Montana Fish, Wildlife & Parks

INVESTIGATIONS INTO THE EFFECTS OF CYANIDE ON TROUT IN THE GERMAN GULCH DRAINAGE, 2003

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1.0. INTRODUCTION AND SCOPE OF WORK

This is a brief summary of field studies conducted in 2003 to investigate the possible effects of cyanide from the Beal Mountain Mine on brook and cutthroat trout in the German Gulch drainage. German Gulch flows into Silver Bow Creek near Anaconda, Montana. Beal Mountain Mine is located at the head of the German Gulch drainage, on the ridge between German Gulch and Minnesota Gulch (called Beal's Hill, see Figure 1). From 1988 through 1997, Beal Mountain Mining Company mined 14,807,100 tons of low-grade gold-silver deposits out of two open pits. Ore was transported to a heap-leach pad where the metals were recovered using cyanide solution. Land application of the cyanide solution captured from the heap-leach pad began in 1994. However, disposal of most of the solution did not occur until a biological treatment plant was built in 2001 to treat the leach pad solution prior to land application. Between July 2001 and November 2003, a total of 152,084,681 gallons of treated solution were land applied using drip lines and sprinklers (Maxim 2004).

Cyanide from the land application process has found its way to groundwater and surface waters. Water quality sampling in early 2003 in German Gulch and Beefstraight Creek revealed total cyanide at levels in exceedence of Montana Water Quality standards. This prompted an investigation into the effects of this cyanide on brook trout and cutthroat trout. We chose to measure several parameters that have been shown to be indicative of chronic or acute cyanide exposure—mortality, plasma thiocyanate, fecundity, egg size, histological changes, and liver weight.

2.0. METHODS

2.1. *In-situ* bioassays using hatchery fish. Westslope cutthroat trout were transported from the Washoe Park State Fish Hatchery to Beefstraight Creek and Norton Gulch (a control stream with no cyanide) in the German Gulch watershed on April 30, 2003. Ten or 11 fish were placed randomly into each of two cages in each stream. The cages were 10x10x12-inch chambers with a wooden frame, constructed with 1/8-inch nylon mesh panels on all sides but one. The cages had Styrofoam collars to provide floatation and to keep one side of the cage above water. This was a solid wood-covered side, which was hinged to provide access to the fish. Cages were placed in the slower, deeper water behind beaver dams. On Beefstraight Creek, the cage location was about ¼ mile above the road crossing and ½ mile upstream from the confluence with German Gulch. Cage location on Norton Gulch was about 100 yards below the road crossing and ¼ mile upstream from the German Gulch confluence (Figure 1).

Fish were inspected daily between April 30 and the end of the exposure on May 9. Beginning on May 4, fish were fed each day with a small, measured amount of hatchery food. At the end of the exposures, five or six fish from each cage were removed, placed in a plastic bag and frozen until May 12 when they were measured for length, weight and liver weight. The remaining fish from each cage were preserved in Davidson's solution (and later transferred to ethanol) for histological examination.

