The toxicity to fathead minnow of seep waters along Whitewood Creek, South Dakota

Final Report
~~December 14, 1999~~
Revised July 7, 2000

Steven J. Hamilton and Kevin J. Buhl

U.S. Geological Survey
Biological Resources Division
Columbia Environmental Research Center
Ecotoxicology Research Station
31247 436th Avenue
Yankton, South Dakota 57078-6364

Prepared for
Cheyenne River Sioux Tribe
212 North Main Street
Eagle Butte, South Dakota 57625

United States Department of the Interior

U. S. GEOLOGICAL SURVEY

Columbia Environmental Research Center
Ecotoxicology Research Station
31247 436th Avenue
Yankton, South Dakota 57078-6364
Comm. No.: 605-665-9217 FTS No. 700-751-9217 FAX No. 605-665-9335
http://www.cerc.usgs.gov/

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Interested parties,

You had previously received a copy of the final report entitled: "Toxicity to fathead minnow of seep water along Whitewood Creek, South Dakota" written by S.J. Hamilton and K.J. Buhl.

Enclosed is a revised version of the report dated July 7, 2000. We recently discovered an error in our calculations of corrected conductivity (i.e., we used a cell constant of 5.0 instead of 1.0) that caused the values to be five times too high. This correction does not change the interpretation of the results, but does change values in Table 1 and Appendix 1.

I regret this error and any inconvenience the error may have caused you or your staff. Please destroy the version of the report dated December 14, 1999.

Steven J. Hamilton, PhD
Leader, Yankton Ecotoxicology Research Station

DISCLAIMER

This study was funded by the Cheyenne River Sioux Tribe. The opinions and recommendations expressed in this report are those of the authors and do not necessarily reflect the views of the Cheyenne River Sioux Tribe or the U.S. Geological Survey.

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TABLE OF CONTENTS

	Page
LIST OF TABLES	4
LIST OF FIGURES	4
LIST OF APPENDICES	5
ACKNOWLEDGMENTS	5
EXECUTIVE SUMMARY	6
INTRODUCTION	7
METHODS	9
Fish acquisition and culture	9
Site description	10
Test procedure	13
General water sample collection	15
Decontamination procedures	15
Water quality characteristics	15
Test chemical analysis of exposure water and fish diet	16
Proximate analysis of brine shrimp	19
Statistics	19
RESULTS	20
Analytical quality control	20
Water quality	22
Inorganic elements	25
Brine shrimp	31
Test procedure and larvae survival	31
DISCUSSION	32
CONCLUSIONS	40
REFERENCES	41

LIST OF TABLES

Page

1. Mean (standard error in parentheses; n=4) water quality characteristics measured on site in Whitewood Creek, South Dakota 23
2. Mean (standard error in parentheses, n=4 except where noted in brackets) water quality characteristics of water collected from various sites in Whitewood Creek, South Dakota, and measured in the laboratory. 26
3. Mean (standard error, number of analyses greater than the limit of detection in brackets) concentrations of inorganic elements (μg/L) in water collected from various sites in Whitewood Creek, South Dakota. 28
4. Mean (standard error in parentheses, n=3) survival of fathead minnow exposed to waters collected from various sites in Whitewood Creek, South Dakota 33
5. Comparisons of inorganic concentrations in water from seep 33L tested with fathead minnow to published individual acute toxicity values for fathead minnow 36

LIST OF FIGURES

1. Map showing locations of the five seeps along Whitewood Creek, South Dakota, used in toxicity tests with fathead minnow 12

LIST OF APPENDICES

Page

1. Water quality characteristics measured in-situ in Whitewood Creek, South Dakota 47
2. Water quality characteristics of water collected from various sites in Whitewood Creek, South Dakota, and measured in the laboratory 50
3. Concentrations of inorganic elements (μg/L) in water collected from various sites in Whitewood Creek, South Dakota 54
4. Dissolved oxygen, pH, and temperature of water in exposure beakers before and after water renewal. Waters were collected from various sites in Whitewood Creek, South Dakota 59
5. Percent survival of fathead minnow exposed to waters collected from various sites in Whitewood Creek, South Dakota 63

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EXECUTIVE SUMMARY

A toxicity test was conducted to determine if waters in Whitewood Creek collected above seeps, at seeps, and in the mixing zone of seeps with the creek cause adverse effects in 5-day-old fathead minnow. Whitewood Creek, located in the Black Hills of southwestern South Dakota, has a long history of contamination from mining activity. No adverse effects on survival of fish were observed in the 96-hour test with waters from five seeps entering Whitewood Creek, creek water collected above the seeps, or water collected from the mixing zone of the seep and the creek. Whitewood Creek was being loaded with arsenic from four seeps, and with nickel from three seeps. Creek water contained higher mercury concentrations than were found in seep waters. Copper concentrations in one seep were lower than in creek water. Similar concentrations of beryllium, cadmium, chromium, lead, and zinc were present in seep and creek waters. Additional research is needed to determine if long-term exposure to inorganic elements in water and in sediments from Whitewood Creek is adversely affecting aquatic organisms.

