ranged from 10.2 ºC (2010) to 15.7 ºC (2011). Specific conductivity ranged from 105 µmhos (2010) to 62 µmhos (2011). The pH ranged from 6.9 (2010) to 6.4 (2011). Dissolved oxygen values were 6.4 ppm in 2010 and 6.8 ppm in 2011.

SD026 – Second Creek

Available habitat types at Second Creek included woody debris, emergent vegetation, undercut banks/overhanging vegetation, and sediment (Table 3-2). The riparian zone was characterized by reed canarygrass, grasses, willows and alder shrubs, birch, and other larger trees. The substrate included boulders, gravel, silt and detritus. The QHEI for the MPCA worksheet was 69/100. The higher Index score reflects the higher diversity of habitat types, substrate and in-stream cover. Discharge (cfs) was slightly lower in 2011 compared to 2010, with a maximum water depth of 1.1 to 1.3 feet. Discharge is controlled at the upstream end of the reach by a beaver dam. The stream shading was similar in 2010 and 2011 for the reach. The water temperature ranged from 10.7 ºC (2010) to 11.5 ºC (2011). Specific conductivity ranged from 1,206 µmhos (2010) to 1,019 µmhos (2011). The pH ranged from 7.7 (2010) to 8.0 (2011). Dissolved oxygen values were 7.3 ppm in 2010 and 8.4 ppm in 2011.

3.4.2 Water Chemistry

Water chemistry data collected from July 2010, September 2010, October 2010 and June 2011 were evaluated.

General Comparison and Evaluation

Bear Creek and Second creek (SD026) were significantly different based on 7 of the 33 measured water chemistry parameters (Table 3-3). The following is noted:

- Of the general chemistry parameters, total hardness, total dissolved solids and sulfate were significantly higher in Second Creek (SD026) compared to Bear Creek.

- Of the metal concentrations, boron, magnesium, molybdenum and sodium were significantly higher in Second Creek (SD026) compared to Bear Creek.

Comparison to Surface Water Standards and Criterion

The average parameter values were compared against the Minnesota Water Quality (WQ) Standards and Aquatic Life Criteria for surface waters. Of the 18 parameters for which criterion values are available for comparison, Bear Creek met the criteria for 17 parameters and Second Creek (SD026) met the criteria for 16 parameters (Table 3-4). No aquatic life criteria were exceeded.

For those parameters that did not meet the relevant surface water standard, the following is noted:

- Average dissolved oxygen (DO) concentration of 4.8 mg/L in Bear Creek was slightly lower than the daily minimum standard of 5.0 mg/L; however, this was not surprising because Bear creek is a low gradient and slow moving stream that drains a wetland complex. Low dissolved oxygen is typical of these stream reaches in the region.

- Average total hardness value of 621 mg/L for Second Creek (SD026) exceeded the standard of 305 mg/L.

- Average specific conductance at Second Creek (SD026) was 1,144 µmhos/cm, exceeding the surface water quality standard of 1,000 µmhos/cm.

Water Quality Classification Index

Based on the water quality classification index (Prati, et al. 1971), results were variable and dependent upon specific parameters evaluated. The following is noted with regard to the index values calculated for Bear Creek and Second Creek (SD026) (Table 3-5):

- The sites were rated as 'excellent' for the following parameters: biological oxygen demand, chlorides, pH and total suspended solids.

- Chemical oxygen demand (C.O.D.) was highest at Bear Creek, classifying the site as 'slightly polluted-polluted; however, by comparison, Second Creek fell under the classification of 'excellent-acceptable' based on C.O.D. values.

- Based on DO values, Second Creek (SD026) was classified as 'acceptable-slightly polluted'. Although the DO values at Bear Creek classified the site as 'slightly polluted-polluted', the physical characteristics of the stream contribute to the comparatively lower DO values and therefore, the classification is not indicative of a disturbance at the reference site.

- Concentrations of iron were relatively higher at Bear Creek, classifying the site as ‘heavily polluted’. By contrast, iron levels at Second Creek placed the site as ‘acceptable-slightly polluted’.

- Manganese concentration at Second Creek was relatively higher than at Bear Creek (classified as “acceptable-slightly polluted”), classifying Second Creek as ‘slightly polluted-polluted’

Overall, in comparison to the reference site (Bear Creek - which was generally classified as ‘excellent’ or ‘acceptable’ for 5 of the 8 parameters evaluated in the index), Second Creek was generally classified as ‘excellent’ or ‘acceptable’ for 7 of the 8 parameters evaluated in the index (Table 3-5).

3.4.3 Whole Effluent Toxicity (WET) Testing

Literature Review

The available literature indicates that toxicity can occur over a range of dissolved solids concentrations: acute toxicity can occur over a range of ~ 325 mg/L to ~ 5,100 mg/L and chronic toxicity has been shown to occur over a narrower range of values, approximately 29 mg/L up to ~ 2,000 mg/L. It is suspected that some other toxicant may have been influencing the study that produced the chronic toxicity value of 29 mg/L, but the study in question did not identify other potential sources of toxicity in the effluent being tested. The difference in toxicity is due largely to the ions that compose the dissolved solids (i.e., sodium, calcium, magnesium, potassium, sulfate, chloride bicarbonate). In general, the most toxic ions to freshwater organisms are potassium and bicarbonate. Several studies have identified that potassium and magnesium can be more toxic than sulfate. However, the mixture of ions is very important in determining the toxicity of any discharge water and the potential contribution of sulfate to toxicity is an important consideration in any WET testing to be conducted.

Because the ion composition of the discharge water is important to assessing potential toxicity, samples of the discharge water from Second Creek (SD026) were collected and analyzed for a number of specific ions to support the Stream Investigation work and the WET testing.

General Toxicological Results

A summary of the chronic WET testing results for outfall SD026 and for tests with Second Creek water from site PM17 (just upstream of County Highway 666) are provided in Table 3-6. Mixtures of SD026 water with Bear Creek, Embarrass River, and synthetic laboratory water were tested (mixtures were 12.5, 25, 50, 75 and 100 percent SD026 water). Test statistics in Table 3-6 include survival in 100 percent effluent, IC25, and NOEC. It can be seen that *C. dubia* survival was 100 percent in 100 percent SD026 water for the October 2010 and June 2011 tests but survival was 80 percent in July 2010. For the July 2010 test, survival was 100 percent when diluted to 75 percent concentration with Bear Creek water. Overall, there appears to be little potential for SD026 water to cause mortality to zooplankton and other invertebrates of similar sensitivity to *C. dubia*. It should also be noted that there was 100 percent survival for water collected downstream of SD026 (Second Creek at PM17).

WET testing endpoints, which are based upon reproduction (see IC25 and NOEC values in Table 3-6), provide more sensitive indicators of the potential for SD026 to affect biota in the downstream receiving water (Second Creek immediately downstream and Partridge River further downstream). Summary results include the following:

- For the first test in June 2010, Bear Creek was used as the diluents as a first screen to provide a direct comparison of SD026 results with control stream results. The IC25 and NOEC for that test was 82.6 and 75 percent, respectively. This indicates that the reproductive potential of *C. dubia* and species of similar sensitivity to *C. dubia* would be hindered by 25 percent compared to Bear Creek until SD026 water is diluted below a concentration of 75 to 82.6 percent.

- For the October 2010 test, two dilution series were run with SD026 water. The first dilution series used laboratory reconstituted water as the diluents (a standard approach for WET tests) and the IC25 was 100 percent and the NOEC was 100 percent when compared to the laboratory reconstituted water. In the second dilution series using Partridge River water as the diluents, the IC25 was 100 percent and the NOEC 50 percent when compared to Partridge River water.

  It is noted that the number of young produced per adult *C. dubia* for SD026 water was similar in the October 2010 test (18.6 young per adult with a NOEC of 100 percent for dilution series #1 and 50 percent for dilution series #2) and the July 2010 test (reproduction rate was 18.2 and a NOEC of 75 percent) (Table 3-6).

One factor affecting the different results for the July 2010 test and the October 2010 test is the reproduction of *C. dubia* in the dilution water. In the July 2010 test, Bear Creek water was used as the diluent and *C. dubia* reproduction was 30.3 young per adult (very high). In that July 2010 test, the C. dubia reproduction rate was 18.2 for SD026 water (Table 3-6). When the WET test statistics were calculated they showed reproduction was hindered in the SD026 water. In the October 2010 test, laboratory reconstituted water was used as the diluent and *C. dubia* reproduction was 18.3 young per adult. The number of young per adult *C. dubia* was 18.6 for SD026 water, 22.2 for Bear Creek water, and 22.1 for Partridge River water, respectively. The WET test statistics for the October 2010 test indicate no hindrance of *C. dubia* reproduction in SD026 waters compared to the laboratory reconstituted water, but the statistics do suggest some affect when compared to Partridge River water (IC25 > 100 percent but NOEC = 50 percent).

The dilution water plays an important role in the WET test statistics. The high reproduction rate in the Bear Creek water in the July 2010 test (30.3 young per adult *C. dubia*) resulted in reproduction in SD026 (18.2 young per adult) to be considered "hindered". Yet, a reproduction rate of 18.6 young per adult in SD026water for the October 2010 test indicated no hindrance of reproduction when compared to the reconstituted dilution water or to Partridge River water (22.1 young per adult). Therefore, there is uncertainty as to whether there was an actual toxicity effect or that reproduction was truly hindered in SD026 water for the July 2010 test.

- For the June 2011 test, the IC25 and NOEC were 91 and 75 percent, respectively (Partridge River water was the diluents). The number of young produced per adult C. dubia was 11.4 for SD026 water, notably lower than in the other two WET tests.

The full laboratory report for each WET Test is provided in Appendix 3-E to this report.

Because the results for the three WET tests were variable, and in particular because the reproduction rate for SD026 water in the spring 2011 test was lower than in the previous two tests, an additional assessment of the WET test data was conducted.

Evaluation of Chemical Drivers of WET Testing Results

For this analysis, water chemistry data and WET test results for SD033 and SD026 were combined to provide a more robust assessment and to provide a better opportunity to identify the chemicals likely influencing the WET test results.

For each WET test, the number of young produced per adult *C. dubia* are counted for the seven day duration of the test. There are some differences in young production for SD026 water compared to all of the receiving waters considered to be background (Bear Creek, Embarrass River, and Partridge River). If all of the WET testing and chemical data collected as part of this study are considered as one group, a statistical analysis can be conducted in an attempt to understand why the receiving waters may behave differently than the outfall waters.

The WET testing and chemical analytical data were organized as shown in Table 3-7 for waters corresponding to outfall SD026. WET test results for SD033 and corresponding background and downstream waters were also organized as in Table 3-7. A regression analysis was then conducted to formulate a relationship between water chemistry and WET results. Four different models were built and the goodness of fit for each model was then evaluated by comparing the observed to the model-predicted young production (see Figure 3-2). These models were then used to identify the relative importance of the different chemical constituents for young production.

There is a clear difference between the chemical composition of outfall SD026 water and the various receiving waters (Table 3-8, Figure 3-3). From Table 3-8 it can be seen that outfall water (SD026 and SD033 are averaged in Table 3-8) is elevated compared to background for alkalinity, magnesium and calcium (note: magnesium and calcium displayed in Table 3-8 as the ratio of magnesium to calcium), sulfate, and potassium. These parameters are traditionally associated and are elevated by iron mining operations in the Iron Range of northern Minnesota. Several constituents are lower in the outfall waters compared to background, for example, barium, cobalt, copper, iron, dissolved or total organic matter, total phosphorus, and total nitrogen.

It is noted that the best regression model with the fewest parameters includes the variables described above that are lower in the outfall water (e.g., iron, dissolved organic matter, etc.) plus nickel (r^2 = 0.79). This finding is supported by simple regression analysis of individual chemical constituents and young production (Figure 3-4 and 3-5, respectively).

Model 4 (r^2 = 0.86; see Figure 3-2) includes constituents that are both higher and lower in the outfall water compared to the background waters – this model was used to evaluate the relative effect of constituents higher in the outfall water compared to constituents that are lower. Table 3-9 shows the results of this analysis. The table shows that if the parameters with lower concentrations in the outfall waters (SD026, SD033) are held constant at monitored concentrations, and the other parameters found to be elevated in the mining water (e.g., sulfate) are reduced to approximately background concentrations, there is no predicted effect on young production. What this indicates is that the parameters at elevated levels in the mining outfall water (e.g., sulfate, Mg/Ca ratio) are not likely responsible for the observed differences in WET testing results (with respect to *C. dubia* young production) between outfall waters and receiving water. Rather, the regression analysis indicates that the chemicals likely having the most effect on WET test results are those parameters at low levels in the outfall discharges (barium, cobalt, copper, iron, dissolved or total organic carbon, total phosphorus, and total nitrogen).

It is noted that copper, phosphorus, and nitrogen are micronutrients for zooplankton and low concentrations of these parameters in SD033 and SD026 water may be influencing the WET test results. If one or more of these low-concentration parameters (e.g., dissolved organic carbon) are increased in the Model 4 inputs there would be a notable increase in predicted number of young. Dissolved organic carbon is singled out here because Figure 3-5 identifies that there is a relatively strong relationship between dissolved organic carbon concentration and number of young produced per adult *C. dubia*.

Mining-related waters have very little dissolved organic carbon (approximately 5 mg/L for SD026 water compared to 22 mg/L for background waters; Table 3-8). The relationship of dissolved organic carbon and young produced (Figure 3-5) is assumed to be influenced by higher concentrations of dissolved organic carbon in background waters (e.g., Embarrass River, Partridge River, Bear Creek) and downstream waters (e.g., Second Creek, PM17). As dissolved organic carbon concentrations increase, the number of young produced increases (Figure 3-5). This relationship is consistent with other data and evaluations conducted for other mining projects in the Aurora-Hoyt Lakes area and it suggests that the WET test results for SD026 may be influenced by a lack of nutrients (i.e., lack of a carbon source for energy).

Studies have shown that higher dissolved or total organic carbon improves growth and reproduction of aquatic life. The analysis results indicate that the mining-related discharge water is low in these important micronutrients, and low in an energy source (such as total organic carbon or dissolved organic carbon). Therefore, the lower number of young produced in the spring (June 2011) test may be more related to oligotrophic conditions in the Tailings Basin (source of the water to SD026) than representing a “toxic effect” from a high dose of a particular parameter. The WET tests suggest a potential seasonality in the data, with lower number of young produced in the spring (June 2011) test as compared to the summer (July 2010) and fall (October 2010) tests (Table 3-6; Table 3-7).

Dilution of mining-related water may be more pronounced in spring time due to further dilution with snowmelt water.

Assuming that the response of WET test species *C. dubia* can act as a surrogate for the expected response of aquatic life in the actual receiving stream, this analysis suggests that a simple reduction in the constituents that currently have elevated concentrations tailings basin seepage will not improve the suitability of water from outfall SD026 for aquatic life. Rather, the analysis is suggesting that a lack of nutrients in the mining-related discharge water may be playing a greater role than previously expected.

Overall, because the chronic WET test results do not indicate mortality of C. dubia, it is unlikely that water from SD026 has, or will, adversely affect aquatic life in downstream waters. Reproduction (which is a much more sensitive indicator than mortality) of the test species *C. dubia* was reduced in two tests compared to the reference site Bear Creek and the Partridge River. However, reproduction was not severely reduced in SD026 water compared to the reference site or receiving water (Partridge River) and for one test there was no significant difference between SD026 and the reference sites. Therefore, the WET test results indicate that the potential for actual adverse effect to aquatic life is low.

3.4.4 Macroinvertebrate Survey Data and Assessment

The total number of macroinvertebrates sampled in each stream segment is provided in Table 3-10. The data presented in Table 3-10 were then used to prepare other tables discussed in this section and related to macroinvertebrate survey results.

Taxa

Reference Stream – Bear Creek

Taxa collected at Bear Creek in 2010 and 2011 represented 6 classes and 14 orders (Tables 3-11 and 3-12, respectively). There were 32 families collected in 2010 and 34 families collected in 2011 (Table 3-2). The **classes** and orders collected in 2010 and 2011 included: **Insecta (insects) –** Coleoptera (beetles), Diptera (true flies), Ephemeroptera (mayflies), Odonata (dragonflies), Megaloptera (alderflies and dobsonflies), Lepidoptera (moths and butterflies), Plecoptera (stoneflies) and Trichoptera (caddisflies); **Crustacea (crustaceans) –** Amphipoda (scuds) and Decapoda (crayfish); **Entoprocta (brozoans)**; **Annelida (segmented worms) –** Oligochaeta (aquatic worms), Arhynchnobdellida (leeches) and Rhynchnobdellida (leeches); **Gastropoda (snails)** – Basommatophora (snails); **Bivalvia (bivalve clams)** – Veneroida (clams); **Malacostraca**

(crustaceans) – Isopoda (pillbugs and sowbugs); **Hydrozoa (hydrozoans) –** Hydroida (hydra)**;** and **Nematoda (roundworms)**.

Classes identified at the site in 2010 and 2011 included insects, crustaceans, segmented worms, snails, and clams. Classes only identified in 2010 and 2011 were bryozoans and hydrozoans, respectively. Dominant classes in 2010 and 2011 were insects, segmented worms and crustaceans.

Orders that were identified at the site in 2010 and 2011 included beetles, true flies, mayflies, dragonflies, moths and butterflies, caddisflies, scuds, aquatic worms, leeches, snails and clams. Orders only identified in 2010 included crayfish, bryozoans and alderflies, dobbonflies and fishflies. Orders only identified in 2011 included stoneflies and hydra. Dominant orders in 2010 were true flies, caddisflies, aquatic worms and scuds; and in 2011 were mayflies, true flies, scuds and aquatic worms.

SD026 – Second Creek

Taxa collected at Second Creek in 2010 and 2011 represented 6 classes and 11 orders (Tables 3-11 and 3-12, respectively). There were 25 families collected in 2010 and 17 families collected in 2011 (Table 3-2). The **classes** and orders collected in 2010 and 2011 included: **Insecta (insects) –** Coleoptera (beetles), Diptera (true flies), Ephemeroptera (mayflies), Odonata (dragonflies), and Trichoptera (caddisflies); **Crustacea (crustaceans) –** Amphipoda (scuds); **Annelida (segmented worms) –** Oligochaeta (aquatic worms) and Rhynchobdellida (leeches); **Gastropoda (snails)** – Basommatophora (snails); **Bivalvia (bivalve clams)** – Veneroida (clams); and **Malacostraca (crustaceans) –** Isopoda (pillbugs and sowbugs).

Classes identified at the site in 2010 and 2011 included insects, crustaceans, segmented worms, snails, and clams. Classes only identified in 2010 and 2011 were bryozoans and hydrozoans, respectively. Dominant classes in 2010 were insects and crustacean; in 2011 were insects.

Orders that were identified at the site in 2010 and 2011 included beetles, true flies, mayflies, dragonflies, caddisflies, scuds, aquatic worms, leeches, snails, clams and pillbugs and sowbugs. Orders only identified in 2010 included beetles, dragonflies and leeches. Orders only identified in 2011 included pillbugs and sowbugs. Dominant orders in 2010 were true flies, caddisflies, aquatic worms and scuds. Dominant orders in 2010 were caddisflies, mayflies, true flies, scuds and clams; and in 2011 were caddisflies, true flies and mayflies.

Abundance and Richness

For Bear Creek (reference stream), the abundance of macroinvertebrates in September 2010 and June 2011 was 2,787 and 1,113, respectively (Table 3-11). By comparison, in Second Creek (SD026), the abundance of macroinvertebrates in September 2010 and June 2011 was 2,534 and 838, respectively (Table 3-11). The difference in abundance reflects the seasonal emergence of adults such as caddisflies, mayflies and black flies.

Richness describes the number of families or genera present within a sampled group.

- For Bear Creek (reference stream), in 2010 there were 32 families and 46 genera collected; in 2011 there were 34 families and 43 genera collected from the site (Tables 3-2 and 3-11).

- For Second Creek (SD026) in 2010 there were 25 families and 32 genera collected; in 2011 there were 17 families and 19 genera collected from the site (Tables 3-2 and 3-11).

Shannon-Wiener Diversity Index (H´) and Evenness

For Bear Creek, the H´ scores were similar in 2010 and in 2011, while for Second Creek (SD026), the H´ score decreased in the second year.

- Bear Creek (reference stream): 2010 H´ = 2.91; and 2011 = 2.42 (Table 3-2).

- Second Creek (SD026): 2010 H´ = 3.15; and 2011 = 1.24 (Table 3-2)

Evenness scores were considered similar for Bear Creek and Second Creek. For Bear Creek, evenness scores were similar for both years, but for Second Creek (SD026) the scores were considered to be different.

- Bear Creek: Evenness scores were 0.75 in 2010 and 0.64 in 2011.

- Second Creek (SD026): Evenness scores were 0.89 in 2010 and 0.41 in 2011.

The index is increased either by having additional unique species or by having a greater evenness. Typically, the value of the index ranges from 1.5 (low species richness and evenness) to 3.5 (high species richness and evenness).

Overall, the H´ and evenness scores indicate similarity between the stream sites.

Hilsenhoff Biotic Index

The HBI values are scaled to **indicate improving biotic condition with decreasing values** (Table 3-14).

- Bear Creek: HBI score was 6.36 (“fairly poor”) in 2010 and 5.94 (“fair”) in 2011 (Tables 3-2 and 3-15). In 2011, the number of tolerant taxa (tolerance score $\geq$ 7) decreased slightly which slightly improved the HBI rating from “fairly poor” to “fair”.

- Second Creek: HBI score was 4.53 (“good”) in 2010 5.11 (“good”) in 2011 (Tables 3-2 and 3-15). In 2011, the number of tolerant taxa (tolerance score $\geq$ 7) decreased slightly however, the number of sensitive taxa (tolerance score $\leq$ 3) decreased over 15 percent which decreased the HBI value, although the rating remained “good”.

Other Measures of Biotic Integrity

The percentage composition of Ephemeroptera, Plecoptera and Trichoptera (% EPT) and Ephemeroptera, Plecoptera, Trichoptera and Odonata (% EPTO) are other methods used to evaluate macroinvertebrate data. These species are generally considered to be in more environmentally sensitive Orders and thus are better indicators of the stream quality or are more sensitive to stress.

Another composition metric used to evaluate macroinvertebrate data includes percentage composition of black flies (Simulidae), non-insects (Non-Insecta), true flies (Diptera) and midges (Chironomids).

Results for the other measures of biotic integrity for each stream site are presented below

Reference Stream – Bear Creek

In 2010, there were 14 EPT and 19 EPTO genera collected in the stream; in 2011, there were 9 EPT and 12 EPTO genera (Table 3-2).

The % EPT and EPTO ranges from 24 percent to 37 percent over the two sampling events (Table 3-2). In 2010 caddisflies were one of the dominant orders, while in 2011; mayflies were a dominant order (Table 3-13). Most of the caddisfly and dragonfly species present at the site tend to be the more tolerant species that can adapt to a wide range of environmental conditions; however, there are species present with tolerance values $\leq$ 3 (Table 3-15). No riffles were present at the site, so most of these organisms were either found on overhanging vegetation or woody debris.

The abundance of black flies (moderately sensitive) was 11 percent in 2010 and 15 percent in 2011 (Table 3-2). The percentage composition of non-insect individuals was lowest at the reference site, Bear Creek, compared to all other sites (Table 3-2). True flies comprised about one-third of the macroinvertebrates at the site, with chironomids (bloodworms) accounting for 20 to 30 percent of the true flies. The higher percentage of chironomids is typically found in slow-moving, low DO streams typically found in this area.

SD026 – Second Creek

In 2010, there were 9 EPT and 12 EPTO genera collected in the stream; in 2011, there were 7 EPT and 7 EPTO genera present (Table 3-2).

The % EPT and EPTO ranges from 72 percent to 77 percent over the two sampling events (Table 3-2). In 2010 and 2011 caddisflies accounted for over 63 percent of the individuals present at the site (Table 3-13). Most of the caddisfly and dragonfly species present at the site tend to be the more tolerant species that can adapt to a wide range of environmental conditions; however, there are species present with tolerance values $\leq$ 3 (Table 3-15). Riffles, with cobbles, were present at the site which provided habitat for caddisfly genera.

The abundance of black flies (moderately sensitive) was 1 percent in 2010 and 13 percent in 2011 (Table 3-2). The percentage composition of non-insect individuals was 83 percent at the site in 2010 and 96 percent in 2011 (Table 3-2). True flies comprised about less than 20 percent of the macroinvertebrates at the site, with chironomids (bloodworms) accounting for 47 percent of the true flies in 2010 with no chironomids collected in 2011.

3.5 Conclusions

Chemistry

The chemical composition of water from the permitted outfall SD026 is different from the composition of the receiving water--Second Creek, and is different from waters that served as reference or background sites for this field investigation. Samples from SD026 had elevated concentrations with respect to total dissolved solids, hardness, sulfate, boron, sodium, magnesium and molybdenum. Copper was also slightly elevated for SD026 compared to background. SD026 was also lower for several constituents, including organic carbon, total nitrogen, total phosphorus, total suspended solids, barium, and iron. Except for the possibility of copper and chloride, constituents found to be elevated at SD026 are not traditionally viewed as “toxicants” and do not have applicable water quality criteria for aquatic life. No water quality criteria for aquatic life were exceeded at Outfall SD026.

Whole Effluent Toxicity (WET) Tests

The chronic WET test results strongly suggest that it is unlikely that the constituents observed and the concentration of the constituents observed will cause any mortality of aquatic life in Second Creek downstream of SD026. Reproduction (which is a much more sensitive indicator than mortality) of the test species *C. dubia* was considered to be reduced in two tests compared to the reference site Bear Creek and the Partridge River. It should be noted that reproduction was not severely reduced in SD026 compared to the reference sites and for one test there was no significant difference between SD026 and the reference sites.

WET testing (particularly chronic tests with *C. dubia*) is a sensitive methodology and the results suggest that the tailings basin water, which was the primary source of water to SD026 during the study period, is lacking any notable toxicant and the additive or cumulative effects of the constituents present are not significant. A statistical analysis of outfall SD026 water and the receiving waters suggest that reduced reproduction for *C. dubia* in some tests is largely due to constituents that are lacking in the SD026 water, including organic carbon, phosphorus, nitrogen, and possibly some trace metals. It does not appear that bicarbonate, hardness, sulfate, and potassium, which are elevated in SD026, are responsible for the WET test results that indicate reproductive differences between water from SD026 and the reference sites.

Macroinvertebrates

Overall, the macroinvertebrate community in Second Creek just downstream of outfall SD026 is comparable to the macroinvertebrate community in Bear Creek (the chosen reference site) and there is no evidence that the macroinvertebrate community in Second Creek is being notably impacted by the discharge from SD026.

In Second Creek just downstream of SD026, there are more sensitive species. It should be noted that Second Creek has better habitat quality (according to the QHEI) compared to Bear Creek. However, Second Creek has a much smaller watershed and flow compared to Bear Creek, and hence it is expected that there will be less diversity simply due to the stream size and order. Again, due to the similarity of the macroinvertebrate communities in Bear Creek and Second Creek, and due to an overall high proportion of sensitive species, it can be concluded that there is no significant effect on the macroinvertebrate community in Second Creek due to the SD026 discharge.

Summary

Overall, the results from the Stream Investigation indicate that while the SD026 discharge water has elevated concentrations of some parameters (e.g., hardness, total dissolved solids, magnesium, sodium), the biological monitoring data for macroinvertebrates indicate no measurable or notable effects in Second Creek compared to the data from the reference stream (Bear Creek).

3.6 Recommendations for Future Work

Based on the biological monitoring data collected for the 2010-2011 Stream Investigation Study, the following is recommended.

1) No fish monitoring. Second Creek immediately downstream of SD026 does not have fish habitat as identified in the initial site reconnaissance that followed MPCA guidance. Therefore, no fish monitoring is proposed.

2) No additional macroinvertebrate monitoring. The available data and calculated indices indicate that the macroinvertebrate community inhabiting Second Creek immediately downstream of the SD026 discharge has not been measurably affected when compared to the control stream (Bear Creek). Because this discharge has been part of the environment for decades and there has been no notable effect to date, there does not seem to be a need to conduct additional macroinvertebrate studies.

3) Additional WET testing. Because of the variability in the WET test results, and in particular the potential seasonality effects on results, additional WET tests are recommended prior to the development of site specific standards. The additional WET tests are recommended for late spring/early summer. Samples for water chemistry analyses and flow data should be collected at the same time water is collected from SD026 for the WET tests to provide support information to better assess WET test results. The additional tests can include some nutrient-related dosing to further elucidate whether the previous WET test results were more influenced by potential nutrient deficiency or by a high dose of a particular chemical constituent. A work plan would be developed prior to any additional WET testing. Because the tailings basin seepage is currently being collected upstream of SD026 and pumped to the tailings basin (as part of Short Term Mitigation under the Consent Decree), most of the seepage no longer reports to SD026. Therefore, the work plan will need to consider an appropriate method for obtaining representative sample(s) for WET testing.

4) Develop site specific standards after additional WET testing is completed.

4.0 Methylmercury Investigation

As described in the *NPDES Field Studies Plan – SD026* (approved by the MPCA on June 16, 2010), it is unlikely that the continued contributions of sulfate to Second Creek from local mining features, including the former LTVSMC tailings basin, will alter the existing relationship between sulfate and methylmercury. Therefore, no additional monitoring or data collection for sulfate and methylmercury in Second Creek was conducted as part of the Field Studies.

5.0 Wild Rice and Sulfate Monitoring

5.1 Background

In 2009, the MPCA requested PolyMet and Mesabi Mining, LLC (Mesabi) provide information and data regarding wild rice stand locations, densities, and surface sulfate levels in waters potentially affected by their projects (correspondence May 28, 2009 regarding the PolyMet - NorthMet and Mesabi Nugget Phase II Projects (study areas)). The request included: 1.) conducting a literature search for the presence of wild rice in downstream receiving waters, 2.) cooperating with tribes in the study areas, 3.) conducting field surveys to determine the presence of wild rice in the study areas, and 4.) determining surface sulfate levels in waters where wild rice is identified. Following the 2009 request, PolyMet and Mesabi carried out multi-phase studies in summers 2009 and 2010. PolyMet and Mesabi carried out the following activities: First, they consulted literature sources as part of determining the study areas. Second, they analyzed historic aerial photographs of the project areas and compared them to results from field surveys. Third, they determined wild rice stand density and calculated average plant height. Finally, they collected and analyzed water samples for sulfate concentrations in the study areas. The study results are documented in *2009 Wild Rice Survey and Sulfate Monitoring Prepared for Steel Dynamics, Inc. and Mesabi Mining, LLC*, October 2009, *2009 Wild Rice and Sulfate Monitoring Prepared for PolyMet Mining Inc. – NorthMet Project*, September 2009, *2010 Wild Rice Survey and Sulfate Monitoring Prepared for Mesabi Mining, LLC*, March 2011, and *2010 Wild Rice and Water Quality Monitoring Report, Prepared for PolyMet Mining Inc. – NorthMet Project*, January 2011.

5.2 Objective

The purpose of the Wild Rice Survey was to determine the presence of wild rice (*Zizania palustris* L.), an annual grass, in waterbodies potentially affected by the SD026 discharge in the study area. The study's purpose was also to determine sulfate levels at the locations where wild rice was found and whether sulfate affects wild rice growth and production. In particular, the objective of the Wild Rice Survey conducted under the Consent Decree was to evaluate the presence of wild rice downstream of SD026, including Second Creek and the Lower Partridge River downstream from its confluence with Second Creek.

5.3 Scope and Methods

Waterbodies potentially affected by the SD026 discharge include Second Creek and the Lower Partridge River. These waterbodies were surveyed for the presence of wild rice and surface water samples were analyzed for sulfate in response to the MPCA request. The results of the multi-phase studies (submitted to the MPCA in 2009 and 2011), and the findings from the MDNR's 2008 Legislative Report on wild rice (February 2008), will form the basis for the MPCA's determination of wild rice waterbodies potentially affected by SD026 seepage.

5.4 2009 Results

A ground survey of a downstream portion of Second Creek was carried out in mid-September 2009. The 2009 survey work identified wild rice on Second Creek beginning from approximately 200 m upstream of its confluence with the Partridge River down to the confluence. No wild rice was identified on Second Creek other than this rice identified at the Second Creek/ Partridge River confluence. Wild rice was identified in downstream portions of the Partridge River to below the Highway 110 bridge crossing (Figure 5-1).

The Partridge River and sulfate concentration results are documented in *2009 Wild Rice and Sulfate Monitoring Prepared for PolyMet Mining Inc. – NorthMet Project, September 2009*.

5.5 2010 Results and Discussion

A ground survey of an upstream portion of Second Creek from the tailings basin to Highway 666 (shown on Figure 5-2) was carried out on September 9, 2010. No wild rice was found in this portion of Second Creek. Wild rice was again identified at the confluence of Second Creek and the Partridge River by field staff standing at the Partridge River and looking upstream in Second Creek.

The Partridge River wild rice survey and sulfate concentration results are documented in *2010 Wild Rice and Water Quality Monitoring Report, Prepared for PolyMet Mining Inc. – NorthMet Project, January 2011*.

Based on this information, it is not possible to determine the effects of sulfate on wild rice growth and populations.

5.6 Recommendations

Based on findings that sparse wild rice was identified along the lowermost reach (final 200 m) of Second Creek in 2009 and 2010 and no wild rice was identified in the upper reaches of Second Creek near the SD026 discharge, no additional wild rice survey work is recommended for the Consent Decree Field Studies. A number of ongoing and potential future studies are being undertaken to address questions regarding sulfate and wild rice. None of these studies are related directly to the Consent Decree.

6.0 Summary

The Field Studies for SD026 were intended to provide a better understanding of the potential impacts of constituents that have been detected at elevated concentrations in water in SD026. The results from the Field Studies were also intended to be used to support either the development of recommendations for long-term mitigation alternatives or the development of site specific standards for SD026.

Briefly, the Field Studies results indicate the following:

- Overall, the results from the Stream Investigation indicate that while the SD026 discharge water has elevated concentrations of some parameters (e.g., hardness, total dissolved solids, magnesium, sodium), the biological monitoring data for macroinvertebrates indicate no measurable or notable effects in Second Creek (SD026) compared to the data from the reference stream (Bear Creek).

- Because the chronic WET test results do not indicate mortality of C. dubia, it is unlikely that water from SD026 has, or will, adversely affect aquatic life in downstream waters. Reproduction (which is a much more sensitive indicator than mortality) of the test species *C. dubia* was reduced in two tests compared to the reference site Bear Creek and the Partridge River. However, reproduction was not severely reduced in SD026 water compared to the reference site or receiving water (Partridge River) and for one test there was no significant difference between SD026 and the reference sites. Therefore, the WET test results indicate that the potential for actual adverse effect to aquatic life is low.

- WET testing (particularly chronic tests with *C. dubia*) is a sensitive methodology and the results suggest that the tailings basin water, which was the primary source of water to SD026 during the study period, is lacking any notable toxicant and the additive or cumulative effects of the constituents present are not significant. A statistical analysis of outfall SD026 water and the receiving waters suggest that reduced reproduction for *C. dubia* in some tests is largely due to constituents that are lacking in the SD026 water, including organic carbon, phosphorus, nitrogen, and possibly some trace metals. It does not appear that bicarbonate, hardness, sulfate, and potassium, which are elevated in SD026, are responsible for the WET test results that indicate reproductive differences between water from SD026 and the reference sites.

- No wild rice was found in the upstream portion of Second Creek surveyed for this study.
- Tailings basin seepage is currently being collected upstream of SD026 and pumped to the tailings basin (as part of Short Term Mitigation under the Consent Decree).

7.0 Recommendations

The following recommendations are based on the results of the Field Studies for SD026:

- Because the results from the Field Studies indicate that the aquatic life in Second Creek downstream of SD026 has not been adversely impacted by the discharge at SD026, no additional macroinvertebrate monitoring is recommended.

- Because of the variability in the WET test results, and in particular the potential seasonality effects on results, additional WET tests are recommended prior to the development of site specific standards. The additional WET tests are recommended for late spring/early summer. Samples for water chemistry analyses and flow data should be collected at the same time water is collected from SD033 for the WET tests to provide support information to better assess WET test results. The additional tests can include some nutrient-related dosing to further elucidate whether the previous WET test results were more influenced by potential nutrient deficiency or by a high dose of a particular chemical constituent. A work plan would be developed prior to any additional WET testing. Because the tailings basin seepage is currently being collected upstream of SD026 and pumped to the tailings basin (as part of Short Term Mitigation under the Consent Decree), most of the seepage no longer reports to SD026. Therefore, the work plan will need to consider an appropriate method for obtaining representative sample(s) for WET testing.

- It is recommended that site specific standards be developed (for parameters other than sulfate) after the additional WET test testing is completed.

- Wild rice is found near the confluence of Second Creek and Partridge River. There are other sulfate sources between SD026 and the rice. A potential compliance point for SD026 should be downstream of SD026 and upstream of the rice and any other sulfate sources. Compliance to wild rice standard is emerging and at the present time, flow from SD026 has been eliminated to the extent practical. Options for passive treatment that could be applied at SD026, if MPCA determines a compliance point is appropriate, are being evaluated. Recent water quality study activities performed for the NorthMet Project in the Embarrass River watershed have indicated that sulfate reduction is occurring in the surface waterbodies downstream from SD033 (i.e., sulfate load tends to decrease in the downstream direction). In order to better understand ramifications of this reduction related to potential long-term mitigation at SD026 (related to sulfate), it is recommended that additional study be conducted into the fate of sulfate that is discharged at SD026. The scope of such a study has not been developed at this time. A detailed work plan would be developed prior to conducting the study into the fate of sulfate in the SD026 discharge.

8.0 References

Section 3

Hilsenhoff, W.L. 1982. Using a biotic index of to evaluate water quality in streams. Technical Bulletin. WI. Department of Natural Resources No 132 22pp.

Hilsenhoff, W.L. 1987. An improved biotic index of organic stream pollution. Great Lakes Entomology 20:31-39.

Prati, L.R., R. Pavanello and F. Pesarin. 1971. Assessment of surface water quality by a single index of pollution. Water Research 5: 741-751.

Section 5

Barr Engineering Co. 2009. Wild Rice Survey and Sulfate Monitoring. Prepared for Steel Dynamics, Inc. and Mesabi Mining, LLC, October 2009.

Barr Engineering Co. 2009. Wild Rice and Sulfate Monitoring. Prepared for PolyMet Mining Inc. – NorthMet Project, September 2009.

Barr Engineering Co. 2010. Wild Rice Survey and Sulfate Monitoring. Prepared for Mesabi Mining, LLC, March 2011.

Barr Engineering Co. 2010. Wild Rice and Water Quality Monitoring Report. Prepared for PolyMet Mining Inc. – NorthMet Project, January 2011.

Tables

Table 3-1 Summary of water chemistry concentrations and parameter values.

Field and laboratory data for Bear Creek (control stream) and Second Creek (SD026) for Summer (July 26, 2010), Fall (mean of Sept 14, 2010 and Oct 26, 2010), and Spring (June 2, 2011).

Site	**Bear Creek (control)**			**Second Creek (SD026)**		
Sampling date	**Summer '10**	**Fall '10**	**Spring '11**	**Summer '10**	**Fall '10**	**Spring '11**
General Parameters (mg/L unless noted)						
Total Alkalinity	39.3	43.75	35.7	5	476.5	429
Biochemical Oxygen Demand (5-day)	2	1.75	1.5	1.5	1.2	1.2
Dissolved Organic Carbon	35.4	16.7	17	5	5.2	5
Total Organic Carbon	35.3	20.6	17.4	4.9	5.3	4.9
Chemical Oxygen Demand	92.7	58.1	56.9	19.2	14.75	19.9
Chloride	1.26	0.745	0.25	11.4	11.9	9.43
Dissolved oxygen	3.8	5.13	5.49	6.53	6.655	7.13
Total Hardness, as CaCO3	51.4	54.35	39.9	652	619	591
Nitrate + Nitrite	0.05	0.05	0.05	0.05	0.05	0.05
Total Nitrogen (kjeldahl)	2.21	2.35	0.25	0.81	1.005	0.68
Total Nitrogen (N2)	2.21	2.45	0.25	0.91	1.055	0.68
pH	6.59	6.61	6.96	8.04	7.93	8.04
Total Phosphorus	0.056	0.0355	0.021	0.042	0.0115	0.016
Total Dissolved Solids	94	81.5	77	747	661	646
Total Suspended Solids	2.5	20.15	1.6	26.5	2.95	5.6
Specific Conductance umhos@ 25°C	90	95.55	55	1231	1146.5	1055
Sulfate	0.5	1.18	0.5	170	156.5	150
Temperature (°C)	20.82	10.71	12.77	20.43	10.205	10.29
Turbidity (NTU)	5.1	3.2	0	3.1	0	0
Metals (µg/L unless noted)						
Antimony			0.25			0.25
Arsenic	1.96	0.82	0.25	1.80	0.80	0.25

Site	Bear Creek (control)			Second Creek (SD026)		
Sampling date	**Summer '10**	**Fall '10**	**Spring '11**	**Summer '10**	**Fall '10**	**Spring '11**
Barium	35.6	35.7	22.7	38.9	16.6	16.4
Beryllium	0.10	0.10	0.10	0.10	0.10	0.10
Metals (µg/L unless noted)						
Boron	25	25	25	262	230	214
Cadmium	0.10	0.02	0.10	0.10	0.08	0.10
Calcium (mg/L)	15.20	17.15	12.80	81.50	80.55	77.60
Chromium	0.50	2.09	0.50	0.50	0.50	0.50
Cobalt	0.53	0.68	0.10	0.89	0.16	0.10
Copper	0.82	1.12	0.35	2.02	0.83	0.35
Iron	6490	2940	1110	1980	232	325
Lead	0.25	0.36	0.25	0.25	0.13	0.25
Magnesium (mg/L)	3.26	2.80	1.93	109.00	101.50	96.40
Manganese	218.0	284.0	140.0	1370.0	157.0	173.0
Molybdenum	0.41	0.15	0.10	36.20	25.05	20.60
Nickel	2.12	1.86	0.67	2.50	2.27	1.58
Potassium	0.55	1.14	0.92	8.86	7.96	6.57
Selenium	0.50	0.20	0.06	0.50	0.27	0.06
Silver	0.10	0.10	0.10	0.10	0.10	0.10
Sodium (mg/L)	1.0	1.0	1.0	46.9	41.6	34.9
Thallium	0.26	0.10	0.10	0.10	0.10	0.10
Tin	0.25	0.25		0.25	0.25	
Zinc	3.00	4.70	3.00	9.76	3.00	3.00

Table 3-2 Habitat characteristics and macroinvertebrate data summary.

Bear Creek (control stream) and Second Creek (SD026).