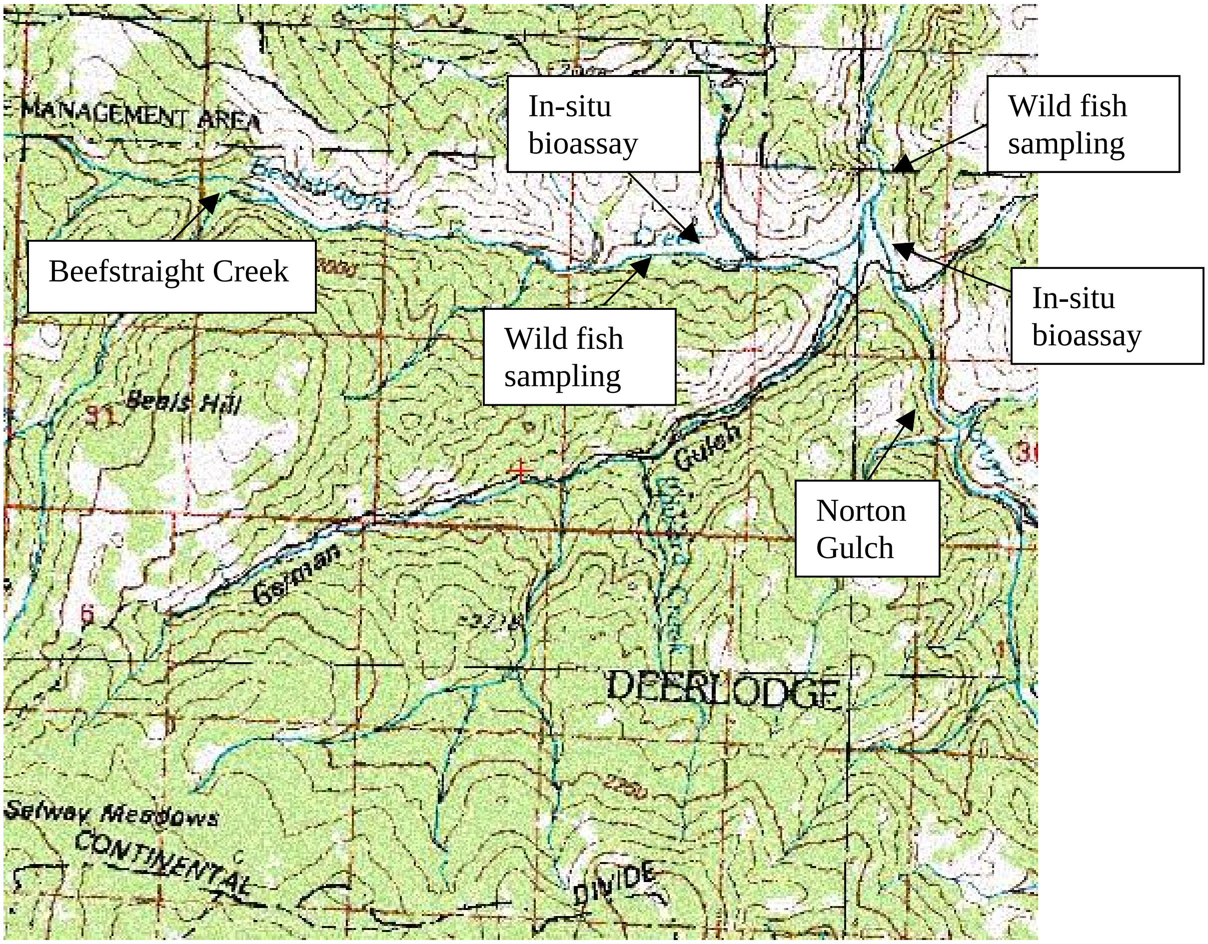

Figure 1. Topographic map showing locations of sampling during this study.

2.2. Water Quality. Levels of total cyanide, weak acid dissociable (WAD) cyanide and thiocyanate in the surface waters of Beefstraight Creek and German Gulch Creek were measured on a routine basis in 2003 by Beal Mountain Mining Company employees. The Beefstraight sampling location (BS-D) was about ¼ mile upstream of the bioassay test location. The German Gulch sampling location (Station 1) was within the section of stream sampled for fish in this study. Additional water samples were taken on a daily basis between April 30 and May 9 on Beefstraight Creek in conjunction with the *in-situ* bioassays.

2.3. Measurements and sampling of wild fish. Brook trout were sampled from German Gulch (below the confluence with Norton Gulch), Beefstraight Creek (about 1 miles above road crossing), and the North Fork of Divide Creek (as a control stream without cyanide, located southwest of the Interstate 15/90 interchange) on August 11-12, 2003 using backpack electroshockers. All captured fish were measured for length and weight. At each site, 19-20 fish were sacrificed for histological analysis and measurement of plasma thiocyanate. First, blood was drawn from the caudal aorta of each fish using a syringe and transferred to Microtainer Brand Plasma Separator Tubes with Lithium Heparin. Tubes were placed on ice for transport to the laboratory. Gill arches and internal organs of each fish were then removed and placed in Davidson's solution for 24 hours, and then transferred to a solution of alcohol. Samples were finally transported to the U.S. Fish and Wildlife Service laboratory in Bozeman for evaluation. An additional 20 fish from each site were sacrificed and livers were removed and weighed immediately for the purpose of calculating hepatosomatic index (HSI) values (liver weight/total body weight x 100).

Fish were collected from the same three sites on September 14, 2003 for the purpose of obtaining eggs from ripe female brook trout. Eggs were removed and enumerated from 20 fish on German Gulch, 13 fish on Beefstraight Creek and 3 fish on Divide Creek. Typically the procedure was to count the number of eggs in one skein, measure the volume of the same eggs, and then calculate the number of eggs/mL. The second skein was then measured volumetrically, and the number of eggs estimated by applying the egg/mL ratio from the first skein. Fish showing any evidence of having lost eggs due to handling or spawning were not used in this procedure.

The laboratory procedure for measuring plasma thiocyanate roughly followed that of Liu and Yun (1993). Upon arrival at the Montana Public Health and Human Services laboratory in Helena, the plasma separator tubes were immediately centrifuged to separate plasma from hematocrit. Then, samples were transported to the Beal Mountain Mining Company laboratory where, within four days of centrifugation, thiocyanate was extracted from the plasma, applied to high-performance liquid chromatography, and quantitated by UV detection.

3.0. RESULTS AND DISCUSSION

3.1. Water Quality. Levels of total cyanide, weak acid dissociable (WAD) cyanide and thiocyanate in the surface waters of Beefstraight Creek and German Gulch Creek were measured on a routine basis in 2003. Concentrations of thiocyanate were always undetectable, except for a detection of 0.3 mg/L in German Gulch on April 23, 2003 (Table 1). Mean levels of total cyanide and WAD cyanide in Beefstraight Creek (0.020 and 0.008 mg/L, respectively) were roughly twice as high as in German Gulch (0.009 and 0.005 mg/L, respectively). The Montana aquatic life standards are based on total cyanide, and the acute and chronic standards are 0.022 and 0.0052 mg/L, respectively. The mean WAD cyanide level in Beefstraight Creek between April 30 and May 9, based on daily measurements, was 0.021 mg/L (range 0.012-0.046).

3.2. *In-situ* bioassays with hatchery fish: Westslope cutthroat trout were exposed to the waters of Beefstraight Creek and Norton Gulch for nine days and the survival was 100% at both sites and no erratic or sluggish behavior was observed to suggest a response to a toxicant. At the end of the exposures, the cutthroat trout exposed to Beefstraight

Table 1. Cyanide measurements in surface waters of German Gulch and Beefstraight Creeks, December 2002-September 2003 (Beal Mountain Mining Company data and Maxim Technologies 2004).