INTRODUCTION

Whitewood Creek, located in the Black Hills of southwestern South Dakota, has a long history of contamination from mining activity. Gold exploration began in the 1870's, and has continued since that time. Whitewood Creek received tailings from 1870 to 1977 from Gold Run Creek in Lead, SD, which carried direct releases of tailings from mining operations. Currently, Homestake Mining Company operates a large gold mine in Lead, SD, near the headwaters of Whitewood Creek.

It has been estimated that approximately 100 million to 1 billion tons of ore have been released into Whitewood Creek by mining activity in the last century (Fox Consultants, Inc. 1984). Mining, milling, and ore processing wastes, consisting of finely ground rock and residual inorganic and organic compounds, have been laid down along the banks of Whitewood Creek to its confluence with the Belle Fourche River and the Belle Fouche River's confluence with the Cheyenne River (Fox Consultants, Inc. 1984, ISSI, Inc. 1998). Tailings deposition has altered the geomorphology of Whitewood Creek, and deposits up to 4.6 m deep in places, have become stabilized in place by vegetation. Gold recovery from ore was accomplished through mercury amalgamation, which was discontinued in 1971 and replaced with the process of cyanide heap-leaching. Several other streams in the Black Hills have also been adversely affected by mining operations (Rahn 1996).

When water leaches through rock strata that are disturbed by surface and subsurface mining, it dissolves inorganic elements and carries them to the groundwater. Groundwater movement through the extensive tailings deposits in the Whitewood Creek valley enter the creek

at various seeps along its downstream course to the Belle Fourche River, and the Belle Fourche River itself, which empties into the Cheyenne River and eventually into Lake Oahe.

Elevated concentrations of inorganic elements have been reported in water, sediment, and biota from Whitewood Creek (Ruelle et al. 1993). Concentrations of inorganic elements such as arsenic, copper, lead, selenium, and zinc had been reported in fish from Whitewood Creek (Sowards et al. 1991) that were elevated above those at the 85th percentile, an arbitrary value distinguishing "high" concentrations, in the National Contaminant Biomonitoring Program (May and McKinney 1981, Lowe et al. 1985, Schmitt and Brumbaugh 1990). Extensive deposits of mining tailings including arsenopyrite compounds occur along Whitewood Creek, with decreasing depositions along the Belle Fourche River and the Cheyenne River. Percolation of water through the tailings has contributed inorganic elements such as arsenic and iron to streams (Stach et al. 1978). Inorganic compounds such as arsenopyrite present in the tailings deposits in Whitewood Creek and the Belle Fourche River have been found in sediments from the Cheyenne River and Lake Oahe (Horowitz et al. 1990). During the period when mercury amalgamation was used, considerable mercury discharge occurred over the years, and has resulted in elevated mercury concentrations in sediment, fish, and fish-eating birds in the Cheyenne River, Cheyenne Arm of Lake Oahe, and Lake Oahe (Walter et al. 1973, Hesse et al. 1975).

In order to determine the potential hazard to aquatic life in Whitewood Creek posed by contaminants in the seep water, ambient concentrations of contaminants in seep water were tested to assess their biological affects on young fish. The purpose of this study was to determine if exposure of larval fathead minnow (*Pimephales promelas*) to water from various seeps in Whitewood Creek affects their survival. The fathead minnow was selected as the test species

because it is a commonly-tested warm water fish, and the lower Whitewood Creek where the test seeps were located is a relatively warm water habitat.

METHODS

Fish acquisition and culture

Fathead minnow (*Pimephales promelas*) 1-day-old were received from Aquatic Bio Systems, Fort Collins, CO, on February 26, 1999. Fish were shipped from Fort Collins to the Yankton facility in water with a hardness of 126 mg/L as $CaCO_3$, alkalinity of 85 mg/L as $CaCO_3$, and pH of 8.37. After arrival at Yankton, fish were acclimated from the shipping water to the reference water that was used in fish culture and the toxicity test (hardness, 668 mg/L as $CaCO_3$; alkalinity, 180 mg/L as $CaCO_3$; and pH, 7.95). This reference water was based on the hardness of two samples of Whitewood Creek water collected on February 21, 1999. The water samples came from near seep locations 23R and 31L (described below). Upon arrival, shipping water was at 18.5°C and fish were acclimated to 20°C.