Parameter	**Bear Creek (reference)**		**Second Creek (SD026)**	
Date Sampled	**9/16/2010**	**6/2/2011**	**9/16/2010**	**6/2/2011**
Watershed	Embarrass River	Embarrass River	Partridge River	Partridge River
UTM coordinate (NAD 83, Zone 15) Upstream End of Reach	5285620, 560384	5285620, 560384	5271774, 565810	5271774, 565810
UTM coordinate (NAD 83, Zone 15) Downstream End of Reach	5285518, 560364	5285518, 560364	5271724, 565775	5271724, 565775
Stream width at cross-section (ft)	13.0	9.5	5.0	6.5
Maximum depth at cross-section (ft)	1.8	1.8	1.1	1.3
Discharge (cfs)	7.06	8.62	1.01	0.89
Water temperature (°C)	10.2	15.7	10.7	10.5
pH	6.9	6.4	7.7	8.0
Specific Conductivity (μmhos)	105	62	1206	1019
Dissolved oxygen (ppm)	6.4	6.8	7.3	8.4
Habitat types (in-stream cover)	undercut bank/overhanging vegetation	undercut bank/overhanging vegetation	woody debris	woody debris
	woody debris	woody debris	emergent vegetation	emergent vegetation
	emergent vegetation	submerged vegetation	undercut bank/overhanging vegetation	undercut bank/overhanging vegetation
	sediment	sediment	sediment	sediment
Substrate	muck	muck	boulder	boulder
	detritus	detritus	gravel	gravel
			silt	silt
			detritus	detritus

Parameter	**Bear Creek (reference)**		**Second Creek (SD026)**	
Date Sampled	**9/16/2010**	**6/2/2011**	**9/16/2010**	**6/2/2011**
Riparian zone vegetation	herbaceous/shrub	herbaceous/shrub	forest/shrub	forest/shrub
Qualitative Habitat Evaluation Index (QHEI)[3]	---	44	---	69
Shannon-Wiener Diversity Index (H')	2.91	2.42	3.15	1.24
Evenness	0.75	0.64	0.89	0.41
Hilsenhoff Biotic Index (HBI)[2]	6.36	5.94	4.53	5.11
	Fairly Poor	Fair	Good	Good
Richness (Family)	32	34	25	17
Richness (Genera)	46	43	32	19
# of Insect Genera	38	33	26	11
% Insects of total individuals present at site	63%	61%	83%	96%
# Ephemeroptera, Plecoptera and Trichoptera (EPT) Genera	14	9	9	7
# Ephemeroptera, Plecoptera and Trichoptera (EPTO) Genera	19	12	12	7
% EPT of total individuals present at site	24%	37%	72%	77%
% EPTO of total individuals present at site	28%	38%	74%	77%
% Diptera (true flies) of total individuals present at site	30%	23%	8%	19%
% Chironomids (bloodworms) of Diptera	53%	31%	47%	0%
% Simulidae of total individuals present at site	11%	15%	1%	13%

[1]The UTM coordinates are given for the furthest downstream point of the sample reach.

[2]See Table 6 for a summary of HBI values and descriptors.

[3]Based on MPCA Stream Habitat Assessment

Table 3-3 Results of Analysis of Variance (F-values and p-values).

Showing variables that were significantly different ($p < 0.0015$, Bonferroni corrected) between the sites Bear Creek (control stream) and Second Creek (SD026).

Parameter	**F-value**	**p-value**
Total hardness, as $CaCO_3$	1164.5	0.0009
Total Dissolved Solids	18783.9	<0.0001
Sulfate	1113.7	0.0009
Boron	1389.7	0.0007
Magnesium	1854.5	0.0005
Molybdenum	1341.7	0.0007
Sodium	1318.8	0.0008

Table 3-4 Comparison of average water chemistry concentrations and parameter values with applicable Minnesota Water Quality (WQ) criteria.

Bear Creek and Second Creek (SD026)

Site	Bear Creek	Second Creek (SD026)	WQ Criterion
General Parameters (mg/L, unless noted)			
Chloride	0.75	10.91	230
Dissolved oxygen	4.81	6.77167	5.0
Total Hardness, as CaCO3	48.55	620.667	305
pH	6.72	8.00333	6.5-8.5
Total Dissolved Solids	84.17	684.667	700
Specific Conductance umhos@ 25°C	80.18	1144.17	1000
Metals (µg/L, unless noted)			
Arsenic	1.01	0.94833	53
Boron	25.00	235.167	500
Cadmium [1]	0.07	0.09167	0.32-3.4
Chromium [1]	1.03	0.5	55.4-644
Cobalt	0.44	0.38167	5
Copper [1]	0.76	1.06667	3.6-23
Lead [1]	0.29	0.21083	0.41-19
Nickel [1]	1.55	2.11667	40.4-509
Selenium	0.25	0.27633	5
Silver	0.10	0.1	1
Thallium	0.15	0.1	0.56
Zinc [1]	3.57	5.25333	27.1-343

[1] For the metals, cadmium, chromium, copper, lead, nickel and zinc, the criteria (listed as a range) are dependent upon hardness. Values marked in red were higher than the WQ criterion.

Table 3-5 Water Quality Classification Index[1].

Bear Creek (control stream), and Second Creek (SD026)

Parameters	Bear Creek index value	Classification	Second creek index value	Classification
Biochemical Oxygen Demand (5-day)	1.16	Excellent-Acceptable	0.86	Excellent
Chemical Oxygen Demand	6.92	Slightly Polluted-Polluted	1.79	Excellent-Acceptable
Chlorides	0.02	Excellent	0.37	Excellent
Dissolved oxygen	4.8	Slightly Polluted-Polluted	2.7	Acceptable-Slightly Polluted
pH, standard units	0.56	Excellent	1.0	Excellent-Acceptable
Total suspended solids	<1	Excellent	0.32	Excellent
Iron	9.49	Heavily Polluted	3.85	Acceptable-Slightly Polluted
Manganese	2.34	Acceptable-Slightly Polluted	4.43	Slightly Polluted-Polluted

[1] Water Quality Classification Index based on Prati et al. (1971)

Table 3-6 Whole Effluent Toxicity (WET) test results.

Outfall SD026 and downstream receiving waters.

Test #	Site/Dilution Water	Sampling Date	WET Report Date	Survival		Reproduction		
				100% Effluent(1)	75% Effluent	Number of young per adult C. dubia	IC25 (%)	NOEC (%)
Test #1	SD026/Bear Creek	7/26/2010	8/12/2010	80%	100%	18.2 / 30.3	82.6%	75.0%
Test #2	SD026/Synthetic Lab Water	10/26/2010	11/8/2010	100%	100%	18.6 / 18.3	>100	100%
	SD026/Partridge River	10/26/2010	11/8/2010	100%	100%	18.6 / 22.1	>100	50%
	Second Creek (PM17)	10/26/2010	11/8/2010	100%	not applicable	20.7	---	---
Test #3	SD026/Synthetic Lab Water	6/3/2011	6/16/2011	100%	100%	11.4 / 19.2	79%	50%
	SD026/Partridge River	6/3/2011	6/16/2011	100%	100%	11.4 / 18.0	91%	75%
	Second Creek (PM17)	6/3/2011	6/16/2011	100%	not applicable	13.3	---	---

(1) 100% effluent = 100% Bear Creek, Laboratory, or Partridge River water.

Table 3-7 Whole Effluent Toxicity (WET) testing results and corresponding chemical anlysis data
(Data related to SD026 and SD033, background water (Bear Creek), downstream waters and receiving waters (Embarrass River and Partridge River))

Site	Sampling Date	Report Date	Young Production per Adult *C. dubia*	Sp Con (us/cm)	TDS (mg/L)	Cl (mg/L)	Alk (mg/L)	SO_4 (mg/L)	Ca (mg/L)	Mg (mg/L)	Na (mg/L)	Hardness (mg/L)	DOC or TOC (mg/L)	TP (mg/L)	TN (mg/L)	As (µg/L)	Ba (µg/L)	B (µg/L)	Co (µg/L)	Cu (ug/L)	Fe (µg/L)	Mn (µg/L)	Mo (ug/L)	Ni (ug/L)	K (mg/L)	Se (µg/L)	Zn (ug/L)
Outfall SD026	7/26/2010	8/12/2010	18.2	1231	747	11.4	548	170	81.5	109	46.9	652	5.0	0.042	0.91	1.80	38.9	260	0.89	2.02	1,980	1,370	36.20	2.50	8.9	**0.500**	9.8
Bear Creek	7/26/2010	8/12/2010	30.3	90	94	1.26	39	0.5	15.2	3.26	1	51.4	35	0.056	2.21	1.96	35.6	25	0.53	0.82	6,490	218	0.41	2.12	0.55	0.5	3.0
Outfall SD026	10/26/2010	11/8/2010	18.6	1125	637	12.8	474	155	79	102	42.1	617	5.4	0.014	0.61	**0.50**	17.6	239	**0.10**	0.91	185	121	24.00	2.46	8.6	0.037	**3.0**
Partridge River	10/26/2010	11/8/2010	22.1	336	185	10.0	70	74.4	36.4	16.2	9.96	158	15	0.013	1.04	**0.50**	12.9	169	0.25	3.15	388	170	1.60	3.64	2.3	0.762	6.4
Second Creek-PM 17	10/26/2010	11/8/2010	20.7	1116	715	17.2	322	303	77.5	111	24.3	651	11	0.02	0.94	1.74	22.9	87.4	**0.10**	0.74	375	148	6.62	3.00	7.3	0.095	**3.0**
Outfall SD026	6/2/2011	6/16/2011	11.4	1059	646	9.43	429	150	77.6	96.4	34.9	591	5	0.016	0.68	0.25	16.4	214	0.1	0.35	325	173	20.6	1.58	6.57	0.061	3
Partridge River	6/2/2011	6/16/2011	18.0	144	134	2.92	28.9	23.8	14.3	6.35	4.14	61.8	29	0.024	1.59	**0.25**	8.9	55.7	0.29	3.96	858	106	0.79	2.55	1.2	0.607	**3.0**
Second Creek-PM 17	6/2/2011	6/16/2011	13.3	1459	1210	5.92	274	613	51.9	188	29.3	904	13	0.022	1.19	0.73	16.7	107	0.32	**0.35**	524	420	5.02	1.82	10.0	0.0605	**3.0**
Outfall SD033	7/26/2010	8/12/2010	20.2	2350	1,880	4.33	336	1,110	99.3	255	95.3	1,300	4	0.025	1.21	**0.50**	3.2	169	0.37	1.61	**25**	326	3.32	3.63	57.4	**0.500**	**3.00**
Bear Creek	7/26/2010	8/12/2010	30.3	90	94	1.26	39.3	0.5	15.2	3.26	1	51.4	35.4	0.056	2.21	1.96	35.6	25	0.53	0.82	6,490	218	0.41	2.12	0.55	0.5	3.00
Outfall SD033	10/26/2010	11/8/2010	17.0	2420	1,880	4.9	363	1,140	98.2	269	95	1,350	4.9	0.013	2.05	1.47	4.61	155	0.58	2.14	150	1700	3.72	5.06	53.4	0.452	**3.00**
Bear Creek	10/26/2010	11/11/2010	22.2	97	56	0.92	39.9	1.35	15.4	2.65	1	49.4	8.3	0.056	1.12	0.5	43.8	25	1.12	1.85	3,270	453	0.1	2.63	1.53	0.102	6.39
Embarrass River-PM12	10/26/2010	11/8/2010	16.7	135	90	4.96	50.3	1.65	13.8	5.4	4.07	56.7	19.4	0.037	1.76	**5.00**	18.1	**25**	0.50	0.58	2150	184	0.25	1.12	1.1	0.085	**3.00**
Lower Spring Mine Creek-PM 12.1	10/26/2010	11/8/2010	20.3	876	551	2.76	159	311	39.6	80.1	32.4	429	9.6	0.024	1.19	**0.50**	20.4	**25**	**0.10**	0.86	172	118	0.39	1.43	17.8	0.096	**3.00**
Outfall SD033	6/2/2011	6/16/2011	8.0	2210	1780	3.88	341	961	85.8	253	89.2	1260	4.9	0.02	1.09	0.93	3.09	158	0.31	1.62	148	344	3.63	2.46	49.5	0.515	**3.00**
Bear Creek	6/2/2011	6/16/2011	22.6	82	77	0.25	35.7	0.5	12.8	1.93	1	39.9	17	0.021	0.25	0.25	22.7	25	0.1	0.35	1110	140	0.1	0.67	0.92	0.0605	3.00
Embarrass River-PM12	6/2/2011	6/16/2011	19.1	71	79	2.33	27	**0.5**	8.36	3.25	2.88	34.2	32.5	0.022	1.56	0.53	10.9	**25**	0.35	1	1420	71.2	**0.10**	1.36	0.3	0.0605	**3.00**
Lower Spring Mine Creek-PM12.1	6/2/2011	6/16/2011	13.7	684	490	1.17	120	235	33	60.2	23	330	16	0.022	1.14	**0.25**	18.5	50.4	**0.10**	**0.35**	320	161	0.46	0.88	12.7	0.0605	**3.00**

Chemical abbreviations in the table defined below:

Bold= below detectioni limit, value set to 1/2 detection limit

Sp Con=	Specific conductance	Co	Cobalt
TDS	Total dissolved solids	Cu	Copper
Cl	Chloride	Fe	Iron
Alk	Alkalinity	Mn	Manganese
SO_4	Sulfate	Mo	Molybdenum
Ca	Calcium	Ni	Nickel
Mg	Magnesium	K	Potassium
Na	Sodium	Se	Selenium
Hardness	Hardness	Zn	Zinc
DOC or TOC	Dissolved or Total Organic Carbon		
TP	Total Phosphorus		
TN	Total Nitrogen		
As	Arsenic		
Ba	Barium		
B	Boron		

Table 3-8 Comparison of mining outfalls to background surface waters.

Average concentrations of constituents monitored which are lower in mining outfalls (SD033 and SD026 combined) and parameters that are higher in mining outfalls compared to background surface waters.

(Averages of these parameters are also provided for background waters (Bear Creek, Partridge River, and Embarrass River--combined) and waters consisting of mixtures of mining and background waters (defined as Mining Influenced Water and includes Trimble Creek and Second Creek))

	Parameters Lower Due to Properties of Mine Pit Waters							Parameters Elevated Due to Mining					
Site	Barium (µg/L)	Cobalt (µg/L)	Copper (ug/L)	Iron (µg/L)	DOC or TOC (mg/L)	TP (mg/L)	Total N (mg/L)	Nickel (ug/L)	Magnesium/ Calcium	Alkalinity (mg/L)	Sulfate (mg/L)	Potassium (mg/L)	Young Production
Permitted Outfalls	14.0	0.39	1.4	469	5	0.022	1.1	2.9	2.0	415	614	31	16
Background Waters	21.8	0.45	1.7	2241	22	0.033	1.4	2.0	0.3	42	15	1	23
Mining Influenced Waters	19.6	0.16	0.6	348	12	0.022	1.1	1.8	2.2	219	366	12	18

Table 3-9 Evaluation of the effect of parameter concentrations elevated by mining operations on C. dubia young production in WET tests.

(Young production predicted using the model equation provided in note 1 and other constituent concentrations provided in note 2.)

Condition	Magnesium/ Calcium	Alkalinity (mg/L)	Sulfate (mg/L)	Potassium (mg/L)	Predicted Number of Young Production
Mining Levels	2.0	415.2	614.3	30.7	15.5
	1.7	352.9	572.9	27.6	15.5
	1.4	294.1	477.4	23.0	15.6
Mining Influenced	1.2	245.1	397.8	19.2	15.7
	1.0	204.2	331.5	16.0	15.7
Background	0.8	170.2	276.3	13.3	15.8
	0.7	141.8	230.2	11.1	15.8
	0.3	42	366	12	15.3

Note 1:
Predictive Model #4; Young Production=31*1/(1+EXP(-(-2.02+0.0435*Ba-1.90*Co-0.225*Cu +0.769*Ni +0.000246*Fe+0.0564*DOC +19.5*TP-0.485*TN +0.0503*Mg/Ca -0.00101*Alk-0.00136*Sulfate +0.0354*Potassium)))

Note 2:
Concentration of other parameters used in the model includes: Barium (μg/L) = 14, Cobalt (μg/L) = 0.39, Copper (μg/L) = 01.4, Iron (μg/L) = 469, TOC or DOC (mg/L) = 4.9, TP (mg/L) = 0.022, Total N (mg/L) = 1.09, Nickel (μg/L) = 2.94.

Table 3-10 Total macroinvertebrates sampled in stream sites related to SD026.

Taxa				HBI Value	Bear Creek (reference)		Second Creek (SD026)	
Class	Order	Family	*Genus species*	(10-0)	2010	2011	2010	2011
Insecta	Coleoptera	Curculionidae	undetermined	5				
		Dystiscidae	*Agabus* adults	5				
			Hydroporus adults	5				
			Dytiscus larvae			1		
			Nebrioporus					
		Elmidae	*Dubiraphia* larvae	6				
			Dubiraphia adults					
			Macronychus		16			
			Macronychus adults	5				
			Optioservus	4	8	2		
			Stenelmis larvae	5	16			
			Stenelmis adult	5				
			undetermined	4				
		Gyrinidae	*Gyrinus* adults		48		8	
		Hydrophilidae	*Tropisternus* adults					
	Diptera		undetermined Diperta larva					
			undetermined Diptera pupae					2
		Chironomidae	undetermined	5				
			Chironomus	10			16	
			Cladopelma					
			Cryptochironomus	8				
			Dicrotendipes					
			Endochironomus	10	8			
			Labrundinia	7				
			Microtendipes	6	64			
			Paratendipes					
			Polypedilum	6	32	6		
			Stenochironomus		136	4		
			Xenochironomus					
		Chironominae	*Pseudochironomus*					
			Microsectra			10		
			Paratanyytarsus					
			Rheotanytarsus	6	60			
			Tanytarsus	6		20	8	
		Diamesinae	*Diamesa*	5				
		Orthocladiinae	*Undetermined*					
			Acricotopus	7				
			Brillia					
			Chaetocladius					
			Cricotopus	7				
			Cricotopus (C.) bicinctus group					
			Eukiefferiella	4				
			Heterotrissocladius	4			8	
			Orthocladius	6		4	16	
			Parametriocnemus	5				
			Psectrocladius					
			Pseudorthocladius	0			8	
			Rheocricotopus	6		4		
			Symposiocladius					
			Thienemanniella	6		2		
			Tvetenia	5				
			Xylotopus	5	32			
		Prodiamesinae	*Prodiamesa*	8				
		Tanypodinae	*Ablabesmyia*	6		16		
			Larsia	6			16	
			Nilotanypus	6				
			Paramerina	6				
			Thienemannimyia group	6	4		16	
			Conchapelopia	6	64	4		
			Procladius	9	52	4	8	
			Zavrelimyia			4		
		Ceratopogonidae	*Bezzia/Palpomyia*	6	64			
			Ceratopogon	6	16			
			Culicoides					
			Probezzia	6				
			undetermined			6		25
		Dixidae	*Dixa*	1			64	
			Dixella			4		
		Empididae	undetermined Empidid larvae	6				
		Simuliidae	*Simulium*	6	308	162	16	108
			Simulium pupae					
		Tabanidae	*undetermined Tabanid*	5	8		8	
		Tipulidae	*Antocha*	3				
			Dicronota	3				
			Limnophila	3				
			Lipsothrix					
			Tipula	6		2	4	
			undetermined Tipulidae				8	18
		Ptychopteridae	*Ptycoptera*			1	8	5
	Ephemeroptera	Ameletidae	*Ameletus*			4		
		Arthropleidae	*Arphroplea*			4		
		Baetidae	*Baetis brunneicolor*	4	12	264	216	111
			Baetis flavistriga	4				
			Baetis intercalaris	6				
			Baetis tricaudatus	6				
			undetermined *Baetis*			4		
			Acentrella	4		68		
			Labiobaetis	na	12			
			Acerpenna macdunnoughi	5	4			

Taxa				HBI Value	Bear Creek (reference)		Second Creek (SD026)	
Class	Order	Family	*Genus species*	(10-0)	2010	2011	2010	2011
			Callibaetis	7				
		Caenidae	*Caenis*	7				
		Ephemerellidae	*Attenella*	3				
		Heptageniidae	*Stenacron*	7	8			
			Maccaffertium		2			
		Leptophlebiidae	*Leptophlebia*		6			
		Siphlonuridae	*Siphlonurus*	4		2		
		Metretopodidae	*undetermined Genus*		16			
	Hemiptera	Corixidae	*Sigara*					
	Odonata	Aeshnidae	*Aeshna*	5	10	8	8	
			Anax	8			2	
			Boyeria		12			
		Calopterygidae	*Calopteryx*	5	54			
		Coenagrionidae	undetermined Immatures					
		Gomphidae	*Gomphus*	6		1		
			immature Gomphus nymph		4			
		Cordulegasteridae	*Cordulegaster*	3			60	
		Corduliidae	*Somatochlora*		32	10		
		Libellulidae	undetermined (immature)					
	Megaloptera	Sialidae	*Sialis*	4	13			
	Lepidoptera	Pyralidae	*Acentria*	5				
			Paraponyx	5	8	1		
	Plecoptera	Perlidae	*Paragnetina*	1				
			Perlesta	5		22		
			immature Perlidae					
		Isoperliidae	*Isoperla*	2				
		Nemouridae	*Amphinemora*					
			Nemoura	1				
		Taeniopterugidae	undetermined earlyi nstar nymph					
	Trichoptera	Arctopsychidae	*Parapsyche*	0				
		Goeridae	*Goera*	3				
		Helicopsychidae	*Helicopsyche*	3				
		Hydropsychidae	*Hydropsyche slossonae*	4			464	217
			Hydropsyche alhydra	4				
			Hydropsyche betteni	6	128	1	144	32
			Hydropsyche betteni pupae					
			undetermined *Hydropsyche*	4			72	
			Cheumatopsyche	5	144	4	80	37
		Hydroptilidae	*Hydroptila*	6				230
			Undet. Pupae					
		Lepidostomatidae	*Lepidostoma*	1		4	24	
		Leptoceridae	*Ceraclea*					
			Oecetis	8				
			Triaenodes	6				
			undetermined pupae					
		Limnephilidae	*Anabolia*	5		17		1
			Hydatophylax	2	8		4	
			Limnephilus	3	4			
			Platycentropus					
			Pycnopsyche	4				
			very immature larva					
			undetermined pupae					
		Molannidae	*Molanna*	6				
		Philopotamidae	*Chimarra*	4			464	12
		Phryganeidae	*Banksiola*					
			Ptilostomis	5	14		40	
			very immature larva					
		Polycentropodidae	*Nyctiophylax*	5				
			Polycentropus	6	208	13	48	
		Psychomiidae	*Lype*	2	112		256	4
		undetermined pupae	undetermined pupae			1		
Crustacea	Amphipoda	Talitridae	*Hyalella*	8	356	218	192	14
		Gammaridae	*Gammarus*	6				
	Decapoda	Astacidae	*Orconectes*	6	2			
Malacostraca	Isopoda	undetermined	*undetermined*					2
Entoprocta	Urnatellida	Urnatellidae	*Urnatella gracilis*		16			
Annelida	Oligochaeta		undetermined	8	588	160	40	5
	Arhynchnobdellida	Erpobdellidae	*Erpobdella punctata*		2	4		
	Rhynchnobdellida	Glossiphoniidae	*Helobdella stagnalis*	6				
			undetermined Leech			1	8	
Gastropoda	Basommatophora	Ancylidae	*Ferrisia*	7	32	4		
		Lymnaeidae	*Pseudosuccinea*	6			8	
			Fossaria	6			8	4
			Stagnicola			1		2
		Planorbidae	*Gyraulus*					1
		Actinommidae	*Helisoma*	6		2		
		Physidae	*Physa*	7	22	3		
	undetermined slug	undetermined slug	undetermined slug					1
Bivalvia/Pelecypoda	Veneroida	Pisidiidae(clams)	*Musculium*	6				
			Pisidium	6		32	168	7
			Sphaerium	6	6			
			very immature Sphaeriidae	6	16		16	
Hydrozoa	Hydroida	Clavidae	*Cordylophora*			4		
Nematoda (phylum)	undetermined	undetermined	undetermined					
Total					**2,787**	**1,113**	**2,534**	**838**

Table 3-11 Classes, orders, families and abundance of macroinvertebrates.

	Bear Creek (reference)		Second Creek (SD026)	
Taxa	**2010**	**2011**	**2010**	**2011**
Class	6	6	5	6
Order	14	14	9	9
Family	32	34	25	17
Genera	46	43	32	19
Total Organisms	2,787	1,113	2,534	838

Table 3-12 Percentage of macroinvertebrate classes collected at each site.

	Bear Creek (reference)		Second Creek (SD026)	
Class	2010	2011	2010	2011
Insecta	**62.7%**	**61.5%**	**82.6%**	**95.7%**
Crustacea	**12.8%**	**19.6%**	**7.6%**	1.7%
Malacostraca	0.0%	0.0%	0.0%	0.2%
Entoprocta (Phylum)	0.6%	0.0%	0.0%	0.0%
Annelida	**21.2%**	**14.8%**	1.9%	0.6%
Gastropoda	1.9%	0.9%	0.6%	1.0%
Bivalvia	0.8%	2.9%	7.3%	0.8%
Hydrozoa	0.0%	0.4%	0.0%	0.0%
Nematoda	0.0%	0.0%	0.0%	0.0%

Table 3-13 Percentage of macroinvertebrate orders collected at each site.

(**bold font** in cells represent dominant orders)

Order	Bear Creek (reference)		Second Creek (SD026)	
	2010	2011	2010	2011
Coleoptera	3.2%	0.3%	0.3%	0.0%
Diptera	**30.4%**	**22.7%**	**8.1%**	**18.9%**
Ephemeroptera	2.2%	**31.1%**	**8.5%**	**13.2%**
Hemiptera	0.0%	0.0%	0.0%	0.0%
Odonata	4.0%	1.7%	2.8%	0.0%
Megaloptera	0.5%	0.0%	0.0%	0.0%
Lepidoptera	0.3%	0.1%	0.0%	0.0%
Plecoptera	0.0%	2.0%	0.0%	0.0%
Trichoptera	**22.2%**	3.6%	**63.0%**	**63.6%**
Amphipoda	**12.8%**	**19.6%**	**7.6%**	1.7%
Decapoda	0.1%	0.0%	0.0%	0.0%
Urnatellida	0.6%	0.0%	0.0%	0.0%
Oligochaeta	**21.1%**	**14.4%**	1.6%	0.6%
Arhynchnobdellida	0.1%	0.4%	0.0%	0.0%
Rhynchnobdellida	0.0%	0.1%	0.3%	0.0%
Basommatophora	1.9%	0.9%	0.6%	1.0%
Veneroida	0.8%	2.9%	**7.3%**	0.8%
Isopoda	0.0%	0.0%	0.0%	0.2%
Hydroida	0.0%	0.4%	0.0%	0.0%
Nematoda-unknown	0.0%	0.0%	0.0%	0.0%

Table 3-14 Hilsenhoff Biotic Index (HBI) values for streams.

HBI Value	**Water Quality**	**Degree of Organic Pollution**
0.00-3.50	Excellent	No apparent organic pollution
3.51-4.50	Very Good	Possible slight organic pollution
4.51-5.50	Good	Some organic pollution
5.51-6.50	Fair	Fairly significant organic pollution
6.51-7.50	Fairly Poor	Significant organic pollution
7.51-8.50	Poor	Very significant organic pollution
8.51-10.00	Very Poor	Severe organic pollution

Table 3-15 Hilsenhoff Biotic Index (HBI) calcuations for each stream sampling site.

Taxa					Bear Creek (reference) 2010			Bear Creek (reference) 2011			Second Creek (SD26) 2010			Second Creek (SD26) 2011		
Class	Order	Family	*Genus species*	Tolerance Value (10-0)	Total	Total with tolerance values	HBI Sum	Total	Total with tolerance values	HBI Sum	Total	Total with tolerance values	HBI Sum	Total	Total with tolerance values	HBI Sum
Insecta	Coleoptera	Curculionidae	undetermined	5												
		Dysticae	*Agabus* adults	5												
			Hydroporus adults	5												
			Dytiscus larvae	na				1								
			Nebrioporus	na												
		Elmidae	*Dubiraphia* larvae	6												
			Dubiraphia adults	6												
			Macronychus	5	16	16	80									
			Macronychus adults	5												
			Optioservus	4	8	8	32	2	2	8						
			Stenelmis larvae	5	16	16	80									
			Stenelmis adult	5												
			undetermined	4												
		Gyrinidae	*Gyrinus* adults	na	48						8					
		Hydrophilidae	*Tropisternus* adults	na												
	Diptera		undetermined Diperta larva	na												
			undetermined Diptera pupae	na										2		
		Chironomidae	undetermined	5												
			Chironomus	10							16	16	160			
			Cladopelma	9												
			Cryptochironomus	8												
			Dicrotendipes	na												
			Endochironomus	10	8	8	80									
			Labrundinia	7												
			Microtendipes	6	64	64	384									
			Paratendipes	8												
			Polypedilum	6	32	32	192	6	6	36						
			Stenochironomus	5	136	136	680	4	4	20						
			Xenochironomus	na												
		Chironominae	*Pseudochironomus*	5												
			Microsectra	na				10								
			Paratanytarsus	6												
		(Tanytarsini)	*Rheotanytarsus*	6	60	60	360									
		(Tanytarsini)	*Tanytarsus*	6				20	20	120	8	8	48			
		Diamesinae	*Diamesa*	5												
		Orthocladiinae	undetermined	na												
			Acricotopus	na												
			Brillia	5												
			Chaetocladius	na												
			Cricotopus (Cricotopus)	7												
			Cricotopus (C.) bicinctus	na												
			Eukiefferiella	4												
			Heterotrissocladius	4							8	8	32			
			Orthocladius	6				4	4	24	16	16	96			
			Parametriocnemus	5												
			Psectrocladius	8												
			Pseudorthocladius	0							8					
			Rheocricotopus	6				4	4	24						
			Symposiocladius	na												
			Thienemanniella	6				2	2	12						
			Tvetenia	5												
			Xylotopus	5	32	32	160									
		Prodiamesinae	*Prodiamesa*	8												
		Tanypodinae	*Ablabesmyia*	na				16								
			Conchapelopia	6	64	64	384	4	4	24						
			Larsia	6							16	16	96			
			Nilotanypus	6												
			Paramerina	na												
			Procladius	9	52	52	468	4	4	36	8	8	72			
			Thienemannimya Group	6	4	4	24	0			16	16	96			
			Zavrelimyia	8				4	4	32						
		Ceratopogonidae	*Bezzia/Palpomyia*	6	64	64	384									
			Ceratopogon	6	16	16	96									
			Probezzia	6												
			undetermined	na				6						25		
		Dixidae	*Dixa*	1							64	64	64			
			Dixella	na				4								
		Empididae	*undetermined Empidid* larvae	6												
		Simuliidae	*Simulium*	6	308	308	1,848	162	162	972	16	16	96	108	108	648
			Simulium pupae	6												
		Tabanidae	*undetermined Tabanid*	5	8	8	40				8	8	40			
		Tipulidae	*Antocha*	3												
			Dicronota	3												
			Limnophila	3												
			Lipsothrix	na												
			Tipula	6				2	2	12	4	4	24			
			undetermined Tipulidae	na							8			18		
		Ptychopteridae	*Ptycoptera*	na				1			8			5		
	Ephemeroptera	Ameletidae	*Ameletus*	na				4								
		Arthropleidae	*Arphroplea*	na				4								
		Baetidae	*Baetis brunneicolor*	4	12	12	48	264	264	1,056	216	216	864	111	111	444
			Baetis flavistriga	4												
			Baetis intercalaris	6												
			Baetis tricaudatus	6												
			undetermined *Baetis*	na				4								
			Acentrella	4				68	68	272						
			Labiobaetis	na	12											
			Acerpenna macdunnoughi	5	4	4	20									
			Callibaetis	7												
		Caenidae	*Caenis*	7												
		Ephemerellidae	*Attenella*	3												
		Heptageniidae	*Stenacron*	7	8	8	56									
			Maccaffertium	na	2											
		Leptophlebiidae	*Leptophlebia*	4	6	6	24									
		Siphlonuridae	*Siphlonurus*	4				2	2	8						
		Metretopodidae	undetermined genus	na	16											
	Hemiptera	Corixidae	*Sigara*	na	0											
	Odonata	Aeshnidae	*Aeshna*	5	10	10	50	8	8	40	8	8	40			
			Anax	8							2	2	16			
			Boyeria	na	12											
		Calopterygidae	*Calopteryx*	5	54	54	270									
		Coenagrionidae	undetermined immatures	na												
		Gomphidae	*Gomphus*	6				1	1	6						
			immature Gomphus nymph	6	4	4	24									
		Cordulegasteridae	*Cordulegaster*	3							60	60	180			
		Corduliidae	*Somatochlora*	1	32	32	32	10	10	10						
		Libellulidae	undetermined (immature)	na												
	Megaloptera	Sialidae	*Sialis*	4	13	13	52									

Taxa					Bear Creek (reference) 2010			Bear Creek (reference) 2011			Second Creek (SD26) 2010			Second Creek (SD26) 2011		
Class	Order	Family	*Genus species*	Tolerance Value (10-0)	Total	Total with tolerance values	HBI Sum	Total	Total with tolerance values	HBI Sum	Total	Total with tolerance values	HBI Sum	Total	Total with tolerance values	HBI Sum
	Lepidoptera	Pyralidae	*Acentria*	5												
			Paraponyx	5	8	8	40	1	1	5						
	Plecoptera	Perlidae	*Paragnetina*	1												
			Perlesta	5				22	22	110						
			immature Perlidae	na												
		Isoperliidae	*Isoperla*	2												
		Nemouridae	*Amphinemora*	na												
			Nemoura	1												
		Taeniopterugidae	undetermined early instar nymph	na												
	Trichoptera	Arctopsychidae	*Parapsyche*	0												
		Goeridae	*Goera*	3												
		Helicopsychidae	*Helicopsyche*	3												
		Hydropsychidae	*Hydropsyche slossonae*	4							464	464	1,856	217	217	868
			Hydropsyche alhydra	4												
			Hydropsyche betteni	6	128	128	768	1	1	6	144	144	864	32	32	192
			Hydropsyche betteni pupae	6												
			undetermined *Hydropsyche*	na							72					
			Cheumatopsyche	5	144	144	720	4	4	20	80	80	400	37	37	185
		Hydroptilidae	*Hydroptila*	6										230	230	1,380
			undetermined pupae	na												
		Lepidostomatidae	*Lepidostoma*	1				4	4	4	24	24	24			
		Leptoceridae	*Ceraclea*	na												
			Oecetis	8												
			Triaenodes	6												
			undetermined pupae	na												
		Limnephilidae	*Anabolia*	5				17	17	85				1	1	5
			Hydatophylax	2	8	8	16				4	4	8			
			Limnephilus	3	4	4	12									
			Platycentropus	na												
			Pycnopsyche	4												
			very immature larva	na												
			undetermined pupae	na												
		Molannidae	*Molanna*	6												
		Philopotamidae	*Chimarra*	4							464	464	1,856	12	12	48
		Phryganeidae	*Banksiola*	na												
		Phryganeidae	*Ptilostomis*	5	14	14	70				40	40	200			
			very immature larva	na												
		Polycentropodidae	*Nyctiophylax*	5												
			Polycentropus	6	208	208	1,248	13	13	78	48	48	288			
		Psychomiidae	*Lype*	2	112	112	224				256	256	512	4	4	8
		undetermined pupae	*undetermined pupae*	na				1								
Crustacea	Amphipoda	Talitridae	*Hyalella*	8	356	356	2,848	218	218	1,744	192	192	1,536	14	14	112
		Gammaridae	*Gammarus*	6												
	Decapoda	Astacidae	*Orconectes*	6	2	2	12									
Malacostraca	Isopoda	undetermined	undetermined	na										2		
Entoprocta	Urnatellida	Urnatellidae	*Urnatella gracilis*	na	16											
Annelida	Oligochaeta		undetermined	8	588	588	4,704	160	160	1,280	40	40	320	5	5	40
	Arhynchnobdellida	Erpobdellidae	*Erpobdella punctata*	na	2			4								
	Rhynchnobdellida	Glossiphoniidae	*Helobdella stagnalis*	6												
			undetermined Leech	na				1			8					
Gastropoda	Basommatophora	Ancylidae	*Ferrisia*	7	32	32	224	4	4	28						
		Lymnaeidae	*Pseudosuccinea*	6							8	8	48			
			Fossaria	6							8	8	48	4	4	24
			Stagnicola	na				1						2		
		Planorbidae	*Gyraulus*	na										1		
		Actinommidae	*Helisoma*	6				2	2	12						
		Physidae	*Physa*	7	22	22	154	3	3	21						
	undetermined slug	undetermined slug	undetermined slug	na										1		
Bivalvia/Pelecypoda	Veneroida	Pisidiidae(clams)	*Musculium*	6												
			Pisidium	6				32	32	192	168	168	1,008	7	7	42
			Sphaerium	6	6	6	36									
			very immature Sphaeriidae	na	16						16					
Hydrozoa	Hydroida	Clavidae	*Cordylophora*	na				4								
Nematoda (phylum)	undetermined	undetermined	undetermined	na												
			TOTAL		**2,787**	2,663	16,944	**1,113**	1,052	6,297	**2,534**	2,406	10,892	**838**	782	3,996
			HBI Value				6.36			5.99			4.53			5.11
			Water Quality Rating (see Table 3-14)				Fairly Poor			Fair			Good			Good

Figures

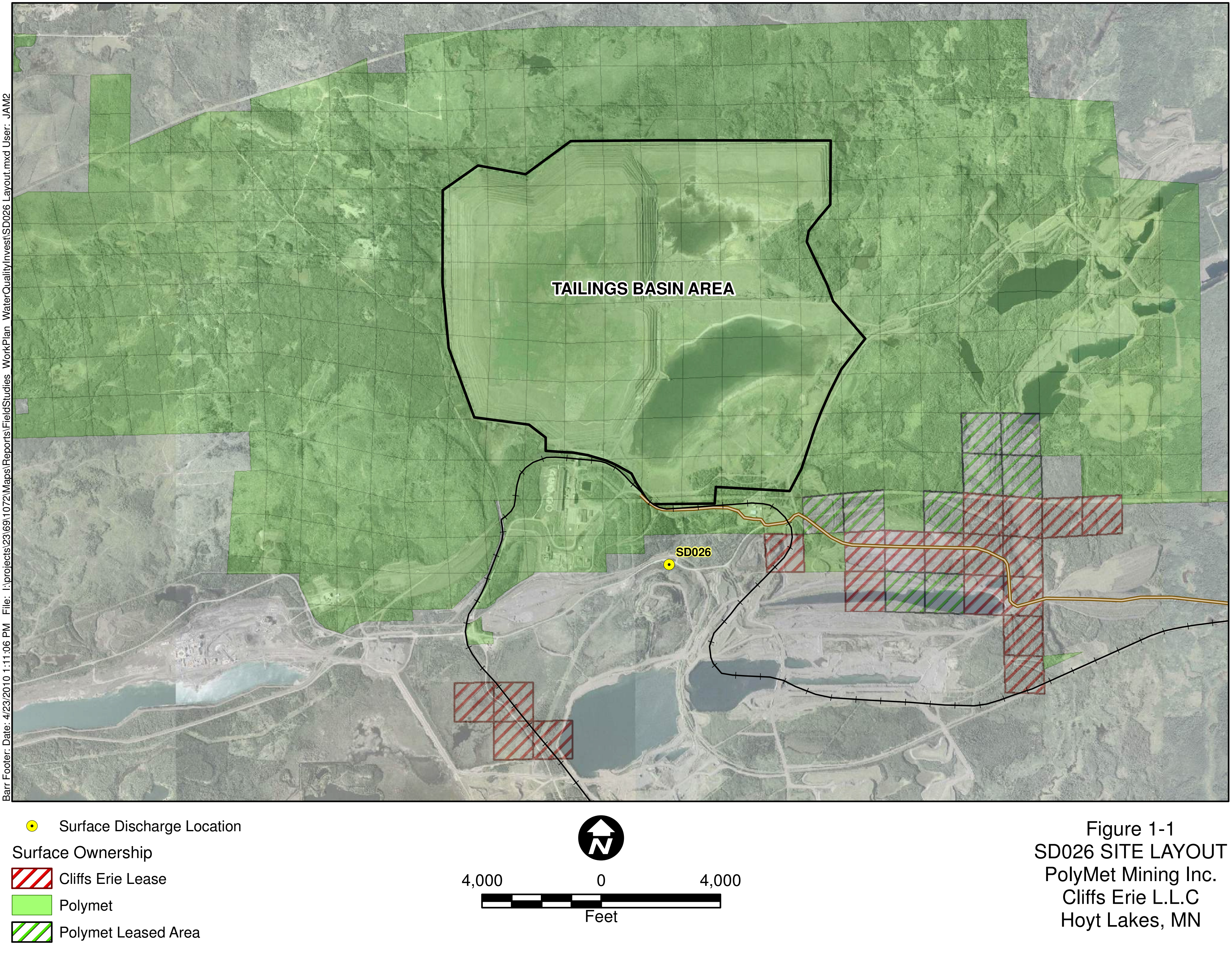
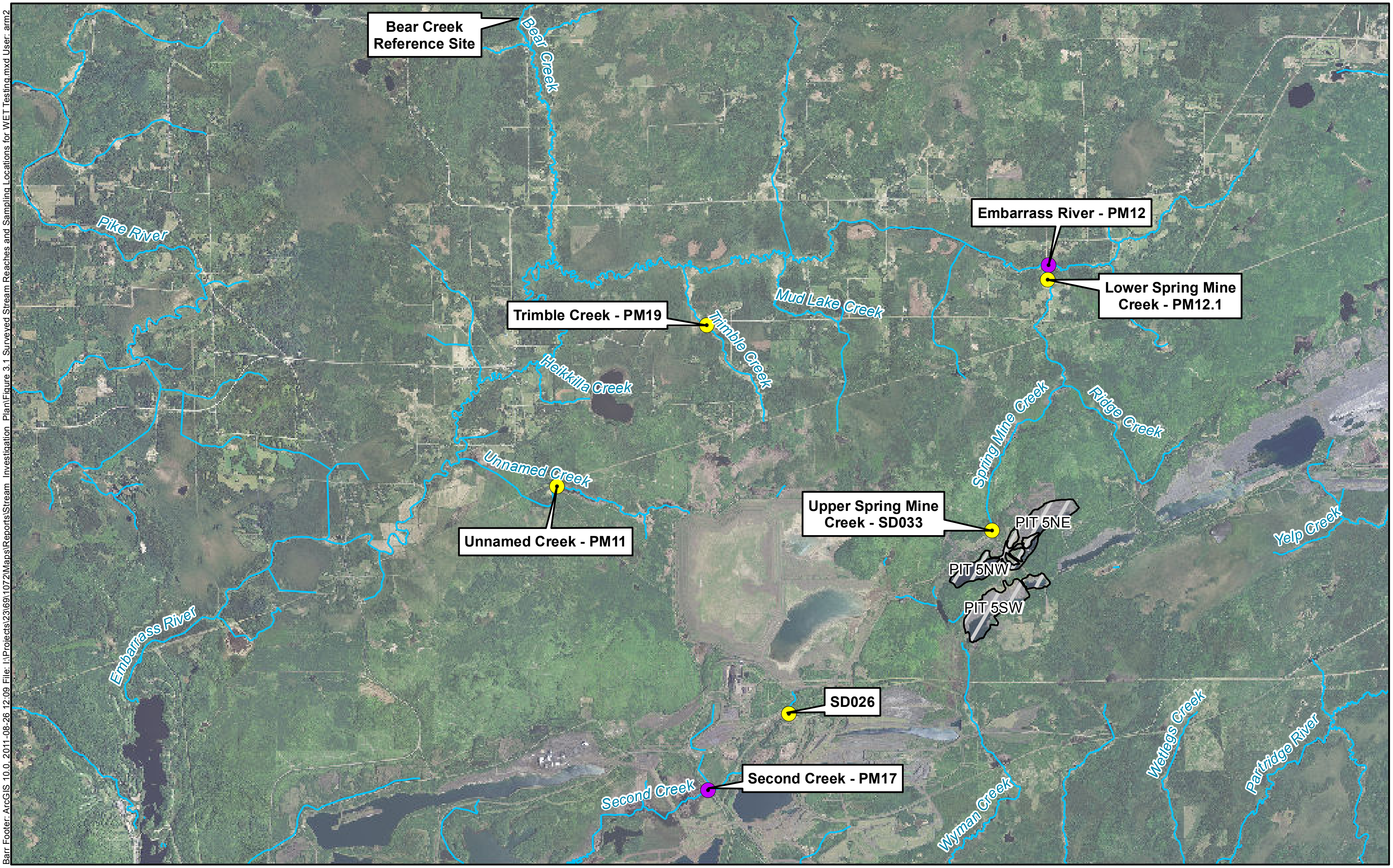