	German Gulch Station 1				Beefstraight Creek, Station BS-D			
Date	Total Cyanide (mg/L)	Thiocyanate (mg/L)	WAD cyanide (mg/L)	Field pH	Total Cyanide (mg/L)	Thiocyanate (mg/L)	WAD cyanide (mg/L)	Field pH
12/10/02	0.005U				0.005U			
1/15/2003	0.005U	0.2U			0.005U	0.2U		
2/12-13/2003	0.005U	0.2U			0.007	0.2U	0.005U	
3/12/2003	0.006	0.2U	0.005U		0.020	0.2U	0.011	
3/25/03	0.020		0.005U		0.077		0.024	
4/23/2003	0.010	0.3	0.005U		0.024	0.2U	0.008	
5/13/2003	0.007	0.2U	0.005U		0.034	0.2U	0.01	
5/21/03					0.037		0.016	
5/29/03					0.02		0.005U	
6/2/03					0.022		0.012	
6/12/03					0.030		0.010	
6/18/2003	0.011	0.2U	0.008		0.020	0.2U	0.008	
7/11/03					0.016		0.005U	
7/18/05					0.014		0.005U	
7/25/2003	0.009	0.2U	0.005U		0.013	0.2U	0.005U	
8/1/03					0.009		0.005U	
8/7/2003	0.021	0.2U	0.009		0.005U			
8/22/2003					0.009	0.05U	0.005U	
9/23/2003	0.012	0.2U	0.008		0.024	0.05U	0.012	
Mean (Dec02-Sep03)	**0.009**		**0.005**		**0.020**		**0.008**	

Creek water had a mean length (TL), body weight and liver weight of 127.9 mm, 21.1 g, and 0.238 g, respectively. The mean length, body weight, and liver weight for cutthroat trout exposed to Norton Gulch water was 127.1 mm, 20.0 g, and 0.251 g, respectively. Livers of the cutthroat trout were slightly smaller in Beefstraight Creek than Norton Gulch after this nine day exposure, as measured by the hepatosomatic index, but the differences were not significant: Norton Gulch—1.256 ($\pm$ 0.063 SEM) and Beefstraight Creek--1.129 ($\pm$ 0.047 SEM).

The finding in this study, that Beefstraight Creek water was not acutely lethal to brook trout, can be compared to studies by Smith et al (1978) and Kovacs and Leduc (1982a) who determined the acute toxicity of HCN to brook and rainbow trout. This comparison must take into account the uncertainties in relating HCN levels to WAD levels. WAD cyanide is probably a close approximation to HCN because it measures any HCN in the water as well as metal complexes that are easily dissociated, but there may be forms of cyanide measured by WAD that are less bioavailable to trout than HCN. Another consideration is the fact that acute toxicity of cyanide to fish depends on water temperature and pH, i.e. toxicity increases as water temperatures and pH decrease. The toxicity of cyanide, however, is little affected when the pH is in the range of 6.8-8.3 (Eisler 1991).

Kovacs and Leduc (1982a) found that when juvenile rainbow trout were exposed to HCN at 6 and 12^{o}C, the 96 hr LC50s were 0.028 mg/L and 0.042 mg/L, respectively. The highest concentrations with no mortality after 96 hours were 0.018 mg/L and 0.032, respectively. The WAD cyanide levels in our bioassays averaged 0.021 mg/L, which falls between the no-effect level and the LC50 at 6^{o}C. If westslope cutthroat trout react similarly to cyanide as the rainbow trout in the Kovacs and Leduc study, then conditions in Beefstraight Creek were probably at or near the threshold for lethality.

Brook trout also live in the German Gulch drainage, and the study by Smith et al (1978) is relevant to understanding the possible effects of the cyanide on this species. They investigated the toxicity of HCN to juvenile brook trout at 4.0 and 6.0^{o}C, and found that the 96 hr LC50s were 0.053 and 0.0618 mg/L, respectively. The pH during these tests was 7.19 and 7.24, respectively. They also calculated a 96 hr LC50 of 0.0844 mg/L at a water temperature of 6.8^{o}C and a pH of 8.06. These water quality conditions were similar to those on Beefstraight Creek, where water temperatures on April 23 and May 13 were 3.4 and 6.6^{o}C respectively, while pH was 8.3 and 8.4. No information was presented by Smith et al (1978) to indicate a no-effect or incipient lethal threshold for cyanide. If we assume the dose-response slope is similar between rainbow trout and brook trout, then we can use the results from Kovacs and Leduc to estimate a no-effect level for brook trout. Kovacs and Leduc calculated the no-effect level for the rainbow trout to be 64% of the LC50 (0.018/0/028), and if it assumed that this fraction would be applicable to the brook trout LC50 in the Smith et al study, then the no-effect level for acute toxicity in brook trout would be estimated to be 0.034, 0.040, and 0.054 mg/L, at 4.0, 6.0 and 6..8^{o}C, respectively. This suggests that the cyanide levels in Beefstraight Creek in early May 2003 were not acutely lethal to brook trout.

The effects of chronic HCN exposure on juvenile rainbow trout were investigated by Kovacs and Leduc (1982b). They measured growth rates over a 20 day period of exposure, and found that the threshold concentrations of HCN that produced no effect on growth were 0.01, 0.015 and 0.030 mg/L at water temperatures of 6, 12 and 18^{o}C. In Beefstraight Creek and German Gulch, water temperatures are typically below 12^{o}C from September-June, and therefore any prolonged periods where WAD cyanide is over 0.010-0.015 mg/L could have a negative effect on cutthroat trout growth. Growth rates of cutthroat trout were not measured in this study.

3.3. Measurements of Wild Fish

3.3.1. Plasma thiocyanate: Most absorbed cyanides are converted to thiocyanate (SCN^-) in the bodies of animals, which is about 120 times less toxic than the parent compound (Eisler 1991). For this reason, we chose to measure plasma SCN^- as a quantitative indication of chronic cyanide exposure. Results showed the highest mean levels in fish from Beefstraight Creek (3.11 mg/L $\pm$ 5.08 SD), followed by Divide Creek (1.76 mg/L $\pm$ 3.09 SD) and finally German Gulch (0.87 mg/L $\pm$ 1.03 SD). No significant differences were found among the three groups. These levels do not correspond well with cyanide levels in the water. Water samples taken in the three months prior to our study showed that mean levels of total cyanide in Beefstraight Creek (0.025 mg/L + 0.012 SD) were almost twice as high as German Gulch at Station 1 (0.013 + 0.005 SD)(Table 1); levels of cyanide in Divide Creek were not measured but assumed to be zero.

It was not expected that fish from Divide Creek would have SCN^- in their blood, as there is no known history of cyanide use by humans in the Divide Creek drainage. Hemming and Blumhagen (1989) encountered a similar situation in a laboratory setting where rainbow trout used as control fish (and exposed to no cyanide) had mean levels of 3.0 mg/L SCN^- in their blood. They attributed the source of SCN^- to dietary precursors. It is possible this is the source of SCN^- in this study, although the possibility of cross-contamination or improper analytical techniques cannot be ruled out. Lanno (1990) also measured SCN^- in the plasma of rainbow trout that were being used as controls. After 12 weeks consuming commercial fish feed, juvenile trout had a mean SCN^- level of 5.1 mg/L, although after 16 weeks exposure there was no SCN^- detected in the blood.

Lanno (1990) measured SCN^- in the plasma of juvenile rainbow trout following exposure to 0, 5 or 40 mg/L SCN^- for 16 weeks. After this time, fish from the three treatment levels had mean plasma SCN^- levels of 0, 10.1 and 63.7 mg/L. The corresponding HSI values were 1.10, 1.05 and 0.80. The last value was significantly different from the control. These results suggest that plasma SCN^- levels would need to be somewhere between 10.1 and 63.7 before significant changes in liver size will occur. Even 10.1 is much higher than the mean plasma SCN^- value on Beefstraight Creek of 3.11 mg/L.

Lanno (1990) also exposed fathead minnows to varying levels of SCN^- for 124 days. He found that egg production dropped significantly as SCN^- concentration increased from 1.1 to 7.3 mg/L. Corresponding plasma SCN^- levels were <0.01 and 2.5 mg/L. The plasma levels in brook trout in Beefstraight Creek (3.11 mg/L) were higher than the 2.5

mg/L level that showed a reproductive effect in fathead minnows. However, the relevance of the fathead minnow study to the present study is uncertain because fathead minnows may have different sensitivities to cyanide than do trout, and the toxicological consequences of an exposure to SCN^- versus exposure to the cyanide forms found in the German Gulch drainage is not well understood.

3.3.2. Hepatosomatic index (HSI) values in wild fish: Brook trout collected in August from the three sites were measured for differences in the HSI. Results showed that Beefstraight Creek had the highest mean HSI (1.11 + 0.10 SEM), followed by Divide Creek (1.095 + 0.04 SEM) and German Gulch (0.98 + 0.03 SEM). No significant differences were found among the three groups. The high mean value for Beefstraight Creek was strongly influenced by the liver from one fish which was a strong outlier and had a HSI of 2.45. The influence of this one value was reduced by comparing the median values (see boxplots in Figure 2). Based on this comparison, both German Gulch and Beefstraight had median HSI values of 1.01, with Divide Creek having a median value of 1.05. Cyanide poisoning causes fish livers to shrink in size, because the body tends to mobilize glycogen stores to compensate for reduced energy production. Therefore, if exposure to cyanide was the only factor affecting liver size, then it would be expected that Divide Creek fish would have the largest livers (and HSI values), followed by German Gulch and Beefstraight Creek.

Da Costa and Ruby (1984) reported on the HSI values in adult rainbow trout following seven days of sublethal exposure to HCN. HSI values in females declined from about 1.4 in the control to about 1.1 following exposure to 0.02 or 0.03 mg/L HCN. The HSI score for females exposed to 0.01 mg/L was only marginally different from the controls. HSI scores for male fish actually increased from 1.2 in the controls to 1.35 after exposure to 0.01 mg/L, but then declined to about 1.1 and 1.0 after exposure to 0.02 and 0.03 mg/L HCN, respectively. Increasing levels of HCN were also accompanied by decreasing levels of serum calcium, which the authors interpreted as potentially being below levels required for the production of exogenous yolk by the liver.

Lanno and Dixon (1996) found that increased exposure to waterborne SCN- was associated with reduced HSI, reduced growth, and increased plasma SCN- in rainbow trout. However, neither these authors or De Costa and Ruby (1984) identified an HSI value that could be considered a threshold for health effects. Consequently, changes in HSI are not considered direct evidence or proof of harm.