Figure 1-1
SD026 SITE LAYOUT
PolyMet Mining Inc.
Cliffs Erie L.L.C
Hoyt Lakes, MN

Barr Footer: Date: 4/23/2010 1:11:06 PM File: I:\projects\23\69\1072\Maps\Reports\FieldStudies_WorkPlan_WaterQualityInvest\SD026 Layout.mxd User: JAM2



Water Sample Collection Points

- Sampling Locations
- WET Tests Only
- Rivers & Streams
- Area 5 Pits

0 1 2

Miles

Figure 3.1
SURVEYED STREAM REACHES
AND SAMPLING LOCATIONS FOR
WHOLE EFFLUENT TOXICITY TESTING
St. Louis County, MN

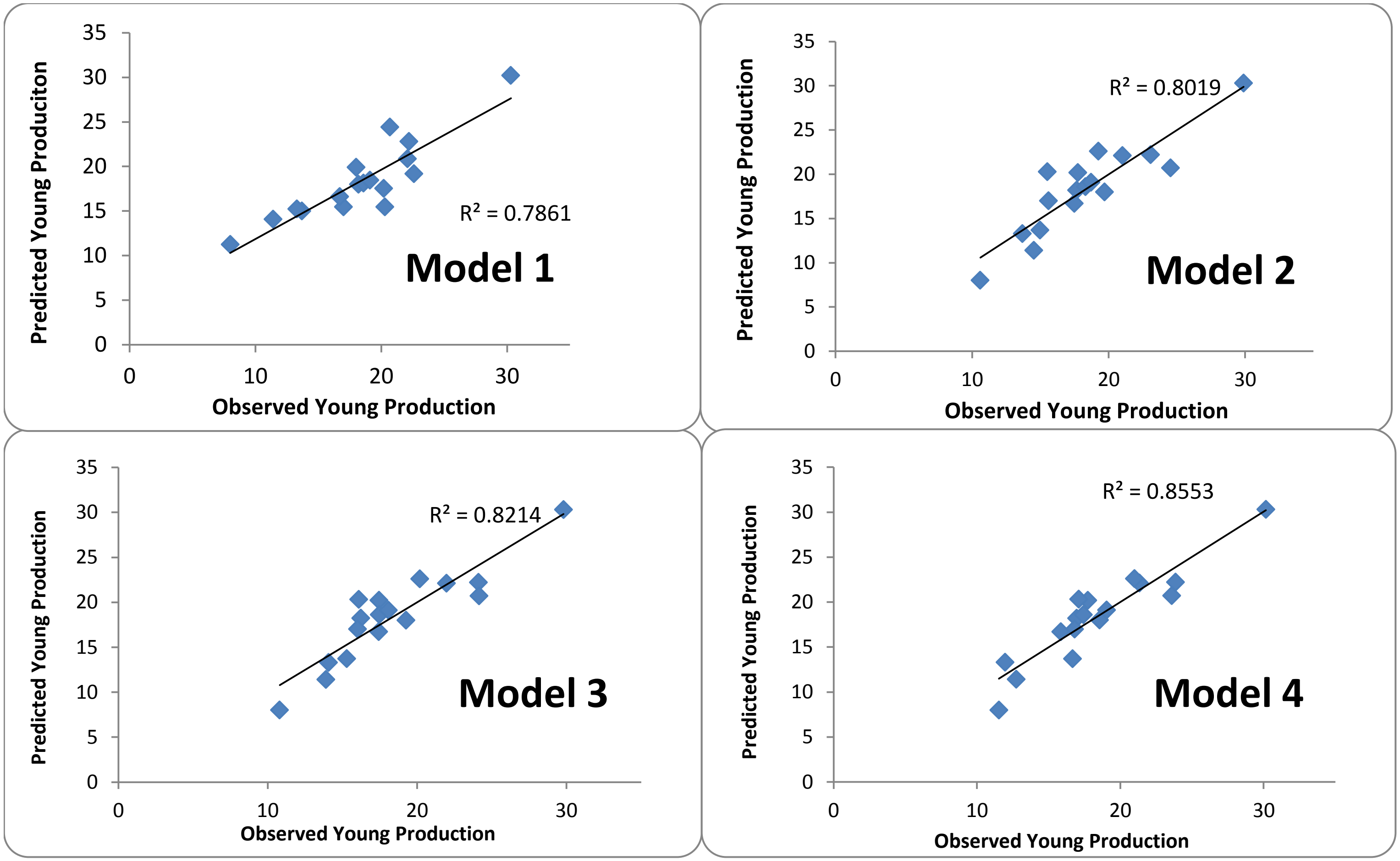


Figure 3-2. Evaluation of the predictive capacity of the multi-parameter logistic model for observed C. dubia young production compared to predicted production (goodness-of-fit assessment)

Model 1 young production =31*1/(1+EXP(-(-2.12+0.0212*Ba-2.22*Co-0.17*Cu+0.75*Ni+0.000247*Fe+0.051*DOC+41.9*TP-0.46*TN)))

Model 2 young production=31*1/(1+EXP(-(-1.96+0.019*Ba-2.11*Co-0.226*Cu+0.761*Ni+0.000130*Fe+0.0468*DOC+46.4*TP -0.366*TN-0.127*Ca/Mg)))

Model 3 young production=31*1/(1+EXP(-(-1.51*Ba-2.02*Co-0.210*Cu+0.752*DOC+0.000199*Fe+0.0336*DOC+36.75*TP-0.395*TN-0.0771*Mg/Ca-0.000969*Alkalinity)))

Model 4 young production=31*1/(1+EXP(-(-2.02+0.0435*Ba-1.90*Co-0.225*Cu+0.769*Ni+0.000246*Fe+0.0564*DOC+19.5*TP-0.485*TN+0.0503*Mg/Ca-0.00101*Alk-0.00136*Sulfate+0.0354*Potassium)))

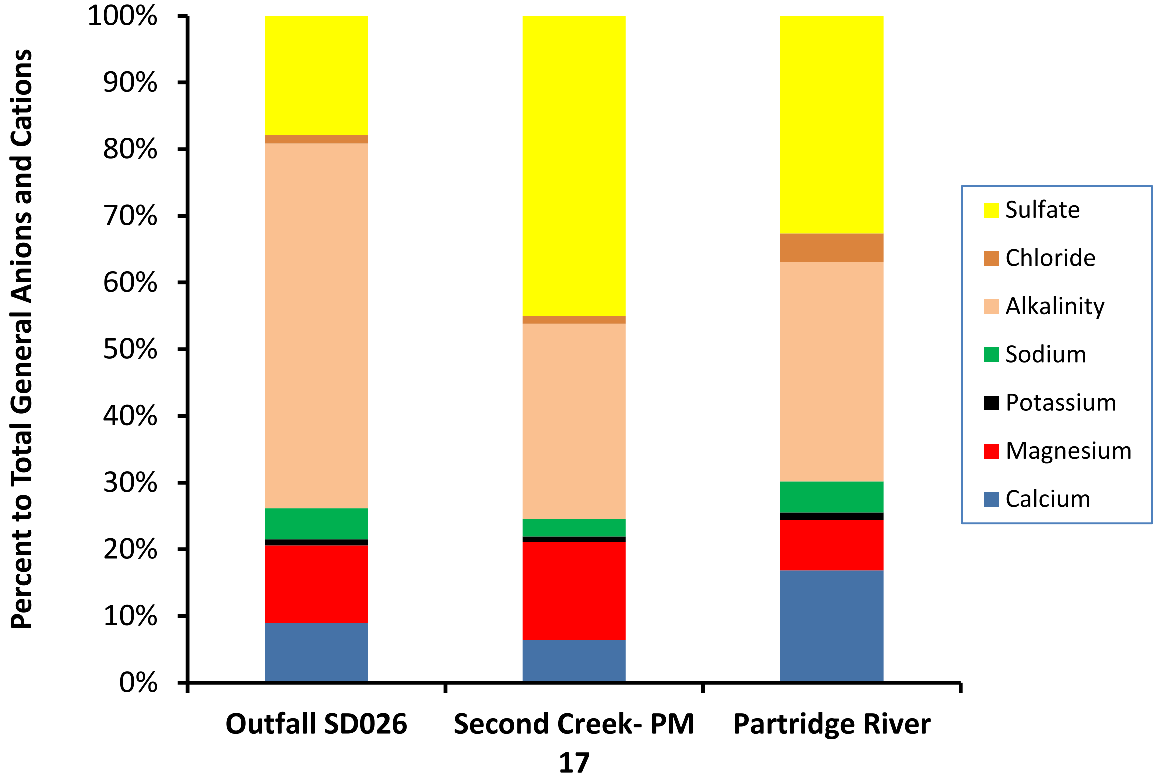


Figure 3-3. Comparison of the relative proportions of major cations and anions in mining outfall waters (SD033, SD026) and background receiving waters

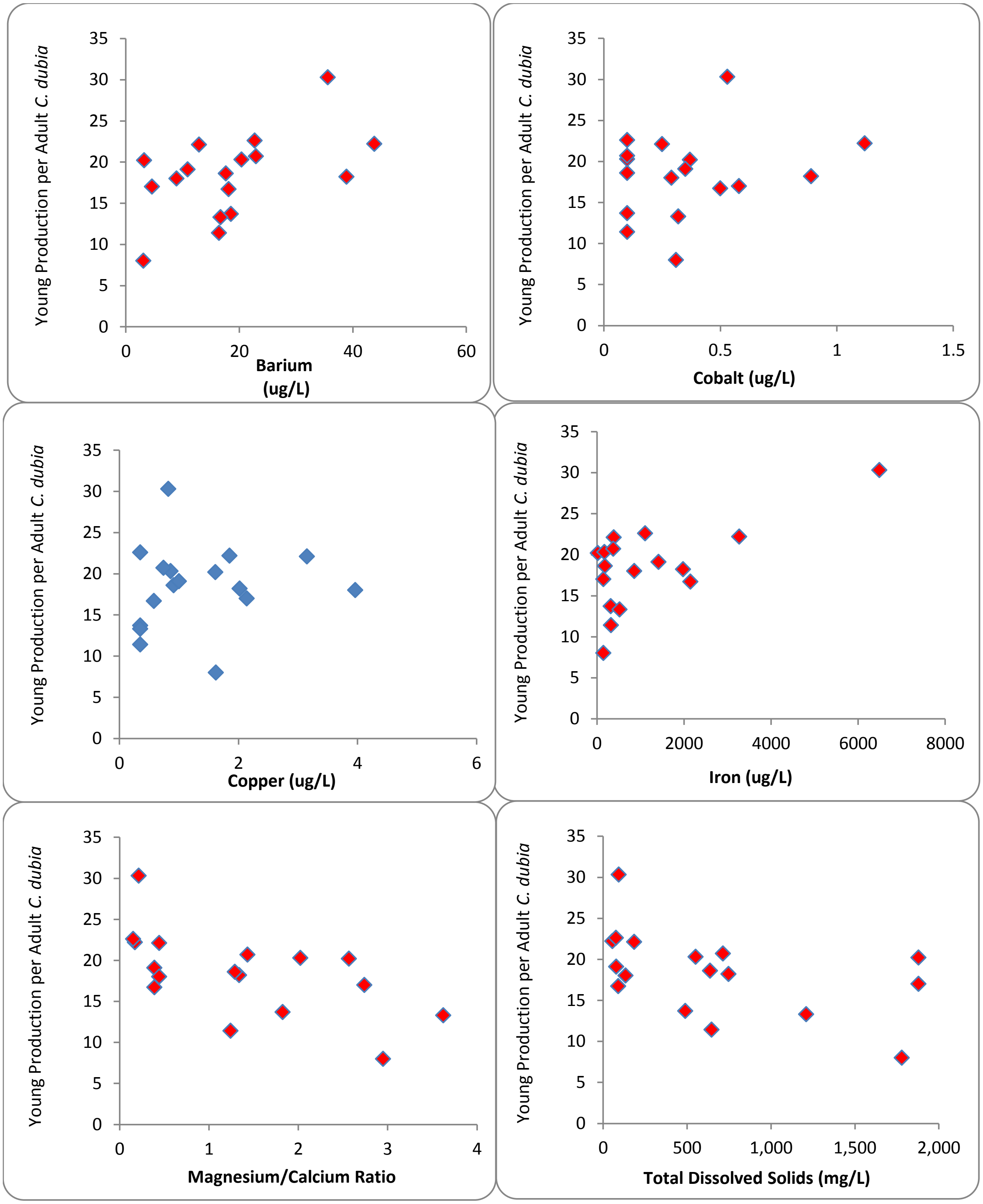


Figure 3-4. Relationship between chemical concentrations in mining outfalls (SD033 and SD026) and background and receiving waters with WET test results (young production per adult C. dubia) (parameters = barium, cobalt, copper, iron, magnesium/calcium ratio, total dissolved solids)

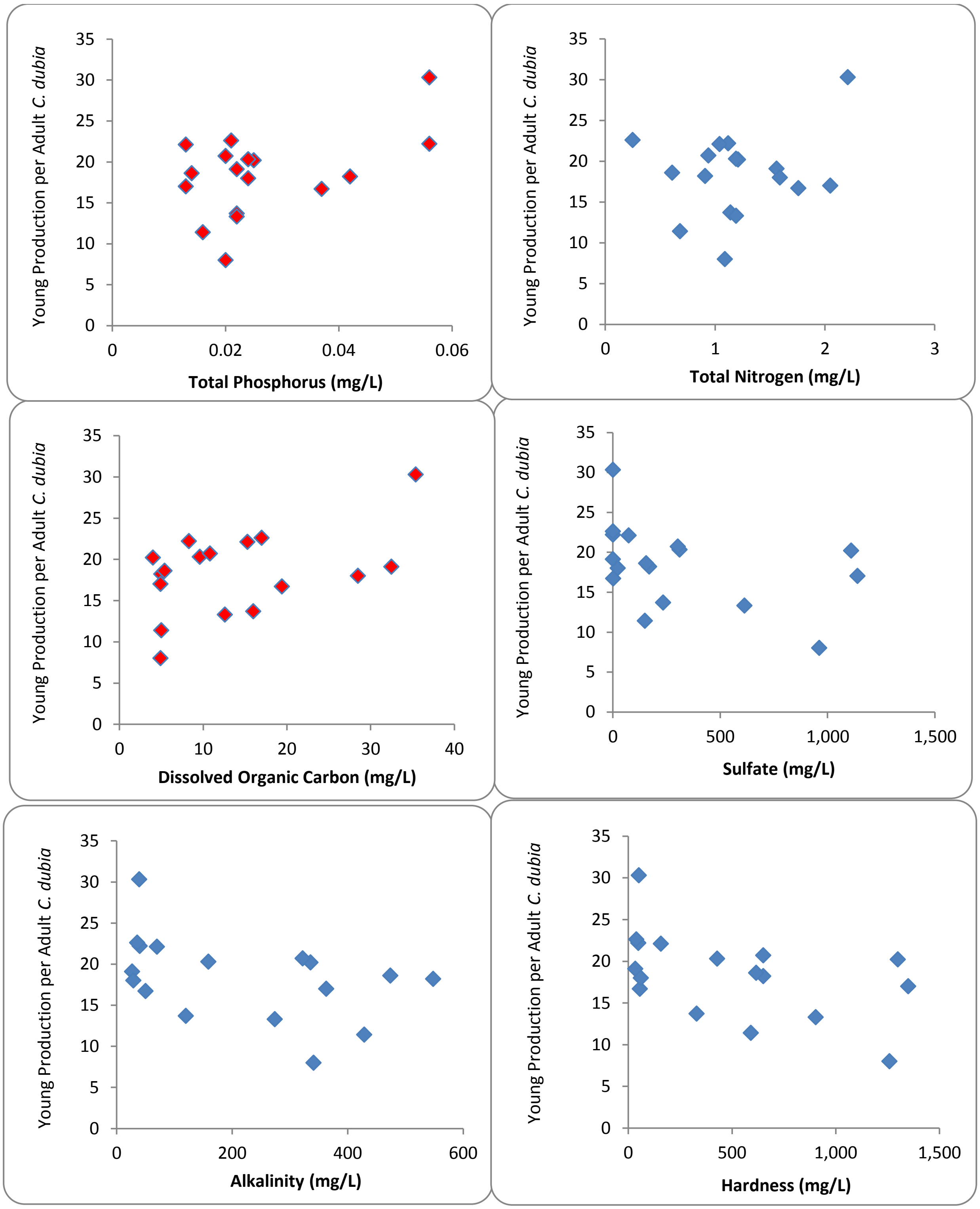
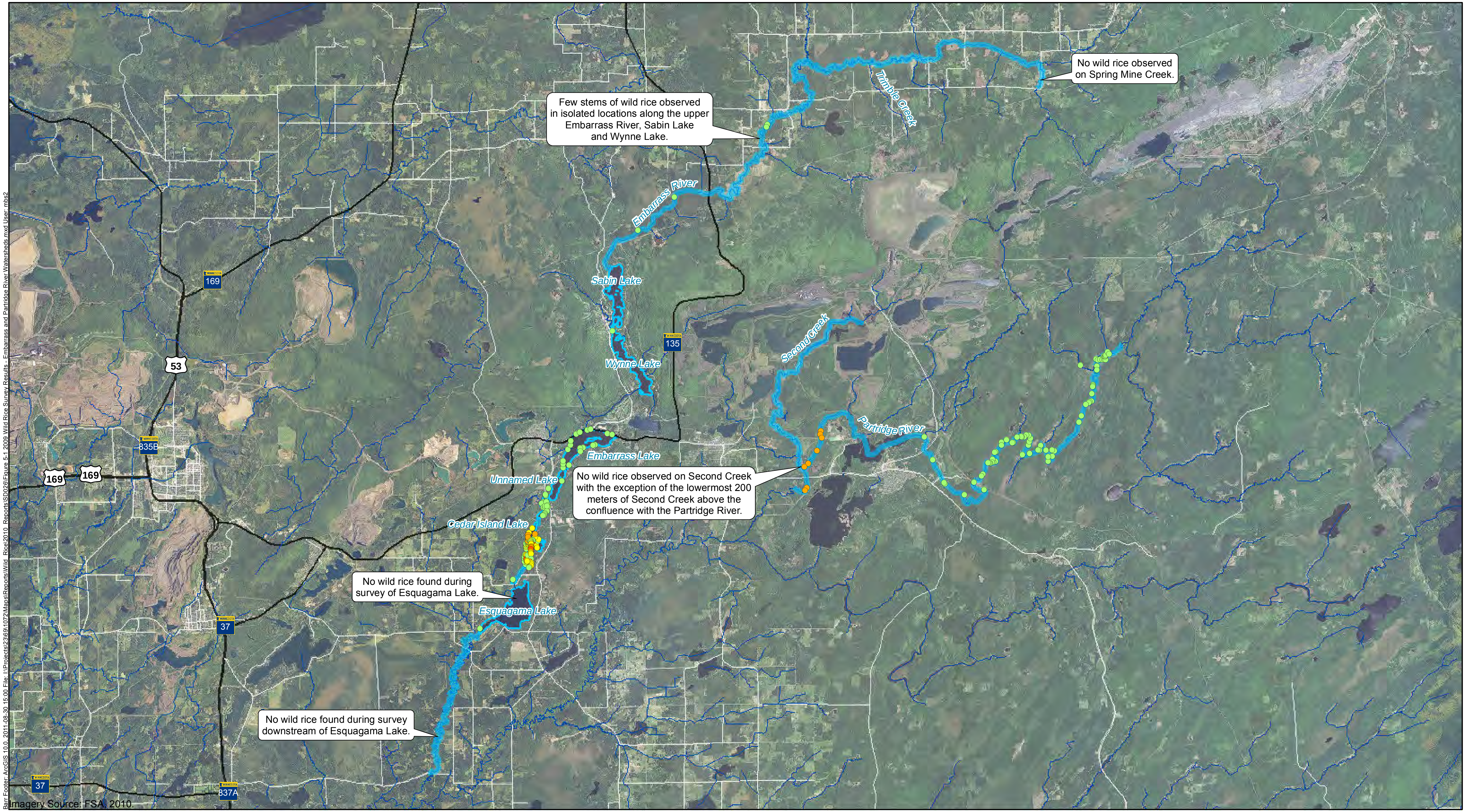


Figure 3-5. Relationship between chemical concentrations in mining outfalls (SD033 and SD026) and background and receiving waters with WET test results (young production per adult C. dubia) (parameters = total phosphorus, total nitrogen, dissolved organic carbon, sulfate, alkalinity, hardness)



Barr Footer: ArcGIS 10.0, 2011-08-30 15:00 File: I:\Projects\23\69\1072\Maps\Reports\Wild_Rice\2010_Reports\SD026\Figure 5-1 2009 Wild Rice Survey Results - Embarrass and Partridge River Watersheds.mxd User: mbs2

Wild Rice Density

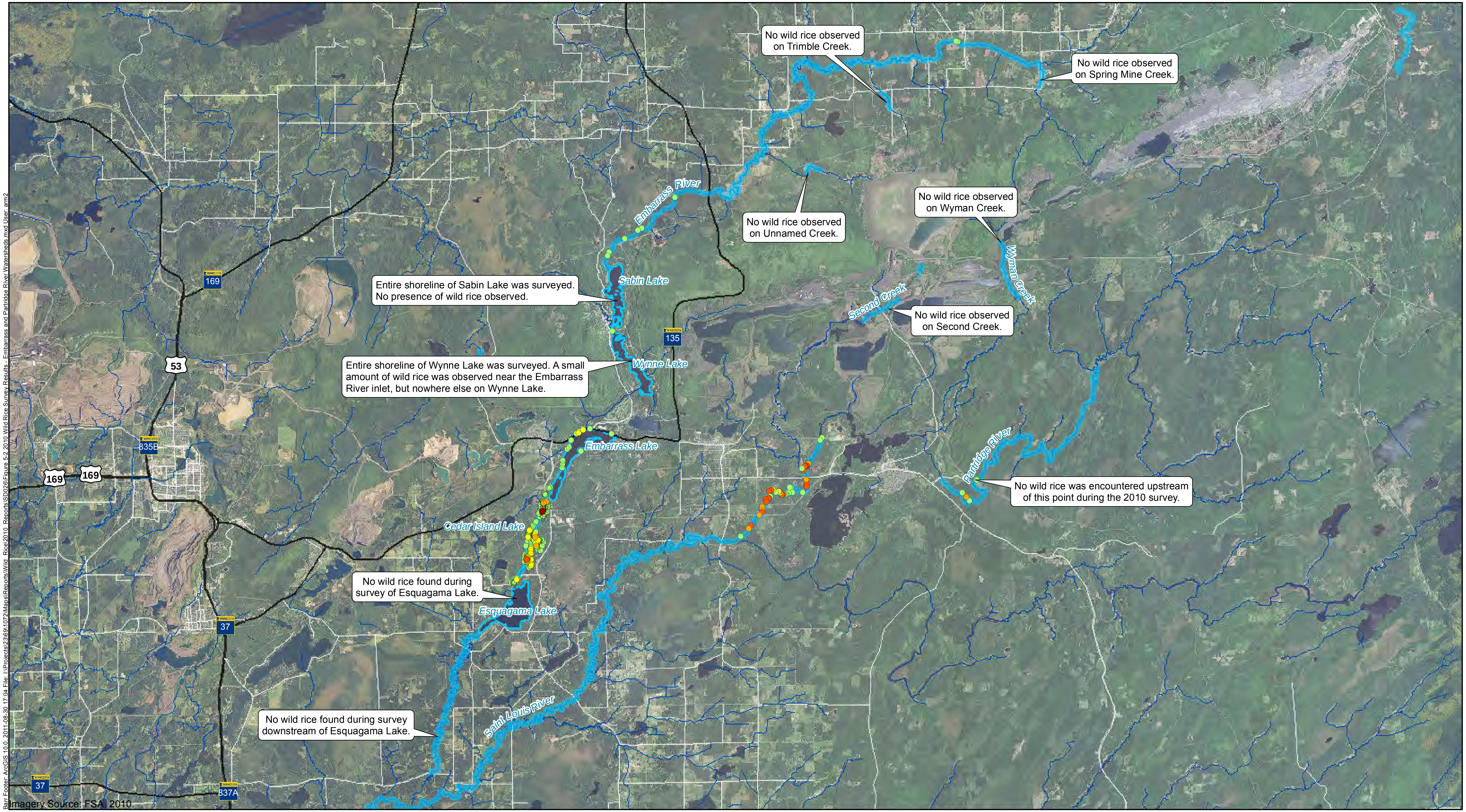
- 1 <10% Wild Rice Coverage
- 2
- 3
- 4
- 5 >75% Wild Rice Coverage

Stream or Shoreline Segment Surveyed in 2009

Data Sources: *2009 Wild Rice Survey and Sulfate Monitoring*
Prepared for Steel Dynamics, Inc. and Mesabi Mining, LLC, October 2009
2009 Wild Rice and Sulfate Monitoring
Prepared for PolyMet Mining Inc. – NorthMet Project, September 2009

0 1 2 4
Miles

Figure 5-1
2009 WILD RICE SURVEY RESULTS -
EMBARRASS AND PARTRIDGE RIVER WATERSHEDS
Cliffs Erie, L.L.C. and
PolyMet Mining, Inc.
Hoyt Lakes, Minnesota



Wild Rice Density

- 1 <10% Wild Rice Coverage
- 2
- 3
- 4
- 5 >75% Wild Rice Coverage
- Stream or Shoreline Segment Surveyed in 2010

Data Sources: *2010 Wild Rice Survey and Sulfate Monitoring*
Prepared for Mesabi Mining, LLC, March 2011
2010 Wild Rice and Water Quality Monitoring Report
Prepared for PolyMet Mining Inc. – NorthMet Project, January 2011

0 1 2 4
Miles

Figure 5-2
2010 WILD RICE SURVEY RESULTS - EMBARRASS AND PARTRIDGE RIVER WATERSHEDS
Cliffs Erie, L.L.C. and
PolyMet Mining, Inc.
Hoyt Lakes, Minnesota

Appendices

Appendix 3-A

Physical Habitat and Water Chemistry Assessment Protocol

PHYSICAL HABITAT AND WATER CHEMISTRY ASSESSMENT PROTOCOL FOR WADEABLE STREAM MONITORING SITES

I. PURPOSE

To describe the methods used by the Minnesota Pollution Control Agency's (MPCA) Biological Monitoring Program to collect physical habitat and water chemistry information at stream monitoring sites for the purpose of assessing water quality and developing biological criteria.

II. SCOPE/LIMITATIONS

This procedure applies to all wadeable monitoring sites for which an integrated assessment of water quality is to be conducted. An integrated assessment involves the collection of biological (fish and macroinvertebrate communities), physical habitat, and chemical information to assess stream condition.

III. GENERAL INFORMATION

Sites may be selected for assessment for a number of reasons including: 1) sites randomly selected for condition monitoring as part of the Environmental Monitoring and Assessment Program (EMAP), 2) sites selected for the development and calibration of biological criteria, and 3) sites selected to evaluate a suspected source of pollution. Although the reasons for monitoring a site vary, the physical habitat and water chemistry assessment protocols outlined in this document apply to all wadeable stream monitoring sites unless otherwise noted. For our purposes, wadeable sites constitute those that are sampled for fish utilizing a backpack electrofisher or stream electrofisher (see SOP--"***Fish Community Sampling Protocol for Stream Monitoring Sites***").

IV. REQUIREMENTS

A. Qualifications of crew leaders: The crew leader must be a professional aquatic biologist with a minimum of a Bachelor of Science degree in aquatic biology or closely related specialization. He or she must have a minimum of six months field experience in physical habitat sampling methodology. Field crew leaders should also possess excellent map reading skills and a demonstrated proficiency in the use of a GPS (Global Positioning System) receiver and orienteering compass.

B. Qualifications of field technicians/interns: A field technician/intern must have at least one year of college education and coursework in environmental and/or biological science.

C. General qualifications: All personnel conducting this procedure must have the ability to perform rigorous physical activity. It is often necessary to wade through streams and/or wetlands, canoe, or hike for long distances to reach a sampling site.

V. RESPONSIBILITIES

A. Field crew leader: Implement the procedures outlined in the action steps and ensure that the data generated meets the standards and objectives of the Biological Monitoring Program.

B. Technicians/interns: Implement the procedures outlined in the action steps, including maintenance and stocking of equipment, data collection and recording.

VI. QUALITY ASSURANCE AND QUALITY CONTROL

Compliance with this procedure will be maintained through annual internal reviews. Technical personnel will conduct periodic self-checks by comparing their results with other trained personnel. Calibration and maintenance of equipment will be conducted according to the guidelines specified in the manufacturer's manuals.

In addition to adhering to the specific requirements of this sampling protocol and any supplementary site specific procedures, the minimum QA/QC requirements for this activity are as follows:

A. Control of deviations: Deviation shall be sufficiently documented to allow repetition of the activity as performed.

B. QC samples: Ten percent of sites sampled in any given year are resampled as a means of determining sampling error and temporal variability.

C. Verification: The field crew leader will conduct periodic reviews of field personnel to ensure that technical personnel are following procedures in accordance with this SOP.

VII. TRAINING

A. All inexperienced personnel will receive instruction from a trainer designated by the program manager. Major revisions in this protocol require that all personnel be re-trained in the revised protocol by experienced personnel.

B. The field crew leader will provide instruction in the field and administer a field test to ensure personnel can execute this procedure.

VIII. ACTION STEPS

A. Equipment list: Verify that all necessary items are present before commencement of this procedure (Table 1).

B. Data collection method: The location and length of the sampling reach is determined during site reconnaissance (see SOP--"***Reconnaissance Procedures for Initial Visit to Stream Monitoring Sites***"). Sampling is conducted during daylight hours within the summer index period of mid-June through mid-September. Sampling should occur when streams are at or near base-flow. Water chemistry is sampled immediately prior to fish sampling. The physical habitat assessment is conducted after fish sampling, so as not to disturb the fish community.

Habitat within a station is quantified utilizing the transect-point method (modified from: Simonson, T.D., Lyons, J., and Kanehl, P.D. 1994. Guidelines for Evaluating Fish Habitat in Wisconsin Streams. Gen. Tech. Rep. NC-164. St. Paul, MN: U.S. Dept. of Agriculture, Forest Service, North Central Experiment Station. 36 p.). Thirteen transects are established within the reach and four equally spaced points plus the thalweg are located along each transect. Measurements or visual estimates are made to characterize key components of the physical habitat structure important in influencing stream ecology. Key components include: channel morphology, substrate, cover, and riparian condition.

Three data sheets are required for the physical habitat and water chemistry assessment. One copy of the **Station Features** and **Visit Summary** form is needed for each site. One copy of the **Transect** form is needed for each of the thirteen transects (or only seven copies if forms are doubled-sided). Copies of these forms are attached. Guidelines for filling out each data sheet are described in the following pages.

C. Station Features Data Sheet

This data sheet describes the length and location of the major morphological features within a sampling station (bends, pools, riffles, runs, log jams, islands, and beaver dams). The **Station Features** data is collected in conjunction with the **Transect** data as you proceed from the downstream end to the upstream end of the station. The variables on this data sheet are as follows:

1) *Field Number* – A seven-digit code that uniquely identifies the station. The first two digits identify the year of sampling, the second two identify the major river basin, and the last three are numerically assigned in sequential order (example: 02UM001).

2) *Date* – The date habitat sampling is conducted in month/day/year format (MM/DD/YY).

3) *Crew* – The personnel who collected the habitat data.

4) *Distance From Start* (column) – The distance from the downstream end of the station to the downstream end of each *stream feature*. Bends, log jams, and beaver dams are measured only to their midpoint because they are features that are located within one of the channel morphology types (i.e. riffle, run, or pool). Measure distances to the nearest tenth of a meter following the center of the stream channel. The first value is always "0" to indicate the *stream feature* at the beginning of the station. As you proceed upstream it is not necessary to continue to measure from the downstream end of the station, as each successive **Transect** data sheet has the distance of that transect from the downstream end of the station recorded. The last value in this column is the total length of the station.

5) *Stream Feature* (column) – Record the major morphological features encountered as you proceed upstream. If a cross-section of stream contains two or more channel morphology types (i.e. riffle, run, or pool) record the dominant type. Stream features recorded include:

   Riffles: Portions of the stream channel where water velocities are fast, water depths are relatively shallow, and substrates are typically coarse. Steeper stream gradient results in obvious surface turbulence. Areas of high gradient that are deep, fast, and turbulent are called **rapids**.

   Runs: Water velocities may be moderately fast to slow but the water surface typically appears smooth with little or no surface turbulence. Generally, runs are deeper than a riffle and shallower than a pool. Runs with very slow water velocities are sometimes called **glides**. For our purposes, if the channel type is not considered a riffle or pool it is defined as a run.

   Pools: Water is slow and generally deeper than a riffle or run. Water surface is smooth, no turbulence. A general rule that can be used to distinguish a pool is if two or more of the following conditions apply; the stream channel is wider, deeper, or slower than average.

   Bends: A change in the direction of the stream channel of at least 60 degrees.

   Islands: Areas of land within the stream channel that is surrounded on all sides by water and is dry even when the stream is experiencing bankfull flow. Areas with nearly all of the stream's flow on one side and just a trickle of water on the other are not considered islands. Islands usually contain vegetation. **Bars**, channel features below the bankfull flow level that are dry during baseflow conditions, are not recorded.

   Log Jams: Woody material that is of sufficient size to appreciably alter the direction of flow or change the morphology within the stream channel. Large log jams can be similar in effect and appearance to beaver dams.

   Beaver Dams: Structures constructed by beavers that span the entire stream channel and block flow. Beaver dams consist of sticks and mud, but older dams may be overgrown with vegetation.

   Other noteworthy features include: **bridges**, **culverts**, **dams**, and **tributaries**. The last feature noted in this column is the upstream **end of the reach**.

6) *Length* (column) – The length, measured to the nearest tenth of a meter, of each *stream feature* encountered within the reach. The length of bends, log jams, and beaver dams are not recorded. It is not necessary to complete this column while in the field as this information is derived from the *Distance from start* and *Stream feature* columns.

7) *Distance Between Bends* – The distance (m) between successive bends contained within the station. The first row is the distance between the mid-point of the first and second bend. The second row is the distance between the second and third, and so forth. These values can be derived using the information contained in the columns *Distance from start* and *Stream feature*. The "sum" and "mean" rows summarize all the distances between bends within the station.

8) *Distance Between Riffles* – The distance (m) between successive riffles contained within the station. The first row is the distance between the upstream end of the first riffle and the downstream end of the next riffle upstream, and so forth. Distances can be derived using the *Distance from start* and *Stream feature* columns. The "sum" and "mean" rows summarize these distances.

9) *Length of Individual Riffles, Pools, and Runs* – The individual length (m) of each riffle, pool, or run within the station, which can be derived using the *Stream feature* and *Length* columns. The sum of their lengths is also recorded here.

D. Transect Data Sheet

Record the data generated from each of the thirteen transects on this data sheet. One data sheet is needed for each transect. To determine the placement of each of the thirteen transects within the station divide the station length (determined during reconnaissance) by thirteen, this number is the *transect spacing* or distance between transects. The first transect is located one half of the transect spacing distance from the downstream end of the station. Each subsequent transect is then the distance of one transect spacing from the previous transect. All numbers are rounded to the nearest half meter.

For example, if the station length is 150 m, 150 ÷ 13 = 11.5 (equals the transect spacing). The first transect would then be located a distance of 6 m from the downstream end of the station, 11.5 ÷ 2 = 5.75 (equals 6 rounded to the nearest half meter). The second transect would then be located a distance of 17.5 m from the downstream end of the station, 6 + 11.5 = 17.5, and so forth for subsequent transects.

Each transect consists of several measurements or visual estimates, made within 0.3 m x 0.3 m quadrates at set intervals, or along the transect line perpendicular to the stream channel. The variables on this data sheet are as follows:

D.1. <u>Location Information</u>

1) *Field Number* – Same as for **Stream Features** data sheet.

2) *Date* – Same as for **Stream Features** data sheet.

3) *Transect Number* – The number (1-13) of the current transect as you proceed upstream. The downstream most transect is number one, the next transect upstream is two, and so on.

4) *Crew* – Same as for **Stream Features** data sheet.

5) *Distance from Start* – The distance from the downstream end of the station to the current transect following the center of the stream channel, rounded to the nearest half meter.

6) *Stream Width* – The wetted width of the stream channel at the transect, measured to the nearest tenth of a meter. Exposed bars and boulders are included in the wetted width of the stream channel, but islands are not. Backwaters not in contact with the stream at the transect are also excluded. If a channel is split by an island(s), the wetted widths of each side channel should be combined so that a single number is recorded in *stream width*. In low gradient streams the wetted width is the defined portion of the stream channel, it does not include adjacent wetlands and areas of emergent vegetation.

7) *Channel Type* – Circle the predominant channel type at the transect. See the **Station Features** section for riffle, pool, and run definitions.

D.2. <u>Transect Point Measurements</u>: At each transect, measurements or visual estimates are made at five points along the transect. Variables quantified include: *water depth, depth of fines and water, embeddedness, substrate, percent algae, and percent macrophytes*. Four points are equally spaced across the stream channel and the fifth point is the thalweg, or deepest point along the transect line. Divide the *stream width* at the transect by five to determine the 1/5, 2/5, 3/5, and 4/5 locations across the wetted width of the stream channel. Measurements are made at each of these four locations moving from the right bank to the left bank along the transect. The right stream bank is on the right as you are facing downstream. For example, if the stream is 10 m wide, measurements are taken at the thalweg and along the transect at 2.0, 4.0, 6.0, and 8.0 m from the right bank. In some instances, the thalweg will occur at the same location as one of the four other points, in which case their measurement values will be the same.

1) *Water Depth* – The depth of the stream channel at each transect point. Measure the vertical distance of the water column from the streambed to the water surface to the nearest centimeter with a calibrated wading rod or meter stick. If the water depth is over 120 cm, record as >120 cm.

2) *Depth of Fines and Water* – The water depth plus the depth of fine sediments at each transect point. Fine sediments are those that are less than 2.0 mm in diameter and generally consist of sand, silt, clay, or detritus. Without using the weight of your body, push a wading rod into the sediment as far as possible, measure to the water surface to the nearest centimeter. This measurement is later converted to depth of fines by subtracting *water depth*.

3) *Embeddedness of Coarse Substrates* – The extent to which coarse substrates are surrounded by or covered with fine sediments. Coarse substrates consist of gravel, rubble/cobble, and boulders. If the dominant substrate within the quadrate is coarse, embeddedness should be visually estimated to the nearest 25%. Estimate the average percent embeddeness of coarse substrates within the 0.3 m x 0.3 m quadrate centered on the channel position. An embeddedness rating of 0% corresponds to very little or no fine sediments surrounding coarse substrates. Course substrate material completely surrounded and covered with sediment is considered 100% embedded. If the dominant substrate within a quadrate is anything other than gravel, rubble/cobble, or boulder then the column should be left null.

4) *Dominant Substrate* – The predominant substrate type within each quadrate. Visually estimate which substrate type is predominant within each quadrate and place a check mark in the appropriate column. If the stream bottom cannot be seen, use your hands and feet to determine the dominant substrate type. Choose from the following substrate types:

   Bedrock: A solid slab of rock, > 4000 mm in length (larger than a car).

   Boulder: Large rocks ranging from 250 mm to 4000 mm in diameter (basketball to car size).

   Rubble/Cobble: Rocks ranging in diameter from 64 mm to 250 mm (tennisball to basketball).

   Gravel: Rocks varying in diameter from 2 mm to 64 mm (BB to tennisball).

   Sand: Inorganic material that is visible as particles and feels gritty between the fingers. 0.06 mm to 2.0 mm in size.

   Silt: Fine inorganic material that is typically dark brown in color. Feels greasy between fingers and does not retain its shape when compacted into a ball. A person's weight will not be supported if the stream bottom consists of silt.

   Clay: Very fine inorganic material. Individual particles are not visible or are barely visible to the naked eye. Will support a person's weight and retains its shape when compacted.

   Detritus: Decaying organic material such as macrophytes, leaves, finer woody debris, etc. that may appear similar to silt when very fine.

   Other: Any substrate type not listed above, specify the type. Possibilities could include woody debris, culverts, tires, or mussel beds.

5) *Algae (%)* – Visually estimate the amount of algae within the quadrate, to the nearest 5 %. Algae can either be attached to the substrate in the form of a mat or crust; or filamentous algae, which forms dense mats of long, hair-like strands and is usually green in color.

6) *Macrophytes (%)* – Visually estimate the amount of aquatic vegetation within the quadrate, to the nearest 5 %. Aquatic macrophytes can be either submergent or emergent and are defined under *cover for fish*.

D.3. Cover and Land Use Characteristics

1) *Cover for Fish (%)* – The amount of cover or shelter available for fish along the transect. Visually estimate the percentage (nearest 5 %) occupied by each cover type along the transect within a 0.3 m band centered on the transect line. If a cover type is absent, enter a zero. In order to be considered cover, the water depth must be at least 15 cm where the cover type occurs. Cover for fish consists of objects or features dense enough to provide complete or partial shelter from the stream current or concealment from predators or prey.

   Undercut Banks: Stream banks where the stream channel has cut underneath the bank. The bank could overhang the water surface when water levels are low. The undercut bank must overhang (horizontally) the wetted stream channel a minimum of 15 cm and the bottom of the bank must be no more than 15 cm above the water level in order to be considered cover for fish.

   Overhanging Vegetation: Terrestrial vegetation overhanging the wetted stream channel that meets the same criteria for cover as undercut banks.

   Woody Debris: Logs, branches, or aggregations of smaller pieces of wood in contact with or submerged in water.

   Boulders: Large rocks as described under *Substrate*.

   Submergent Macrophytes: Vascular plants that have all of their biomass (except flowers) at or below the surface of the water. Examples include *Vallisneria*, *Elodea*, *Potamogeton*, *Nymphaea* and *Ceratophyllum*.

   Emergent Macrophytes: Vascular plants that typically have a significant portion of their biomass above the water surface. Examples include *Typha, Scirpus,* and *Zizania.*

   Other Debris: Additional objects that meet the criteria of cover, typically of human origin. Examples would include filamentous algae, culverts, docks, tires, discarded appliances, etc. Specify the type.

2) *Bank Erosion* – The amount of the stream bank that is exposed soil and therefore, susceptible to erosion. For each bank, along the transect line, use a wading rod or measuring tape to quantify the length (nearest 0.1 m) of bare soil. Measure the amount of exposed soil from the waters edge to the top of the stream bank, up to a maximum of 5 m. If there is no bare soil, record 0.

3) *Riparian Land Use* – The predominant land use within the riparian zone. For each bank, extending along the transect line, visually estimate the predominant land use within 30 m of the waters edge and place a check mark in the corresponding column. Repeat this same procedure for the riparian zone 30 – 100 m from the waters edge. Land use categories are as follows:

   Cropland: Land that is cultivated with crops for forage or cover. Includes those areas under intensive cropping or rotation, or that are regularly mowed for hay.

   Pasture: Land that is regularly grazed by livestock.

   Barnyard: Land associated with farmsteads and the adjoining farmyard area. Includes grain storage facilities, barns, farmhouses, and feedlots (areas used to confine and feed high densities of livestock).

   Developed: Land that has been modified (rural or urban) for commercial, industrial, or residential use. Includes commercial buildings/structures, parking lots, all roads, railroads, and power utilities. Also includes residential buildings, lawns, parks, golf courses, ball fields, etc. Specify the type in the space provided.

   Exposed Rock: Natural areas of rock outcrops that lack appreciable soil development or vegetative cover.

Meadow: Land dominated by grasses and forbs with little woody vegetation, which is not subject to regular mowing or grazing.

Shrub: Land consisting primarily of woody vegetation less than 3 m in height. Typical shrubs include alder, dogwood, and willows.

Woodland: Land dominated by deciduous or coniferous tree species, generally taller than 3 m.

Wetland: Low-lying areas that are saturated or inundated with water frequently or for considerable periods of time on an annual basis. Wetlands include bogs, marshes, and swamps and contain vegetation adapted for life in saturated conditions.

Other: If a land use category other than one of those listed above is predominant, specify the type.

4) *Riparian Buffer Width* – The amount of contiguous undisturbed land use within a 10 m buffer zone. For each bank, starting from the waters edge and extending out along the transect line 10 m, measure the width (nearest meter) of contiguous land that is considered undisturbed. Meadow, shrub, woodland, wetland, and exposed rock are considered undisturbed. If no undisturbed land uses are directly adjacent to the stream, then the riparian buffer width is 0 m. If more than 10 m is present, record it as >10 m.

5) *Canopy/Shading* – A measure of overhead canopy cover that is shading the stream channel. A concave spherical crown densiometer is utilized for this measurement. The densiometer must be taped as shown in Figure 1 to limit the number of grid intersections to 17. Hold the densiometer at elbow level in front of you, making sure the instrument is level using the bubble level, count and record the number (0 to 17) of grid intersections that have vegetation covering them. If the reflection of a tree, branch, or leaf overlies any of the intersection points, that particular intersection is counted as having cover. Perform this measurement from the center of the stream channel along the transect line in each of four directions; facing upstream, downstream, towards the left bank, and towards the right bank. In addition, perform the measurement at the wetted edge of both the left and right banks facing the stream bank.

E. Visit Summary Data Sheet

This data sheet contains location information, water chemistry data, and channel characteristics of the station. Some of the data is derived from maps or from the other data sheets. Record the following information on this data sheet:

E.1. <u>Location Information</u>

1) *Field Number* – Same as for **Station Features** data sheet.

2) *Date* – Same as for **Station Features** data sheet.

3) *Stream Name* – The name of the stream as shown on the most recent USGS 7.5" topographic map. Include all parts of the name (i.e. "North Branch", "Creek", "River", "Co. Ditch", etc.).

4) *Location* – A general description of where the sampling station is located. Usually includes the nearest road crossing and town. For example, "0.5 mi. downstream of C.R. 30, 4 mi. SW of Northome".

5) *County* – The county in which the station is located.

6) *Visit Result* – The result of the sampling trip, typically as it pertains to fish collection. Circle only one of the available choices. A visit or sampling trip is considered "reportable" when sampling is conducted for the first time at a station and no problems are encountered that would render the data questionable. If subsequent sampling trips are made to the same station and no sampling problems occur, the *visit result* is considered a "replicate". Circle "other", and explain in the space provided, in the event that the data generated is questionable or unsuitable for use. Reasons might include equipment problems, poor sampling efficiency, excessive water velocity, poor fish taxis, or other sampling deficiencies.

7) *GPS File Name* – The unique identifier of a rover file assigned by the GPS unit. If a GPS file is taken (to record the location of a sampling site), the unit will assign an eight-digit code consisting of a file prefix, date stamp, and time stamp that uniquely identifies that file. In most instances, it is not necessary to take a GPS file during the sampling visit because sampling sites are located and flagged during site reconnaissance. However, circumstances may occur that necessitate a file be taken during the sampling visit. These include but are not limited to: original reconnaissance file unreliable or inaccurate, flagging cannot be located, initial site location determined to be incorrect, and GPS file not obtained during initial site reconnaissance. If sampling and initial site reconnaissance are conducted at the same time, the GPS information should be recorded as part of the reconnaissance protocol. Consult the GPS user's manual and SOP--"***Reconnaissance Procedures for Initial Site Visit to Stream Monitoring Sites***" for additional guidance on GPS operation and protocol.

8) *Type of GPS Fix* – If a GPS file is taken during the sampling visit, indicate the position mode (3D or 2D) in which the GPS file was recorded.

9) *PDOP* – If a GPS file is taken during the sampling visit, record the approximate Position Dilution of Precision (PDOP) value that was observed while the GPS file was being recorded.

10) *Data Source* – The source or entity that generated the data. For Minnesota Pollution Control Agency (MPCA) staff within the Biological Monitoring Unit this field should be recorded as "MPCA".

11) *Project* – The specific project that the data collection effort is associated with. Some possibilities include EMAP, biocriteria development, problem investigation, and longitudinal survey.

E.2. Field Water Chemistry: Water chemistry parameters should be sampled immediately prior to fish sampling. All water chemistry parameters are measured from the same general location at a representative stream cross-section within the sampling reach. Samples are taken at a point that is judged to represent the water quality of the total instantaneous flow at the cross-section. Avoid sampling areas that are poorly mixed, contain springs, or are upstream of or immediately adjacent to tributaries within the sampling reach. Water chemistry measurements and water samples are taken at an intermediate depth in the water column without disturbing substrate materials or collecting floating materials and constituents from the water surface. Refer to the manufacturer's owners manual for guidance concerning the calibration and operation of water quality meters.

1) *Time* – The time of day (24-hour clock) that field water chemistry parameters are measured.

2) *Air Temp* – The ambient air temperature (°C) at the time of sampling, measure to the nearest degree with a dry thermometer.

3) *Water Temp* – The water temperature (°C) of the station at the time of sampling, measure to the nearest tenth of a degree with a thermometer or water quality meter.

4) *Conductivity* – Temperature compensated conductivity, or *specific conductance*, is the parameter actually being determined and is a measure of the ability of water to carry an electrical current. Consult your conductivity meter's manual for guidance measuring specific conductance (measured in μmhos/cm) compensated for temperature to 25 °C.

5) *Dissolved Oxygen* – The amount of oxygen present in a water sample, expressed as milligrams of oxygen per liter of water (mg/L). Two water samples should be taken and measured for dissolved oxygen concentrations using a DO meter or the Winkler Titration Method.

6) *Turbidity* – The light scattering property associated with suspended particles in the water, measured with a turbidimeter in nephelometric turbidity units (NTUs). A turbid sample will appear cloudy. A water sample is taken in a 500-ml plastic bottle rinsed with stream water three times. Due to the sensitivity of the turbidimeter to road dust and other conditions encountered while in the field, place the sample on wet ice until days end and measure turbidity in a more suitable environment (office or hotel room).

7) *pH* – A measure of the negative log of the hydrogen ion [H^+] concentration in the water. Pure water has a pH of 7.00 and is considered neutral. Measure pH utilizing a temperature compensating pH meter.

8) *Stream Flow* – Also known as discharge, it is the volume of water moving downstream per unit time, and is the product of current velocity and the dimensions of the stream channel. Measure the instantaneous flow rate (cubic meters/second) at a suitable stream cross-section using a current meter. Detailed guidelines for determining stream flow at a station are available from the USGS.

9) *Transparency* – A measure of water clarity, an indicator of the water's ability to transmit light. Stream transparency serves as an indirect measure of the amount of dissolved and suspended materials present. Measure (nearest cm) with a transparency tube, a clear tube 60 cm in length with a secci-type disk at the bottom.

10) *Water Level* – An estimation of water level as it relates to summer base flow expectations. Check the appropriate category and measure the vertical distance (nearest 0.1 m) above or below the normal water line. In most streams, the "normal" water level can be determined with relative ease by observing channel characteristics.

E.3 Lab Water Chemistry: Water samples taken for laboratory analyses typically include total phosphorus (P), total suspended solids (TSS), ammonia nitrogen (NH^3+NH^4), and nitrite-nitrate (NO^2+NO^3). Additional parameters may be measured in special circumstances. Samples taken for laboratory analyses are subject to the same general guidelines concerning sampling location and time as outlined above under *field water chemistry*. Sterilized sample bottles are obtained from the Minnesota Department of Health. Before collecting samples, label the containers with the *date* and *field number* with a waterproof pen or pencil. Collect a 250 ml nutrients sample and a one-liter general chemistry sample for laboratory analysis. The bottles should be lowered mouth down to an intermediate depth and then turned upstream to collect the sample, the Dept. of Health does not recommend rinsing their sample bottles. Immediately after sample collection, 5 ml of 10% sulfuric acid preservative solution is added to the nutrients sample. Both sample bottles must be stored at 4 °C and shipped to the Dept. of Health Water Lab within the minimum holding times.

1) *Collection Time (field sample)* – The time of day (24-hour clock) that water samples for laboratory analysis are collected.

2) *Collection Time (field duplicate)* – A field duplicate is a second sample taken immediately following an initial sample in the same manner and location. Duplicate samples are taken at 10% of all sampling sites for quality assurance and control (QA/QC) purposes. If a duplicate water sample is taken, record the time (24 hour clock) here.

E.4 Channel Characteristics

1) *Transect Spacing* – Document the distance (m) that was used to space transects from one another (see **Transect** data sheet section).

2) *Station Length* – The actual length (m) of the sampling reach as determined during the physical habitat assessment. The station length should be recorded directly from the **Stream Features** data sheet, as measured from the start of the station to the upstream end of the reach, rounded to the nearest meter. This measurement of station length is considered more accurate than the measurement conducted during the initial site reconnaissance.

3) *Channel Condition* – The condition of the stream channel at the station, check the category that best describes the state of the stream channel: natural channel, old channelization, recent channelization, or concrete channel.

4) *Mean Distance Between Bends* – The average distance (m) between successive bends contained within the station. Obtained from the **Station Features** data sheet.

5) *Mean Distance Between Riffles* – The average distance (m) between successive riffles contained within the station. Obtained from the **Station Features** data sheet
6) *Total Length of Riffles, Pools, and Runs* – The sum of the lengths (m) for all riffles, pools, and runs contained within the station. Obtained from the **Station Features** data sheet.

7) *Total Number of Riffles, Pools, Runs, Bends, and Log Jams* – The number of each of these stream features contained within the station. Obtained from the **Station Features** data sheet.

E.5. <u>Comments/Notes</u>: Record any additional information about the station in the space provided.

Table 1. Equipment List – This table identifies all equipment needed in the field in order to implement the sampling protocol as described.

Physical Habitat Sampling

Measuring tape (m) – for measuring distances

Wading rod – for measuring depths and short distances

Spherical crown densiometer (concave) – to measure canopy cover

Water Chemistry Sampling

Thermometer – for measuring air and water temperature

Conductivity meter – for measuring conductivity

Turbidimeter – for measuring turbidity

D.O. meter or Winkler-Titration kit – for measuring dissolved oxygen

pH meter – for measuring pH

Current meter – for measuring stream discharge

Transparency tube – for measuring stream water transparency

1-L plastic bottle – to collect general chemistry sample for lab analysis

250-ml plastic bottle – to collect nutrients sample for lab analysis

500-ml plastic bottle – to collect turbidity sample

5-ml of 10% sulfuric acid – for preserving nutrients sample

Cooler and ice – for holding and preserving water samples

Miscellaneous

Clipboard – to store forms and record data

Forms – for recording data

Pencil – for filling out forms

GPS – to locate and document sampling location (if necessary)

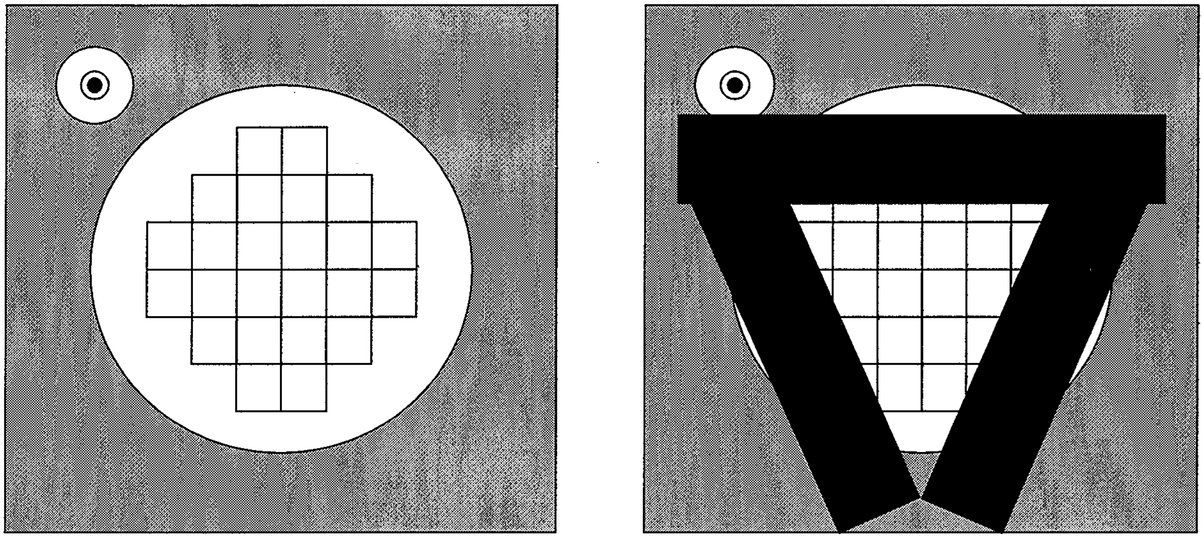


Figure 1. Illustration depicting how a spherical crown densiometer should be taped to limit the number of grid intersections to 17.

STATION FEATURES

MPCA

Field Number:________________ Date(mm/dd/yy):______________ Crew:__________________

DISTANCE FROM START (m)	STREAM FEATURE (Riffle, Pool, Run, Bend Log Jam, etc.) *	LENGTH (m)
0		

DISTANCE SUMMARY

Distance Between Bends(m):

1st - 2nd:____________
2nd - 3rd:____________
3rd - 4th:____________
4th - 5th:____________
5th - 6th:____________
6th - 7th:____________
7th - 8th:____________
8th - 9th:____________
9th - 10th:____________
10th - 11th:____________
11th - 12th:____________
12th - 13th:____________
13th - 14th:____________
14th - 15th:____________

Sum:____________

Mean:____________

Distance Between Riffles(m):

1st - 2nd:____________
2nd - 3rd:____________
3rd - 4th:____________
4th - 5th:____________
5th - 6th:____________
6th - 7th:____________
7th - 8th:____________
8th - 9th:____________
9th - 10th:____________
10th - 11th:____________
11th - 12th:____________
12th - 13th:____________
13th - 14th:____________
14th - 15th:____________

Sum:____________

Mean:____________

Length (m) Of Individual Riffles, Pools, And Runs:

1st Riffle:________	1st Pool:________	1st Run:________
2nd Riffle:________	2nd Pool:________	2nd Run:________
3rd Riffle:________	3rd Pool:________	3rd Run:________
4th Riffle:________	4th Pool:________	4th Run:________
5th Riffle:________	5th Pool:________	5th Run:________
6th Riffle:________	6th Pool:________	6th Run:________
7th Riffle:________	7th Pool:________	7th Run:________
8th Riffle:________	8th Pool:________	8th Run:________
9th Riffle:________	9th Pool:________	9th Run:________
10th Riffle:________	10th Pool:________	10th Run:________
11th Riffle:________	11th Pool:________	11th Run:________
12th Riffle:________	12th Pool:________	12th Run:________
13th Riffle:________	13th Pool:________	13th Run:________
14th Riffle:________	14th Pool:________	14th Run:________
15th Riffle:________	15th Pool:________	15th Run:________
Sum:________	Sum:________	Sum:________

* For riffles, runs, and pools note distance from start at beginning of feature. For bends, log jams, etc., note center-point.

(Revised Dec. 2002)

Station Features Continued:

DISTANCE FROM START (m)	STREAM FEATURE (Bend, Riffle, Pool, Run, Log Jam, etc.) *	LENGTH (m)
0		

TRANSECT MPCA

Field Number:________________ Date (mm/dd/yy):____________ Transect Number (1-13):________

Crew:____ Distance from Start (m):___________

Stream Width (m):________________ Channel Type (circle one): Riffle Pool Run

Channel Position (fifths of wetted stream width and deepest point, 0 = rightbank *)	1/5	2/5	3/5	4/5	Deep
Water Depth (cm)					
Depth of Fines and Water (cm)					
Embeddedness of Coarse Substrates (nearest 25%)					

Check Dominant Substrate Type in Quadrate:

Channel Position (fifths of wetted stream width and deepest point, 0 = rightbank *)	1/5	2/5	3/5	4/5	Deep
Bedrock (solid slab)					
Boulder (basketball or bigger)					
Rubble/Cobble (tennis ball to basketball)					
Gravel (BB to tennis ball)					
Sand (gritty, visible, < BB)					
Silt					
Clay					
Detritus					
Other (specify)					

Note Amount Observed on Quadrate:

Channel Position (fifths of wetted stream width and deepest point, 0 = rightbank *)	1/5	2/5	3/5	4/5	Deep
Algae (attached & filamentous., nearest 5%)					
Macrophytes (nearest 5%)					

Cover for Fish: *Percent length of transect* ***(over at least 15 cm water depth)*** *with:*
___Undercut Banks ____Overhanging Vegetation ____Woody Debris ____Boulders
___Submergent Macrophytes ____Emergent Macrophytes ____Other (specify):__________________

Bank Erosion: *Length (nearest 0.1 m) of bare soil,* ***within 5 m of waters edge****, along transect:*
LEFT BANK *: _________(m) RIGHT BANK *: _________ (m)

Riparian Land Use: *Dominant land use* ***within 30 m of stream edge*** *(along transect):* ***(L / R)*** *
___/___Cropland ___/___Pasture ___/___Barnyard ___/___Developed ___/___Exposed Rock
___/___Meadow ___/___Shrubs ___/___Woodland ___/___Wetland ___/___Other (specify):__________

Riparian Land Use: *Dominant land use* ***from 30 to 100 m of stream edge*** *(along transect):* ***(L / R)*** *
___/___Cropland ___/___Pasture ___/___Barnyard ___/___Developed ___/___Exposed Rock
___/___Meadow ___/___Shrubs ___/___Woodland ___/___Wetland ___/___Other (specify):__________

Riparian Buffer Width: *Length (nearest meter) of undisturbed land use along transect,* ***within 10 m of stream:***
LEFT BANK *: _________(m) RIGHT BANK *: _________ (m)

Canopy/Shading (Densiometer reading, note #/17 that are shaded):

___Center Upstream ___Center Left ___Center Downstream ___Center Right ___Left Bank * ___Right Bank *

* Right Bank and Left Bank identified while facing downstream.

(Revised Dec 2002)

VISIT SUMMARY — MPCA

LOCATION INFORMATION ==

Field Number:___________ Date (mm/dd/yy):__________ Stream Name:_________________________

Location:____ County:____________________

Visit Result (circle one): Reportable - Replicate - Other (explain)_________________________

GPS File Name:______________________ Type of GPS Fix: ☐ 2D ☐ 3D PDOP:_________
(only if GPS taken during visit)

Data Source:______________________________ Project:_________________________________

FIELD WATER CHEMISTRY ==

Time (24 hr clock):______________ Air Temp.(°C):_____________ Water Temp.(°C):____________

Conductivity (umhos@25°C):__________________ Dissolved Oxygen (mg/l):__________________

Turbidity (ntu):_________________ pH:_______________ Stream Flow (m^3/s):________________

Transparency Tube (cm):______ Water Level: ☐ Normal ☐ Below______(m) ☐ Above______(m)

LAB WATER CHEMISTRY ==

Collection Time (field sample):_______________ Collection Time (field duplicate):_______________

CHANNEL CHARACTERISTICS ==

Transect Spacing (m):___________ Station Length (m) (from stream features form):_____________

Channel Condition (check appropriate box):

☐ Natural Channel ☐ Old Channelization ☐ Recent Channelization ☐ Concrete Channel

Mean Distance Between Bends (m):____________ Mean Distance Between Riffles (m):____________

Total Length (Sum) of All (m): Riffles:_____________ Pools:_____________ Runs:_____________

Total Number of: Riffles:_______ Pools:_______ Runs:_______ Bends:_______ Log Jams:______

COMMENTS/NOTES: ____

____

____

(Revised Dec. 2002)

Appendix 3-B

Stream Habitat and Evaluation Form

MPCA STREAM HABITAT ASSESSMENT (MSHA) PROTOCOL FOR STREAM MONITORING SITES

I. PURPOSE

To describe the methods used by the Minnesota Pollution Control Agency's (MPCA) Biological Monitoring Program to collect qualitative physical habitat information at stream monitoring sites for the purpose of assessing water quality and developing biological criteria.

II. SCOPE/LIMITATIONS

This procedure applies to all river and stream monitoring sites for which an integrated assessment of water quality is to be conducted. An integrated assessment involves the collection of biological (fish and macroinvertebrate communities), physical habitat, and chemical information to assess stream condition.

III. GENERAL INFORMATION

Sites may be selected for assessment for a number of reasons including: 1) sites randomly selected for condition monitoring as part of the Environmental Monitoring and Assessment Program (EMAP), 2) sites selected for the development and calibration of biological criteria, and 3) sites selected to evaluate a suspected source of pollution. Although the reasons for monitoring a site vary, the MSHA protocol described in this document applies to all monitoring sites unless otherwise noted.

IV. REQUIREMENTS

A. <u>Qualifications of crew leaders</u>: The crew leader must be a professional aquatic biologist with a minimum of a Bachelor of Science degree in aquatic biology or closely related specialization. He or she must have a minimum of six months field experience in physical habitat sampling methodology. Field crew leaders should also possess excellent map reading skills and a demonstrated proficiency in the use of a GPS (Global Positioning System) receiver and orienteering compass.

B. <u>Qualifications of field technicians/interns</u>: A field technician/intern must have at least one year of college education and coursework in environmental and/or biological science.

C. <u>General qualifications</u>: All personnel conducting this procedure must have the ability to perform rigorous physical activity. It is often necessary to wade through streams and/or wetlands, canoe, or hike for long distances to reach a sampling site.

V. RESPONSIBILITIES

A. <u>Field crew leader</u>: Implement the procedures outlined in the action steps and ensure that the data generated meets the standards and objectives of the Biological Monitoring Program.

B. <u>Technicians/interns</u>: Implement the procedures outlined in the action steps, including maintenance and stocking of equipment, data collection and recording.

VI. QUALITY ASSURANCE AND QUALITY CONTROL

Compliance with this procedure will be maintained through annual internal reviews. Technical personnel will conduct periodic self-checks by comparing their results with other trained personnel.

In addition to adhering to the specific requirements of this sampling protocol and any supplementary site specific procedures, the minimum QA/QC requirements for this activity are as follows:

A. Control of deviations: Deviation shall be sufficiently documented to allow repetition of the activity as performed.

B. QC samples: Ten percent of sites sampled in any given year are resampled as a means of determining sampling error and temporal variability.

C. Verification: The field crew leader will conduct periodic reviews of field personnel to ensure that technical personnel are following procedures in accordance with this SOP.

VII. TRAINING

A. All inexperienced personnel will receive instruction from a trainer designated by the program manager. Major revisions in this protocol require that all personnel be re-trained in the revised protocol by experienced personnel.

B. The field crew leader will provide instruction in the field and administer a field test to ensure personnel can execute this procedure.

VIII. ACTION STEPS

A. Equipment list: Verify that either a form and pencil, or a field computer is present before commencement of this procedure.

B. Data collection method: The location and length of the sampling reach is determined during site reconnaissance (see SOP--"***Reconnaissance Procedures for Initial Visit to Stream Monitoring Sites***"). Unless otherwise instructed, observations of physical habitat characteristics should be limited to the sampling reach. Sampling is conducted during daylight hours within the summer index period of mid-June through mid-September. Sampling should occur when streams are at or near base-flow. The habitat evaluation is conducted immediately after fish sampling in order to provide the evaluator a perspective of the fish habitat within the reach.

Habitat characteristics are recorded using a qualitative, observation based method (modified from: Rankin 1989. The Qualitative Habitat Evaluation Index (QHEI): Rationale, Methods, and Application. Ohio EPA, Division of Water Quality Planning and Assessment, Ecological Analysis Section, Columbus, Ohio.). The Ohio QHEI is a physical habitat index designed to provide an empirical evaluation of the lotic macrohabitat characteristics that are important to fish communities and which are generally important to other aquatic life. Although similar to the Ohio QHEI, the MSHA has been modified to more adequately assess important characteristics influencing Minnesota streams. The MSHA incorporates measures of watershed land use, riparian quality, bank erosion, substrate type and quality, instream cover, and several characteristics of channel morphology.

Observations are recorded on the **MPCA Stream Habitat Assessment Worksheet**. A copy is attached and guidelines for filling out this data sheet are described in the following pages.

C. MPCA Stream Habitat Assessment Data Sheet

This data sheet describes the presence and abundance of instream and riparian characteristics within the sampling reach. The variables recorded are as follows:

C.1. Stream Documentation

A) *Stream* – The name of the stream as shown on the most recent USGS 7.5" topographic map. Include all parts of the name (i.e. South Branch Wild Rice River).

B) *County* – The county in which the station is located.

C) Date – The date habitat sampling is conducted in month/day/year format (MM/DD/YY).

D) Field Number – A seven-digit code that uniquely identifies the station. The first two digits identify the year of sampling, the second two identify the major river basin, and the last three are numerically assigned in sequential order (example: 02UM001).

E) Person Scoring – The personnel completing the MSHA. This person(s) should have walked or boated the entire stream reach paying particular attention to habitat features.

F) Site Location – A general description of where the sampling station is located. Usually includes the nearest road crossing and town. For example, "0.5 mi. downstream of C.R. 30, 4 mi. SW of Northome".

C.2. Surrounding Land Use: Record the predominant land use on each bank within approximately 2 to 3 square miles, not just the surrounding area of the site. The emphasis should be on upstream land use. Check either the most predominant land use, or choose two and average the scores. A land use or aerial map can be used for this assessment if available. Land use categories are as follows:

Forest, Wetland, Prairie, Shrub: Land that is dominated by trees, low-lying areas saturated with water, grasses and forbs, or woody vegetation less than 3 m. in height.

Old Field/Hay Field: Land that is used for agricultural purposes other than row crops or pasture.

Fenced Pasture: Land that is regularly grazed by livestock, but is fenced to prevent livestock from entering streams.

Conservation Tillage, No Till: Land that is currently in agricultural production, but retains the vegetative material from the previous year's crop to protect the soil.

Residential/Park: Land that has been modified for residential use (i.e. backyards, city parks).

Urban/Industrial: Land that has been modified for commercial or industrial use (i.e. parking lots, malls).

Open Pasture: Land that is regularly grazed by livestock, but is not fenced to prevent livestock from entering streams.

Row Crop: Land that is currently in intensive agricultural production, and doesn't use any conservation tactics (i.e. corn, soybeans, beets, potatoes).

C.3. Riparian Zone (Check the most appropriate category for each bank)

A) Riparian Width – Estimate the width of the undisturbed vegetative zone adjacent to the stream. Beneficial vegetation types include stable grasses, trees, and shrubs with low runoff potential. Disturbed vegetation is not included in the riparian width (i.e. mowed grass).

B) Bank Erosion – Estimate the percentage of the stream bank that is actively eroding. To be considered as erosion, the banks must be actively eroding through break down, soil sloughing, or false banks. False banks are natural banks that have been cut back, usually by livestock trampling.

C) Shade – Estimate the percentage of overhead canopy cover that is shading the stream channel. Professional judgment may be required to rate stream shading characteristics in larger streams and rivers as 100% shade cover would not be expected in these systems even in the absence of disturbance. The general intent of the rating is to evaluate the condition of stream canopy characteristics.

C.4. Instream Zone

A) Substrate – Document the two predominant substrate types for each channel type present within the reach. One substrate type may be recorded where > 80% of the channel is dominated by a single substrate type. For each channel type present within the reach, estimate the percent of the stream channel represented by that channel type. The percentages should add up to 100. For example, if the majority of your reach was a run, with a few pools and one riffle, the percentage could be 75% run, 20% pool, and 5% riffle. The definitions for each channel and substrate type are as follows:

Channel Types

Pool: Water is slow and generally deeper than a riffle or run. Water surface is smooth, no turbulence. A general rule that can be used to distinguish a pool from a run or riffle is if two or more of the following conditions apply; the stream channel is wider, deeper, or slower than average.

Riffle: Higher gradient areas where the water is fast and turbulent, water depths are relatively shallow, and substrates are typically coarse. Water surface is visibly broken.

Run: The water may be moderately fast to slow but the water surface typically appears smooth with little or no surface turbulence. Generally, runs are deeper than a riffle and shallower than a pool.

Glide: Similar to a run, but where there is no visible flow and the channel is too shallow for a pool. Examples include a channelized stream with a uniform depth and flow. This term should not be used in conjunction with pools, riffles, and runs in a natural stream setting.

Substrate Types

Boulder: Large rocks ranging from 250 mm to 4000 mm in diameter (basketball to car size).

Cobble: Rocks ranging in diameter from 64 mm to 250 mm (tennisball to basketball).

Gravel: Rocks varying in diameter from 2 mm to 64 mm (BB to tennisball).

Sand: Inorganic material that is visible as particles and feels gritty between the fingers, 0.06 to 2.0 mm in size.

Clay: Very fine inorganic material. Individual particles are not visible or are barely visible to the naked eye. Will support a person's weight and retains its shape when compacted.

Bedrock: A solid slab of rock, > 4000 mm in length (larger than a car).

Silt: Fine inorganic material that is typically dark brown in color. Feels greasy between fingers and does not retain its shape when compacted into a ball. A person's weight will not be supported if the stream bottom consists of silt.

Muck: A fine layer of black completely decomposed vegetative organic matter.

Detritus: Decaying organic material such as macrophytes, leaves, finer woody debris, etc. that may appear similar to silt when very fine.

Sludge: A thick layer of organic matter of animal or human origin, often originating from wastewater.

B) *Embeddedness* – Indicate the percentage to which coarse substrates are surrounded by or covered with fine sediments throughout the reach. Coarse substrates consist of gravel, cobble, and boulders. An embeddedness rating of 0% corresponds to very little or no fine sediments surrounding coarse substrates. Course substrate material completely surrounded and covered with sediment is considered 100% embedded. If course substrates are not present in the reach, check "no course substrate".

C) *Substrate Types* – Record the number of substrate types present within the reach, either less than or equal to 4, or greater then 4.

D) *Water Color* – Record the predominant color of the water by checking the appropriate category. Definitions are as follows:

Clear: Water is transparent, and objects are clearly visible underwater.

Stained: Water is colored due to minerals in the water, but objects are still visible.

Turbid: Water is colored and not transparent; brown due to silt, green due to algae, or other.

E) *Cover Type* – Indicate the types of cover available to fish within the reach (check all that apply). Cover for fish consists of objects or features dense enough to provide complete or partial shelter from the stream current or concealment from predators or prey. In order to be considered cover, the water depth must be at least 10 cm where the cover type occurs. Definitions are as follows:

Undercut Banks: Stream banks where the stream channel has cut underneath the bank. The bank could overhang the water surface when water levels are low. The undercut bank must overhang (horizontally) the wetted stream channel a minimum of 15 cm and the bottom of the undercut bank must be no more than 15 cm above the water level in order to be considered cover for fish.

Overhanging Vegetation: Terrestrial vegetation overhanging the wetted stream channel. Vegetation must be no more than 15 cm above the water level to be considered cover for fish.

Deep Pools: Area where the channel is particularly deep, often near a bend.

Logs or Woody Debris: Logs, branches, or aggregations of smaller pieces of wood in contact with or submerged in water.

Boulders: Large rocks as described under *Substrate Types.*

Rootwads: Aggregation of tree roots that extend into the stream.

Emergent Macrophytes: Vascular plants that typically have a significant portion of their biomass above the water surface. Examples include *Typha, Scirpus,* and *Zizania*.

Floating Leaf Macrophytes: Vascular plants with a significant amount of their biomass floating on the water in the form of leaves and flowers. Examples include duckweed and water lily.

Submergent Macrophytes: Vascular plants that have all of their biomass (except flowers) at or below the surface of the water. Examples include *Vallisneria*, *Elodea*, *Potamogeton*, *Nymphaea* and *Ceratophyllum*.

F) *Cover Amount* – Estimate the total percentage of fish cover within the reach. If the channel is completely filled with aquatic vegetation, check the "choking vegetation only" option.

C.5. Channel Morphology (Check the most appropriate category for each)

A) *Depth Variability* – The difference in thalweg depth between the shallowest stream cross section and the deepest stream cross section. The thalweg depth is the deepest point along a stream cross section. Indicate the degree to which the thalweg depths vary within the stream reach.

B) *Channel Stability* – The ability of a stream channel to maintain its bed and banks, without eroding or moving particles downstream. A riffle that forms diagonally across the channel and has a high amount of fine substrates that change location is indicative of an unstable stream bed. Channelized streams often have high bank stability but low bed stability as the substrate is typically comprised of fine materials that are susceptible to moving downstream. Ratings are as follows:

High: Channel with stable banks and substrates, little or no erosion of the banks, and little or no bedload within the stream. Artificial channels (i.e. concrete) exhibit a high degree of stability even though they typically have a negative effect on biological communities.

Moderate/High: Channel has the ability to maintain stable riffle, run, and pool characteristics. A minor amount of bank erosion and/or bedload is present.

Moderate: Channel that exhibits some instability, characterized by erosion, bedload, or shows the effects of wide fluctuations in water level.

Low: Channels that have a high degree of bedload and severely eroding banks. A homogenous stream bed characterized by shifting sand substrates has low stability.

C) *Velocity Types* – Indicate which flow types are present within the reach (check all that apply). The definitions are as follows:

Torrential: Extremely turbulent and fast flow; water surface is broken, usually limited to gorges and dam spillways.

Fast: Mostly non-turbulent flow with small standing waves in riffle-run areas, water surface may be partially broken.

Moderate: Non-turbulent flow that is detectable (i.e. floating objects are visibly moved downstream).

Slow: Water flow is detectable, but barely perceptible.

Eddies: Areas of circular motion within the current, usually formed in pools immediately downstream of riffles/runs.

Interstitial: Water flow that infiltrates a streambed, and moves through gravel substrates in riffle-run areas.

Intermittent: No flow is present, with standing pools separated by dry reaches.

D) *Sinuosity* – Indicate the degree to which the stream meanders. Sinuosity is defined as the ratio of stream channel distance to straight line distance between two points on a stream. For wide streams or rivers it may be necessary to consider a longer stream reach, as the true meander cycle is often not adequately represented in these systems within the sampling reach. Ratings are as follows:

Excellent: Streams exhibiting a high degree of meandering. Presence of 2 or more well defined bends (deep areas outside and shallow areas on the inside of the bend).

Good: Stream with more than 2 bends, with at least one well defined bend.

Fair: Channel with 1 or 2 poorly defined outside bends, or slight meandering within a modified reach.

Poor: Straight channel with no bends in the reach. Channelized streams or ditches are often rated as poor.

E) *Pool Width/Riffle Width* – Indicate the ratio of pool width to riffle width within the reach. If there is no riffle at the site select “no riffle”.

F) *Channel Development* – Indicate the complexity of the stream channel or the degree to which the stream has developed different channel types, creating sequences of riffles, runs, and pools. In small streams, riffles, runs, and pools must occur more than once within the sampling reach. The ratings of channel development are as follows:

Excellent: Well defined riffles present with gravel, cobble, or boulder substrates; pools vary in depth, and there is a clear transition between pools, riffles, and runs. Multiple sequences of riffles, runs, and pools are present within the reach.

Good: Riffles, runs, and pools are all present, but with less frequency, and are less distinct. Riffles have large substrates (gravel, rubble, or boulder), and pools have variation in depth.

Fair: Riffles are absent or poorly developed (shallow with sand and fine gravel substrates). Some deeper pools may exist, but transitions are generally not abrupt.

Poor: Riffles are absent; pools if present are shallow or lack variation in depth. Channelized streams generally have poor channel development.

G) *Present Water Level* – An estimation of water level as it relates to summer base flow expectations. In most streams, the "normal" water level can be determined with relative ease by observing channel characteristics.

D. Scoring the MSHA

Following are instructions on how to score the completed MSHA form. The maximum score is 100.

D.1. Surrounding Land Use: Average the scores of the two banks. For example, if residential/park was the land use selected on the left bank, and forest, wetland, prairie, shrub was selected on the right bank, then the land use score would be (2+5)/2=3.5. In the case of two land uses selected for one bank, the two scores are averaged together, and then averaged with the score of the other bank. The maximum land use score is 5.

D.2. Riparian Zone: Average the scores of the two banks for Riparian Width, Bank Erosion, and Shade; then add the three scores. For example, if moderate riparian width (3) was chosen for the left bank and very narrow (1) on the right bank; little bank erosion (4) on the left bank, and moderate (3) on the right bank; heavy shade (5) on the left bank, and substantial (4) on the right bank; the riparian zone score would be: [(3+1)/2] + [(4+3)/2] + [(5+4)/2] = 10. The maximum riparian score is 15.

D.3. Instream Zone

A) *Substrate, Embeddedness, and Substrate Types* – Add the scores of substrate, embeddedness, and substrate type. The substrate score is calculated by adding the two substrate scores for each channel type, multiplying by the percentage of the channel type, and adding the scores for each channel type present. If only one substrate type is chosen because it makes up more than 80% of the channel type, multiply the one substrate score by 2 before multiplying it by the percentage of the channel type. The maximum substrate score is 27.

B) *Cover Type and Cover Amount* – Add the scores of cover type and cover amount. The cover score can range from 1 to 8. The highest macrophyte score is 1, even if all three macrophyte types are present. The maximum cover score is 17.

D.4. Channel Morphology: Add the scores of Depth Variability, Channel Stability, Velocity Types, Sinuosity, Pool Width/Riffle Width, and Channel Development. The maximum channel morphology score is 36.

D.5. Total Score: Add the Surrounding Land Use, Riparian Zone, Instream Zone, and Channel Morphology scores together to get the total MSHA score for the site.

MPCA STREAM HABITAT ASSESSMENT

(revised 3-07)

1. Stream Documentation

Stream ______________________

County ______________________ Date ______________________

Field Number ______________________ Person Scoring ______________________

Site Location ______________________

MSHA SCORE ☐ **Max = 100**

2. Surrounding Land Use (check the most predominant or check two and average scores) [L=left bank/R =right bank, facing downstream]

L	R			L	R		
☐	☐	Forest, Wetland, Prairie, Shrub	[5]	☐	☐	Residential/Park	[2]
☐	☐	Old Field/Hay Field	[3]	☐	☐	Urban/Industrial	[0]
☐	☐	Fenced Pasture	[2]	☐	☐	Open Pasture	[0]
☐	☐	Conservation Tillage, No Till	[2]	☐	☐	Row Crop	[0]

Land Use ☐ **Max=5**

3. Riparian Zone (check the most predominant)

A. Riparian Width

L	R			
☐	☐	Extensive	> 300’	[5]
☐	☐	Wide	150’-300’	[4]
☐	☐	Moderate	30’-150’	[3]
☐	☐	Narrow	15’-30’	[2]
☐	☐	Very Narrow	3’-15’	[1]
☐	☐	None		[0]

B. Bank Erosion

L	R			
☐	☐	None		[5]
☐	☐	Little	5-25%	[4]
☐	☐	Moderate	25-50%	[3]
☐	☐	Heavy	50-75%	[1]
☐	☐	Severe	75-100%	[0]

C. Shade

L	R			
☐	☐	Heavy	>75%	[5]
☐	☐	Substantial	50-75%	[4]
☐	☐	Moderate	25-50%	[2]
☐	☐	Light	5-25%	[1]
☐	☐	None		[0]

Riparian ☐ **Max=15**

4. Instream Zone

A. Substrate (check two for each channel type)

	Boulder [10]	Cobble [9]	Gravel [8]	Sand [7]	Clay [5]	Bedrock [5]	Silt [2]	Muck [1]	Detritus [1]	Sludge [0]	Channel Type %
Pool	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	______
Riffle	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	______
Run	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	______
Glide	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	______

B. Embeddedness

- ☐ None [5]
- ☐ Light 25-50% [3]
- ☐ Moderate 50-75% [1]
- ☐ Severe 75-100% [-1]
- ☐ No coarse substrate [0]

C. Substrate Types

- ☐ >4 [2]
- ☐ <=4 [0]

D. Water Color

- ☐ Clear
- ☐ Stained

Turbid
- ☐ Brown
- ☐ Green
- ☐ Other

Substrate ☐ **Max=27**

E. Cover Type (check all that apply)

- ☐ Undercut Banks [1]
- ☐ Overhanging Vegetation [1]
- ☐ Deep Pools [1]
- ☐ Logs or Woody Debris [1]
- ☐ Boulders [1]
- ☐ Rootwads [1]
- ☐ Macrophytes: [1]
  - ☐ Emergent
  - ☐ Floating Leaf
  - ☐ Submergent

F. Cover Amount (check one)

- ☐ Extensive >50% [10]
- ☐ Moderate 25-50% [7]
- ☐ Sparse 5-25% [3]
- ☐ Nearly Absent [0]
- ☐ Choking Vegetation only [-1]

Cover ☐ **Max=17**

5. Channel Morphology

A. Depth Variability

- ☐ Greatest Depth >4X Shallow Depth [6]
- ☐ Greatest Depth 2-4X Shallow Depth [3]
- ☐ Greatest Depth <2X Shallow Depth [0]

B. Channel Stability

- ☐ High [9]
- ☐ Moderate/High [6]
- ☐ Moderate [3]
- ☐ Low [0]

C. Velocity Types (check all that apply)

- ☐ Torrential [-1]
- ☐ Fast [1]
- ☐ Moderate [1]
- ☐ Slow [1]
- ☐ Eddies [1]
- ☐ Intermittent [-2]
- ☐ Interstitial [-1]

D. Sinuosity

- ☐ Excellent [6]
- ☐ Good [4]
- ☐ Fair [2]
- ☐ Poor [0]

E. Pool Width/Riffle Width

- ☐ Pool Width > Riffle Width [2]
- ☐ Pool Width = Riffle Width [1]
- ☐ Pool Width < Riffle Width [0]
- ☐ No Riffle [0]

F. Channel Development

- ☐ Excellent [9]
- ☐ Good [6]
- ☐ Fair [3]
- ☐ Poor [0]

G. Present Water Level

- ☐ Flood
- ☐ High
- ☐ Normal
- ☐ Low
- ☐ Interstitial

Channel Morphology ☐ **Max=36**

Appendix 3-C

EMAP SOP4
Invertebrate Sampling Procedures

Subject: Invertebrate Sampling Procedures

I. PURPOSE

To describe methods used in the collection of stream invertebrates for the purpose of developing biological criteria used in assessing water quality.

II. REFERENCES

A. <u>Source Documents</u>

U.S. Environmental Protection Agency (USEPA). 1994. Environmental Monitoring and Assessment Program - Surface Waters and Region 3 Regional Environmental Monitoring and Assessment Program: 1994 pilot field operations and methods manual for streams. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory. Cincinnati, OH. EPA/620/5-94/004.

Barbour, M. T., J. Gerritsen, and J. S. White. 1996. Development of the Stream Condition Index (SCI) for Florida. Florida Department of Environmental Protection, Tallahassee, Florida. 105 pp.

B. <u>Other References</u>

U.S. Environmental Protection Agency (USEPA). 1996. Biological Criteria: Technical Guidance for Streams and Small Rivers. Revised Edition. Office of Water, Washington DC. EPA/822/B-96/001.

U.S. Environmental Protection Agency (USEPA). 1997. Revision to Rapid Bioassessment Protocols for Use in Streams and Rivers (Draft). Office of Water, Washington D.C. EPA/841/D-97/002.

III. SCOPE/LIMITATIONS

This procedure applies to all site visits in which stream invertebrates are to be collected for the development of biological criteria and/or the assessment of water quality.

IV. DEFINITIONS

Integrated monitoring A stream monitoring technique to assess water quality using chemical, biological and physical indicators.

Environmental Monitoring and Assessment Program (EMAP): U.S. Environmental Protection Agency program designed to determine the status, extent, changes, and trends in the condition of our national ecological resources on regional and national scales.

Biological Criteria: Narrative expressions or numerical values that describe the reference biological integrity of a specified habitat. Biological criteria are the benchmarks for judging the condition of aquatic communities.