3.3.3. Histology of wild fish: In August 2003, brook trout were captured from all three sites and the tissues that were processed and examined included liver, kidney, gill, gastrointestinal tract, spleen, gonads, and thyroid. Notable differences among sites were seen in the liver, thyroid and kidney. The livers from the fish in Divide Creek were unique in showing mild to moderately severe nuclear vacuolation of hepatocytes (a condition often associated with toxicant exposure)(Figure 3). Both Divide Creek and Beefstraight Creek fish displayed no glycogen vacuolation, while German Gulch fish ranged from no glycogen vacuolation to moderate vacuolation. A lack of vacuolation would be consistent with cyanide exposure where glycogen stores are depleted to meet increased energy needs.

Fish in Beefstraight fish showed the highest incidence of degeneration and necrosis of kidney tubules; also observed was mild to moderately severe accumulation of melanomacrophages (Figure 4); kidneys of German Gulch and Divide Creek fish had a greater number of normal kidney tubules.

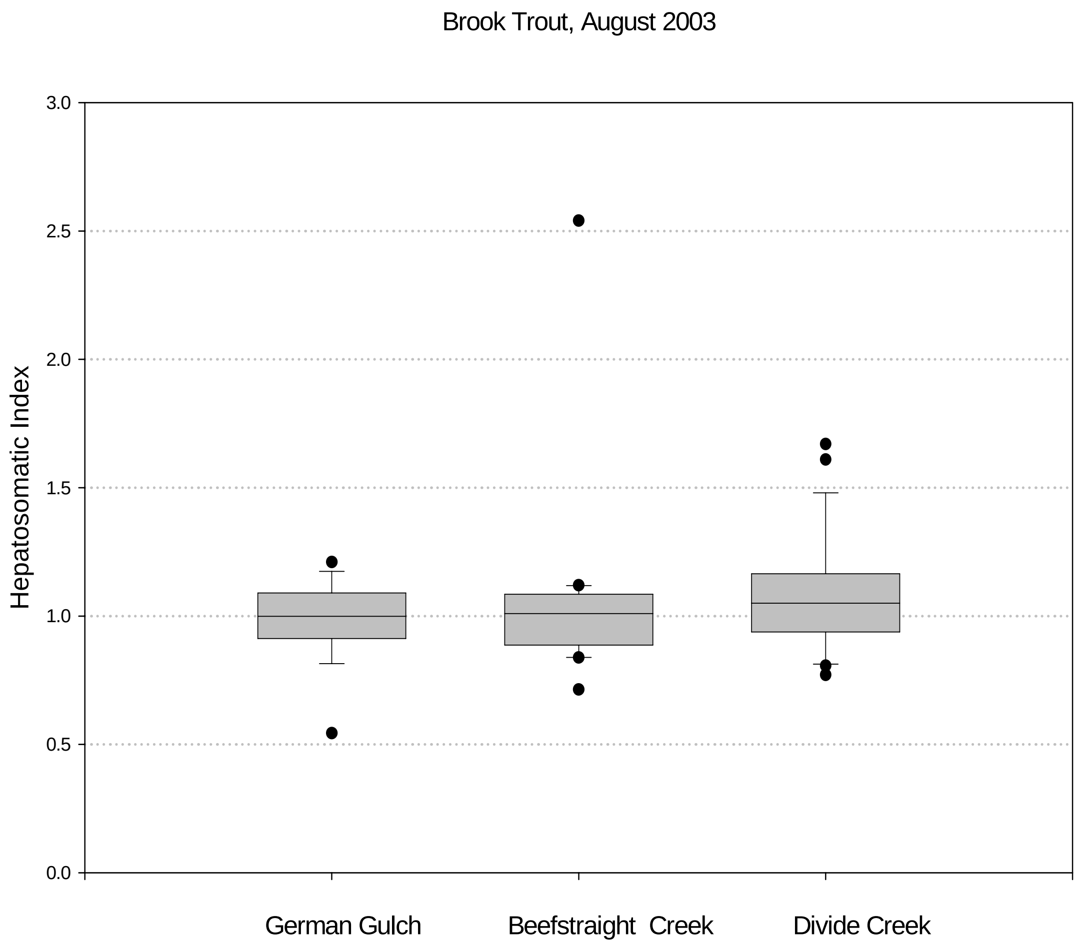

Figure 2. Box plot of hepatosomatic index values for brook trout from German Gulch, Beefstraight Creek and Divide Creek, August 2003. Line in box represent median value, upper and lower borders of box indicate 75th and 25th percentile values, upper and lower error bars represent 90th and 10th percentile values. Outliers indicated with dots.

German Gulch fish showed the greatest impairment to thyroid tissue. Most showed moderate to moderately severe hyperplasia of follicles (Figure 5). Most fish in Beefstraight Creek showed widely dispersed follicles along with a mild proliferation of follicular epithelia that did not contain colloid. Only two specimens were available from Divide Creek, but they showed numerous large follicles with colloid. The entire case file prepared by Beth MacConnell is presented as Attachment A.