Qualitative Multihabitat Sample (QMH): A method of sampling invertebrates which involves sampling a variety of invertebrate habitats, including the following substrata: rocky substrates, vegetation, undercut banks, snags, leafpacks, and soft sediment.

V. GENERAL INFORMATION

The methods described herein are to be applied to all wadeable streams included in the MPCA's integrated stream condition monitoring program. This document is not meant to be used by itself, consult one of the documents indicated in the box below if any of the described situations apply. For most efficient use of time and resources, crew leaders must be in constant communication with crews sampling for fish, preventing duplication of effort. It must be understood that this method is not to be applied to streams sampled for fish that are not wadeable.

Data generated from samples collected using the described method can be used for any of the following reasons: 1) Development of regional biological criteria, 2) Calibration of biological criteria, 3) Ambient water quality assessment, 4) Water quality assessment of sites suspected of a having a problematic source of pollution.

NOTE

SOP1 - Site Reconnaissance: A site reconnaissance should be done by the first crew to visit a site. After the initial recon has been done, no more are required. One must be done before any sampling can take place.

SOP2 - Chemical Assessment: A chemical assessment should be done by the first crew to visit a site following a site reconnaissance. These procedures can be completed during a single site visit.

VI. REQUIREMENTS

SOP3 - Habitat Assessment: A habitat assessment should be done during the same visit as the chemical assessment. If a habitat assessment is to be done during the same visit as an invertebrate collection, the invertebrate collection should be done first.

A. Qualifications of Crew Leaders
A crew leader must be a professional aquatic biologist with a minimum of a Bachelor of Science degree in biology with an aquatic entomology, invertebrate, zoology, fisheries, or closely related specialization. Additionally, they must have at least 6 months experience working under a macroinvertebrate biologist in the areas of invertebrate sampling methodology and taxonomy.

B. Qualifications of field technicians/interns
A field technician/intern must have at least one year of college education and had coursework in environmental and/or biological science.

C. General Qualifications
All personnel conducting this procedure must have excellent map reading skills and a demonstrated proficiency in the use of a GPS receiver and an orienteering compass.
Because sites may be located miles from the nearest vehicle assessable road, it is often necessary to wade through streams and/or wetlands, canoe, or hike for long distances to reach a site. Personnel conducting this procedure must have the physical ability to accomplish this.

VII. RESPONSIBILITIES

A. Field Crew Leader
Ensures that data generated using this procedure meet the standards and objectives of the integrated condition monitoring program. Carries out the procedures outlined in the action steps.

B. Technical personnel
Carries out the procedures outlined in the action steps, including maintenance and stocking of equipment, date collection and recording.

VII. QUALITY ASSURANCE AND QUALITY CONTROL

Compliance with this procedure will be maintained through annual internal reviews. Technical personnel will conduct periodic self-checks by comparing their results with other trained personnel. Calibration and maintenance of equipment will be conducted according to the guidelines specified in the manufacturer manuals.

VII. QUALITY ASSURANCE AND QUALITY CONTROL (continued)

In addition to adhering to the specific requirements of this sampling protocol and any supplementary site specific procedures, the QA/QC requirements for this protocol are as follows:

A. Control of Deviations
Deviations from the procedure shall be sufficiently documented to allow repetition of the activity as actually performed.

B. QC Samples
Ten percent of all sites sampled on any given year are resampled as a means of determing sampling error.

C. Verification
The field crew leader will conduct periodic reviews of field personnel to ensure that technical personnel are following the procedures according to this SOP.

IX. TRAINING

A. All personnel will receive training annually from a trainer designated by the program manager. Major revisions in this procedure will require that all personnel be retrained in the revised procedure by an authorized trainer.

B. Training activities will include instruction in the field as well as a field test to ensure that personnel can implement this procedure.

X. ACTION STEPS

A. Equipment List

Ensure that all of the following items are presents before implementing this procedure:

Two D-frame dipnets with 500 micron mesh nets, preferably Wildco, turtox design
Two sieve buckets with 500 micron sieves
Stream Invertebrate Visit Form
Stream verification form, previously completed with attached copies of 1:24,000 USGS topographical map
Minnesota Atlas and Gazateer (Delorme)
Pencils
Permanent/Alcohol proof markers

A. Equipment List (continued)

Labeling tape
Invertebrate sample identification labels
100% reagent alcohol, enough to preserve one days worth of samples, ca. 1 gallon/site
Waterproof notebook
Chest-high waders
Rain-gear
Jars or bottles in which sample is to be preserved; preferably non-breakable synthetic, minimum 1 litre capacity
Box or crate to store sample bottles
Canoe
Backpack

B. Method

The multihabitat method entails collecting a composite sample from up to five different habitat types. The goal of this method is to get a sample representative of the invertebrate community of a particular sampling reach, it is also to collect and process that sample in a time and cost effective manner. For that reason the habitats described below are relatively non-specific, being chosen to represent broad categories rather than microhabitats. Every broad category includes numerous microhabitats, some of which will not be sampled. It is to the discretion of the sampler which microhabitats are to be sampled. As a general rule, sample in manner that reflects the most common microhabitat of any given broad habitat category. The habitats to be sampled include:

Hard bottom (riffle/cobble/boulder)
This category is intended to cover all hard, rocky substrates, not just riffles. Runs and wadable pools often have suitable "hard" substrates, and should not be excluded from sampling. The surfaces of large boulders and areas of flat, exposed bedrock are generally quite unproductive, avoid including these habitats in the sampling area if possible. This is a general rule, if a particular stream has productive exposed bedrock, or boulder surfaces, those habitats should be considered sampleable.

Aquatic Macrophytes (submerged/emergent vegetation)
Any vegetation found at or below the water surface should be considered in this category. Emergent vegetation is included because all emergent plants have stems that extend below the water surface, serving as suitable substrate for macroinvertebrates. Do not sample the emergent portion of any plant.

B. Method (continued)

Undercut Banks (undercut banks/overhanging veg)
This category is meant to cover in-bank or near-bank habitats, shaded areas away from the main channel that typically are buffered from high water velocities.

Snags (snags/rootwads)
Snags include any piece of large woody debris found in the stream channel. Logs, tree trunks, entire trees, tree branches, large pieces of bark, and dense accumulations of twigs should all be considered snags. Rootwads are masses of roots extending from the stream bank.

Leaf Packs
Leaf packs are dense accumulations of leaves typically present in the early spring and late fall They are found in deposition zones, generally near stream banks, around logjams, or in current breaks behind large boulders.

Sampling consists of dividing 20 sampling efforts equally among the dominant, productive habitats present in the reach. If 2 habitats are present, each habitat should receive 10 sampling efforts. If 3 habitats are present, the two most dominant habitats should receive 7 jabs, the third should receive 6 jabs. If a productive habitat is present in a reach but not in great enough abundance to receive an equal proportion of sampling efforts, it should be thoroughly sampled and the remaining samples should be divided among the remaining habitat types present.

A sample effort is defined as taking a single dip or sweep in a common habitat. A sweep is taken by placing the D-net on the substrate and disturbing the area directly in front of the net opening equal to the net width, ca. $1ft^2$. The net should be swept several times over the same area to ensure that an adequate sample is collected. Each effort should cover approximately $.09m^2$ of substrate. Total area sampled is ca. $1.8m^2$.

Once a site reach has been found or newly established, invertebrate sampling should follow. If a habitat assessment and chemical analysis is to be done it should follow invertebrate sampling.

NOTE
Before leaving the vehicle be sure that the following equipment is brought to the site: two d-frame dipnets, one (or two) sieve buckets, habitat partition form, site file, compass, GPS receiver, backpack filled with sample bottles (optional), alcohol (optional)

B. Method (continued)

1. Before sampling can begin, the Crew Leader and field tech must determine which habitats are present in the reach. This should be a cooperative effort. This is done by walking the length of the stream and determining which productive habitats dominate the stream reach. A site visit form should be filled out during this process. Ideally the stream should be viewed from the top of the stream bank, but this is generally the exception rather than the rule. For this reason, great care must be taken to walk gingerly along the stream edge, or any streamside exposed areas. If this is not possible, stay to one side of the stream so as to disturb as little substrate as possible.

NOTE
Since sampling should be conducted in a downstream to upstream fashion, it will save time to start the initial visual inspection of the stream from the upstream end of the sampling reach, and walk downstream. This will allow you to start sampling at the down stream end of the reach as soon the inspection is completed.

It is difficult to estimate total stream coverage of certain habitats due to their linear or three dimensional natures. Undercut banks and overhanging vegetation appear linear, snags are three dimensional, as are vegetation mats, and emergent vegetation. For these reasons best professional judgment must be used to determine what level of effort is adequate to equal one “sample effort” for any given substrate. Keep in mind that this method is considered semiquantitative, rulers and grids are not necessary to effectively implement this procedure. Following are some suggestions as to how approach each habitat for the perspective of

Hard bottom: Riffles are basically two dimensional areas, and should be thought of as such when trying to determine how dominant the riffle habitat is in a stream. It must be kept in mind that the riffle is likely to be the most productive and diverse habitat in the reach, relatively speaking. The field personnel must not get overzealous, the purpose of this method is to get a representative sample. The temptation will undoubtedly exist to spend all day in the riffle areas, this must be avoided. Sampling in this habitat type is relatively simple. The D-net should be place firmly, and squarely on the substrate downstream of the area to be sampled. If the water is shallow enough, the area directly in front of the net should be disturbed with the hands, taking care to wash large rock off directly into the net. If the water

is too deep for this, kicking the substrate in front of the net is adequate. Watch for stoneflies trying to crawl out of the net!

Vegetation: Aquatic vegetation is either completely submerged, mostly submerged and partially floating on the waters surface, or partially submerged and mostly extended above the waters surface. Things like Potamageton sp., coontail, and milfoil tend to clump and float at the waters surface. These types of plants should be sampled with an upward sweep of the net. If the net fills with weeds, the weeds should be hand washed vigorously or jostled in the net for a few moments and then discarded. Emergent plants such as reed canary grass and various plants in the rush family, should be sampled with horizontal and vertical sweeps of the net until it is felt that the area being swept has been adequately sampled. Plants like floating bur reed, and water celery tend to float in long strands with the current. They can be floating on the surface of completely submerged. These plants should be sample as emergent plants with horizontal and vertical sweeps in a downstream to upstream motion.

Undercut banks/ Overhanging Vegetation: Undercut banks and overhanging vegetation follow the line of the stream bank. Undercut banks can vary in how undercut they are. An additional problem is that many banks appear undercut, but when investigated prove not to be. For these reasons banks should be prodded to determine how deeply they are undercut. Overhanging vegetation should be treated the same way. Sampling should consist of upward thrusts of the net, beating the undercut portion of the bank or the overhanging vegetation, so as to dislodge any clinging organisms.

Snags: Snags and rootwads can be large or small, long or wide, simple or twisted masses of logs or twigs that don't have any consistent shape. Best professional judgment must be used to determine what a "sampling effort" is. Approximating the amount of sampleable surface area is a sensible method with larger tree trunks or branches. Where as masses of smaller branches and twigs must be given a best guess. Given their variable nature, there is not one best method for sampling snags. Using something like a toilet brush works well for large pieces of wood, whereas kicking and beating with the net works best for masses of smaller branches. The person taking the sample must determine the best method for each particular situation.

B. Method (continued)

Leaf packs: Leaf packs are simple, but messy to sample. One square foot of leaf pack surface area that has two cubic feet of leaf underneath should be sampled near the surface. Whereas a shallow leafpack can be sampled in it's entirety. Sweeping to the bottom of every leafpack could create a disproportionately large amount of sample volume being collected for relatively small sample area. In most situations leaf packs will not be dominate enough to be included in a sample. If leaf packs are sampled, it is suggested that time be spent streamside washing invertebrates off of leaves and discarding the leaves, as a leaf pack sample can easily become overwhelmingly large.

2. After the number of productive, sampleable habitats have been determined, the sampling team should proceed in a downstream to upstream manner, sampling the various habitats present.

NOTE

In order to get complete samples, the contents of the D-net should be emptied into a sieve bucket frequently. This prevents the back flow of water resulting from a clogged net. In larger streams it is convenient for each sampler to have a sieve bucket. This allows samplers to sample independent of each other, avoiding frequent stream crossings which can alter the stream bed.

NOTE

While sampling it may become necessary to clean the sample of muddy, fine sediment. This can be done by filling the sieve bucket with clean water and allowing the resulting mucky water to drain. Care must be taken not twist and turn the bucket to much, this creates a washing machine action which separates insects from their delicate parts quite effectively.

B. Method (continued)

3. Once sampling is complete the sample material should be preserved as quickly as possible. Transfer the sample material from the sieve bucket to the sample containers. Fill sample containers to the top with 100% reagent alcohol. Be sure to thoroughly clean the bucket as well as sampling nets of all invertebrates. The use of forceps might be necessary to dislodge some of the smaller organisms.

4. With labeling tape, label the outside of the container with field number, date, site name, initials of those who collected samples, and number of containers, i.e 1 of 3, and Place a properly filled out sample label in each sample container.

XI. REQUIRED RECORDS

Stream Invertebrate Visit Form

A. The Stream Invertebrate Visit Form should be filled out during the streamside survey, or notes should be taken on field note books and transferred to visit form. This information will be placed in the biological database.

Quantitative Riffle Sample (optional):

These samples are being taken by the MPCA as a means to determining the best method for sampling streams with dominant riffle/run features.

If a riffle is present in the sampling reach, or in close proximity to the reach, a riffle sample should be taken. This should be a "quality" riffle, that is, a riffle that consists of gravel and/or cobble of varying sizes, and has adequate flow for sampling. The flow should be fast enough to wash dislodged organisms into the sampling net.

Three quantitative riffle samples should be taken. They do not need to be side by side. They should be spread throughout the riffle area.

Appendix 3-D

Invertebrate Identification and Enumeration

SOP BMIP03
Invertebrate Identification and Enumeration

STEP

Materials:

1. Waterproof paper labels and water/solvent proof marker
2. 80 percent ethanol
3. Squeeze bottles (for ethanol and water)
4. 4 oz. jars, with plastic or foam-line cap
5. Dissecting scope with a 10x minimum power
6. Fine tipped forceps, watchmaker type
7. Vials, with polyseal caps -2,4, and 8 dram

Methods:

Sort sample according to SOP BMIP03, placing the picked organisms in 2 or 4 dram vials

Mulit-habitat sub-sample / quantitative sample:

Empty contents of vial(s) into a petri-dish

To facilitate identification, sort organisms according to major taxonomic groups, i.e. stoneflies, caddisflies, bottles. Different groups can be placed in separate, 60mm petri-dishes or kept separate in several larger petri-dishes.

Identify organisms to the lowest practical taxonomic level. The desired level is genus. Organisms should be counted as they are identified, and removed to another dish or placed back in the sample vial to avoid miscounting.

When sorting, chironomids should be counted and separated into their own individual vial. Chironmids are not identified past the family level, they are sent to an external lab for identification. It is imperative that they be enumerated correctly. In the chironomid vial include a label with a Site ID number, site name, latitude, longitude, collection date. An additional label including taxonomic identification, and number of individuals in the vial should also be included

Final identifications are to be made by experienced taxonomists. Preliminary identifications made by interns, or inexperienced taxonomists must be verified by a staff member whose name appears on the invertebrate QC list. The lab maintains a library of taxonomic reference materials. When making identifications, the taxonomist should refer to the taxonomic reference list for the preferred reference for each major group. The lab also maintains a reference collection the can be used to check identifications. Many taxonomic references contain high quality pictures, **identifications are never to be made using pictures alone**. The proper way to make an identification includes taking a specimen through a dichotomous key, checking range distribution, checking habitat preference, and checking for seasonal emergence and growth patterns. If any questions remain about the identity of a specimen, consult another staff taxonomist, or a regional or taxonomic group specialist. A list of regional and group specialists is maintained in the lab.

When large numbers of individual taxa are present a laboratory counter should be used to keep a running total. Counters should be labeled to avoid confusion if using more than one counter.

If an organism is encountered for the first time in the laboratory, remove it to it's own vial for inclusion in the reference collection. Make a note of this on the Invertebrate Identification and Enumeration Sheet.

When adding an organism to the reference collection, place it in a 4 dram vial with two labels. One label including a taxonomic identification, taxonomist name and date of identification. The other including, Site ID number, site name, state, county, latitude and longitude - or a brief location description- and collection date.

Large/Rare Sample:

The Large/Rare sample should be identified and enumerated separate from the main sub-sample.

Sort organisms according to major taxonomic groups, i.e. stoneflies, caddisflies, beetles Different groups can be placed in separate, 60mm petri dishes or kept separate in several larger petri-dishes.

Identify organisms to the lowest practical taxonomic level. The goal is to identify organisms to Genus. Organisms should be counted as they are identified, and removed to another dish or placed back in the sample vial to avoid miscounting.

Record numbers of Large/Rare organisms in the Large/Rare column of the Invertebrate Identification and Enumeration Sheet.

It is imperative that organisms which are a part of the large/rare sample are kept separate from the multihabitat subsample, and quantitative sample.
Large/rare organisms are only used in taxa richness measures, so it is most important that there presence is noted.

Macroinvertebrate Identification Lab Bench Sheet

Field Number	**Sample Date**
Site Name	**Taxonomist:**
Sample Type QMH* QR HD other________	**Date of Sample ID:** ____/____/____

*A processed QMH sample consists of 2 parts, the subsample(ss) and large/rare (l/r), both parts must be identified

Order/Family	Genus	Species/Notes	ss	l/r
Ephemeroptera				
Baetiscidae	Baetisca			
Caenidae	Bracycercus			
	Caenis			
Ephemerellidae	Attenella			
	Ephemerella			
	Serratella			
Ephemeridae	Ephemera			
	Hexagenia			
Leptohyphidae	Tricorythodes			
Leptophlebiidae	Leptophlebia			
	Paraleptophlebia			
Polymitarcidae	Ephoron			
Potamanthidae	Anthopotamus			
Heptageniidae	Epeorus			
	Heptagenia			
	Stenacron			
	Stenonema			
Isonychiidae	Isonychia			
Ametropodidae	Ametropus			
Baetidae	Acerpenna			
	Baetis			
	Callibaetis			
	Heterocloeon			

notes/additional taxa

Order/Family	Genus	Species/Notes	ss	l/r
Plecoptera				
Leuctridae				
Taeniopterygidae				
Perlidae	Acroneuria			
	Agnetina			
	Attaneuria			
	Neoperla			
	Paragnetina			
	Perlinella			
Perlodidae				
Pteronarcyidae	Pteronarcys			

notes/additional taxa

Order/Family	Genus	Species/Notes	ss	l/r
Lepidoptera				
Pyralidae	Paraponyx			
	Petrophila			

notes/additional taxa

Order/Family	Genus	Species/Notes	ss	l/r
Megaloptera				
Corydalidae	Chauliodes			
	Corydalus			
	Nigronia			
Sialidae	Sialis			

notes/additional taxa

Order/Family	Genus	Species/Notes	ss	l/r
Isopoda				
Asselidae	Asselus			

notes/additional taxa

Order/Family	Genus	Species/Notes	ss	l/r
Odonata				
Calopterygidae	Calopteryx			
	Hetaerina			
Coenagrionidae	Argia			
	Enallagma			
	Nehalennia			
Lestidae	Lestes			
Aeshnidae	Aeschna			
	Anax			
	Basiaeschna			
	Boyeria			
Cordulegastridae	Cordulegaster			
Corduliidae	Cordulia			
	Dorocordulia			
	Epitheca			
	Somatochlora			
Gomphidae	Dromogomphus			
	Gomphurus			
	Gomphus			
	Hagenius			
	Ophiogomphus			
	Phanogomphus			
	Progomphus			

notes/additional taxa

Order/Family	Genus	Species/Notes	ss	l/r
Hemiptera				
Belostomatidae	Belstoma			
	Corixidae			
Corixidae	Hesperocorixa			
	Sigara			
	Trichocorixa			
Nepidae	Ranatra			
Notonectidae	Buenoa			
	Notonecta			

notes/additional taxa

Order/Family	Genus	Species/Notes	ss	l/r
Amphipoda				
Talitridae	Hyallela	azteca		
Gammaridae	Gammarus			

notes/additional taxa

Order/Family	Genus	Species/Notes	ss	l/r
Decapoda				
Cambaridae	Cambarus			
	Orconectes			
	Procambarus			

notes/additional taxa

Order/Family	Genus	Species/Notes	ss	l/r
Pelecypoda				
Sphaeriidae				
Corbiculidae				
Unionidae				

notes/additional taxa

Entered into DataInverts by______ --- (initials) date__________

Trichoptera				
Dipseudopsidae	Phylocentropus			
Hydropsycidae	Ceratopsyche			
	Cheumatopsyche			
	Diplectrona			
	Hydropsyche			
	Potamyia			
Philopotamidae	Chimarra			
	Dolophilodes			
Polycentropodidae	Cernotina			
	Cymellus			
	Neureclipsis			
	Paranyctiophylax			
	Polycentropus			
Psychomyiidae	Lype			
	Psychomyia			
Glossosomatidae	Agapetus			
	Glossosoma			
	Protoptila			
Hydroptilidae	Hydroptila			
	Leucotrichia			
	Mayatrichia			
	Oxyethira			
	Orthotrichia			
Rhyacophilidae	Rhyacophila			
Brachyecentridae	Brachycentrus			
	Micrasema			
Helicopsychidae	Helicopsyche			
Lepidostomatidae	Lepidostoma			
Leptoceridae	Ceraclea			
	Leptocerus			
	Mystacides			
	Nectopsyche			
	Oecetis			
	Trianodes			
Limnephilidae	Limnephilus			
	Hydatophylax			
Molannidae	Molanna			
Phryganeidae	Phryganea			
	Ptilostomis			
Sericostomatidae	Agarodes			

notes/additional taxa

Gastropoda				
ncylidae	Ferrissia			
lanorbidae	Helisoma			
	Promentus			
	Planorbula			
	Gyraulus			
ivaparidae	Campeloma			
ymnaeidae	Lymnaea			
	Bulimnea			
	Fossaria			
ydrobiidae	Amnicola			
leuroceridae	Pleurocera			
hysidae	Physa			

otes/additional taxa

Diptera		Species/Notes	SS	
Ceratopogonidae	Alluaudomyia			
	Atrichopogon			
	Bezzia			
	Ceratopogon			
	Culicoides			
	Nilobezzia			
	Palpomyia			
	Probezzia			
	Sphaeromias			
Chironomidae	G.			
Dixidae	Dixa			
	Dixella			
Simuliidae	Simulium			
Tipulidae	Antocha			
	Dicranota			
	Hexatoma			
	Limnophila			
	Limonia			
	Pilaria			
	Tipula			
Athericidae	Atherix			
Empididae	Hemerodromia			
Tabanidae	Chrysops			
	Tabanus			

notes/additional taxa

Coleoptera				
Dytiscidae	Agabus			
	Laccophilus			
	Liodessus			
Gyrinidae	Dineutus			
	Gyrinus			
Elmidae	Ancyronyx			
	Dubiraphia			
	Macronychus			
	Optioservus			
	Stenelmis			
Hydrophilidae	Berosus			
	Helocombus			
	Laccobius			
	Sperchopsis			
	Tropisternus			
Annelida	Oligochaeta			
	Hirudinea			

notes/additional taxa

Hydracarina (trombidoformes, acarina)				
Nematoda				

notes/additional taxa

-MPCA Biological Monitoring Program-

Macroinvertebrate Identification QC Form

Field Number	Sample Date	Identifiers' Initials		Discrepancies		Comments	Total # of Conflicts	Total # of Taxa	Precision	
		Original ID	QC ID	Original Identification	QC Identification				Original ID	QC ID

Appendix 3-E1

WET Test Results, July 2010, Report 10-151

TOXICITY TEST RESULTS

POLYMET MINING

Report Date: August 12, 2010

Project No. 10-151

Prepared for:

Barr Engineering
4700 W. 77th Street
Minneapolis, MN 55435

ENVIRONMENTAL TOXICITY CONTROL INC.

6265 Applewood Road • Woodbury, Minnesota 55125
Phone 651 501-2075 • Fax 651 501-2076

PROJECT: <u>WHOLE EFFLUENT TOXICITY TESTING POLYMET MINING</u>

PROJECT NUMBER: 10-151

TOXICITY TEST RESULTS

INTRODUCTION:

This report presents the results of toxicity testing on water samples received by Environmental Toxicity Control (ETC) on July 28, 2010. The samples identified as SD026 and SD033 were from the PolyMet Mining facility and were collected by employees from Northeast Technical Services. Chronic toxicity testing was conducted on the water samples using Bear Creek water as dilution water. The scope of our services was limited to conducting chronic toxicity tests on the invertebrate, *Ceriodaphnia dubia*, in the laboratory.

TEST METHODS:

Tests were conducted in accordance with the procedures outlined in <u>Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms</u>, Fourth Edition, EPA-821-R-02-013.

Testing was started on 7/28/10, approximately 24 hours after sample collection.

RESULTS:

Toxicity test results are summarized in Tables 1and 2, test conditions are summarized in Table 3.

Both SD026 and SD033 were toxic to *Ceriodaphnia dubia* reproduction.

In the SD026 test, the number of *C. dubia* young produced in the 100% concentration (18.2) was significantly less than the number produced in the control (30.3). The 25% Inhibition Concentration (IC25), the calculated concentration which would exhibit a 25% decrease in the measured effect from the control, for reproduction was 82.6% effluent resulting in 1.21 TUc (Chronic Toxic Units). The NOEC (No-Observable Effect Concentration) was 75% effluent.

In the SD033 test, the number of *C. dubia* young produced in the 100% concentration (20.2) and 75% concentration (22.4) was significantly less than the number produced in the control (30.3). The IC25 for reproduction was 72.5% effluent resulting in 1.38 TUc (Chronic Toxic Units). The NOEC (No-Observable Effect Concentration) was 50% effluent.

Both water samples were not toxic to *C. dubia* survival.

QUALITY ASSURANCE AND QUALITY CONTROL:

Satisfactory laboratory performance on an ongoing basis is demonstrated by conducting at least one acceptable toxicity test per month with a reference toxicant. Control charts for a reference toxicant and successive endpoints (LC50 and IC25) are plotted to determine if results are within prescribed limits. Results from our most recent reference tests are shown in the following table:

Reference Toxicity Test		
Species	IC_{25}	Test Date
Ceriodaphnia dubia	0.661 g/l NaCl	7/16/10

Our results are within range of EPA expected results for the type of tests conducted.

Test methods and procedures are documented in ETC's Standard Operating Procedures (SOPs). Test and analysis protocols are reviewed by ETC's Quality Assurance/Quality Control Officer. Procedures are documented and followed as written. Any deviation from a QA/QC procedure is documented and kept in the project file. During this project, no deviation in method was warranted.

ENVIRONMENTAL TOXICITY CONTROL

Walter Koenst
Bioassay Manager

Table 1. Survival and Reproduction of *Ceriodaphnia dubia* Tested With SD026 Water.

Concentration (%)	% Survival	Mean # of Young Produced
Control	100	30.3
12.5%	100	34.1
25%	100	28.1
50%	100	23.9
75%	100	29.6
100%	80	18.2
IC25		82.6%
NOEC	100%	75%
TUc		1.21

Table 2. Survival and Reproduction of *Ceriodaphnia dubia* Tested With SD033 Water.

Concentration (%)	% Survival	Mean # of Young Produced
Control	100	30.3
12.5%	100	30.3
25%	90	29.2
50%	90	25.6
75%	90	22.4
100%	100	20.2
IC25		72.5%
NOEC	100%	50%
TUc		1.38

Table 3. Summary of Chemical and Physical Data of Toxicity Tests

Sample: SD026						
% effluent	**pH**	**Dissolved Oxygen (mg/L)**	**Temperature (°C)**	**Total Hardness (mg/L)**	**Total Alkalinity (mg/L)**	**Conductivity (µmhos/cm)**
Control	6.95 - 8.04	8.1 - 9.0	25	68	52	95
12.5	7.41 - 8.18	8.1 - 9.0	25			
25	7.73 - 8.40	8.1 - 9.0	25			
50	8.04 - 8.61	8.0 - 9.2	25			
75	8.14 - 8.73	8.0 - 9.4	25			
100	8.16 - 8.62	8.0 - 10.0	25	640	548	1186

Sample: SD033						
% effluent	**pH**	**Dissolved Oxygen (mg/L)**	**Temperature (°C)**	**Total Hardness (mg/L)**	**Total Alkalinity (mg/L)**	**Conductivity (µmhos/cm)**
Control	6.95 - 8.04	8.1 - 9.0	25	68	52	95
12.5	7.36 - 8.23	8.1 - 9.0	25			
25	7.55 - 8.27	8.1 - 9.1	25			
50	7.84 - 8.46	8.0 - 9.2	25			
75	7.99 - 8.59	8.0 - 9.4	25			
100	8.00 - 8.65	7.9 - 9.9	25	1236	360	2360

EPA Methods:

Parameter	EPA Method Number
Dissolved Oxygen (mg/L)	360.1
pH	150.1
Total Hardness (as mg/$CaCO_3$/L)	130.2
Total Alkalinity (as mg/$CaCO_3$/L)	310.2
Specific Conductivity (µmhos/cm)	120.1

BIOASSAY TEST CONDITIONS

Client: BARR Engineering	Project No.: 10-151	
Type of sample: Grab	Test type: Chronic	
Test length: 6 days Species: Ceriodaphnia dubia		Organism age: <24 h
# of treatments: 6	# of replicates: 10	mL/replicate: 15
Organisms/rep.: 1	Organisms/treatment: 10	
Temperature (°C): 25	Light intensity: 60 ft-c	Photoperiod: 16/8
Type of dilution water: Receiving	Source: Bear Creek	
Collection date/time of sample/effluent:		

TEST SOLUTION PREPARATION

Nominal conc. or % effluent	0	12.5	25	50	75	100	
mL of effluent or stock	0	25	50	100	150	200	
mL of dilution water	200	175	150	100	50	0	
TOTAL mL	200	200	200	200	200	200	

Comments:

Analyst: KM

Reviewed by: [signature]

CHRONIC TOXICITY TEST
CERIODAPHNIA REPRODUCTION AND SURVIVAL

Client: Barr Engineering - SD 026 Project No.: 10-151
Test Dates/Time ● Initiation: 1440 7/28/10 Termination: 1045 8/3/10

Concentration	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Remarks
0	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	4	4	4	4	0	4	0	4	3	0	
	4	0	0	9	0	4	0	0	0	0	3	
	5	10	10	0	11	9	8	11	11	0	6	
	6	20	18	20	20	16	16	20	17	19	18	
	Total	34	32	33	35	~~38~~ 29	28	31	32	22	27	
12.5	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	3	0	3	0	0	6	5	0	3	0	
	4	0	3	0	4	2	0	0	3	0	4	
	5	13	13	12	8	11	13	10	10	10	11	
	6	19	16	22	22	18	20	21	21	17	18	
	Total	35	32	37	34	31	39	36	34	30	33	
25	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	6	0	0	0	3	3	0	3	4	
	4	4	0	5	2	0	0	8	6	8	0	
	5	10	10	11	10	4	10	17	11	0	8	
	6	18	16	15	14	4	17	0	18	17	19	
	Total	32	32	31	26	8	30	28	35	28	31	

✓ = Alive # = No. of Live Young 0 = No Young X = Dead y = Male M= Missing
(-#) = No. of Dead Young

Analyst: KM Reviewed By:

CHRONIC TOXICITY TEST
CERIODAPHNIA REPRODUCTION AND SURVIVAL

Client: Barr Engineering - SD026 Project No.: 10-151
Test Dates/Time ● Initiation: 1440 7/28/10 Termination: 1045 8/3/10

Concentration	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Remarks
50	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	2	3	3	0	0	0	0	4	4	3	
	4	0	0	0	3	5	0	5	0	0	0	
	5	8	11	0	10	10	7	7	5	4	10	
	6	0	15	14	14	15	12	11	16	20	18	
	Total	10	29	17	27	30	19	23	25	28	31	
75	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	2	3	3	3	2	3	1	2	0	3	
	4	0	0	0	0	0	0	0	0	4	0	
	5	10	7	7	10	9	12	10	10	11	10	
	6	17	17	20	18	18	16	18	16	16	18	
	Total	29	27	30	31	29	31	29	28	31	31	
100	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	0	0	0	0	0	4	
	4	6	2	4	0	0	4	4	X	0	0	
	5	11	8	6	4	6	7	8		5	9	
	6	13	0	13	14	10	12	12		6	~~5X~~	12
	Total	30	10	23	20	16	23	24	0	11	25	

✓ = Alive # = No. of Live Young 0 = No Young X = Dead y = Male M= Missing
(-#) = No. of Dead Young

Analyst: KM Reviewed By: Walter Kreutz

Conc. ID	1	2	3	4	5	6
Conc. Tested	0	12.5	25	50	75	100
Response 1	34	35	32	10	29	30
Response 2	32	32	32	29	27	10
Response 3	33	37	31	17	30	23
Response 4	35	34	26	27	31	20
Response 5	29	31	8	30	29	16
Response 6	28	39	30	19	31	23
Response 7	31	36	28	23	29	24
Response 8	32	34	35	25	28	0
Response 9	22	30	28	28	31	11
Response 10	27	33	31	31	31	25

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: PolyMet SD026
Test Start Date: 7/28/10 Test Ending Date: 8/3/10
Test Species: Ceriodaphnia dubia
Test Duration: 6 Days
DATA FILE:
OUTPUT FILE: ICPout.i25

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	10	0.000	30.300	3.889	32.200
2	10	12.500	34.100	2.767	32.200
3	10	25.000	28.100	7.505	28.100
4	10	50.000	23.900	6.724	26.750
5	10	75.000	29.600	1.430	26.750
6	10	100.000	18.200	8.967	18.200

The Linear Interpolation Estimate: 82.6023 Entered P Value: 25

Number of Resamplings: 80
The Bootstrap Estimates Mean: 81.8037 Standard Deviation: 7.6860
Original Confidence Limits: Lower: 49.0252 Upper: 89.1500
Resampling time in Seconds: 0.00 Random_Seed: 373956

Ceriodaphnia Reproduction
File: PolyMet SD026 Transform: NO TRANSFORMATION

STEELS MANY-ONE RANK TEST - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	0	30.300				
2	12.5	34.100	133.50	75.00	10.00	
3	25	28.100	95.50	75.00	10.00	
4	50	23.900	73.00	75.00	10.00	*
5	75	29.600	91.50	75.00	10.00	
6	100	18.200	63.00	75.00	10.00	*

Critical values use k = 5, are 1 tailed, and alpha = 0.05

Ceriodaphnia Reproduction
File: PolyMet SD026   Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	4.020	14.520	22.920	14.520	4.020
OBSERVED	5	10	23	21	1

Calculated Chi-Square goodness of fit test statistic = 6.8069
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Ceriodaphnia Reproduction
File: PolyMet SD026   Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 30.56
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 9.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data FAIL homogeneity test at 0.01 level. Try another transformation.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

Toxicity Test
Daily Chemistries

Client: Barr Engineering	Project Number: 10-151
Test Type: Chronic - SD026	Species: Ceriodaphnia dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 0	pH	7.01	7.54	7.83	8.07	8.19	8.23	
	Dissolved Oxygen (mg/l)	8.6	8.6	8.7	8.7	8.8	9.6	
Date:	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
7/28/10	Conductivity (µmhos)	95					1186	
Analyst:	Total Alkalinity (mg/l)	52					548	
Km	Total Hardness (mg/l)	68					640	
	Total Ammonia (mg/l)							
Day: 1 OLD	pH	7.74	8.18	8.40	8.60	8.71	8.54	
	Dissolved Oxygen (mg/l)	8.6	8.6	8.6	8.5	8.7	8.6	
Date:	Temperature (°C)	24.8	24.8	24.8	24.8	24.8	24.8	
7/29/10	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
WK	Total Hardness (mg/l)							
Day: 1 New	pH	6.95	7.41	7.73	8.04	8.14	8.16	
	Dissolved Oxygen (mg/l)	8.8	8.9	9.0	9.1	9.4	10.0	
Date:	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
7/29/10	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
Km	Total Hardness (mg/l)							
Day: 2 OLD	pH	7.86	8.15	8.39	8.60	8.73	8.60	
	Dissolved Oxygen (mg/l)	8.6	8.5	8.5	8.5	8.5	8.6	
Date:	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
7/30/10	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
WK	Total Hardness (mg/l)							
Day: 2 NEW	pH	7.07	7.63	7.90	8.22	8.23	8.22	
	Dissolved Oxygen (mg/l)	8.8	9.0	9.0	9.2	9.4	9.8	
Date:	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
7/30/10	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
WK	Total Hardness (mg/l)							

Reviewed by: Walter Fornt Date: 8/11/10

Toxicity Test
Daily Chemistries

Client: BARR Engineering	Project Number: 10-151
Test Type: Chronic - SD-026	Species: C. dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 3 Old	pH	7.90	8.16	8.34	8.60	8.72	8.58	
	Dissolved Oxygen (mg/l)	8.2	8.1	8.1	8.1	8.2	8.3	
Date: 7/31/10	Temperature (°C)	25.1	25.1	25.1	25.1	25.1	25.1	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
	Total Ammonia (mg/l)							
Day: 3 New	pH	7.19	7.65	7.89	8.15	8.20	8.22	
	Dissolved Oxygen (mg/l)	8.8	8.7	8.8	8.8	8.9	9.2	
Date: 7/31/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 4 Old	pH	7.99	8.18	8.38	8.61	8.70	8.55	
	Dissolved Oxygen (mg/l)	8.1	8.2	8.1	8.0	8.0	8.0	
Date: 8/1/10	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 4 New	pH	6.99	7.59	7.78	8.09	8.18	8.19	
	Dissolved Oxygen (mg/l)	9.0	8.9	8.8	8.9	9.1	9.5	
Date: 8/1/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 5 Old	pH	8.04	8.16	8.36	8.59	8.70	8.62	
	Dissolved Oxygen (mg/l)	8.30	8.1	8.1	8.2	8.4	8.4	
Date: 8/2/10	Temperature (°C)	25.1	25.1	25.1	25.1	25.1	25.1	
	Conductivity (µmhos)							
Analyst: JK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Kurant

Date: 8/11/10

Toxicity Test
Daily Chemistries

Client: BARR Engineering	Project Number: 10-151
Test Type: Chronic - SD-026	Species: C. dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 5	pH	7.30	7.78	8.04	8.26	8.32	8.32	
New	Dissolved Oxygen (mg/l)	8.9	8.8	8.6	8.7	8.8	8.9	
Date:	Temperature (°C)	25.1	25.1	25.1	25.1	25.1	25.1	
8/12/10	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
JK	Total Hardness (mg/l)							
	Total Ammonia (mg/l)							
Day: 6	pH	7.87	8.07	8.30	8.55	8.68	8.53	
Final	Dissolved Oxygen (mg/l)	8.1	8.1	8.1	8.1	8.0	8.1	
Date:	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
8/13/10	Conductivity (µmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day:	pH							
	Dissolved Oxygen (mg/l)							
Date:	Temperature (°C)							
/ /	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day:	pH							
	Dissolved Oxygen (mg/l)							
Date:	Temperature (°C)							
/ /	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day:	pH							
	Dissolved Oxygen (mg/l)							
Date:	Temperature (°C)							
/ /	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Koenig (signature)   Date: 8/11/10

ENVIRONMENTAL TOXICITY CONTROL

CHRONIC TOXICITY TEST
CERIODAPHNIA REPRODUCTION AND SURVIVAL

Client: Barr Engineering - SD 033 Project No.: 10-151

Test Dates/Time • Initiation: 1445 7/28/10 Termination: 1130 8/3/10

Concentration	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Remarks
0	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	4	4	4	4	0	4	0	4	3	0	
	4	0	0	9	0	4	0	0	0	0	3	
	5	10	10	0	11	9	8	11	11	0	6	
	6	20	18	20	20	16	16	20	17	19	18	
	Total	34	32	33	35	29	28	31	32	22	27	
12.5	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	5	0	0	0	0	3	0	3	3	3	
	4	0	3	0	3	4	8	4	0	0	0	
	5	7	10	7	11	11	0	10	11	10	9	
	6	21	17	16	18	16	14	20	18	21	17	
	Total	33	30	23	32	31	25	34	32	34	29	
25	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	4	0	5	2	4	5	0	0	
	4	4	5X	0	4	0	0	0	0	4	4	
	5	13		11	9	12	10	7	11	9	10	
	6	16		19	17	18	18	19	19	17	16	
	Total	33	5	34	30	35	30	30	35	30	30	

✓ = Alive # = No. of Live Young (-#) = No. of Dead Young 0 = No Young X = Dead ♂ = Male M= Missing

Analyst: KM

Reviewed By: [signature]

Bio.105

ENVIRONMENTAL TOXICITY CONTROL

CHRONIC TOXICITY TEST
CERIODAPHNIA REPRODUCTION AND SURVIVAL

Client: Barr Engineering - SD 033 Project No.: 10-151
Test Dates/Time ● Initiation: 1445 7/28/10 Termination: 1130 8/3/10

Concentration	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Remarks
50	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	4	0	3	5	0	3	2	4	3	
	4	0	0	4	0	0	4	X	4	0	0	
	5	8	10	10	9	9	9		0	12	11	
	6	16	17	15	13	16	13		17	17	18	
	Total	24	31	29	25	30	26	3	23	33	32	
75	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	2	0	3	4	5	3	0	4	0	4	
	4	0	5	0	0	6	0	3	0	3	8	
	5	6	5	5	0	X	3	8	6	6	18	
	6	18	14	16	14		12	15	16	12	0	
	Total	26	24	24	18	11	18	26	26	21	30	
100	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	3	0	0	0	0	0	0	0	2	0	
	4	0	1	0	3	2	2	0	3	0	5	
	5	8	4	11	5	8	5	6	5	2	6	
	6	14	12	8	12	10	12	12	15	12	14	
	Total	25	17	19	20	20	19	18	23	16	25	

✓ = Alive # = No. of Live Young (-#) = No. of Dead Young 0 = No Young X = Dead y = Male M= Missing

Analyst: KM Reviewed By: Walter Trent

Bio.105

Conc. ID	1	2	3	4	5	6
Conc. Tested	0	12.5	25	50	75	100
Response 1	34	33	33	24	26	25
Response 2	32	30	5	31	24	17
Response 3	33	23	34	29	24	19
Response 4	35	32	30	25	18	20
Response 5	29	31	35	30	11	20
Response 6	28	25	30	26	18	19
Response 7	31	34	30	3	26	18
Response 8	32	32	35	23	26	23
Response 9	22	34	30	33	21	16
Response 10	27	29	30	32	30	25

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: PolyMet SD033
Test Start Date: 7/28/10 Test Ending Date: 8/3/10
Test Species: Ceriodaphnia dubia
Test Duration: 6 Days
DATA FILE:
OUTPUT FILE: ICPout.i25

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	10	0.000	30.300	3.889	30.300
2	10	12.500	30.300	3.713	30.300
3	10	25.000	29.200	8.779	29.200
4	10	50.000	25.600	8.669	25.600
5	10	75.000	22.400	5.502	22.400
6	10	100.000	20.200	3.155	20.200

The Linear Interpolation Estimate: 72.4609 Entered P Value: 25

Number of Resamplings: 80Those resamples not used had estimates above the highest concentration/ %Effluent.

The Bootstrap Estimates Mean: 68.5090 Standard Deviation: 13.0316

No Confidence Limits can be produced since the number of resamples generated is not a multiple of 40.
Resampling time in Seconds: 0.05 Random_Seed: 24746844

Ceriodaphnia Reproduction
File: PolyMet SD033 Transform: NO TRANSFORMATION

STEELS MANY-ONE RANK TEST - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	0	30.300				
2	12.5	30.300	105.50	75.00	10.00	
3	25	29.200	110.00	75.00	10.00	
4	50	25.600	84.50	75.00	10.00	
5	75	22.400	64.00	75.00	10.00	*
6	100	20.200	58.00	75.00	10.00	*

Critical values use k = 5, are 1 tailed, and alpha = 0.05

Ceriodaphnia Reproduction
File: PolyMet SD033 Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	4.020	14.520	22.920	14.520	4.020
OBSERVED	5	8	27	18	2

Calculated Chi-Square goodness of fit test statistic = 5.7420
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Ceriodaphnia Reproduction
File: PolyMet SD033 Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 16.70
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 9.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data FAIL homogeneity test at 0.01 level. Try another transformation.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

Toxicity Test
Daily Chemistries

Client: Barr Engineering	Project Number: 10-151
Test Type: Chronic - SD 033	Species: Ceriodaphnia dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 0	pH	7.01	7.43	7.63	7.92	8.05	8.00	
	Dissolved Oxygen (mg/l)	8.6	8.6	8.7	8.9	8.9	9.3	
Date: 7/28/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)	95					2360	
Analyst: KM	Total Alkalinity (mg/l)	52					360	
	Total Hardness (mg/l)	68					1236	
	Total Ammonia (mg/l)							
Day: 1 OLD	pH	7.74	8.13	8.25	8.46	8.57	8.65	
	Dissolved Oxygen (mg/l)	8.6	8.6	8.5	8.5	8.5	8.6	
Date: 7/29/10	Temperature (°C)	24.8	24.8	24.8	24.8	24.8	24.8	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 1 New	pH	6.95	7.36	7.55	7.84	7.99	8.04	
	Dissolved Oxygen (mg/l)	8.8	8.9	9.0	9.1	9.3	9.9	
Date: 7/29/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 2 old	pH	7.86	8.09	8.21	8.43	8.54	8.63	
	Dissolved Oxygen (mg/l)	8.6	8.5	8.4	8.4	8.4	8.5	
Date: 7/30/10	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
	Conductivity (µmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 2 NEW	pH	7.07	7.48	7.66	7.89	8.05	8.08	
	Dissolved Oxygen (mg/l)	8.8	9.0	9.1	9.2	9.4	9.8	
Date: 7/30/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: WK.	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Kowalt   Date: 8/11/10

Toxicity Test
Daily Chemistries

Client: Barr Engineering	Project Number: 10-151
Test Type: Chronic - SD-033	Species: C. dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 3 Old	pH	7.90	8.23	8.27	8.44	8.57	8.65	
	Dissolved Oxygen (mg/l)	8.2	8.1	8.2	8.1	8.2	8.3	
Date: 7/31/10	Temperature (°C)	25.1	25.1	25.1	25.1	25.1	25.1	
	Conductivity (µmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
	Total Ammonia (mg/l)							
Day: 3 New	pH	7.19	7.64	7.71	7.99	8.09	8.10	
	Dissolved Oxygen (mg/l)	8.8	8.8	8.9	9.0	9.0	9.3	
Date: 7/31/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 4 Old	pH	7.99	8.20	8.23	8.43	8.59	8.65	
	Dissolved Oxygen (mg/l)	8.1	8.1	8.1	8.0	8.0	7.9	
Date: 8/1/10	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 4 New	pH	6.99	7.55	7.71	7.95	8.07	8.08	
	Dissolved Oxygen (mg/l)	9.0	8.9	8.9	9.0	9.1	9.5	
Date: 8/1/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 5 Old	pH	8.04	8.17	8.27	8.43	8.56	8.65	
	Dissolved Oxygen (mg/l)	8.3	8.3	8.3	8.3	8.4	8.3	
Date: 8/2/10	Temperature (°C)	25.1	25.1	25.1	25.1	25.1	25.1	
	Conductivity (µmhos)							
Analyst: JK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Kunt Date: 8/11/10

Toxicity Test
Daily Chemistries

Client: Barr Engineering	Project Number: 10-151
Test Type: Chronic - SD-033	Species: C. dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 5 New	pH	7.30	7.77	7.80	8.07	8.18	8.20	
	Dissolved Oxygen (mg/l)	8.9	8.7	8.8	8.8	8.8	8.9	
Date: 8/12/10	Temperature (°C)	25.1	25.1	25.1	25.1	25.1	25.1	
	Conductivity (µmhos)							
Analyst: JK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
	Total Ammonia (mg/l)							
Day: 6 Final	pH	7.87	8.15	8.18	8.39	8.52	8.60	
	Dissolved Oxygen (mg/l)	8.1	8.2	8.2	8.3	8.1	8.2	
Date: 8/13/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day:	pH							
	Dissolved Oxygen (mg/l)							
Date: / /	Temperature (°C)							
	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day:	pH							
	Dissolved Oxygen (mg/l)							
Date: / /	Temperature (°C)							
	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day:	pH							
	Dissolved Oxygen (mg/l)							
Date: / /	Temperature (°C)							
	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Koenst

Date: 8/11/10

Appendix 3-E2

WET Test Results, October 2010, Report 10-234

TOXICITY TEST RESULTS

POLYMET MINING

Report Date: November 8, 2010

Project No. 10-234

Prepared for:

Barr Engineering
4700 W. 77th Street
Minneapolis, MN 55435

6265 Applewood Road • Woodbury, Minnesota 55125
Phone 651 501-2075 • Fax 651 501-2076

PROJECT: **CHRONIC TOXICITY TESTING**
POLYMET MINING

PROJECT NUMBER: 10-234

TOXICITY TEST RESULTS

INTRODUCTION:

This report presents the results of toxicity testing on water samples received by Environmental Toxicity Control (ETC) on October 27, 2010. The samples identified as SD026, SD033, Bear Creek, PM 12.1, and PM 17 were from the PolyMet Mining facility and were collected by employees from Northeast Technical Services on October 26, 2010. Chronic toxicity testing was conducted on the water samples using Reconstituted Water, Embarrass River water and Partridge River water as dilution water. The scope of our services was limited to conducting chronic toxicity tests on the invertebrate, *Ceriodaphnia dubia*, in the laboratory.

TEST METHODS:

Tests were conducted in accordance with the procedures outlined in Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition, EPA-821-R-02-013.

SD026, SD033, and Bear Creek were tested using Reconstituted Water as dilution water. Additionally, SD033 and SD026 were tested using Embarrass River and Partridge River water, respectively.

Testing was started on 10/27/10, approximately 24 hours after sample collection.

RESULTS:

Toxicity test results are summarized in Tables 1, test conditions are summarized in Table 2.

The samples were not toxic to *Ceriodaphnia dubia* reproduction and survival.

QUALITY ASSURANCE AND QUALITY CONTROL:

Satisfactory laboratory performance on an ongoing basis is demonstrated by conducting at least one acceptable toxicity test per month with a reference toxicant. Control charts for a reference toxicant and successive endpoints (LC50 and IC25) are plotted to determine if results are within prescribed limits. Results from our most recent reference tests are shown in the following table:

Reference Toxicity Test		
Species	IC_{25}	Test Date
Ceriodaphnia dubia	0.836 g/l NaCl	10/12/10

Our results are within range of EPA expected results for the type of tests conducted.

Test methods and procedures are documented in ETC's Standard Operating Procedures (SOPs). Test and analysis protocols are reviewed by ETC's Quality Assurance/Quality Control Officer. Procedures are documented and followed as written. Any deviation from a QA/QC procedure is documented and kept in the project file. During this project, no deviation in method was warranted.

ENVIRONMENTAL TOXICITY CONTROL

Walter Koenst

Walter Koenst
Bioassay Manager

Table 1. Survival and Reproduction of *Ceriodaphnia dubia*.

Test: Reconstituted Water/SD033		
Concentration (%)	**% Survival**	**Mean # of Young Produced**
Control	100	18.3
12.5%	100	16.8
25%	100	18.4
50%	100	15.4
75%	100	15.3
100%	100	17.0
IC25		>100%
NOEC	100%	100%
TUc		<1.0

Test: Reconstituted Water/SD026		
Concentration (%)	**% Survival**	**Mean # of Young Produced**
Control	100	18.3
12.5%	100	17.9
25%	100	16.3
50%	100	16.7
75%	100	21.5
100%	100	18.6
IC25		>100%
NOEC	100%	100%
TUc		<1.0

Table 1(Continued). **Survival and Reproduction of *Ceriodaphnia dubia*.**

Test: Reconstituted Water/Bear Creek		
Concentration (%)	**% Survival**	**Mean # of Young Produced**
Control	100	18.3
12.5%	100	19.2
25%	100	19.4
50%	100	22.7
75%	100	20.9
100%	100	22.2
IC25		>100%
NOEC	100%	100%
TUc		<1.0

Test: Embarrass River/SD033		
Concentration (%)	**% Survival**	**Mean # of Young Produced**
Control	100	16.7
12.5%	100	16.2
25%	100	17.4
50%	90	13.9
75%	100	14.0
100%	100	17.0
IC25		>100%
NOEC	100%	100%
TUc		<1.0

Table 1(Continued). Survival and Reproduction of *Ceriodaphnia dubia*.

Test: Partridge River/SD026		
Concentration (%)	**% Survival**	**Mean # of Young Produced**
Control	100	22.1
12.5%	100	22.5
25%	100	20.7
50%	100	20.1
75%	100	18.8
100%	100	18.6
IC25		>100%
NOEC	100%	50%
TUc		<1.0

Screen Test: PM 12.1, PM 17		
Sample ID	**% Survival**	**Mean # of Young Produced**
Control	100	18.3
PM 12.1	100	20.3
PM 17	100	20.7

Table 2. Summary of Chemical and Physical Data of Toxicity Tests

Test: Reconstituted Water/SD033

% effluent	pH	Dissolved Oxygen (mg/L)	Temperature (°C)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Conductivity (µmhos/cm)
Control	7.95 - 8.20	8.0 - 8.6	25	92	88	286
12.5	7.90 - 8.29	8.1 - 8.8	25			
25	7.88 - 8.43	8.0 - 8.7	25			
50	7.83 - 8.57	8.0 - 8.8	25			
75	7.81 - 8.66	8.0 - 8.9	25			
100	7.74 - 8.73	7.9 - 9.2	25	1288	384	2420

Test: Reconstituted Water/SD026

% effluent	pH	Dissolved Oxygen (mg/L)	Temperature (°C)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Conductivity (µmhos/cm)
Control	7.95 - 8.20	8.0 - 8.6	25	92	88	286
12.5	8.09 - 8.49	8.1 - 8.7	25			
25	8.07 - 8.54	8.0 - 8.8	25			
50	8.04 - 8.71	8.0 - 8.8	25			
75	8.01 - 8.76	8.0 - 8.9	25			
100	7.95 - 8.69	7.9 - 9.2	25	608	504	1125

Test: Reconstituted Water/Bear Creek

% effluent	pH	Dissolved Oxygen (mg/L)	Temperature (°C)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Conductivity (µmhos/cm)
Control	7.95 - 8.20	8.0 - 8.6	25	92	88	286
12.5	7.90 - 8.14	7.9 - 8.8	25			
25	7.75 - 8.13	7.9 - 8.8	25			
50	7.54 - 8.06	7.8 - 8.9	25			
75	7.37 - 8.00	7.9 - 9.0	25			
100	7.13 - 7.97	7.8 - 9.3	25	56	44	97

Table 2 (Continued). Summary of Chemical and Physical Data of Toxicity Tests

Test: Embarrass River/SD033						
% effluent	**pH**	**Dissolved Oxygen (mg/L)**	**Temperature (°C)**	**Total Hardness (mg/L)**	**Total Alkalinity (mg/L)**	**Conductivity (µmhos/cm)**
Control	7.04 - 8.00	7.9 - 9.3	25	80	52	135
12.5	7.29 - 8.24	7.9 - 9.3	25			
25	7.54 - 8.37	7.8 - 9.3	25			
50	7.72 - 8.57	7.9 - 9.2	25			
75	7.81 - 8.69	7.9 - 9.2	25			
100	7.74 - 8.73	7.9 - 9.2	25	1288	384	2420

Test: Partridge River/SD026						
% effluent	**pH**	**Dissolved Oxygen (mg/L)**	**Temperature (°C)**	**Total Hardness (mg/L)**	**Total Alkalinity (mg/L)**	**Conductivity (µmhos/cm)**
Control	7.78 - 8.13	7.9 - 9.5	25	156	72	336
12.5	7.92 - 8.39	7.9 - 9.5	25			
25	7.98 - 8.57	7.9 - 9.5	25			
50	8.00 - 8.70	7.9 - 9.4	25			
75	8.01 - 8.77	7.8 - 9.3	25			
100	7.95 - 8.69	7.9 - 9.2	25	608	504	1125

Screen Test: PM 12.1, PM 17						
% effluent	**pH**	**Dissolved Oxygen (mg/L)**	**Temperature (°C)**	**Total Hardness (mg/L)**	**Total Alkalinity (mg/L)**	**Conductivity (µmhos/cm)**
Control	7.95 - 8.20	8.0 - 8.6	25	92	88	286
PM 12.1	7.86 - 8.53	8.0 - 9.3	25	408	180	876
PM 17	7.87 - 8.74	8.0 - 9.3	25	632	356	1116

CHRONIC TOXICITY TEST
CERIODAPHNIA REPRODUCTION AND SURVIVAL

Client: Polymet- Recon | SD033 Project No.: 10-234

Test Dates/Time ● Initiation: 1505 10/27/10 Termination: 1015 11/3/10

Concentration	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Remarks
0	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	0	0	0	0	0	0	
	4	2	3	4	3	3	4	4	4	4	4	
	5	5	7	5	7	4	8	7	6	6	6	
	6	0	12	6	8	10	0	0	0	0	10	
	7	10	0	0	0	0	9	6	8	8	0	
Total		17	22	15	18	17	21	17	18	18	20	
12.5	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	0	0	0	0	0	0	
	4	4	4	3	4	3	3	3	4	2	4	
	5	5	6	6	7	3	2	5	5	6	5	
	6	9	7	9	0	0	0	0	0	8	0	
	7	0	0	0	8	10	9	8	9	0	7	
Total		18	17	18	19	16	14	16	18	16	16	
25	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	0	3	2	0	0	0	
	4	0	4	3	4	5	0	0	4	3	4	
	5	6	7	6	6	6	8	8	6	8	6	
	6	9	9	7	11	6	8	0	10	0	6	
	7	0	0	0	0	0	0	10	0	9	0	
Total		15	20	16	21	17	19	20	20	20	16	

✓ = Alive # = No. of Live Young (-#) = No. of Dead Young 0 = No Young X = Dead y = Male M= Missing

Analyst: KM Reviewed By: WK

CHRONIC TOXICITY TEST
CERIODAPHNIA REPRODUCTION AND SURVIVAL

Client: Polymet - Recon / SD033 Project No.: 10-234

Test Dates/~~Time~~ • Initiation: 1505 10/27/10 Termination: 1015 11/3/10

Concentration	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Remarks
50	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	0	0	0	0	0	0	
	4	2	3	3	0	4	2	4	2	3	4	
	5	6	7	5	0	7	6	6	5	7	5	
	6	0	0	8	0	0	0	8	10	0	6	
	7	9	6	0	0	10	6	0	0	10	0	
Total		17	16	16	0	21	14	18	17	20	15	
75	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	0	0	0	0	0	0	
	4	2	2	4	2	3	2	3	4	1	4	
	5	4	5	6	6	6	7	6	0	5	6	
	6	6	0	0	8	6	1X	8	8	8	10	
	7	0	10	0	0	0		0	10	0	0	
Total		12	17	10	16	15	10	17	22	14	20	
100	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	0	0	0	0	0	0	
	4	3	1	0	0	2	3	3	4	3	0	
	5	5	8	6	4	7	7	5	6	5	5	
	6	0	10	0	5	0	8	0	0	10	10	
	7	6	0	9	0	7	0	9	7	0	12	
Total		14	19	15	9	16	18	17	17	18	27	

✓ = Alive # = No. of Live Young 0 = No Young X = Dead y = Male M= Missing
(-#) = No. of Dead Young

Analyst: KM Reviewed By: WK

Conc. ID	1	2	3	4	5	6
Conc. Tested	0	12.5	25	50	75	100
Response 1	17	18	15	17	12	14
Response 2	22	17	20	16	17	19
Response 3	15	18	16	16	10	15
Response 4	18	19	21	0	16	9
Response 5	17	16	17	21	15	16
Response 6	21	14	19	14	10	18
Response 7	17	16	20	18	17	17
Response 8	18	18	20	17	22	17
Response 9	18	16	20	20	14	18
Response 10	20	16	16	15	20	27

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Recon SD033
Test Start Date: 10/27/10 Test Ending Date: 11/3/10
Test Species: Ceriodaphnia dubia
Test Duration: 7 days
DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	10	0.000	18.300	2.111	18.300
2	10	12.500	16.800	1.476	17.600
3	10	25.000	18.400	2.171	17.600
4	10	50.000	15.400	5.816	15.900
5	10	75.000	15.300	3.974	15.900
6	10	100.000	17.000	4.522	15.900

*** No Linear Interpolation Estimate can be calculated from the input data since none of the (possibly pooled) group response means were less than 75% of the control response mean.

Ceriodaphnia reproduction
File: Recon SD033 Transform: NO TRANSFORMATION

STEELS MANY-ONE RANK TEST - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	0	18.300				
2	12.5	16.800	85.00	75.00	10.00	
3	25	18.400	105.50	75.00	10.00	
4	50	15.400	84.00	75.00	10.00	
5	75	15.300	78.50	75.00	10.00	
6	100	17.000	89.50	75.00	10.00	

Critical values use k = 5, are 1 tailed, and alpha = 0.05

Toxicity Test
Daily Chemistries

Client: Poly Met	Project Number: 10-234
Test Type: Chronic - Recon/SD033	Species: Ceriodaphnia dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 0	pH	8.05	7.91	7.89	7.83	7.81	7.75	
	Dissolved Oxygen (mg/l)	8.0	8.1	8.1	8.2	8.3	8.6	
Date: 10/27/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)	286					2420	
Analyst: Km	Total Alkalinity (mg/l)	88					384	
	Total Hardness (mg/l)	92					1288	
	Total Ammonia (mg/l)							
Day: 1 old	pH	8.00	8.13	8.39	8.54	8.63	8.69	
	Dissolved Oxygen (mg/l)	8.3	8.3	8.3	8.3	8.3	8.4	
Date: 10/28/10	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
	Conductivity (µmhos)							
Analyst: Km	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 1 New	pH	7.95	7.90	7.88	7.83	7.81	7.74	
	Dissolved Oxygen (mg/l)	8.2	8.2	8.2	8.3	8.3	8.5	
Date: 10/28/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 2 old	pH	7.98	8.16	8.37	8.53	8.63	8.67	
	Dissolved Oxygen (mg/l)	8.6	8.6	8.6	8.6	8.6	8.6	
Date: 10/29/10	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 2 New	pH	8.02	8.08	7.97	7.90	7.87	7.80	
	Dissolved Oxygen (mg/l)	8.5	8.8	8.7	8.8	8.9	9.2	
Date: 10/29/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Trent [signature] Date: 11/6/10

Toxicity Test
Daily Chemistries

Client: PolyMet	Project Number: 10-234
Test Type: Chronic- Recon / SD033	Species: C. dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 3 OLD	pH	8.06	8.24	8.38	8.53	8.60	8.65	
	Dissolved Oxygen (mg/l)	8.5	8.5	8.4	8.4	8.4	8.3	
Date: 10/30/10	Temperature (°C)	25.2	25.2	25.2	25.2	25.2	25.2	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
	Total Ammonia (mg/l)							
Day: 3 New	pH	8.01	8.11	7.97	7.91	7.89	7.82	
	Dissolved Oxygen (mg/l)	8.4	8.5	8.6	8.7	8.8	9.2	
Date: 10/30/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: Km	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 4 OLD	pH	8.06	8.25	8.39	8.55	8.63	8.69	
	Dissolved Oxygen (mg/l)	8.1	8.1	8.1	8.1	8.1	8.0	
Date: 10/31/10	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 4 New	pH	8.12	8.12	8.02	7.92	7.88	7.80	
	Dissolved Oxygen (mg/l)	8.1	8.1	8.3	8.3	8.7	9.2	
Date: 10/31/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 5 OLD	pH	8.13	8.29	8.41	8.57	8.66	8.73	
	Dissolved Oxygen (mg/l)	8.4	8.5	8.5	8.3	8.3	8.3	
Date: 11/01/10	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
	Conductivity (µmhos)							
Analyst: [illegible]	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: [signature] Date: 11/6/10

Toxicity Test
Daily Chemistries

Client: PolyMet	Project Number: 10-234
Test Type: Chronic - Recon / SD033	Species: C. dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 5 New	pH	8.20	8.05	8.04	7.91	7.90	7.85	
	Dissolved Oxygen (mg/l)	8.2	8.1	8.3	8.3	8.4	8.6	
Date: 11/01/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (μmhos)							
Analyst: DL	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
	Total Ammonia (mg/l)							
Day: 6 Old	pH	8.09	8.25	8.59	8.57	8.66	8.73	
	Dissolved Oxygen (mg/l)	8.6	8.6	8.6	8.5	8.4	8.5	
Date: 11/2/10	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
	Conductivity (μmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 6 New	pH	8.12	8.06	8.00	7.90	7.88	7.82	
	Dissolved Oxygen (mg/l)	8.5	8.5	8.6	8.6	8.8	8.9	
Date: 11/2/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (μmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 7 Final	pH	8.09	8.28	8.43	8.56	8.65	8.69	
	Dissolved Oxygen (mg/l)	8.1	8.1	8.0	8.0	8.0	7.9	
Date: 11/3/10	Temperature (°C)	25.1	25.1	25.1	25.1	25.1	25.1	
	Conductivity (μmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day:	pH							
	Dissolved Oxygen (mg/l)							
Date: / /	Temperature (°C)							
	Conductivity (μmhos)							
Analyst:	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Kraut

Date: 11/6/10

CHRONIC TOXICITY TEST
CERIODAPHNIA REPRODUCTION AND SURVIVAL

Client: PolyMet - Recon | SD026 Project No.: 10-234

Test Dates/~~Time~~ • Initiation: 1510 10/27/10 Termination: 1030 11/3/10

Concentration	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Remarks
0	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	0	0	0	0	0	0	
	4	2	3	4	3	3	4	4	4	4	4	
	5	5	7	5	7	4	8	7	6	6	6	
	6	0	12	6	8	10	0	0	0	0	10	
	7	10	0	0	0	0	9	6	8	8	0	
Total		17	22	15	18	17	21	17	18	18	20	
12.5	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	4	0	0	0	0	0	
	4	4	2	4	0	0	3	2	4	4	2	
	5	7	6	8	5	5	6	5	7	7	8	
	6	10	0	0	4	8	0	0	8	8	0	
	7	0	9	13	8	0	9	7	0	0	2	
Total		21	17	25	17	17	18	14	19	19	12	
25	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	0	0	0	0	0	0	
	4	2	4	0	3	4	3	2	3	2	4	
	5	5	4	4	6	7	7	6	7	5	6	
	6	0	0	11	8	0	0	6	0	0	10	
	7	0	10	6	0	9	8	0	10	7	0	
Total		7	18	15	17	20	18	14	20	14	20	

✓ = Alive # = No. of Live Young (-#) = No. of Dead Young 0 = No Young X = Dead y = Male M= Missing

Analyst: KM

Reviewed By: WK

CHRONIC TOXICITY TEST
CERIODAPHNIA REPRODUCTION AND SURVIVAL

Client: PolyMet - Recon / SD026 Project No.: 10-234

Test Dates/Time ● Initiation: 1510 10/27/10 Termination: 1030 11/3/10

Concentration	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Remarks
50	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	4	0	0	0	0	0	0	
	4	2	2	4	0	3	2	3	4	2	3	
	5	3	7	8	6	5	6	8	6	7	6	
	6	8	0	0	10	10	10	0	0	8	8	
	7	0	0	0	0	0	0	12	10	0	0	
Total		13	9	12	20	18	18	23	20	17	17	
75	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	4	0	0	4	0	0	
	4	4	0	4	4	0	2	4	0	4	4	
	5	8	7	8	9	7	5	8	7	8	7	
	6	0	10	0	12	11	0	12	7	12	10	
	7	10	0	12	0	0	11	0	0	0	0	
Total		22	17	24	25	22	18	24	18	24	21	
100	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	0	0	3	2	0	0	
	4	3	3	4	3	2	4	0	0	2	4	
	5	4	6	9	6	6	8	7	6	7	6	
	6	0	10	11	8	11	9	8	4	11	9	
	7	8	0	0	0	0	0	0	0	0	0	
Total		15	19	24	17	19	21	18	14	20	19	

✓ = Alive # = No. of Live Young (-#) = No. of Dead Young 0 = No Young X = Dead y = Male M= Missing

Analyst: Km Reviewed By: WK

Conc. ID	1	2	3	4	5	6
Conc. Tested	0	12.5	25	50	75	100
Response 1	17	21	7	13	22	15
Response 2	22	17	18	9	17	19
Response 3	15	25	15	12	24	24
Response 4	18	17	17	20	25	17
Response 5	17	17	20	18	22	19
Response 6	21	18	18	18	18	21
Response 7	17	14	14	23	24	18
Response 8	18	19	20	20	18	14
Response 9	18	19	14	17	24	20
Response 10	20	12	20	17	21	19

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Recon SD026
Test Start Date: 10/27/10   Test Ending Date: 11/3/10
Test Species: Ceriodaphnia dubia
Test Duration: 7 days
DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	10	0.000	18.300	2.111	18.300
2	10	12.500	17.900	3.573	18.200
3	10	25.000	16.300	4.029	18.200
4	10	50.000	16.700	4.218	18.200
5	10	75.000	21.500	2.915	18.200
6	10	100.000	18.600	2.875	18.200

*** No Linear Interpolation Estimate can be calculated from the input data since none of the (possibly pooled) group response means were less than 75% of the control response mean.

Ceriodaphnia reproduction
File: Recon SD026   Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	170.083	34.017	3.001
Within (Error)	54	612.100	11.335	
Total	59	782.183		

Critical F value = 2.45 (0.05,5,40)
Since F > Critical F REJECT Ho:All groups equal

Ceriodaphnia reproduction
File: Recon SD026   Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2   Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	0	18.300	18.300		
2	12.5	17.900	17.900	0.266	
3	25	16.300	16.300	1.328	
4	50	16.700	16.700	1.063	
5	75	21.500	21.500	-2.125	
6	100	18.600	18.600	-0.199	

Dunnett table value = 2.31   (1 Tailed Value, P=0.05, df=40,5)

Ceriodaphnia reproduction
File: Recon SD026   Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2   Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	0	10			
2	12.5	10	3.478	19.0	0.400
3	25	10	3.478	19.0	2.000
4	50	10	3.478	19.0	1.600
5	75	10	3.478	19.0	-3.200
6	100	10	3.478	19.0	-0.300

Ceriodaphnia reproduction
File: Recon SD026 Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	4.020	14.520	22.920	14.520	4.020
OBSERVED	6	12	25	14	3

Calculated Chi-Square goodness of fit test statistic = 1.8788
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Ceriodaphnia reproduction
File: Recon SD026 Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 5.25
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 9.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

ENVIRONMENTAL TOXICITY CONTROL

Toxicity Test
Daily Chemistries

Client: PolyMet	Project Number: 10-234
Test Type: Chronic - Recon / SD026	Species: Ceriodaphnia dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 0	pH	8.05	8.11	8.07	8.04	8.03	8.79	
	Dissolved Oxygen (mg/l)	8.0	8.2	8.2	8.2	8.4	8.8	
Date: 10/27/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)	286					1125	
Analyst: WK	Total Alkalinity (mg/l)	88					504	
	Total Hardness (mg/l)	92					608	
	Total Ammonia (mg/l)							
Day: 1 old	pH	8.00	8.49	8.53	8.67	8.74	8.63	
	Dissolved Oxygen (mg/l)	8.3	8.4	8.4	8.4	8.4	8.4	
Date: 10/28/10	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 1 New	pH	7.95	8.10	8.10	8.06	8.05	7.98	
	Dissolved Oxygen (mg/l)	8.2	8.4	8.4	8.4	8.4	8.6	
Date: 10/28/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 2 old	pH	7.98	8.30	8.52	8.69	8.75	8.69	
	Dissolved Oxygen (mg/l)	8.6	8.6	8.5	8.5	8.6	8.6	
Date: 10/29/10	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
	Conductivity (µmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 2 New	pH	8.02	8.09	8.07	8.06	8.01	7.96	
	Dissolved Oxygen (mg/l)	8.5	8.7	8.8	8.8	8.9	9.2	
Date: 10/29/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: [signature] Date: 11/6/10

Toxicity Test
Daily Chemistries

Client: PolyMet	Project Number: 10-234
Test Type: Chronic - Recon / SD026	Species: C. dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 3 Old	pH	8.06	8.36	8.50	8.66	8.73	8.59	
	Dissolved Oxygen (mg/l)	8.5	8.4	8.4	8.4	8.4	8.3	
Date: 10/30/10	Temperature (°C)	25.2	25.2	25.2	25.2	25.2	25.2	
	Conductivity (μmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
	Total Ammonia (mg/l)							
Day: 3 New	pH	8.01	8.14	8.10	8.07	8.04	7.99	
	Dissolved Oxygen (mg/l)	8.4	8.6	8.7	8.7	8.8	9.2	
Date: 10/30/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (μmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 4 Old	pH	8.06	8.34	8.47	8.65	8.73	8.62	
	Dissolved Oxygen (mg/l)	8.1	8.1	8.1	8.1	8.0	7.9	
Date: 10/31/10	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
	Conductivity (μmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 4 New	pH	8.12	8.23	8.20	8.17	8.16	8.09	
	Dissolved Oxygen (mg/l)	8.1	8.2	8.2	8.2	8.2	8.2	
Date: 10/31/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (μmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 5 Old	pH	8.13	8.35	8.49	8.67	8.73	8.64	
	Dissolved Oxygen (mg/l)	8.4	8.3	8.2	8.2	8.1	8.2	
Date: 11/01/10	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
	Conductivity (μmhos)							
Analyst: [illegible]	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Ford Date: 11/6/10

Toxicity Test
Daily Chemistries

Client: Polymet	Project Number: 10-234
Test Type: Chronic Recon / SD 026	Species: C. dubia

Day/Date/Analyst	Parameter	0	12.5	25	50	75	100	Remarks
		Concentration						
Day: 5	pH	8.20	8.28	8.22	8.18	8.19	8.15	
New	Dissolved Oxygen (mg/l)	8.2	8.5	8.4	8.5	8.6	8.4	
Date:	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
11/1/10	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
WK	Total Hardness (mg/l)							
	Total Ammonia (mg/l)							
Day: 6	pH	8.09	8.35	8.50	8.67	8.76	8.65	
OLD	Dissolved Oxygen (mg/l)	8.6	8.6	8.5	8.5	8.5	8.5	
Date:	Temperature (°C)	25.3	25.3	25.3	8.3	8.3	8.3	
11/2/10	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
WK	Total Hardness (mg/l)							
Day: 6	pH	8.12	8.22	8.21	8.18	8.18	8.14	
New	Dissolved Oxygen (mg/l)	8.5	8.6	8.6	8.6	8.7	8.7	
Date:	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
11/2/10	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
WK	Total Hardness (mg/l)							
Day: 7	pH	8.09	8.39	8.54	8.71	8.75	8.62	
Final	Dissolved Oxygen (mg/l)	8.1	8.1	8.0	8.0	8.0	8.0	
Date:	Temperature (°C)	25.1	25.1	25.1	25.1	25.1	25.1	
11/3/10	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
WK	Total Hardness (mg/l)							
Day:	pH							
	Dissolved Oxygen (mg/l)							
Date:	Temperature (°C)							
/ /	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Kount

Date: 11/6/10

ENVIRONMENTAL TOXICITY CONTROL

CHRONIC TOXICITY TEST
CERIODAPHNIA REPRODUCTION AND SURVIVAL

Client: PolyMet - Recon / Bear Creek Project No.: 10-234

Test Dates/Time ● Initiation: 1515 10/27/10 Termination: 1040 11/3/10

Concentration	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Remarks
0	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	0	0	0	0	0	0	
	4	2	3	4	3	3	4	4	4	4	4	
	5	5	7	5	7	4	8	7	6	6	6	
	6	0	12	6	8	10	0	0	0	0	10	
	7	10	0	0	0	0	9	6	8	8	0	
Total		Ø	22	15	18	17	21	17	18	18	20	
		17										
12.5	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	3	0	0	0	0	0	0	0	0	0	
	4	0	3	3	1	3	4	2	3	2	4	
	5	5	5	6	6	7	6	8	5	7	7	
	6	12	0	8	11	10	0	10	8	0	0	
	7	0	11	0	0	0	11	0	0	10	11	
Total		20	19	17	18	20	21	20	16	19	22	
25	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	0	0	0	0	0	0	
	4	3	1	2	3	3	4	2	4	3	4	
	5	8	8	7	6	7	7	8	6	9	6	
	6	0	12	12	8	0	0	8	0	0	10	
	7	10	0	0	0	12	7	0	2	12	0	
Total		21	21	21	17	22	18	18	12	24	20	

✓ = Alive # = No. of Live Young (-#) = No. of Dead Young 0 = No Young X = Dead y = Male M= Missing

Analyst: KM Reviewed By: WK

CHRONIC TOXICITY TEST
CERIODAPHNIA REPRODUCTION AND SURVIVAL

Client: Polymet - Recon | Bear Creek Project No.: 10-234

Test Dates/~~Time~~ • Initiation: 1515 10/27/10 Termination: 1040 11/3/10

Concentration	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Remarks
50	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	0	0	0	0	0	0	
	4	3	3	0	3	0	2	2	1	0	4	
	5	10	7	4	7	6	8	9	8	8	10	
	6	0	9	12	0	10	0	10	12	14	0	
	7	14	0	0	14	14	10	0	0	0	13	
Total		27	19	16	24	30	20	21	21	22	27	
75	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	0	0	0	0	0	0	
	4	2	5	2	2	2	3	1	2	4	1	
	5	10	8	8	6	5	7	7	7	7	9	
	6	0	0	10	10	0	10	14	11	12	15	
	7	0	15	0	0	14	0	0	0	0	0	
Total		12	28	20	18	21	20	22	20	23	25	
100	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	3	0	0	0	0	2	
	4	0	3	0	1	0	4	1	2	2	0	
	5	8	7	6	7	8	9	10	7	7	9	
	6	14	0	12	10	11	0	0	0	14	14	
	7	0	14	0	0	0	14	10	13	0	0	
Total		22	24	18	18	22	27	21	22	23	25	

✓ = Alive # = No. of Live Young (-#) = No. of Dead Young 0 = No Young X = Dead y = Male M= Missing

Analyst: KM Reviewed By: WK

Conc. ID	1	2	3	4	5	6
Conc. Tested	0	12.5	25	50	75	100
Response 1	17	20	21	27	12	22
Response 2	22	19	21	19	28	24
Response 3	15	17	21	16	20	18
Response 4	18	18	17	24	18	18
Response 5	17	20	22	30	21	22
Response 6	21	21	18	20	20	27
Response 7	17	20	18	21	22	21
Response 8	18	16	12	21	20	22
Response 9	18	19	24	22	23	23
Response 10	20	22	20	27	25	25

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Recon Bear Creek
Test Start Date: 10/27/10 Test Ending Date: 11/3/10
Test Species: Ceriodaphnia dubia
Test Duration: 7 days
DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	10	0.000	18.300	2.111	20.450
2	10	12.500	19.200	1.814	20.450
3	10	25.000	19.400	3.340	20.450
4	10	50.000	22.700	4.270	20.450
5	10	75.000	20.900	4.254	20.450
6	10	100.000	22.200	2.821	20.450

*** No Linear Interpolation Estimate can be calculated from the input data since none of the (possibly pooled) group response means were less than 75% of the control response mean.

Ceriodaphnia reproduction
File: Recon Bear Creek Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	156.150	31.230	2.966
Within (Error)	54	568.700	10.531	
Total	59	724.850		

Critical F value = 2.45 (0.05,5,40)
Since F > Critical F REJECT Ho:All groups equal

Ceriodaphnia reproduction
File: Recon Bear Creek Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	0	18.300	18.300		
2	12.5	19.200	19.200	-0.620	
3	25	19.400	19.400	-0.758	
4	50	22.700	22.700	-3.032	
5	75	20.900	20.900	-1.792	
6	100	22.200	22.200	-2.687	

Dunnett table value = 2.31 (1 Tailed Value, P=0.05, df=40,5)

Ceriodaphnia reproduction
File: Recon Bear Creek Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	0	10			
2	12.5	10	3.352	18.3	-0.900
3	25	10	3.352	18.3	-1.100
4	50	10	3.352	18.3	-4.400
5	75	10	3.352	18.3	-2.600
6	100	10	3.352	18.3	-3.900

Ceriodaphnia reproduction
File: Recon Bear Creek   Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	4.020	14.520	22.920	14.520	4.020
OBSERVED	5	11	29	10	5

Calculated Chi-Square goodness of fit test statistic = 4.3510
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Ceriodaphnia reproduction
File: Recon Bear Creek   Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 9.98
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 9.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

ENVIRONMENTAL TOXICITY CONTROL

Toxicity Test
Daily Chemistries

Client: Polymet	Project Number: 10-234
Test Type: Chronic - Recon / Bear Creek	Species: Ceriodaphnia dubia

Day/Date/Analyst	Parameter	0	12.5	25	50	75	100	Remarks
		Concentration						
Day: 0	pH	8.05	8.02	7.75	7.54	7.37	7.13	
	Dissolved Oxygen (mg/l)	8.0	8.1	8.1	8.2	8.3	8.4	
Date:	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
10/27/10	Conductivity (µmhos)	286					97	
Analyst:	Total Alkalinity (mg/l)	88					44	
Km	Total Hardness (mg/l)	92					56	
	Total Ammonia (mg/l)							
Day: 1 old	pH	8.00	8.06	8.08	8.06	8.00	7.93	
	Dissolved Oxygen (mg/l)	8.3	8.4	8.4	8.4	8.3	8.3	
Date:	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
10/28/10	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
Km	Total Hardness (mg/l)							
Day: 1 New	pH	7.95	7.90	7.79	7.60	7.47	7.27	
	Dissolved Oxygen (mg/l)	8.2	8.3	8.3	8.4	8.5	8.6	
Date:	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
10/28/10	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
WK	Total Hardness (mg/l)							
Day: 2 OLD	pH	7.98	8.05	8.13	8.00	7.95	7.91	
	Dissolved Oxygen (mg/l)	8.6	8.8	8.8	8.8	8.7	8.8	
Date:	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
10/29/10	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
WK	Total Hardness (mg/l)							
Day: 2 New	pH	8.02	8.10	7.85	7.60	7.42	7.14	
	Dissolved Oxygen (mg/l)	8.5	8.7	8.8	8.9	9.0	9.3	
Date:	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
10/29/10	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
Km	Total Hardness (mg/l)							

Reviewed by: Walt Kont Date: 11/6/10

ENVIRONMENTAL TOXICITY CONTROL

Toxicity Test
Daily Chemistries

Client: PolyMet	Project Number: 10-234
Test Type: Chronic - Recon / Bear Creek	Species: C.dubia

Day/Date/Analyst	Parameter	0	12.5	25	50	75	100	Remarks
Day: 3 OLD	pH	8.06	8.10	8.05	7.97	7.91	7.86	
	Dissolved Oxygen (mg/l)	8.5	8.4	8.4	8.4	8.3	8.2	
Date: 10/30/10	Temperature (°C)	25.2	25.2	25.2	25.2	25.2	25.2	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
	Total Ammonia (mg/l)							
Day: 3 New	pH	8.01	7.98	7.87	7.74	7.59	7.36	
	Dissolved Oxygen (mg/l)	8.4	8.6	8.6	8.7	8.8	9.0	
Date: 10/30/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 4 OLD	pH	8.06	8.10	8.07	8.00	7.96	7.90	
	Dissolved Oxygen (mg/l)	8.1	8.1	8.1	8.1	8.0	8.0	
Date: 10/31/10	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 4 New	pH	8.12	8.03	7.88	7.74	7.56	7.34	
	Dissolved Oxygen (mg/l)	8.1	8.4	8.4	8.5	8.5	8.6	
Date: 10/31/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 5 Old	pH	8.13	7.99	7.94	7.99	7.94	7.91	
	Dissolved Oxygen (mg/l)	8.4	8.1	8.0	8.0	8.1	8.1	
Date: 11/01/10	Temperature (°C)	25.2	25.2	25.2	25.2	25.2	25.2	
	Conductivity (µmhos)							
Analyst: [illegible]	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Koenst Date: 11/6/10

Toxicity Test
Daily Chemistries

Client: Polymit	Project Number: 10-234
Test Type: Chronic Recon/Bear Creek	Species: C. dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 5 New	pH	8.20	8.02	7.86	7.65	7.47	7.27	
	Dissolved Oxygen (mg/l)	8.2	8.3	8.3	8.4	8.5	8.6	
Date: 11/1/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
	Total Ammonia (mg/l)							
Day: 6 Old	pH	8.09	8.14	8.11	8.01	7.97	7.90	
	Dissolved Oxygen (mg/l)	8.6	8.5	8.5	8.4	8.4	8.3	
Date: 11/2/10	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 6 New	pH	8.12	8.02	7.86	7.63	7.40	7.26	
	Dissolved Oxygen (mg/l)	8.5	8.6	8.6	8.7	8.9	9.0	
Date: 11/2/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 7 Final	pH	8.09	8.13	8.07	8.01	7.99	7.97	
	Dissolved Oxygen (mg/l)	8.1	7.9	7.9	7.8	7.9	7.8	
Date: 11/3/10	Temperature (°C)	25.1	25.1	25.1	25.1	25.1	25.1	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day:	pH							
	Dissolved Oxygen (mg/l)							
Date: / /	Temperature (°C)							
	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Kount Date: 11/6/10

CHRONIC TOXICITY TEST
CERIODAPHNIA REPRODUCTION AND SURVIVAL

Client: PolyMet - Embarrass/SD033 Project No.: 10-234
Test Dates/Time • Initiation: 1520 10/27/10 Termination: 1045 11/3/10

Concentration	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Remarks
0	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	0	2	0	0	0	0	
	4	3	3	4	3	2	0	4	4	2	3	
	5	2	4	3	6	3	6	5	5	3	2	
	6	0	0	0	0	5	8	0	8	0	0	
	7	10	10	12	10	0	0	10	0	10	15	
Total		15	17	19	19	10	16	19	17	15	20	
12.5	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	0	0	0	0	0	0	
	4	3	4	4	4	4	3	3	1	3	0	
	5	4	4	5	4	4	3	5	4	3	5	
	6	0	0	0	0	0	0	8	0	0	0	
	7	10	12	10	11	11	10	0	4	10	6	
Total		17	20	19	19	19	16	16	9	16	11	
25	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	0	0	0	0	0	0	
	4	3	4	2	5	4	3	3	2	0	2	
	5	4	3	5	4	4	5	3	4	7	4	
	6	8	0	0	0	0	0	0	8	9	0	
	7	0	7	9	12	10	9	9	0	10	12	
Total		15	14	16	21	18	17	15	14	26	18	

✓ = Alive # = No. of Live Young (-#) = No. of Dead Young 0 = No Young X = Dead y = Male M= Missing

Analyst: Km Reviewed By: WK

CHRONIC TOXICITY TEST
CERIODAPHNIA REPRODUCTION AND SURVIVAL

Client: PolyMet - Embarrass / SD033 Project No.: 10-234
Test Dates/Time ● Initiation: 1520 10/27/10 Termination: 1045 11/3/10

Concentration	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Remarks
50	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	0	0	0	0	0	0	
	4	3	2	3	4	1	4	3	4	2	2	
	5	7X	4	3	5	2	6	5	3	6	3	
	6		9	0	8	0	8	8	0	8	0	
	7		0	10	0	0	0	0	8	0	8	
Total		10	15	16	17	3	18	16	15	16	13	
75	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	0	0	0	0	0	0	
	4	2	4	3	3	4	3	5	3	3	4	
	5	6	4	4	2	3	5	4	0	4	4	
	6	10	7	8	0	0	10	0	0	0	0	
	7	0	0	0	8	8	0	7	8	0	4	
Total		18	15	15	13	15	18	16	11	7	12	
100	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	0	0	0	0	0	0	
	4	3	1	0	0	2	3	3	4	3	0	
	5	5	8	6	4	7	7	5	6	5	5	
	6	0	10	0	5	0	8	0	0	10	10	
	7	6	0	9	0	7	0	9	7	0	12	
Total		14	19	15	9	16	18	17	17	18	27	

✓ = Alive # = No. of Live Young 0 = No Young X = Dead y = Male M= Missing
(-#) = No. of Dead Young

Analyst: KM Reviewed By: WK

Conc. ID	1	2	3	4	5	6
Conc. Tested	0	12.5	25	50	75	100
Response 1	15	17	15	10	18	14
Response 2	17	20	14	15	15	19
Response 3	19	19	16	16	15	15
Response 4	19	19	21	17	13	9
Response 5	10	19	18	3	15	16
Response 6	16	16	17	18	18	18
Response 7	19	16	15	16	16	17
Response 8	17	9	14	15	11	17
Response 9	15	16	26	16	7	18
Response 10	20	11	18	13	12	27

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Embarass SD033
Test Start Date: 10/27/10 Test Ending Date: 11/3/10
Test Species: Ceriodaphnia dubia
Test Duration: 7 days
DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	10	0.000	16.700	2.946	16.767
2	10	12.500	16.200	3.615	16.767
3	10	25.000	17.400	3.718	16.767
4	10	50.000	13.900	4.433	14.967
5	10	75.000	14.000	3.367	14.967
6	10	100.000	17.000	4.522	14.967

*** No Linear Interpolation Estimate can be calculated from the input data since none of the (possibly pooled) group response means were less than 75% of the control response mean.