Lanno and Dixon (1996) described changes in rainbow trout tissues after exposure to 35-154 mg/L potassium thiocyanate for 16 weeks. Changes were most prominent in the thyroid, but were also evident in the liver, kidney, spleen and gills. The changes in the thyroid included hyperplasia of follicular epithelia and follicular lumina with low (or no) levels of colloid. In the livers, they found moderate numbers of necrotic hepatocytes, which were absent in the controls. Livers of control fish also had a large number of lipid vesicles whereas SCN^- exposed fish had no vesicular lipid deposition. The spleen, head kidney, interrenal and chromaffin tissues appeared normal relative to controls, although "there was a tendency toward early asynchrony of red cell production, as well as a reduced myeloid/erythroid ratio in head kidney of treated fish."

Our observations of thyroid tissue were similar to those described by Lanno and Dixon (1996), and provide circumstantial evidence that the condition of thyroid tissue in German Gulch and Beefstraight fish (follicle proliferation, deficiency in colloid) was caused by cyanide exposure. Histological observations of changes in liver and kidney tissue in this study were not similarly reported by Lanno and Dixon (1996). However, the lack of glycogen vacuolation in Beefstraight fish is a condition that could translate to a decrease in liver weight and a lower HSI score (which was documented by Lanno and Dixon 1996).

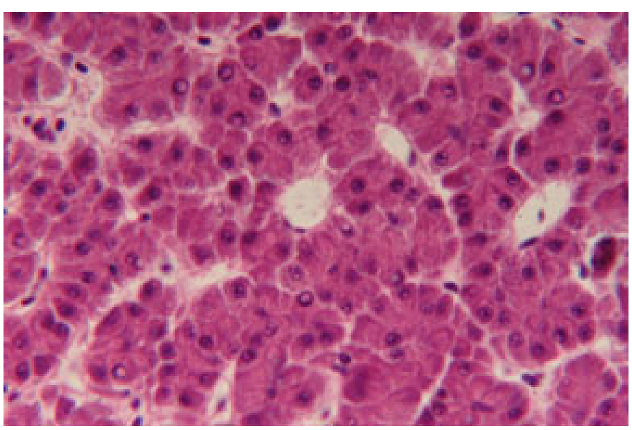

Figure 3. Microscopic slide of liver of Divide Creek fish. Vacuolation of cell nucleus is evident by whitish areas inside cell nuclei.

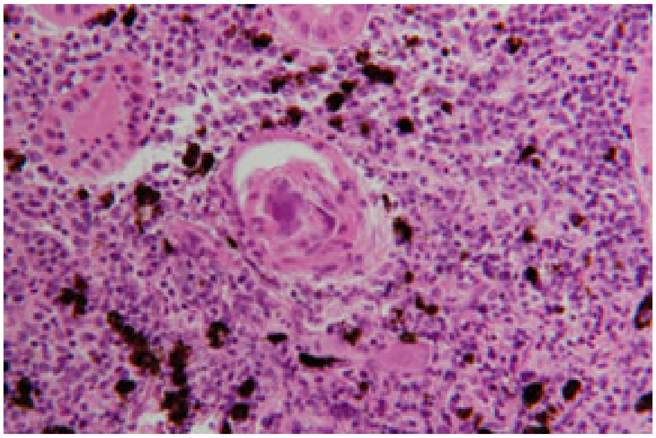

Figure 4. Microscopic slide of kidney of Beefstraight fish. At center is a tubule that has been destroyed, and calcified dying cells can be seen in the center of the tubule. Black spots are melanomacrophages.

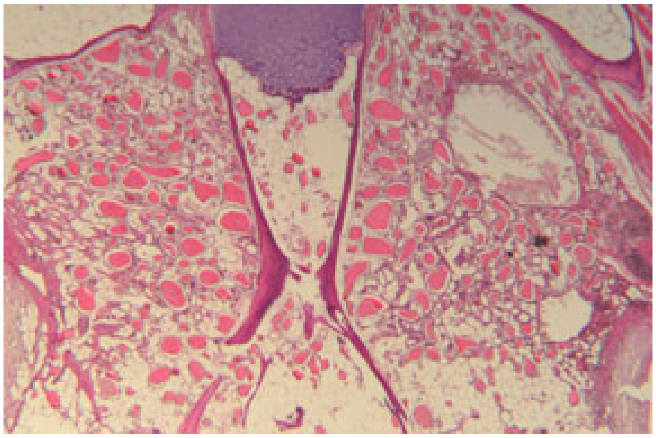

Figure 5. Microscopic slide of thyroid tissue of German Gulch fish. Hyperplasia of follicles is evident between long bones in center of photo**.**

3.3.4. Fecundity of wild fish: Fish in German Gulch had the greatest number of eggs (400 $\pm$ 29 SEM), followed by Beefstraight Creek (348 $\pm$ 21 SEM) and Divide Creek (211 $\pm$ 33 SEM). However, because fecundity is largely size-dependent, we normalized the results using the linear relationship between number of eggs per fish and fish weight. Correlation was good (r^2=0.668), with the relationship described as:

Number of eggs=58.32 + 4.67 (weight, g).

We used this equation to calculate the residual variance of number of eggs for each fish from this calculated relationship (Figure 3). Using this method of comparison, Beefstraight Creek fish had a higher median fecundity than German Gulch, although the 10^{th} and 25^{th} percentiles were much lower than German Gulch, showing that some fish were markedly lower in egg production.