Ceriodaphnia reproduction
File: Embarrass SD033 Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	117.933	23.587	1.627
Within (Error)	54	783.000	14.500	
Total	59	900.933		

Critical F value = 2.45 (0.05,5,40)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

Ceriodaphnia reproduction
File: Embarrass SD033 Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	0	16.700	16.700		
2	12.5	16.200	16.200	0.294	
3	25	17.400	17.400	-0.411	
4	50	13.900	13.900	1.644	
5	75	14.000	14.000	1.585	
6	100	17.000	17.000	-0.176	

Dunnett table value = 2.31 (1 Tailed Value, P=0.05, df=40,5)

Ceriodaphnia reproduction
File: Embarrass SD033 Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	0	10			
2	12.5	10	3.934	23.6	0.500
3	25	10	3.934	23.6	-0.700
4	50	10	3.934	23.6	2.800
5	75	10	3.934	23.6	2.700
6	100	10	3.934	23.6	-0.300

Ceriodaphnia reproduction
File: Embarrass SD033   Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	4.020	14.520	22.920	14.520	4.020
OBSERVED	5	11	28	14	2

Calculated Chi-Square goodness of fit test statistic = 3.2518
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Ceriodaphnia reproduction
File: Embarrass SD033   Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 2.28
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 9.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

Toxicity Test
Daily Chemistries

Client: Polymet	Project Number: 10-234
Test Type: Chronic- Embarrass / SD033	Species: Ceriodaphnia dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 0	pH	7.25	7.43	7.55	7.72	7.81	7.75	
	Dissolved Oxygen (mg/l)	8.5	8.6	8.5	8.5	8.5	8.6	
Date:	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
10/27/10	Conductivity (µmhos)	135					2420	
Analyst:	Total Alkalinity (mg/l)	52					384	
Km	Total Hardness (mg/l)	80					1288	
	Total Ammonia (mg/l)							
Day: 1 OLD	pH	7.95	8.24	8.37	8.53	8.63	8.69	
	Dissolved Oxygen (mg/l)	8.3	8.3	8.3	8.2	8.3	8.4	
Date:	Temperature (°C)	25.3	25.3	25.3	25.5	25.3	25.3	
10/28/10	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 1 New	pH	7.30	7.57	7.68	7.79	7.84	7.74	
	Dissolved Oxygen (mg/l)	8.6	8.6	8.6	8.6	8.5	8.5	
Date:	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
10/28/10	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
Km	Total Hardness (mg/l)							
Day: 2 Old	pH	7.88	8.20	8.34	8.52	8.63	8.67	
	Dissolved Oxygen (mg/l)	8.7	8.6	8.6	8.6	8.7	8.6	
Date:	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
10/29/10	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
Km	Total Hardness (mg/l)							
Day: 2 New	pH	7.10	7.40	7.57	7.77	7.86	7.80	
	Dissolved Oxygen (mg/l)	9.3	9.3	9.2	9.2	9.2	9.2	
Date:	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
10/29/10	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
WK	Total Hardness (mg/l)							

Reviewed by: [signature] Date: 11/6/10

Toxicity Test
Daily Chemistries

Client: PolyMet	Project Number: 10-234
Test Type: Chronic - Embarrass R. / SD033	Species: C. dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 3 Old	pH	7.97	8.18	8.35	8.54	8.64	8.65	
	Dissolved Oxygen (mg/l)	8.4	8.4	8.3	8.3	8.3	8.3	
Date: 10/30/10	Temperature (°C)	25.2	25.2	25.2	25.2	25.2	25.2	
	Conductivity (μmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
	Total Ammonia (mg/l)							
Day: 3 New	pH	7.32	7.48	7.63	7.80	7.84	7.82	
	Dissolved Oxygen (mg/l)	9.1	9.1	9.1	9.0	9.0	9.2	
Date: 10/30/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (μmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 4 Old	pH	7.94	8.13	8.32	8.51	8.61	8.69	
	Dissolved Oxygen (mg/l)	8.1	8.0	8.0	8.0	8.0	8.0	
Date: 10/31/10	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
	Conductivity (μmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 4 New	pH	7.20	7.39	7.60	7.78	7.86	7.80	
	Dissolved Oxygen (mg/l)	8.7	8.7	8.8	8.8	8.8	9.2	
Date: 10/31/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (μmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 5 Old	pH	7.98	8.17	8.30	8.49	8.60	8.73	
	Dissolved Oxygen (mg/l)	8.1	8.0	7.9	7.9	8.0	8.3	
Date: 11/1/10	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
	Conductivity (μmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Kount

Date: 11/6/10

Toxicity Test
Daily Chemistries

Client: Polymer	Project Number: 10.234
Test Type: Chronic- Embarrass R/SD033	Species: C. dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 5 NEW	pH	7.46	7.60	7.73	7.92	7.96	7.85	
	Dissolved Oxygen (mg/l)	8.6	8.7	8.7	8.6	8.6	8.6	
Date: 11/01/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (μmhos)							
Analyst: [illegible]	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
	Total Ammonia (mg/l)							
Day: 6 OLD	pH	7.98	8.19	8.31	8.54	8.69	8.73	
	Dissolved Oxygen (mg/l)	8.1	8.1	8.2	8.3	8.4	8.5	
Date: 11/2/10	Temperature (°C)	25.3	25.3	25.3	25.3	25.5	25.3	
	Conductivity (μmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 6 New	pH	7.04	7.29	7.54	7.74	7.84	7.82	
	Dissolved Oxygen (mg/l)	9.1	9.2	9.3	9.1	9.0	8.9	
Date: 11/2/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (μmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 7 FINAL	pH	8.00	8.16	8.37	8.57	8.66	8.69	
	Dissolved Oxygen (mg/l)	7.9	7.9	7.8	7.9	7.9	7.9	
Date: 11/3/10	Temperature (°C)	25.1	25.1	25.1	25.1	25.1	25.1	
	Conductivity (μmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day:	pH							
	Dissolved Oxygen (mg/l)							
Date: / /	Temperature (°C)							
	Conductivity (μmhos)							
Analyst:	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Kvench Date: 11/6/10

CHRONIC TOXICITY TEST
CERIODAPHNIA REPRODUCTION AND SURVIVAL

Client: PolyMet - Partridge / SD026 Project No.: 10-234
Test Dates/~~Time~~ • Initiation: 1525 10/27/10 Termination: 1100 11/3/10

Concentration	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Remarks
0	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	0	0	0	0	0	0	
	4	4	4	3	4	2	4	3	4	4	3	
	5	8	8	9	9	6	6	6	7	8	8	
	6	0	10	0	11	11	10	10	0	0	12	
	7	13	0	10	0	0	0	0	10	14	0	
Total		25	22	22	24	19	20	19	21	26	23	
12.5	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	0	0	0	0	0	0	
	4	2	4	1	0	3	4	5	4	0	3	
	5	6	7	7	8	6	6	10	9	7	7	
	6	10	10	10	12	10	0	0	12	9	0	
	7	0	0	0	0	0	12	14	0	16	11	
Total		18	21	18	20	19	22	29	25	32	21	
25	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	0	0	0	0	0	0	
	4	3	0	4	0	2	3	3	2	4	4	
	5	7	7	9	8	6	6	6	10	8	10	
	6	9	10	0	14	12	9	0	0	12	12	
	7	0	0	12	0	0	0	1	14	0	0	
Total		19	17	25	22	20	18	10	26	24	26	

✓ = Alive # = No. of Live Young 0 = No Young X = Dead y = Male M= Missing
(-#) = No. of Dead Young

Analyst: KM Reviewed By: WK

CHRONIC TOXICITY TEST
CERIODAPHNIA REPRODUCTION AND SURVIVAL

Client: PolyMet - PaRtRidge / SD026 Project No.: 10-234
Test Dates/Time ● Initiation: 1525 10/27/10 Termination: 1100 11/3/10

Concentration	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Remarks
50	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	0	0	0	0	0	0	
	4	4	3	2	3	3	4	3	3	4	3	
	5	5	7	7	7	6	5	7	6	7	8	
	6	10	0	12	10	12	0	0	0	11	0	
	7	0	9	0	0	0	12	9	9	0	10	
Total		19	19	21	20	21	21	19	18	22	21	
75	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	0	0	0	0	0	0	
	4	1	3	4	3	2	4	4	2	0	3	
	5	8	5	7	7	9	6	5	6	7	7	
	6	0	8	0	10	8	10	8	10	10	0	
	7	12	0	8	0	0	0	0	0	0	11	
Total		21	16	19	20	19	20	17	18	17	21	
100	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	0	0	3	2	0	0	
	4	3	3	4	3	2	4	0	0	2	4	
	5	4	6	9	6	6	8	7	6	7	6	
	6	0	10	11	8	11	9	8	6	11	9	
	7	8	0	0	0	0	0	0	0	0	0	
Total		15	19	24	17	19	21	18	14	20	19	

✓ = Alive # = No. of Live Young 0 = No Young X = Dead y = Male M= Missing
(-#) = No. of Dead Young

Analyst: KM Reviewed By: WK

Conc. ID	1	2	3	4	5	6
Conc. Tested	0	12.5	25	50	75	100
Response 1	25	18	19	19	21	15
Response 2	22	21	17	19	16	19
Response 3	22	18	25	21	19	24
Response 4	24	20	22	20	20	17
Response 5	19	19	20	21	19	19
Response 6	20	22	18	21	20	21
Response 7	19	29	10	19	17	18
Response 8	21	25	26	18	18	14
Response 9	26	32	24	22	17	20
Response 10	23	21	26	21	21	19

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Partridge SD026
Test Start Date: 10/27/10 Test Ending Date: 11/3/10
Test Species: Ceriodaphnia dubia
Test Duration: 7 days
DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	10	0.000	22.100	2.424	22.300
2	10	12.500	22.500	4.743	22.300
3	10	25.000	20.700	5.012	20.700
4	10	50.000	20.100	1.287	20.100
5	10	75.000	18.800	1.751	18.800
6	10	100.000	18.600	2.875	18.600

*** No Linear Interpolation Estimate can be calculated from the input data since none of the (possibly pooled) group response means were less than 75% of the control response mean.

Ceriodaphnia reproduction
File: Partridge SD026 Transform: NO TRANSFORMATION

STEELS MANY-ONE RANK TEST - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	0	22.100				
2	12.5	22.500	99.00	75.00	10.00	
3	25	20.700	98.50	75.00	10.00	
4	50	20.100	79.50	75.00	10.00	
5	75	18.800	69.00	75.00	10.00	*
6	100	18.600	71.50	75.00	10.00	*

Critical values use k = 5, are 1 tailed, and alpha = 0.05

Ceriodaphnia reproduction
File: Partridge SD026   Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	4.020	14.520	22.920	14.520	4.020
OBSERVED	4	16	19	18	3

Calculated Chi-Square goodness of fit test statistic = 1.9142
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Ceriodaphnia reproduction
File: Partridge SD026   Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 22.31
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 9.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data FAIL homogeneity test at 0.01 level. Try another transformation.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

Toxicity Test
Daily Chemistries

Client: Polymet	Project Number: 10-234
Test Type: Chronic- Partridge/SD026	Species: Ceriodaphnia dubia

Day/Date/Analyst	Parameter	0	12.5	25	50	75	100	Remarks
		Concentration						
Day: 0	pH	7.80	7.94	7.98	8.00	8.02	7.95	
	Dissolved Oxygen (mg/l)	9.1	9.0	8.9	8.8	8.6	8.8	
Date:	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
10/27/10	Conductivity (µmhos)	336					1125	
Analyst:	Total Alkalinity (mg/l)	72					504	
WK	Total Hardness (mg/l)	156					608	
	Total Ammonia (mg/l)							
Day: 1 Old	pH	8.13	8.37	8.51	8.66	8.75	8.63	
	Dissolved Oxygen (mg/l)	8.2	8.3	8.3	8.3	8.3	8.4	
Date:	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
10/28/10	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 1 New	pH	7.79	8.00	8.02	8.03	8.01	7.98	
	Dissolved Oxygen (mg/l)	9.0	8.9	8.8	8.7	8.6	8.6	
Date:	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
10/28/10	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
WK	Total Hardness (mg/l)							
Day: 2 Old	pH 8.02	~~7.94~~	8.31	8.46	8.65	8.74	8.69	
	Dissolved Oxygen (mg/l)	8.5	8.3	8.3	8.4	8.4	8.6	
Date:	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
10/29/10	Conductivity (µmhos)							
Analyst: Km	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 2 New	pH	7.92	7.99	8.08	8.12	8.12	7.96	
	Dissolved Oxygen (mg/l)	9.5	9.5	9.5	9.4	9.3	9.2	
Date:	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
10/29/10	Conductivity (µmhos)							
Analyst: Km	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Koenst   Date: 11/6/10

Toxicity Test
Daily Chemistries

Client: Polymet	Project Number: 10-234
Test Type: Chronic - Partridge R./SD024	Species: C.dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 3 OLD	pH	8.10	8.35	8.48	8.64	8.71	8.59	
	Dissolved Oxygen (mg/l)	8.4	8.4	8.3	8.3	8.3	8.3	
Date: 10/30/10	Temperature (°C)	25.2	25.2	25.2	25.2	25.2	25.2	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
	Total Ammonia (mg/l)							
Day: 3 New	pH	7.78	7.92	8.00	8.00	8.02	7.99	
	Dissolved Oxygen (mg/l)	9.3	9.3	9.2	9.1	9.1	9.2	
Date: 10/30/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 4 Old	pH	8.07	8.33	8.48	8.65	8.76	8.62	
	Dissolved Oxygen (mg/l)	8.1	8.0	8.0	8.0	8.0	7.9	
Date: 10/31/10	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
	Conductivity (µmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 4 New	pH	7.83	8.03	8.12	8.15	8.17	8.09	
	Dissolved Oxygen (mg/l)	9.1	9.1	9.0	8.7	8.4	8.2	
Date: 10/31/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 5 OLD	pH	8.10	8.35	8.43	8.63	8.70	8.64	
	Dissolved Oxygen (mg/l)	8.1	8.1	8.1	8.1	8.1	8.2	
Date: 11/1/10	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Koenst Date: 11/6/10

Toxicity Test
Daily Chemistries

Client: Polymet	Project Number: 10.234
Test Type: Chronic. Partridge R/ SD026	Species: C. dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 5 NEW	pH	7.87	8.00	8.06	8.10	8.09	8.15	
	Dissolved Oxygen (mg/l)	8.9	8.9	8.9	8.8	8.8	8.4	
Date: 11/01/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: [illegible]	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
	Total Ammonia (mg/l)							
Day: 6 OLD	pH	8.11	8.37	8.51	8.69	8.77	8.65	
	Dissolved Oxygen (mg/l)	8.9	8.8	8.5	8.5	8.5	8.5	
Date: 11/2/10	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 6 NEW	pH	7.81	8.10	8.16	8.19	8.18	8.14	
	Dissolved Oxygen (mg/l)	9.2	9.2	9.1	8.9	8.8	8.7	
Date: 11/2/10	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 7 FINAL	pH	8.13	8.39	8.57	8.70	8.74	8.62	
	Dissolved Oxygen (mg/l)	7.9	7.9	7.9	7.9	7.8	8.0	
Date: 11/3/10	Temperature (°C)	25.1	25.1	25.1	25.1	25.1	25.1	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day:	pH							
	Dissolved Oxygen (mg/l)							
Date:	Temperature (°C)							
/ /	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Koenst　　　　Date: 11/6/10

CHRONIC TOXICITY TEST
CERIODAPHNIA REPRODUCTION AND SURVIVAL

Client: PolyMet Project No.: 10-234

Test Dates/Time • Initiation: 1455 10/27/10 Termination: 1110 11/3/10

Concentration	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Remarks
0	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	0	0	0	0	0	0	0	0	
	4	2	3	4	3	3	4	4	4	4	4	
	5	5	7	5	7	4	8	7	6	6	6	
	6	0	12	6	8	10	0	0	0	0	10	
	7	10	0	0	0	0	9	6	8	8	0	
Total		17	22	15	18	17	21	17	18	18	20	$\bar{x}$ = 18.3
PM 12.1	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	4	0	0	0	0	3	0	0	0	0	
	4	0	4	4	4	4	0	4	1	2	0	
	5	7	6	8	5	8	7	7	7	6	8	
	6	11	12	10	4	0	8	0	8	9	10	
	7	0	0	0	0	10	0	10	0	0	12	
total		22	22	22	13	22	18	21	16	17	30	$\bar{x}$ = 20.3
PM 17	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	2	2	2	0	0	0	2	0	0	0	
	4	0	0	0	3	4	2	0	3	3	1	
	5	7	8	8	7	7	6	8	6	7	6	
	6	11	12	10	11	12	12	12	0	11	12	
	7	0	0	0	0	0	0	0	10	0	0	
total		20	22	20	21	23	20	22	19	21	19	$\bar{x}$ = 20.7

✓ = Alive # = No. of Live Young (-#) = No. of Dead Young 0 = No Young X = Dead y = Male M= Missing

Analyst: Km

Reviewed By: [signature]

Toxicity Test
Daily Chemistries

Client: PolyMet	Project Number: 10-234
Test Type: Chronic	Species: Ceriodaphnia dubia

Day/Date/Analyst	Parameter	Concentration 0	PM 12.1	PM 17	Remarks
Day: 0	pH	8.05	8.07	8.09	
	Dissolved Oxygen (mg/l)	8.0	8.4	8.3	
Date:	Temperature (°C)	25.0	25.0	25.0	
10/27/10	Conductivity (μmhos)	286	876	1116	
Analyst:	Total Alkalinity (mg/l)	88	180	356	
KM	Total Hardness (mg/l)	92	408	632	
	Total Ammonia (mg/l)				
Day: 1 Old	pH	8.00	8.48	8.71	
	Dissolved Oxygen (mg/l)	8.3	8.2	8.3	
Date:	Temperature (°C)	25.3	25.3	25.3	
10/28/10	Conductivity (μmhos)				
Analyst:	Total Alkalinity (mg/l)				
WK	Total Hardness (mg/l)				
Day: 1 New	pH	7.95	8.14	8.24	
	Dissolved Oxygen (mg/l)	8.2	8.4	8.4	
Date:	Temperature (°C)	25.0	25.0	25.0	
10/28/10	Conductivity (μmhos)				
Analyst:	Total Alkalinity (mg/l)				
WK	Total Hardness (mg/l)				
Day: 2 Old	pH	7.98	8.44	8.70	
	Dissolved Oxygen (mg/l)	8.6	8.5	8.4	
Date:	Temperature (°C)	25.3	25.3	25.3	
10/29/10	Conductivity (μmhos)				
Analyst:	Total Alkalinity (mg/l)				
KM	Total Hardness (mg/l)				
Day: 2 New	pH	8.02	8.08	8.18	
	Dissolved Oxygen (mg/l)	8.5	9.0	9.2	
Date:	Temperature (°C)	25.0	25.0	25.0	
10/29/10	Conductivity (μmhos)				
Analyst:	Total Alkalinity (mg/l)				
KM	Total Hardness (mg/l)				

Reviewed by: Walter Koenst   Date: 11/6/10

Toxicity Test
Daily Chemistries

Client: PolyMet	Project Number: 10-234
Test Type: Chronic	Species: C. dubia

Day/Date/Analyst	Parameter	Concentration 0	PM 12.1	PM 17	Remarks
Day: 3 old	pH	8.06	8.44	8.68	
	Dissolved Oxygen (mg/l)	8.5	8.4	8.4	
Date: 10/30/10	Temperature (°C)	25.2	25.2	25.2	
	Conductivity (µmhos)				
Analyst: KM	Total Alkalinity (mg/l)				
	Total Hardness (mg/l)				
	Total Ammonia (mg/l)				
Day: 3 New	pH	8.01	8.14	8.20	
	Dissolved Oxygen (mg/l)	8.4	9.0	9.0	
Date: 10/30/10	Temperature (°C)	25.0	25.0	25.0	
	Conductivity (µmhos)				
Analyst: WK	Total Alkalinity (mg/l)				
	Total Hardness (mg/l)				
Day: 4 OLD	pH	8.06	8.53	8.68	
	Dissolved Oxygen (mg/l)	8.1	8.0	8.1	
Date: 10/31/10	Temperature (°C)	25.3	25.3	25.3	
	Conductivity (µmhos)				
Analyst: WK	Total Alkalinity (mg/l)				
	Total Hardness (mg/l)				
Day: 4 New	pH	8.12	8.09	8.20	
	Dissolved Oxygen (mg/l)	8.1	9.0	9.1	
Date: 10/31/10	Temperature (°C)	25.0	25.0	25.0	
	Conductivity (µmhos)				
Analyst: KM	Total Alkalinity (mg/l)				
	Total Hardness (mg/l)				
Day: 5 OLD	pH	8.13	8.46	8.69	
	Dissolved Oxygen (mg/l)	8.4	8.0	8.0	
Date: 11/1/10	Temperature (°C)	25.3	25.3	25.3	
	Conductivity (µmhos)				
Analyst: WK.	Total Alkalinity (mg/l)				
	Total Hardness (mg/l)				

Reviewed by: Walter Kord   Date: 11/6/10

Toxicity Test
Daily Chemistries

Client: Polymet	Project Number: 10-234
Test Type: Chronic	Species: C. dubia

Day/Date/Analyst	Parameter	Concentration 0	PM 12.1	PM 17	Remarks
Day: 5 NEW	pH	8.20	8.07	8.13	
	Dissolved Oxygen (mg/l)	8.2	8.6	8.6	
Date: 11/01/10	Temperature (°C)	25.0	25.0	25.0	
	Conductivity (µmhos)				
Analyst: KM	Total Alkalinity (mg/l)				
	Total Hardness (mg/l)				
	Total Ammonia (mg/l)				
Day: 6 old	pH	8.09	8.50	8.74	
	Dissolved Oxygen (mg/l)	8.6	8.5	8.5	
Date: 11/2/10	Temperature (°C)	25.3	25.3	25.3	
	Conductivity (µmhos)				
Analyst: KM	Total Alkalinity (mg/l)				
	Total Hardness (mg/l)				
Day: 6 New	pH	8.12	7.86	7.87	
	Dissolved Oxygen (mg/l)	8.5	9.3	9.3	
Date: 11/2/10	Temperature (°C)	25.0	25.0	25.0	
	Conductivity (µmhos)				
Analyst: WK	Total Alkalinity (mg/l)				
	Total Hardness (mg/l)				
Day: 7 FINAL	pH	8.09	8.50	8.71	
	Dissolved Oxygen (mg/l)	8.1	8.0	8.0	
Date: 11/3/10	Temperature (°C)	25.1	25.1	25.1	
	Conductivity (µmhos)				
Analyst: WK	Total Alkalinity (mg/l)				
	Total Hardness (mg/l)				
Day:	pH				
	Dissolved Oxygen (mg/l)				
Date: / /	Temperature (°C)				
	Conductivity (µmhos)				
Analyst:	Total Alkalinity (mg/l)				
	Total Hardness (mg/l)				

Reviewed by: Walter Kourt Date: 11/6/10

Appendix 3-E3

WET Test Results, June 2011, Report 11-145

TOXICITY TEST RESULTS

POLYMET MINING

Report Date: June 16, 2011

Project No. 11-145

Prepared for:

Barr Engineering
4700 W. 77th Street
Minneapolis, MN 55435

ENVIRONMENTAL TOXICITY CONTROL INC.

6265 Applewood Road • Woodbury, Minnesota 55125
Phone 651 501-2075 • Fax 651 501-2076

QUALITY ASSURANCE AND QUALITY CONTROL:

Satisfactory laboratory performance on an ongoing basis is demonstrated by conducting at least one acceptable toxicity test per month with a reference toxicant. Control charts for a reference toxicant and successive endpoints (LC50 and IC25) are plotted to determine if results are within prescribed limits. Results from our most recent reference tests are shown in the following table:

Reference Toxicity Test		
Species	IC_{25}	Test Date
Ceriodaphnia dubia	0.637 g/l NaCl	05/27/11

Our results are within range of EPA expected results for the type of tests conducted.

Test methods and procedures are documented in ETC's Standard Operating Procedures (SOPs). Test and analysis protocols are reviewed by ETC's Quality Assurance/Quality Control Officer. Procedures are documented and followed as written. Any deviation from a QA/QC procedure is documented and kept in the project file. During this project, no deviation in method was warranted.

ENVIRONMENTAL TOXICITY CONTROL

Walter Koenst
Bioassay Manager

Table 1. **Survival and Reproduction of *Ceriodaphnia dubia*.**

Test: Reconstituted Water/SD033		
Concentration (%)	**% Survival**	**Mean # of Young Produced**
Control	100	19.2
12.5%	100	13.6
25%	100	15.4
50%	100	14.4
75%	100	12.0
100%	100	8.0
IC25		50.0%
NOEC	100%	<12.5%
TUc		2.0

Test: Reconstituted Water/SD026		
Concentration (%)	**% Survival**	**Mean # of Young Produced**
Control	100	19.2
12.5%	100	18.8
25%	100	17.6
50%	100	16.2
75%	100	15.0
100%	100	11.4
IC25		79.2%
NOEC	100%	50%
TUc		1.26

Table 1(Continued). Survival and Reproduction of *Ceriodaphnia dubia*.

Test: Reconstituted Water/Bear Creek		
Concentration (%)	**% Survival**	**Mean # of Young Produced**
Control	100	19.2
12.5%	100	18.4
25%	100	19.3
50%	100	20.1
75%	100	20.5
100%	100	22.6
IC25		>100%
NOEC	100%	100%
TUc		<1.0

Test: Embarrass River/SD033		
Concentration (%)	**% Survival**	**Mean # of Young Produced**
Control	100	19.1
12.5%	100	20.3
25%	100	17.7
50%	90	18.6
75%	100	17.8
100%	100	8.0
IC25		82.7%
NOEC	100%	75%
TUc		1.21

Table 1(Continued). Survival and Reproduction of *Ceriodaphnia dubia*.

Test: Partridge River/SD026		
Concentration (%)	**% Survival**	**Mean # of Young Produced**
Control	100	18.0
12.5%	100	16.8
25%	100	18.3
50%	100	21.5
75%	100	18.5
100%	100	11.4
IC25		90.9%
NOEC	100%	75%
TUc		1.10

Screen Test: Spring Mine Creek, PM 17		
Sample ID	**% Survival**	**Mean # of Young Produced**
Control	100	19.2
Spring Mine Creek	100	13.7
PM 17	100	13.3

Table 2. **Summary of Chemical and Physical Data of Toxicity Tests**

Test: Reconstituted Water/SD033						
% Effluent	**pH**	**Dissolved Oxygen (mg/L)**	**Temperature (°C)**	**Total Hardness (mg/L)**	**Total Alkalinity (mg/L)**	**Conductivity (µmhos/cm)**
Control	7.97 - 8.50	8.0 - 8.4	25	88	60	306
12.5	8.08 - 8.31	7.9 - 8.4	25			
25	8.11 - 8.43	8.0 - 8.6	25			
50	8.10 - 8.56	7.9 - 8.9	25			
75	8.08 - 8.64	7.8 - 9.2	25			
100	8.03 - 8.73	7.8 - 10.0	25	1176	352	2210

Test: Reconstituted Water/SD026						
% Effluent	**pH**	**Dissolved Oxygen (mg/L)**	**Temperature (°C)**	**Total Hardness (mg/L)**	**Total Alkalinity (mg/L)**	**Conductivity (µmhos/cm)**
Control	7.97 - 8.50	8.0 - 8.4	25	88	60	306
12.5	8.07 - 8.39	8.0 - 8.5	25			
25	8.04 - 8.51	7.8 - 8.5	25			
50	8.00 - 8.66	7.8 - 9.0	25			
75	7.99 - 8.75	7.9 - 9.2	25			
100	7.92 - 8.69	7.9 - 9.9	25	572	448	1059

Test: Reconstituted Water/Bear Creek						
% Effluent	**pH**	**Dissolved Oxygen (mg/L)**	**Temperature (°C)**	**Total Hardness (mg/L)**	**Total Alkalinity (mg/L)**	**Conductivity (µmhos/cm)**
Control	7.97 - 8.50	8.0 - 8.4	25	88	60	306
12.5	7.96 - 8.18	7.9 - 8.5	25			
25	7.75 - 8.09	7.9 - 8.6	25			
50	7.41 - 8.02	7.8 - 8.8	25			
75	7.25 - 7.96	7.8 - 8.9	25			
100	6.96 - 7.89	7.8 - 9.6	25	44	40	82

Table 2 (Continued). **Summary of Chemical and Physical Data of Toxicity Tests**

Test: Embarrass River/SD033						
% Effluent	pH	Dissolved Oxygen (mg/L)	Temperature (°C)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Conductivity (µmhos/cm)
Control	6.69 - 7.81	7.8 - 9.3	25	48	44	71
12.5	7.19 - 8.01	7.8 - 9.3	25			
25	7.48 - 8.30	7.8 - 9.3	25			
50	7.87 - 8.53	7.8 - 9.4	25			
75	8.03 - 8.64	7.8 - 9.4	25			
100	8.03 - 8.73	7.8 - 10.0	25	1176	352	2210

Test: Partridge River/SD026						
% Effluent	pH	Dissolved Oxygen (mg/L)	Temperature (°C)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Conductivity (µmhos/cm)
Control	7.41 - 7.93	8.0 - 9.5	25	76	44	144
12.5	7.78 - 8.22	8.0 - 9.4	25			
25	7.92 - 8.38	7.9 - 9.5	25			
50	7.99 - 8.66	7.8 - 9.5	25			
75	8.02 - 8.75	7.8 - 9.5	25			
100	7.92 - 8.69	7.9 - 9.9	25	572	448	1059

Screen Test: Spring Mine Creek, PM 17						
% Effluent	pH	Dissolved Oxygen (mg/L)	Temperature (°C)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Conductivity (µmhos/cm)
Control	7.97 - 8.50	8.0 - 8.4	25	88	60	306
Spring Mine Cr.	7.60 - 8.37	7.9 - 9.8	25	312	128	684
PM 17	7.98 - 8.62	7.8 - 9.8	25	888	280	1459

ENVIRONMENTAL TOXICITY CONTROL

CHRONIC TOXICITY TEST
CERIODAPHNIA REPRODUCTION AND SURVIVAL

Client: PolyMet - Recon / SD033 Project No.: 11-145

Test Dates/Time • Initiation: 1115 6/3/11 Termination: 0900 6/9/11

Concentration	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Remarks
0	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	3	3	2	3	3	2	2	4	
	4	6	6	6	0	6	0	0	6	6	0	
	5	10	9	0	6	0	7	7	0	0	6	
	6	0	0	12	9	12	14	10	13	12	7	
Total		16	15	21	18	20	24	20	21	20	17	
12.5	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	2	0	0	1	0	0	3	0	0	2	
	4	0	3	4	6	4	6	0	6	4	6	
	5	5	7	0	0	6	6	6	4	7	0	
	6	10	0	7	8	0	0	13	0	0	10	
Total		17	10	11	15	10	12	22	10	11	18	
25	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	4	3	0	4	3	3	0	0	3	0	
	4	0	0	6	6	0	0	1	3	0	5	
	5	7	0	9	0	6	7	3	5	5	8	
	6	9	10	0	9	11	6	8	0	10	0	
Total		20	13	15	19	20	16	12	8	18	13	

✓ = Alive # = No. of Live Young (-#) = No. of Dead Young 0 = No Young X = Dead y = Male M= Missing

Analyst: KM / WK Reviewed By: WK

Bio.105

CHRONIC TOXICITY TEST
CERIODAPHNIA REPRODUCTION AND SURVIVAL

Client: Polymet - Recon | SD033 Project No.: 11-145
Test Dates/Time ● Initiation: 1115 6/3/11 Termination: 0900 6/9/11

Concentration	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Remarks
50	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	4	0	0	2	2	0	3	2	0	3	
	4	0	4	5	0	5	7	0	4	4	6	
	5	6	8	5	4	0	10	5	0	6	0	
	6	8	0	0	6	7	0	8	11	0	9	
Total		18	12	10	12	14	17	16	17	10	18	
75	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	2	2	4	2	0	0	3	0	0	
	4	0	4	6	0	4	4	4	0	5	7	
	5	3	0	0	5X	0	6	6	4	6	0	
	6	7	9	6		7	0	0	9	0	5	
Total		10	15	14	9	13	10	10	16	11	12	
100	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	1	0	0	0	0	0	0	0	2	0	
	4	0	0	3	2	0	3	2	4	0	2	
	5	4	3	5	5	3	4	3	4	4	3	
	6	9	8	0	0	4	0	0	0	2	0	
Total		14	11	8	7	7	7	5	8	8	5	

✓ = Alive # = No. of Live Young (-#) = No. of Dead Young 0 = No Young X = Dead y = Male M= Missing

Analyst: KM/WK Reviewed By: WK

Conc. ID	1	2	3	4	5	6
Conc. Tested	0	12.5	25	50	75	100
Response 1	16	17	20	18	10	14
Response 2	15	10	13	12	15	11
Response 3	21	11	15	10	14	8
Response 4	18	15	19	12	9	7
Response 5	20	10	20	14	13	7
Response 6	24	12	16	17	10	7
Response 7	20	22	12	16	10	5
Response 8	21	10	8	17	16	8
Response 9	20	11	18	10	11	8
Response 10	17	18	13	18	12	5

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Recon/SDO33
Test Start Date: 6/3/11 Test Ending Date: 6/9/11
Test Species: Ceriodaphnia dubia
Test Duration: 6 days
DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	10	0.000	19.200	2.700	19.200
2	10	12.500	13.600	4.195	14.500
3	10	25.000	15.400	3.950	14.500
4	10	50.000	14.400	3.204	14.400
5	10	75.000	12.000	2.404	12.000
6	10	100.000	8.000	2.708	8.000

The Linear Interpolation Estimate: 50.0000 Entered P Value: 25

Number of Resamplings: 80
The Bootstrap Estimates Mean: 30.2622 Standard Deviation: 20.1528
Original Confidence Limits: Lower: 9.8763 Upper: 59.1994
Resampling time in Seconds: 0.06 Random_Seed: 42286686

Ceriodaphnia reproduction
File: RECON SD033 Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	689.933	137.987	12.964
Within (Error)	54	574.800	10.644	
Total	59	1264.733		

Critical F value = 2.45 (0.05,5,40)
Since F > Critical F REJECT Ho:All groups equal

Ceriodaphnia reproduction
File: RECON SD033 Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	0	19.200	19.200		
2	12.5	13.600	13.600	3.838	*
3	25	15.400	15.400	2.604	*
4	50	14.400	14.400	3.290	*
5	75	12.000	12.000	4.935	*
6	100	8.000	8.000	7.676	*

Dunnett table value = 2.31 (1 Tailed Value, P=0.05, df=40,5)

Ceriodaphnia reproduction
File: RECON SD033 Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	0	10			
2	12.5	10	3.370	17.6	5.600
3	25	10	3.370	17.6	3.800
4	50	10	3.370	17.6	4.800
5	75	10	3.370	17.6	7.200
6	100	10	3.370	17.6	11.200

Ceriodaphnia reproduction
File: RECON SD033 Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	4.020	14.520	22.920	14.520	4.020
OBSERVED	2	20	19	15	4

Calculated Chi-Square goodness of fit test statistic = 3.7696
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Ceriodaphnia reproduction
File: RECON SD033 Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 4.43
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 9.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

Toxicity Test
Daily Chemistries

Client: Polymet	Project Number: 11-145
Test Type: Chronic - Recon / SD033	Species: Ceriodaphnia dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 0	pH	8.03	8.08	8.11	8.10	8.08	8.03	
	Dissolved Oxygen (mg/l)	8.3	8.3	8.5	8.7	9.0	9.9	
Date: 6/3/11	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)	306					2210	
Analyst: KM	Total Alkalinity (mg/l)	60					352	
	Total Hardness (mg/l)	88					1176	
	Total Ammonia (mg/l)							
Day: 1 old	pH	8.14	8.28	8.39	8.51	8.59	8.64	
	Dissolved Oxygen (mg/l)	8.3	8.1	8.1	8.1	8.1	8.1	
Date: 6/4/11	Temperature (°C)	25.4	25.4	25.4	25.4	25.4	25.4	
	Conductivity (µmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 1 New	pH	8.16	8.15	8.14	8.11	8.11	8.04	
	Dissolved Oxygen (mg/l)	8.2	8.2	8.4	8.5	8.8	9.5	
Date: 6/4/11	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 2 old	pH	8.19	8.31	8.43	8.56	8.64	8.73	
	Dissolved Oxygen (mg/l)	8.0	7.9	8.0	7.9	7.8	7.8	
Date: 6/5/11	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
	Conductivity (µmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 2 New	pH	8.22	8.23	8.22	8.18	8.15	8.10	
	Dissolved Oxygen (mg/l)	8.2	8.1	8.3	8.6	8.9	9.8	
Date: 6/5/11	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Ford Date: 6/15/11

Toxicity Test
Daily Chemistries

Client: Polymet	Project Number: 11-145
Test Type: Chronic-Recon/SD033	Species: Ceriodaphnia dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 3 Old	pH	8.04	8.19	8.30	8.43	8.53	8.64	
	Dissolved Oxygen (mg/l)	8.3	8.1	8.0	8.0	7.9	7.9	
Date: 6/6/11	Temperature (°C)	25.4	25.4	25.4	25.4	25.4	25.4	
	Conductivity (µmhos)							
Analyst: SW	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
	Total Ammonia (mg/l)							
Day: 3 New	pH	8.19	8.21	8.22	8.20	8.17	8.13	
	Dissolved Oxygen (mg/l)	8.2	8.2	8.3	8.4	8.6	9.1	
Date: 6/6/11	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: SW	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 4 OLD	pH	7.97	8.09	8.27	8.44	8.53	8.60	
	Dissolved Oxygen (mg/l)	8.3	8.1	8.1	8.0	8.0	8.0	
Date: 6/7/11	Temperature (°C)	25.2	25.2	25.2	25.2	25.2	25.2	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 4 New	pH	8.14	8.24	8.20	8.20	8.17	8.14	
	Dissolved Oxygen (mg/l)	8.4	8.4	8.6	8.9	9.2	10.0	
Date: 6/7/11	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 5 Old	pH	7.97	8.13	8.23	8.43	8.53	8.62	
	Dissolved Oxygen (mg/l)	8.2	8.0	8.0	8.0	8.1	8.1	
Date: 6/8/11	Temperature (°C)	24.9	24.9	24.9	24.9	24.9	24.9	
	Conductivity (µmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Kornt

Date: 6/15/11

Toxicity Test
Daily Chemistries

Client: Polymet	Project Number: 11-145
Test Type: Chronic - Recon | SD033	Species: C. dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 5 New	pH	8.22	8.25	8.20	8.15	8.12	8.09	
	Dissolved Oxygen (mg/l)	8.3	8.2	8.5	8.6	9.0	9.4	
Date: 6/8/11	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (μmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
	Total Ammonia (mg/l)							
Day: 6 final	pH	8.50	8.21	8.35	8.43	8.57	8.67	
	Dissolved Oxygen (mg/l)	8.2	8.1	8.1	8.0	8.0	8.1	
Date: 6/~~8~~9/11	Temperature (°C)	24.9	24.9	24.9	24.9	24.9	24.9	
	Conductivity (μmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day:	pH							
	Dissolved Oxygen (mg/l)							
Date: / /	Temperature (°C)							
	Conductivity (μmhos)							
Analyst:	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day:	pH							
	Dissolved Oxygen (mg/l)							
Date: / /	Temperature (°C)							
	Conductivity (μmhos)							
Analyst:	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day:	pH							
	Dissolved Oxygen (mg/l)							
Date: / /	Temperature (°C)							
	Conductivity (μmhos)							
Analyst:	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Konat   Date: 6/13/11

CHRONIC TOXICITY TEST
CERIODAPHNIA REPRODUCTION AND SURVIVAL

Client: PolyMet - Recon | SD026 Project No.: 11-145
Test Dates/Time ● Initiation: 1125 6/3/11 Termination: 0915 6/9/11

Concentration	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Remarks
0	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	3	3	2	3	3	2	2	4	
	4	6	6	6	0	6	0	0	6	6	0	
	5	10	9	0	6	0	7	7	0	0	6	
	6	0	0	12	9	12	14	10	13	12	7	
Total		16	15	21	18	20	24	20	21	20	17	
12.5	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	3	2	2	3	1	0	0	3	4	4	
	4	0	6	6	5	4	5	4	6	0	6	
	5	7	0	0	0	0	9	8	0	3	0	
	6	12	10	12	14	12	1	0	12	12	12	
Total		22	18	20	22	17	15	12	21	19	22	
25	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	2	WK ~~0~~ 1	2	4	0	4	0	3	0	3	
	4	0	3	0	6	4	4	6	0	7	6	
	5	6	0	2	0	8	0	6	5	0	0	
	6	8	14	15	14	0	11	0	11	9	12	
Total		16	18	19	24	12	19	12	19	16	21	

✓ = Alive # = No. of Live Young 0 = No Young X = Dead y = Male M= Missing
(-#) = No. of Dead Young

Analyst: KM/WK Reviewed By: WK

CHRONIC TOXICITY TEST
CERIODAPHNIA REPRODUCTION AND SURVIVAL

Client: Polymet - Recon | SD026 Project No.: 11-145
Test Dates/Time ● Initiation: 1125 6/3/11 Termination: 0915 6/9/11

Concentration	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Remarks
50	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	4	4	3	2	0	2	4	0	2	3	
	4	0	5	5	4	4	5	0	5	7	6	
	5	7	0	0	0	7	0	6	6	6	0	
	6	9	7	12	3	0	13	12	0	0	9	
Total		20	16	20	9	11	20	22	11	15	18	
75	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	3	0	2	4	0	3	4	4	3	2	
	4	0	5	0	6	4	0	0	0	2	0	
	5	6	8	5	7	5	7	4	5	0	6	
	6	10	0	2	10	0	9	10	7	10	3	
Total		19	13	9	21	9	19	18	16	15	11	
100	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	4	3	0	0	2	0	3	2	0	
	4	4	2	0	4	2	0	3	0	0	4	
	5	8	0	6	5	3	6	3	6	4	6	
	6	0	7	7	0	0	9	0	7	4	0	
Total		12	13	16	9	5	17	6	16	10	10	