German Gulch fish had the largest eggs (42.4 egg/mL $\pm$ 4.1 SEM), followed by Beefstraight Creek (41.9 $\pm$ 3.9 SEM) and Divide Creek 35.6 $\pm$ 3.0 SEM). The differences between the three sites were not statistically significant. It was not possible to normalize the eggs/mL data, because there was a poor correlation (r^2=0.2437) with fish weight.

Koenst et al (1977) exposed brook trout to HCN for 144 days prior to spawning, and reported that females spawned 42% fewer eggs after exposure to 0.011 mg/L HCN compared to controls and a lower level (0.006 mg/L) of HCN. The mean value of WAD cyanide (roughly equivalent to HCN) in German Gulch and Beefstraight Creek for the nine month period before we collected eggs was 0.005 mg/L and 0.008 mg/L, respectively. Consequently, fecundity of brook trout in German Gulch was probably unaffected, but Beefstraight Creek brook trout could have experienced some reductions in spawning performance.

Lesniak and Ruby (1982) found that, for rainbow trout, exposure to 0.010 or 0.020 mg/L HCN resulted in smaller eggs (due to inhibition of secondary yolk deposition) and an increased frequency of atresia (egg resorption). They directly measured of egg diameters and the relative frequency of eggs in different size classes, whereas we simply measured egg volume (number/milliliter) as a surrogate for egg size. This may have limited our ability to detect effects of cyanide exposure. The concentration of HCN that caused effects in the Lesniak and Ruby study (0.010-0.020 mg/L) were slightly higher than the mean values of WAD cyanide for Beefstraight Creek and German Gulch (0.008 and 0.005 mg/L).

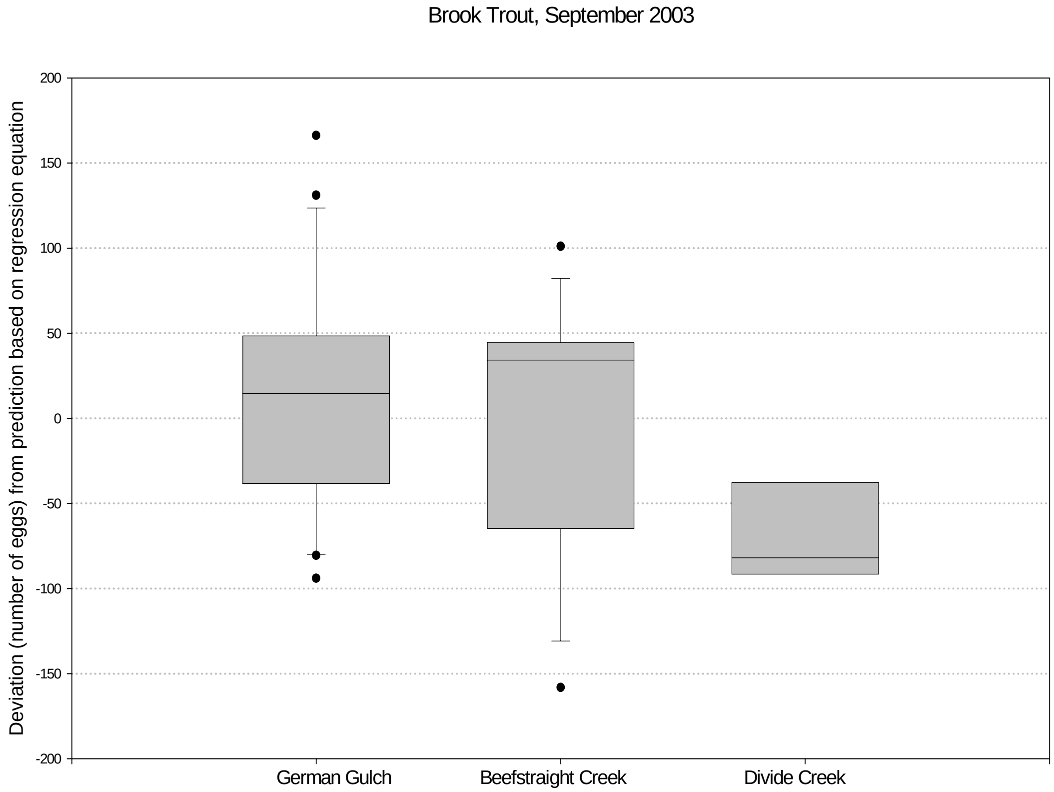

Figure 3. Boxplot of number of eggs per fish at three sites, expressed as the deviation from predicted number of eggs based on the regression equation. Meaning of symbols the same as in Figure 2.

4.0. SUMMARY.

Results from this study provide evidence that brook trout exhibit a physiological reaction to cyanide exposure in German Gulch and Beefstraight Creek. However, none of the measurements support the conclusion that cyanide is causing significant or lasting damage to brook trout. The evidence is somewhat confusing in that responses do not always correlate well with cyanide levels in the water from each site (Table 2). Three of the six parameters in Beefstraight fish were consistent with a response to exposure to cyanide, whereas only two of the six parameters in fish from German Gulch were consistent with exposure to cyanide. The low number of control samples for fecundity and egg size greatly decreases our level of certainty and limits our ability to interpret these data.