✓ = Alive # = No. of Live Young 0 = No Young X = Dead y = Male M= Missing
(-#) = No. of Dead Young

Analyst: Km / WK Reviewed By: WK

Conc. ID	1	2	3	4	5	6
Conc. Tested	0	12.5	25	50	75	100
Response 1	16	22	16	20	19	12
Response 2	15	18	18	16	13	13
Response 3	21	20	19	20	9	16
Response 4	18	22	24	9	21	9
Response 5	20	17	12	11	9	5
Response 6	24	15	19	20	19	17
Response 7	20	12	12	22	18	6
Response 8	21	21	19	11	16	16
Response 9	20	19	16	15	15	10
Response 10	17	22	21	18	11	10

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Recon/SD026
Test Start Date: 6/3/11   Test Ending Date: 6/9/11
Test Species: Ceriodaphnia dubia
Test Duration: 6 days
DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	10	0.000	19.200	2.700	19.200
2	10	12.500	18.800	3.360	18.800
3	10	25.000	17.600	3.748	17.600
4	10	50.000	16.200	4.566	16.200
5	10	75.000	15.000	4.346	15.000
6	10	100.000	11.400	4.169	11.400

The Linear Interpolation Estimate: 79.1667 Entered P Value: 25

Number of Resamplings: 80
The Bootstrap Estimates Mean: 76.0246 Standard Deviation: 10.1619
Original Confidence Limits: Lower: 50.4808 Upper: 89.8077
Resampling time in Seconds: 0.06 Random_Seed: 349432308

Ceriodaphnia reproduction
File: RECON SD026 Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	420.333	84.067	5.621
Within (Error)	54	807.600	14.956	
Total	59	1227.933		

Critical F value = 2.45 (0.05,5,40)
Since F > Critical F REJECT Ho:All groups equal

Ceriodaphnia reproduction
File: RECON SD026 Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	0	19.200	19.200		
2	12.5	18.800	18.800	0.231	
3	25	17.600	17.600	0.925	
4	50	16.200	16.200	1.735	
5	75	15.000	15.000	2.428	*
6	100	11.400	11.400	4.510	*

Dunnett table value = 2.31 (1 Tailed Value, P=0.05, df=40,5)

Ceriodaphnia reproduction
File: RECON SD026 Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	0	10			
2	12.5	10	3.995	20.8	0.400
3	25	10	3.995	20.8	1.600
4	50	10	3.995	20.8	3.000
5	75	10	3.995	20.8	4.200
6	100	10	3.995	20.8	7.800

Ceriodaphnia reproduction
File: RECON SD026 Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	4.020	14.520	22.920	14.520	4.020
OBSERVED	4	13	23	18	2

Calculated Chi-Square goodness of fit test statistic = 2.0086
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Ceriodaphnia reproduction
File: RECON SD026 Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 3.00
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 9.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

Toxicity Test
Daily Chemistries

Client: PolyMet	Project Number: 11-145
Test Type: Chronic- Recon / SD026	Species: Ceriodaphnia dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 0	pH	8.03	8.07	8.04	8.00	7.99	7.92	
	Dissolved Oxygen (mg/l)	8.3	8.2	8.2	8.3	8.4	8.5	
Date: 6/3/11	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)	306					1059	
Analyst: KM	Total Alkalinity (mg/l)	60					448	
	Total Hardness (mg/l)	88					572	
	Total Ammonia (mg/l)							
Day: 1 Old	pH	8.14	8.34	8.43	8.57	8.63	8.64	
	Dissolved Oxygen (mg/l)	8.3	8.0	8.1	8.1	8.1	8.1	
Date: 6/4/11	Temperature (°C)	25.4	25.4	25.4	25.4	25.4	25.4	
	Conductivity (µmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 1 New	pH	8.16	8.15	8.08	8.04	8.01	7.92	
	Dissolved Oxygen (mg/l)	8.2	8.3	8.2	8.4	8.5	9.0	
Date: 6/4/11	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 2 Old	pH	8.19	8.39	8.51	8.66	8.75	8.69	
	Dissolved Oxygen (mg/l)	8.0	8.1	8.0	8.0	7.9	7.9	
Date: 6/5/11	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
	Conductivity (µmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 2 New	pH	8.22	8.24	8.18	8.10	8.06	8.00	
	Dissolved Oxygen (mg/l)	8.2	8.3	8.2	8.4	8.5	9.1	
Date: 6/5/11	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Kount Date: 6/15/11

Toxicity Test
Daily Chemistries

Client: Polymet	Project Number: 11-145
Test Type: Chronic - Recon 15D026	Species: Ceriodaphnia dubia

Day/Date/Analyst	Parameter	0	12.5	25	50	75	100	Remarks
Day: 3 Old	pH	8.04	8.31	8.39	8.56	8.65	8.64	
	Dissolved Oxygen (mg/l)	8.3	8.0	7.8	7.8	7.9	7.9	
Date: 6/6/11	Temperature (°C)	25.4	25.4	25.4	25.4	25.4	25.4	
	Conductivity (μmhos)							
Analyst: SW	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
	Total Ammonia (mg/l)							
Day: 3 New	pH	8.19	8.25	8.20	8.16	8.12	8.06	
	Dissolved Oxygen (mg/l)	8.2	8.2	8.2	8.3	8.4	8.8	
Date: 6/6/11	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (μmhos)							
Analyst: SW	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 4 Old	pH	7.97	8.25	8.36	8.54	8.64	8.61	
	Dissolved Oxygen (mg/l)	8.3	8.5	8.5	8.4	8.3	8.0	
Date: 6/7/11	Temperature (°C)	25.2	25.2	25.2	25.2	25.2	25.2	
	Conductivity (μmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 4 New	pH	8.14	8.19	8.17	8.14	8.12	8.07	
	Dissolved Oxygen (mg/l)	8.4	8.5	8.5	9.0	9.2	9.9	
Date: 6/7/11	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (μmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 5 Old	pH	7.97	8.20	8.33	8.50	8.59	8.54	
	Dissolved Oxygen (mg/l)	8.2	8.2	8.2	8.1	8.1	8.1	
Date: 6/8/11	Temperature (°C)	24.9	24.9	24.9	24.9	24.9	24.9	
	Conductivity (μmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Kont Date: 6/15/11

Toxicity Test
Daily Chemistries

Client: Polymet	Project Number: 11-145
Test Type: Chronic - Recon / SP026	Species: C. dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 5 New	pH	8.22	8.18	8.13	8.09	8.07	8.02	
	Dissolved Oxygen (mg/l)	8.3	8.4	8.4	8.5	8.6	8.7	
Date: 6/8/11	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (μmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
	Total Ammonia (mg/l)							
Day: 6 Final	pH	8.50	8.34	8.42	8.58	8.68	8.63	
	Dissolved Oxygen (mg/l)	8.2	8.2	8.1	8.1	8.0	8.0	
Date: 6/9/11	Temperature (°C)	24.9	24.9	24.9	24.9	24.9	24.9	
	Conductivity (μmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day:	pH							
	Dissolved Oxygen (mg/l)							
Date: / /	Temperature (°C)							
	Conductivity (μmhos)							
Analyst:	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day:	pH							
	Dissolved Oxygen (mg/l)							
Date: / /	Temperature (°C)							
	Conductivity (μmhos)							
Analyst:	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day:	pH							
	Dissolved Oxygen (mg/l)							
Date: / /	Temperature (°C)							
	Conductivity (μmhos)							
Analyst:	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Krenk (signature)   Date: 6/15/11

CHRONIC TOXICITY TEST
CERIODAPHNIA REPRODUCTION AND SURVIVAL

Client: Polymet - Recon / Bear Creek Project No.: 11-145
Test Dates/~~Time~~ ● Initiation: 1135 6/3/11 Termination: 0930 6/9/11

Concentration	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Remarks
0	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	3	3	2	3	3	2	2	4	
	4	6	6	6	0	6	0	0	6	6	0	
	5	10	9	0	6	0	7	7	0	0	6	
	6	0	0	12	9	12	14	10	13	12	7	
Total		16	15	21	18	20	24	20	21	20	17	
12.5	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	4	4	3	2	4	4	0	4	2	
	4	6	7	0	6	6	0	0	4	4	6	
	5	10	0	7	0	8	8	6	4	0	0	
	6	0	11	8	14	0	8	13	0	13	8	
Total		16	22	19	23	16	20	23	8	21	16	
25	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	4	4	3	4	3	3	4	2	0	
	4	4	7	7	9	6	7	0	5	8	5	
	5	7	0	0	0	0	0	7	0	0	10	
	6	0	13	12	11	3	12	10	13	10	0	
Total		11	24	23	23	13	22	20	22	20	15	

✓ = Alive # = No. of Live Young (-#) = No. of Dead Young 0 = No Young X = Dead y = Male M= Missing

Analyst: Km / WK Reviewed By: WK

CHRONIC TOXICITY TEST
CERIODAPHNIA REPRODUCTION AND SURVIVAL

Client: PolyMet - Recon/Bear Creek Project No.: 11-145

Test Dates/~~Time~~ ● Initiation: 1135 6/3/11 Termination: 0930 6/9/11

Concentration	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Remarks
50	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	3	0	3	4	4	3	3	0	2	5	
	4	8	4	0	8	0	0	0	6	4	0	
	5	0	10	10	0	5	9	8	9	0	11	
	6	12	0	1	9	4	16	10	0	12	16	
Total		23	14	14	21	13	28	21	15	20	32	
75	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	3	2	0	3	4	4	5	1	2	1	
	4	0	7	5	7	8	0	0	7	8	6	
	5	7	0	8	0	0	6	9	0	0	0	
	6	14	11	0	14	13	3	13	12	10	12	
Total		24	20	13	24	25	13	27	20	20	19	
100	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	3	4	3	4	4	2	4	2	4	3	
	4	0	7	7	0	5	6	8	6	3	6	
	5	6	0	0	9	0	0	0	0	0	0	
	6	13	14	14	13	12	12	12	14	12	14	
Total		22	25	24	26	21	20	24	22	19	23	

✓ = Alive # = No. of Live Young (-#) = No. of Dead Young 0 = No Young X = Dead y = Male M= Missing

Analyst: km/WK Reviewed By: WK

Ceriodaphnia reproduction
File: RECON BEAR CREEK   Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	107.083	21.417	1.091
Within (Error)	54	1059.900	19.628	
Total	59	1166.983		

Critical F value = 2.45 (0.05,5,40)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

Ceriodaphnia reproduction
File: RECON BEAR CREEK   Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2   Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	0	19.200	19.200		
2	12.5	18.400	18.400	0.404	
3	25	19.300	19.300	-0.050	
4	50	20.100	20.100	-0.454	
5	75	20.500	20.500	-0.656	
6	100	22.600	22.600	-1.716	

Dunnett table value = 2.31   (1 Tailed Value, P=0.05, df=40,5)

Ceriodaphnia reproduction
File: RECON BEAR CREEK   Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2   Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	0	10			
2	12.5	10	4.577	23.8	0.800
3	25	10	4.577	23.8	-0.100
4	50	10	4.577	23.8	-0.900
5	75	10	4.577	23.8	-1.300
6	100	10	4.577	23.8	-3.400

Ceriodaphnia reproduction
File: RECON BEAR CREEK   Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	4.020	14.520	22.920	14.520	4.020
OBSERVED	6	13	19	19	3

Calculated Chi-Square goodness of fit test statistic = 3.4458
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Ceriodaphnia reproduction
File: RECON BEAR CREEK   Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 11.65
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 9.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

Toxicity Test
Daily Chemistries

Client: PolyMet	Project Number: 11-145
Test Type: Chronic - Recon / Bear Creek	Species: Ceriodaphnia dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 0	pH	8.03	8.05	7.75	7.41	7.25	6.96	
	Dissolved Oxygen (mg/l)	8.3	8.2	8.1	8.0	7.9	7.9	
Date: 6/3/11	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)	306					82	
Analyst: Km	Total Alkalinity (mg/l)	60					40	
	Total Hardness (mg/l)	88					44	
	Total Ammonia (mg/l)							
Day: 1 Old	pH	8.14	8.12	8.04	7.96	7.88	7.68	
	Dissolved Oxygen (mg/l)	8.3	8.1	8.0	8.1	8.1	8.0	
Date: 6/4/11	Temperature (°C)	25.4	25.4	25.4	25.4	25.4	25.4	
	Conductivity (µmhos)							
Analyst: Km	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 1 New	pH	8.16	8.03	7.81	7.49	7.26	6.97	
	Dissolved Oxygen (mg/l)	8.2	8.2	8.1	8.2	8.2	8.2	
Date: 6/4/11	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: Km	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 2 Old	pH	8.19	8.18	8.09	8.02	7.96	7.89	
	Dissolved Oxygen (mg/l)	8.0	7.9	8.0	8.0	8.0	8.0	
Date: 6/5/11	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
	Conductivity (µmhos)							
Analyst: Km	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 2 New	pH	8.22	8.16	7.87	7.57	7.35	7.10	
	Dissolved Oxygen (mg/l)	8.2	8.1	8.1	8.2	8.3	8.4	
Date: 6/5/11	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: Km	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Kowd   Date: 6/15/11

Toxicity Test
Daily Chemistries

Client: Polymet	Project Number: 11-145
Test Type: Chronic - Recon/Bear Creek	Species: Ceriodaphnia dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 3 OLD	pH	8.04	7.96	7.82	7.69	7.81	7.77	
	Dissolved Oxygen (mg/l)	8.3	7.9	7.9	7.8	7.8	7.8	
Date: 6/6/11	Temperature (°C)	25.4	25.4	25.4	25.4	25.4	25.4	
	Conductivity (μmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
	Total Ammonia (mg/l)							
Day: 3 New	pH	8.19	8.17	7.95	7.76	7.63	7.25	
	Dissolved Oxygen (mg/l)	8.2	8.2	8.2	8.3	8.4	8.5	
Date: 6/6/11	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (μmhos)							
Analyst: SW	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 4 OLD	pH	7.97	7.98	7.90	7.90	7.86	7.72	
	Dissolved Oxygen (mg/l)	8.3	8.2	8.3	8.2	8.3	8.2	
Date: 6/7/11	Temperature (°C)	25.2	25.2	25.2	25.2	25.2	25.2	
	Conductivity (μmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 4 New	pH	8.14	8.07	7.80	7.66	7.51	7.20	
	Dissolved Oxygen (mg/l)	8.4	8.5	8.6	8.7	8.9	9.6	
Date: 6/7/11	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (μmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 5 OLD	pH	7.97	8.00	7.90	7.93	7.80	7.70	
	Dissolved Oxygen (mg/l)	8.2	8.3	8.2	8.1	8.1	8.1	
Date: 6/8/11	Temperature (°C)	24.9	24.9	24.9	24.9	24.9	24.9	
	Conductivity (μmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Kowert Date: 6/15/11

Toxicity Test
Daily Chemistries

Client:	Polymet	Project Number:	11-145
Test Type:	Chronic- Recon | Bear Creek	Species:	C-dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 5 New	pH	8.22	8.00	7.90	7.74	7.60	7.40	
	Dissolved Oxygen (mg/l)	8.3	8.5	8.6	8.8	8.9	9.1	
Date: 6/8/11	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
	Total Ammonia (mg/l)							
Day: 6 Final	pH	8.50	8.09	7.99	7.92	7.87	7.83	
	Dissolved Oxygen (mg/l)	8.2	8.1	8.1	8.1	8.2	8.1	
Date: 6/9/11	Temperature (°C)	24.9	24.9	24.9	24.9	24.9	24.9	
	Conductivity (µmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day:	pH							
	Dissolved Oxygen (mg/l)							
Date: / /	Temperature (°C)							
	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day:	pH							
	Dissolved Oxygen (mg/l)							
Date: / /	Temperature (°C)							
	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day:	pH							
	Dissolved Oxygen (mg/l)							
Date: / /	Temperature (°C)							
	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Kennedy   Date: 6/15/11

CHRONIC TOXICITY TEST
CERIODAPHNIA REPRODUCTION AND SURVIVAL

Client: PolyMet - Embarrass River / SD033 Project No.: 11-145

Test Dates/Time ● Initiation: 1145 6/3/11 Termination: 0945 6/9/11

Concentration	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Remarks
0	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	3	2	4	3	1	3	4	2	3	
	4	4	7	5	8	7	5	7	7	6	7	
	5	4	0	0	0	0	0	0	0	0	0	
	6	0	14	4	13	13	9	11	12	10	13	
Total		8	24	11	25	23	15	21	23	18	23	
12.5	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	3	3	3	2	4	4	2	2	2	2	
	4	6	9	5	6	0	6	7	6	5	6	
	5	0	0	0	0	6	0	10	8	0	12	
	6	16	13	11	11	5	14	0	1	13	0	
Total		25	25	19	19	15	24	19	17	20	20	
25	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	3	4	0	0	3	2	0	0	2	2	
	4	6	6	4	4	6	5	6	7	8	6	
	5	0	9	0	12	0	0	0	0	0	0	
	6	13	0	10	0	12	11	11	10	6	9	
Total		22	19	14	16	21	18	17	17	16	17	

✓ = Alive # = No. of Live Young (-#) = No. of Dead Young 0 = No Young X = Dead y = Male M= Missing

Analyst: Km / WK Reviewed By: WK

ENVIRONMENTAL TOXICITY CONTROL

CHRONIC TOXICITY TEST
CERIODAPHNIA REPRODUCTION AND SURVIVAL

Client: Polymet - Embarrass River / SD033 Project No.: 11-145
Test Dates/Time ● Initiation: 1145 6/3/11 Termination: 0945 6/9/11

Concentration	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Remarks
50	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	3	4	4	0	2	2	3	3	0	2	
	4	6	8	4	3	6	3	4	0	4	6	
	5	0	0	0	11	0	0	0	7	9	0	
	6	12	15	11	0	11	11	9	11	0	12	
Total		21	27	19	14	19	16	16	21	13	20	
75	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	3	2	2	3	2	4	0	0	3	4	
	4	4	6	0	0	0	2	2	0	6	0	
	5	0	10	5	7	6	0	9	4	0	8	
	6	8	0	7	10	13	14	12	7	7	8	
Total		15	18	14	20	21	20	23	11	16	20	
100	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	1	0	0	0	0	0	0	0	2	0	
	4	0	0	3	2	0	3	2	4	0	2	
	5	4	3	5	5	3	4	3	4	4	3	
	6	9	8	0	0	4	0	0	0	2	0	
Total		14	11	8	7	7	7	5	8	8	5	

✓ = Alive # = No. of Live Young (-#) = No. of Dead Young 0 = No Young X = Dead y = Male M= Missing

Analyst: KM / WK Reviewed By: WK

Conc. ID	1	2	3	4	5	6
Conc. Tested	0	12.5	25	50	75	100
Response 1	8	25	22	21	15	14
Response 2	24	25	19	27	18	11
Response 3	11	19	14	19	14	8
Response 4	25	19	16	14	20	7
Response 5	23	15	21	19	21	7
Response 6	15	24	18	16	20	7
Response 7	21	19	17	16	23	5
Response 8	23	17	17	21	11	8
Response 9	18	20	16	13	16	8
Response 10	23	20	17	20	20	5

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Embarrass River/SD033
Test Start Date: 6/3/11   Test Ending Date: 6/9/11
Test Species: Ceriodaphnia dubia
Test Duration: 6 days
DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	10	0.000	19.100	5.915	19.700
2	10	12.500	20.300	3.368	19.700
3	10	25.000	17.700	2.406	18.150
4	10	50.000	18.600	4.088	18.150
5	10	75.000	17.800	3.706	17.800
6	10	100.000	8.000	2.708	8.000

The Linear Interpolation Estimate: 82.7168   Entered P Value: 25

Number of Resamplings: 80
The Bootstrap Estimates Mean: 79.9222 Standard Deviation: 9.1263
Original Confidence Limits: Lower: 63.9423 Upper: 87.3018
Resampling time in Seconds: 0.06 Random_Seed: 11075006

Ceriodaphnia reproduction
File: EMBARRASS RIVER SD033 Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	999.483	199.897	13.342
Within (Error)	54	809.100	14.983	
Total	59	1808.583		

Critical F value = 2.45 (0.05,5,40)
Since F > Critical F REJECT Ho:All groups equal

Ceriodaphnia reproduction
File: EMBARRASS RIVER SD033 Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	0	19.100	19.100		
2	12.5	20.300	20.300	-0.693	
3	25	17.700	17.700	0.809	
4	50	18.600	18.600	0.289	
5	75	17.800	17.800	0.751	
6	100	8.000	8.000	6.412	*

Dunnett table value = 2.31 (1 Tailed Value, P=0.05, df=40,5)

Ceriodaphnia reproduction
File: EMBARRASS RIVER SD033 Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	0	10			
2	12.5	10	3.999	20.9	-1.200
3	25	10	3.999	20.9	1.400
4	50	10	3.999	20.9	0.500
5	75	10	3.999	20.9	1.300
6	100	10	3.999	20.9	11.100

Ceriodaphnia reproduction
File: EMBARRASS RIVER SD033   Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	4.020	14.520	22.920	14.520	4.020
OBSERVED	4	13	22	18	3

Calculated Chi-Square goodness of fit test statistic = 1.2890
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Ceriodaphnia reproduction
File: EMBARRASS RIVER SD033   Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 9.26
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 9.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

ENVIRONMENTAL TOXICITY CONTROL

Toxicity Test
Daily Chemistries

Client: PolyMet		Project Number:	11-145
Test Type: Chronic-Embarrass River / SD033		Species:	Ceriodaphnia dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 0	pH	6.73	7.23	7.48	7.87	8.05	8.03	
	Dissolved Oxygen (mg/l)	7.8	7.8	8.0	8.4	8.6	9.9	
Date:	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
6/3/11	Conductivity (µmhos)	71					2210	
Analyst:	Total Alkalinity (mg/l)	44					352	
KM	Total Hardness (mg/l)	48					1176	
	Total Ammonia (mg/l)							
Day: 1 Old	pH	7.76	7.99	8.23	8.48	8.55	8.64	
	Dissolved Oxygen (mg/l)	8.4	8.1	8.1	8.1	8.1	8.1	
Date:	Temperature (°C)	25.4	25.4	25.4	25.4	25.4	25.4	
6/4/11	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
KM	Total Hardness (mg/l)							
Day: 1 New	pH	6.69	7.19	7.56	7.92	8.03	8.04	
	Dissolved Oxygen (mg/l)	8.2	8.3	8.4	8.6	8.8	9.5	
Date:	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
6/4/11	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
KM	Total Hardness (mg/l)							
Day: 2 Old	pH	7.67	8.01	8.30	8.53	8.64	8.73	
	Dissolved Oxygen (mg/l)	8.3	8.2	8.1	8.1	8.1	7.8	
Date:	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
6/5/11	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
KM	Total Hardness (mg/l)							
Day: 2 New	pH	6.85	7.25	7.64	7.94	8.12	8.10	
	Dissolved Oxygen (mg/l)	8.5	8.4	8.5	8.6	8.9	9.8	
Date:	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
6/5/11	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
KM	Total Hardness (mg/l)							

Reviewed by: Walter Kronk Date: 6/15/11

Toxicity Test
Daily Chemistries

Client: Polymet	Project Number: 11-145
Test Type: Chronic - Embarrass River / SD033	Species: Ceriodaphnia dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 3 OLD	pH	7.74	7.92	8.14	8.36	8.50	8.64	
	Dissolved Oxygen (mg/l)	7.8	7.9	7.8	7.8	7.8	7.9	
Date: 6/6/11	Temperature (°C)	25.4	25.4	25.4	25.4	25.4	25.4	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
	Total Ammonia (mg/l)							
Day: 3 New	pH	6.98	7.46	7.83	8.10	8.18	8.13	
	Dissolved Oxygen (mg/l)	8.3	8.3	8.4	8.5	8.5	9.1	
Date: 6/6/11	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: SW	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 4 OLD	pH	7.72	7.89	8.11	8.38	8.51	8.60	
	Dissolved Oxygen (mg/l)	8.4	8.3	8.1	8.2	8.0	8.0	
Date: 6/7/11	Temperature (°C)	25.2	25.2	25.2	25.2	25.2	25.2	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 4 New	pH	7.00	7.37	7.80	8.09	8.18	8.14	
	Dissolved Oxygen (mg/l)	9.3	9.3	9.3	9.4	9.4	10.0	
Date: 6/7/11	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 5 OLD	pH	7.64	7.83	8.10	8.37	8.51	8.62	
	Dissolved Oxygen (mg/l)	8.3	8.2	8.1	8.1	8.1	8.1	
Date: 6/8/11	Temperature (°C)	24.9	24.9	24.9	24.9	24.9	24.9	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Kroeker Date: 6/15/11

Toxicity Test
Daily Chemistries

Client: Polymet	Project Number: 11-145
Test Type: Chronic - Embarrass River / SD033	Species: C. dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 5 New	pH	7.22	7.60	7.91	8.09	8.14	8.09	
	Dissolved Oxygen (mg/l)	9.0	9.0	9.0	8.9	9.0	9.4	
Date: 6/8/11	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (μmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
	Total Ammonia (mg/l)							
Day: 6 Final	pH	7.81	7.95	8.22	8.47	8.59	8.67	
	Dissolved Oxygen (mg/l)	8.5	8.4	8.3	8.4	8.4	8.1	
Date: 6/9/11	Temperature (°C)	24.9	24.9	24.9	24.9	24.9	24.9	
	Conductivity (μmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day:	pH							
	Dissolved Oxygen (mg/l)							
Date: / /	Temperature (°C)							
	Conductivity (μmhos)							
Analyst:	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day:	pH							
	Dissolved Oxygen (mg/l)							
Date: / /	Temperature (°C)							
	Conductivity (μmhos)							
Analyst:	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day:	pH							
	Dissolved Oxygen (mg/l)							
Date: / /	Temperature (°C)							
	Conductivity (μmhos)							
Analyst:	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Koord Date: 6/15/11

CHRONIC TOXICITY TEST
CERIODAPHNIA REPRODUCTION AND SURVIVAL

Client: PolyMet - Partridge River | SD026 Project No.: 11-145
Test Dates/~~Time~~ ● Initiation: 1155 6/3/11 Termination: 1015 6/9/11

Concentration	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Remarks
0	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	3	4	2	2	2	2	4	2	2	3	
	4	6	0	7	5	7	5	7	7	0	0	
	5	0	7	11	0	0	0	0	0	3	7	
	6	12	3	0	11	11	13	10	2	10	10	
Total		21	14	20	18	20	20	21	11	15	20	
12.5	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	2	2	4	3	3	2	4	2	2	2	
	4	4	0	6	7	6	6	7	6	5	5	
	5	0	6	0	0	0	0	0	0	0	0	
	6	12	6	3	10	11	2	11	9	10	10	
Total		18	14	13	20	20	10	22	17	17	17	
25	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	4	3	0	4	3	4	2	4	1	4	
	4	6	0	3	6	7	7	6	4	6	8	
	5	0	7	7	0	0	0	0	0	0	0	
	6	12	8	0	9	10	9	10	11	9	9	
Total		22	18	10	19	20	20	18	19	16	21	

✓ = Alive # = No. of Live Young 0 = No Young X = Dead y = Male M= Missing
(-#) = No. of Dead Young

Analyst: KM | WK Reviewed By: WK

CHRONIC TOXICITY TEST
CERIODAPHNIA REPRODUCTION AND SURVIVAL

Client: Polymet - Partridge River / SD026 Project No.: 11-145

Test Dates/Time ● Initiation: 1155 6/3/11 Termination: 1015 6/9/11

Concentration	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Remarks
50	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	3	0	4	3	4	4	4	3	5	4	
	4	6	5	5	0	6	4	5	0	0	8	
	5	0	11	0	10	0	0	0	6	9	0	
	6	10	0	10	12	11	9	15	11	16	12	
Total		19	16	19	25	21	17	24	20	30	24	
75	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	2	4	4	2	2	0	4	4	4	3	
	4	4	0	0	5	0	4	0	6	7	0	
	5	0	9	7	0	4	8	5	0	0	6	
	6	8	13	11	9	9	0	11	13	10	7	
Total		14	26	22	16	15	12	20	23	21	16	
100	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	4	3	0	0	2	0	3	2	0	
	4	4	2	0	4	2	0	3	0	0	4	
	5	8	0	6	5	3	6	3	6	4	6	
	6	0	7	7	0	0	9	0	7	4	0	
Total		12	13	16	9	5	17	6	~~18~~ 16 km	10	10	

✓ = Alive # = No. of Live Young 0 = No Young X = Dead y = Male M= Missing
(-#) = No. of Dead Young

Analyst: km / WK Reviewed By: WK

Conc. ID	1	2	3	4	5	6
Conc. Tested	0	12.5	25	50	75	100
Response 1	21	18	22	19	14	12
Response 2	14	14	18	16	26	13
Response 3	20	13	10	19	22	16
Response 4	18	20	19	25	16	9
Response 5	20	20	20	21	15	5
Response 6	20	10	20	17	12	17
Response 7	21	22	18	24	20	6
Response 8	11	17	19	20	23	16
Response 9	15	17	16	30	21	10
Response 10	20	17	21	24	16	10

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Partridge River/SD026
Test Start Date: 6/3/11   Test Ending Date: 6/9/11
Test Species: Ceriodaphnia dubia
Test Duration: 6 days
DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	10	0.000	18.000	3.464	18.650
2	10	12.500	16.800	3.615	18.650
3	10	25.000	18.300	3.368	18.650
4	10	50.000	21.500	4.249	18.650
5	10	75.000	18.500	4.528	18.500
6	10	100.000	11.400	4.169	11.400

The Linear Interpolation Estimate: 90.8891   Entered P Value: 25

Number of Resamplings: 80Those resamples not used had estimates above the highest concentration/ %Effluent.

The Bootstrap Estimates Mean: 90.1171 Standard Deviation: 3.0369

No Confidence Limits can be produced since the number of resamples generated is not a multiple of 40.
Resampling time in Seconds: 0.06 Random_Seed: -295203832

Ceriodaphnia reproduction
File: PARTRIDGE RIVER SD026   Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	555.483	111.097	7.218
Within (Error)	54	831.100	15.391	
Total	59	1386.583		

Critical F value = 2.45 (0.05,5,40)
Since F > Critical F REJECT Ho:All groups equal

Ceriodaphnia reproduction
File: PARTRIDGE RIVER SD026   Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2   Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	0	18.000	18.000		
2	12.5	16.800	16.800	0.684	
3	25	18.300	18.300	-0.171	
4	50	21.500	21.500	-1.995	
5	75	18.500	18.500	-0.285	
6	100	11.400	11.400	3.762	*

Dunnett table value = 2.31   (1 Tailed Value, P=0.05, df=40,5)

Ceriodaphnia reproduction
File: PARTRIDGE RIVER SD026   Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2   Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	0	10			
2	12.5	10	4.053	22.5	1.200
3	25	10	4.053	22.5	-0.300
4	50	10	4.053	22.5	-3.500
5	75	10	4.053	22.5	-0.500
6	100	10	4.053	22.5	6.600

Ceriodaphnia reproduction
File: PARTRIDGE RIVER SD026   Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	4.020	14.520	22.920	14.520	4.020
OBSERVED	4	16	16	22	2

Calculated Chi-Square goodness of fit test statistic =   7.1086
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Ceriodaphnia reproduction
File: PARTRIDGE RIVER SD026   Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic =   1.29
Table Chi-square value =   15.09 (alpha = 0.01)
Table Chi-square value =   11.07 (alpha = 0.05)

Average df used in calculation ==>   df (avg n - 1) =   9.00
Used for Chi-square table value ==>   df (#groups-1) =   5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

ENVIRONMENTAL TOXICITY CONTROL

Toxicity Test
Daily Chemistries

Client:	PolyMet	Project Number:	11-145
Test Type:	Chronic- Partridge River / SD026	Species:	Ceriodaphnia dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 0	pH	7.43	7.78	7.92	7.99	8.02	7.92	
	Dissolved Oxygen (mg/l)	8.5	8.4	8.5	8.4	8.4	8.5	
Date: 6/3/11	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)	144					1059	
Analyst: WK	Total Alkalinity (mg/l)	44					448	
	Total Hardness (mg/l)	76					572	
	Total Ammonia (mg/l)							
Day: 1 old	pH	7.79	8.18	8.36	8.57	8.66	8.64	
	Dissolved Oxygen (mg/l)	8.2	8.0	8.1	8.1	8.1	8.1	
Date: 6/4/11	Temperature (°C)	25.4	25.4	25.4	25.4	25.4	25.4	
	Conductivity (µmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 1 New	pH	7.41	7.83	7.93	8.03	8.06	7.92	
	Dissolved Oxygen (mg/l)	8.7	8.7	8.7	8.6	8.6	9.0	
Date: 6/4/11	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 2 old	pH	7.85	8.22	8.37	8.66	8.75	8.69	
	Dissolved Oxygen (mg/l)	8.1	8.1	8.0	8.0	8.0	7.9	
Date: 6/5/11	Temperature (°C)	25.3	25.3	25.3	25.3	25.3	25.3	
	Conductivity (µmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 2 New	pH	7.64	8.01	8.08	8.15	8.14	8.00	
	Dissolved Oxygen (mg/l)	8.7	8.7	8.8	8.8	8.7	9.1	
Date: 6/5/11	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Kornt Date: 6/15/11

Toxicity Test
Daily Chemistries

Client: Polymet	Project Number: 11-145
Test Type: Chronic- Partridge River/SD026	Species: Ceriodaphnia dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 3 old	pH	7.82	8.13	8.25	8.50	8.61	8.61	
	Dissolved Oxygen (mg/l)	8.0	8.0	7.9	7.8	7.8	7.9	
Date: 6/6/11	Temperature (°C)	25.4	25.4	25.4	25.4	25.4	25.4	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
	Total Ammonia (mg/l)							
Day: 3 New	pH	7.63	8.05	8.13	8.19	8.17	8.06	
	Dissolved Oxygen (mg/l)	8.6	8.5	8.4	8.4	8.5	8.8	
Date: 6/6/11	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: SW	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 4 OLD	pH	7.77	8.07	8.26	8.52	8.63	8.61	
	Dissolved Oxygen (mg/l)	8.2	8.2	8.1	8.1	8.0	8.0	
Date: 6/7/11	Temperature (°C)	25.2	25.2	25.2	25.2	25.2	25.2	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 4 New	pH	7.63	7.99	8.07	8.13	8.14	8.07	
	Dissolved Oxygen (mg/l)	9.5	9.4	9.5	9.5	9.5	9.9	
Date: 6/7/11	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day: 5 OLD	pH	7.75	8.00	8.21	8.46	8.56	8.54	
	Dissolved Oxygen (mg/l)	8.4	8.1	8.0	8.0	8.0	8.1	
Date: 6/8/11	Temperature (°C)	24.9	24.9	24.9	24.9	24.9	24.9	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Kraut (signature) Date: 6/15/11

Toxicity Test
Daily Chemistries

Client: Polymet	Project Number: 11-145
Test Type: Chronic - Partridge River / SD026	Species: C. dubia

Day/Date/Analyst	Parameter	Concentration 0	12.5	25	50	75	100	Remarks
Day: 5 New	pH	7.93	8.13	8.13	8.15	8.13	8.02	
	Dissolved Oxygen (mg/l)	9.0	8.9	8.9	8.8	8.7	8.7	
Date: 6/8/11	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0	
	Conductivity (µmhos)							
Analyst: WK	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
	Total Ammonia (mg/l)							
Day: 6 Final	pH	7.88	8.17	8.38	8.60	8.73	8.63	
	Dissolved Oxygen (mg/l)	8.3	8.2	8.2	8.2	8.3	8.0	
Date: 6/9/11	Temperature (°C)	24.9	24.9	24.9	24.9	24.9	24.9	
	Conductivity (µmhos)							
Analyst: KM	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day:	pH							
	Dissolved Oxygen (mg/l)							
Date: / /	Temperature (°C)							
	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day:	pH							
	Dissolved Oxygen (mg/l)							
Date: / /	Temperature (°C)							
	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							
Day:	pH							
	Dissolved Oxygen (mg/l)							
Date: / /	Temperature (°C)							
	Conductivity (µmhos)							
Analyst:	Total Alkalinity (mg/l)							
	Total Hardness (mg/l)							

Reviewed by: Walter Kondt   Date: 6/15/11

CHRONIC TOXICITY TEST
CERIODAPHNIA REPRODUCTION AND SURVIVAL

Client: PolyMet Project No.: 11-145

Test Dates/Time ● Initiation: 1200 6/3/11 Termination: 1025 6/9/11

Concentration	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Remarks
Recon.	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	0	0	3	3	2	3	3	2	2	4	
	4	6	6	6	0	6	0	0	6	6	0	
	5	10	9	0	6	0	7	7	0	0	6	
	6	0	0	12	9	12	14	10	13	12	7	
Total		16	15	21	18	20	24	20	21	20	17	$\bar{x} = 19.2$
Lower	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Spring	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Mine	3	M	0	2	3	0	2	0	4	2	2	
Creek	4		5	0	6	3	5	0	0	0	4	
	5		8	6	0	5	0	5	6	9	0	
	6		0	6	8	0	8	9	11	0	4	
Total			13	14	17	8	15	14	21	11	10	$\bar{x} = 13.7$
PM17	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	3	3	4	3	0	2	3	2	0	0	2	
	4	4	4	6	2	6	5	5	5	4	5	
	5	0	0	0	7	0	0	0	6	8	0	
	6	7	9	8	0	0	7	8	0	0	8	
Total		14	17	17	9	8	15	15	11	12	15	$\bar{x} = 13.3$

✓ = Alive # = No. of Live Young 0 = No Young X = Dead y = Male M= Missing
(-#) = No. of Dead Young

Analyst: KM / WK Reviewed By: WK

Toxicity Test
Daily Chemistries

Client: Paymet	Project Number: 11-145
Test Type: Chronic	Species: Ceriodaphnia dubia

Day/Date/Analyst	Parameter	Concentration Recon	Lower Spring Mine Creek	PM17	Remarks
Day: 0	pH	8.03	7.65	8.05	
	Dissolved Oxygen (mg/l)	8.3	8.9	8.8	
Date: 6/3/11	Temperature (°C)	25.0	25.0	25.0	
	Conductivity (µmhos)	306	684	1459	
Analyst: KM	Total Alkalinity (mg/l)	60	128	280	
	Total Hardness (mg/l)	88	312	888	
	Total Ammonia (mg/l)				
Day: 1 old	pH	8.14	8.35	8.60	
	Dissolved Oxygen (mg/l)	8.3	8.4	8.4	
Date: 6/4/11	Temperature (°C)	25.4	25.4	25.4	
	Conductivity (µmhos)				
Analyst: KM	Total Alkalinity (mg/l)				
	Total Hardness (mg/l)				
Day: 1 New	pH	8.16	7.65	8.06	
	Dissolved Oxygen (mg/l)	8.2	9.5	9.7	
Date: 6/4/11	Temperature (°C)	25.0	25.0	25.0	
	Conductivity (µmhos)				
Analyst: KM	Total Alkalinity (mg/l)				
	Total Hardness (mg/l)				
Day: 2 old	pH	8.19	8.37	8.62	
	Dissolved Oxygen (mg/l)	8.0	7.9	7.8	
Date: 6/5/11	Temperature (°C)	25.3	25.3	25.3	
	Conductivity (µmhos)				
Analyst: KM	Total Alkalinity (mg/l)				
	Total Hardness (mg/l)				
Day: 2 New	pH	8.22	7.60	7.98	
	Dissolved Oxygen (mg/l)	8.2	8.9	9.3	
Date: 6/5/11	Temperature (°C)	25.0	25.0	25.0	
	Conductivity (µmhos)				
Analyst: KM	Total Alkalinity (mg/l)				
	Total Hardness (mg/l)				

Reviewed by: Walter Kord Date: 6/15/11

Toxicity Test
Daily Chemistries

Client: Polymet	Project Number: 11-145
Test Type: Chronic	Species: Ceriodaphnia dubia

Day/Date/Analyst	Parameter	Concentration: Recon	Concentration: Lower Spring Mine Creek	Concentration: PM17	Remarks
Day: 3 OLD	pH	8.04	8.25	8.53	
	Dissolved Oxygen (mg/l)	8.3	7.9	7.9	
Date: 6/6/11	Temperature (°C)	25.4	25.4	25.4	
	Conductivity (µmhos)				
Analyst: WK.	Total Alkalinity (mg/l)				
	Total Hardness (mg/l)				
	Total Ammonia (mg/l)				
Day: 3 New	pH	8.19	7.95	8.21	
	Dissolved Oxygen (mg/l)	8.2	8.7	8.6	
Date: 6/6/11	Temperature (°C)	25.0	25.0	25.0	
	Conductivity (µmhos)				
Analyst: SW	Total Alkalinity (mg/l)				
	Total Hardness (mg/l)				
Day: 4 OLD	pH	7.97	8.24	8.56	
	Dissolved Oxygen (mg/l)	8.3	7.9	8.0	
Date: 6/7/11	Temperature (°C)	25.2	25.2	25.2	
	Conductivity (µmhos)				
Analyst: WK	Total Alkalinity (mg/l)				
	Total Hardness (mg/l)				
Day: 4 New	pH	8.14	7.95	8.14	
	Dissolved Oxygen (mg/l)	8.4	9.8	9.8	
Date: 6/7/11	Temperature (°C)	25.0	25.0	25.0	
	Conductivity (µmhos)				
Analyst: WK	Total Alkalinity (mg/l)				
	Total Hardness (mg/l)				
Day: 5 OLD	pH	7.97	8.18	8.51	
	Dissolved Oxygen (mg/l)	8.2	7.9	8.1	
Date: 6/8/11	Temperature (°C)	24.9	24.9	24.9	
	Conductivity (µmhos)				
Analyst: WK.	Total Alkalinity (mg/l)				
	Total Hardness (mg/l)				

Reviewed by: Walter Kornd   Date: 6/15/11

Toxicity Test
Daily Chemistries

Client: Polymet	Project Number: 11-145
Test Type: Chronic	Species: C. dubia

Day/Date/Analyst	Parameter	Concentration: Recon	Lower Spring Mine Creek	PM17	Remarks
Day: 5 New	pH	8.23	8.06	8.21	
	Dissolved Oxygen (mg/l)	8.3	8.9	9.0	
Date: 6/8/11	Temperature (°C)	25.0	25.0	25.0	
	Conductivity (μmhos)				
Analyst: WK	Total Alkalinity (mg/l)				
	Total Hardness (mg/l)				
	Total Ammonia (mg/l)				
Day: 6 Final	pH	8.50	8.36	8.57	
	Dissolved Oxygen (mg/l)	8.2	8.3	8.1	
Date: 6/9/11	Temperature (°C)	24.9	24.9	24.9	
	Conductivity (μmhos)				
Analyst: KM	Total Alkalinity (mg/l)				
	Total Hardness (mg/l)				
Day:	pH				
	Dissolved Oxygen (mg/l)				
Date: / /	Temperature (°C)				
	Conductivity (μmhos)				
Analyst:	Total Alkalinity (mg/l)				
	Total Hardness (mg/l)				
Day:	pH				
	Dissolved Oxygen (mg/l)				
Date: / /	Temperature (°C)				
	Conductivity (μmhos)				
Analyst:	Total Alkalinity (mg/l)				
	Total Hardness (mg/l)				
Day:	pH				
	Dissolved Oxygen (mg/l)				
Date: / /	Temperature (°C)				
	Conductivity (μmhos)				
Analyst:	Total Alkalinity (mg/l)				
	Total Hardness (mg/l)				

Reviewed by: Walter Kornd [signature] Date: 6/15/11