Table 2. Response by fish in Beefstraight Creek and German Gulch relative to control streams (Divide Creek or Norton Gulch). A + indicates a response consistent with cyanide exposure. A – indicates a response not consistent with cyanide exposure.			
Parameter	Beefstraight Creek	German Gulch	Control
Mortality	0	Not Measured	0
Plasma thiocyanate	+	-	0
Hepatosomatic index	+	+	0
Histology	+	+	0
Fecundity	-	-	0
Egg size	-	-	0

Recommendation for future investigations include: 1) re-examine fecundity by sampling a large number of gravid females from each site (20-30) and performing detailed analysis of egg size and egg histology; 2) spawn brook trout from each stream and incubate the eggs in a hatchery environment to evaluate hatch success and fry survival; and 3) use scales and otoliths to evaluate growth patterns of rainbow trout in German Gulch. Such an evaluation would compare growth in streams before and during periods of land application of cyanide at the mine.

6.0. ACKNOWLEDGEMENTS

Kurt Hill (Montana Fish, Wildlife and Parks) helped with field collection of fish and processing of livers and eggs in the laboratory. Tim LaMarr, Kirk Forkan and Michael Wagner (Beaverhead-Deer Lodge National Forest) helped with the field collection of fish. Fred Stone (Beal Mountain Mining Company) performed laboratory analyses of plasma thiocyanate.

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ATTACHMENT A

DRAFT REPORT
February 23, 2004

Bozeman Fish Health Center Case No: 03-421
Pathologist: Beth MacConnell

Lower jaw tissue was sectioned 4 times – basically thru the entire tissue – and the slides read each time, but thyroid tissue sections were only obtained from the lower jaw tissue in 17 of the 59 fish.

Tissue examined from all 59 fish - lower jaw, kidney, liver, gill, GI, spleen and some gonad tissue processed and examined.

Group B 1-20 = 03-421 1-20
Group D 1-19 = 03-421 21-39
Group G 1-20 = 03 421 40-59

Scoring system 0 = none
1 = minimal
2 = mild
3 = moderate
4 = moderately severe
5 = severe

Group B (Beefstraight Creek)

Thyroid – sections from 8 of 20 fish. Most were insufficient. Most thyroid sections showed widely dispersed thyroid follicles and mild proliferation of follicular epithelia that did not contain colloid.

Kidney – degeneration and necrosis of tubule epithelia was noted in this group. Moderate numbers of sloughed cells ere seen in tubule lumen. Dystrophic calcification was seen in 2 fish from this group. Numbers of regenerating tubules were mild to moderate.
Hematopoietic tissue showed mild to moderate proliferation and mild to moderately severe accumulation of melanomacrophages.

Liver – no glycogen vacuolation. Mild atrophy (increased cellularity). Mild to moderate numbers of megahepatocytes. Mild to moderate, one fish showing moderately severe, nuclear vacuolation of hepatocytes. Moderate cytoplasmic degeneration of hepatocytes. Moderate numbers of cystic foci.

GI – most stomachs contained feed. Mild to moderately severe numbers of rodlet cells and lymphocytes infiltrated the mucosal epithelia of pyloric caeca. Increased in mucus was moderate.
Fat (visceral) ranged from none to mild.

Group D (Divide Creek)

Thyroid – only 2 sections from this group. Both showed numerous large follicles containing colloid (normal)

Kidney – Mostly normal kidney tubules. One fish showed moderate hydropic degeneration of tubule epithelia.

Hematopoietic - tissue showed mostly moderate, one fish moderately severe, proliferation and moderate to moderately severe accumulation of melanomacrophages.

Liver - no glycogen vacuolation. Moderate scattered degeneration and mild to moderately severe nuclear vacuolation of hepatocytes. Moderate numbers of megahepatocytes and mild to moderate melnomacrophges. Moderate to moderately severe cystic foci.

GI – stomachs empty to distended with abundant feed. Mild to moderate numbers of lymphocytes infiltrated the mucosal epithelia of pyloric caeca. Increased in mucus was mild to moderate.

Fat (visceral) ranged from minimal to mild.

Group G (German Gulch)

Thyroid - sections from 8 of 20 fish. Most showed moderate to moderately severe hyperplasia of follicles. One fish showed no hyperplasia and degenerate colloid was seen in another fish.

Kidney – Mostly normal but there were mild to moderate numbers of regenerating tubules.

Hematopoietic tissue showed mild to moderate proliferation and mild to moderately severe accumulation of melanomacrophages. Section of one head kidney (most samples did not include head kidney tissue) showed moderately severe hyperplasia of adrenal tissue.

Liver – glycogen vacuolation (good) of hepatocytes ranged from none to moderate levels. Mild scattered degenerative changes were noted.

GI – almost all the fish is this group had numerous parasitic worms in GI. Stomachs ranged from empty to filled with feed. All showed a moderately severe in sloughed mucus. Mild to moderate l rodlet cell numbers and lymphocytic infiltration of the mucosal epithelia was seen.

Fat (visceral) ranged from minimal to moderate.

In summary each group showed notable degenerative cellular changes in one tissue; B group was kidney(degeneration of kidney tubules), D liver (nuclear vacuolation), and G thyroid (hyperplasia). Variability in tissue changes seen within each group may or may not be related to age and or size of fish.

<u>Histological description and significance</u>

regenerating tubules - indicative of repair.

accumulation of melanomacrophages - indicative of cell turnover – increased accumulation common in older fish, but in younger fish indicative of accelerated cell damage.

nuclear vacuolation of hepatocytes – indicative of toxicant exposure

atrophy – indicative of starvation

hyperplasia of adrenal tissue.- indicative of stress