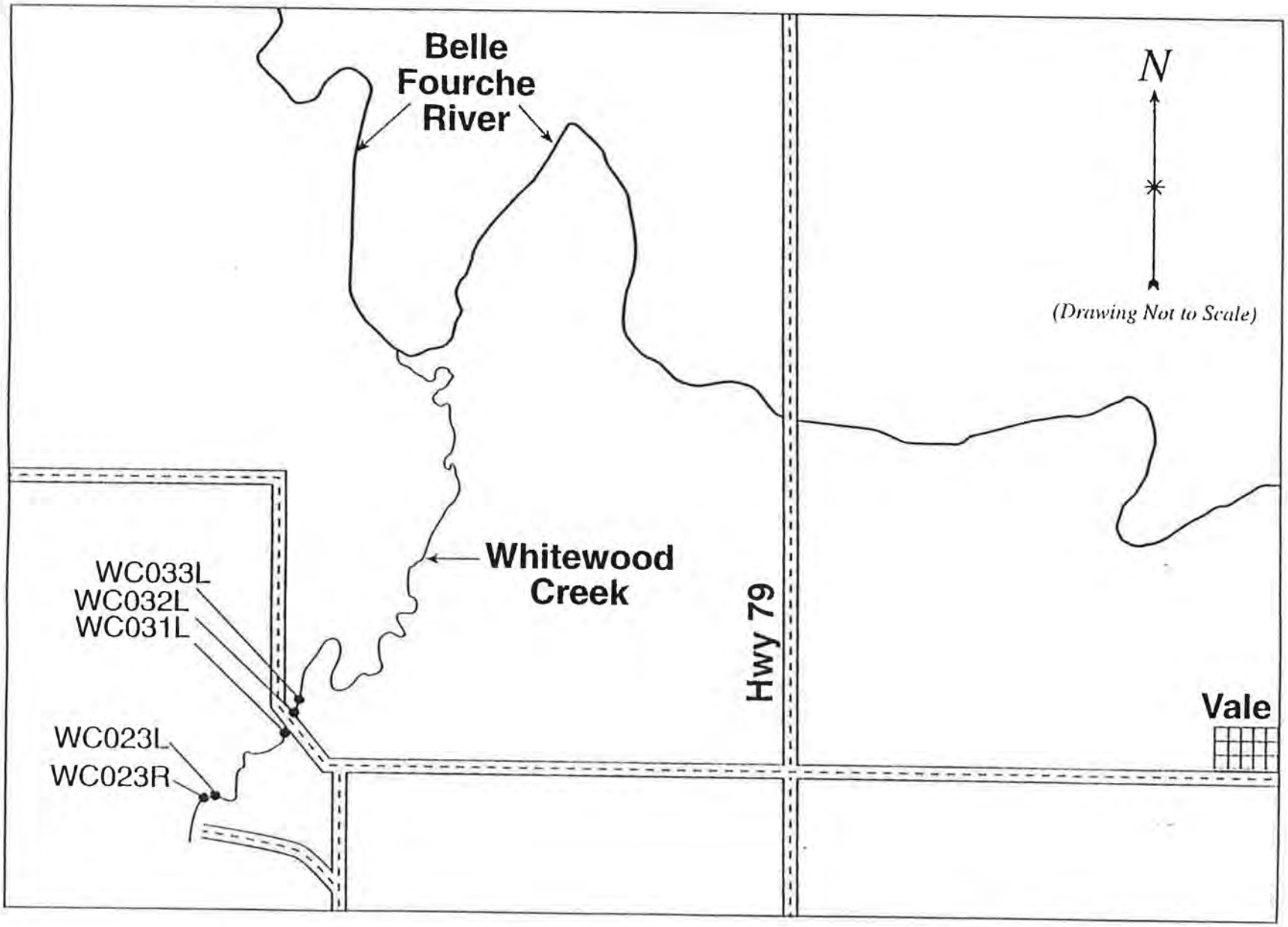
After acclimation to the water quality of the reference water, fish were cultured under static renewal conditions by daily renewal of fifty percent of the tank water with fresh reference water. Dissolved oxygen concentrations in the culture tank were supplemented with compressed air delivered from an oil-less compressor to airstones through latex tubing. Fish were fed 1-day-old nauplii of brine shrimp (*Artemia* sp.) during culture. Brine shrimp cysts (Aquarium Products, Glen Burnie, MD; source Columbia, South America) were hatched in reconstituted seawater at 35 g/L salinity prepared by adding Instant Ocean seasalts to deionized water. Fish were transported from Yankton in the reference water to the testing facility in Rapid City, SD.

Site description

The acute toxicity test was conducted by exposing 5-day-old fish for 96-hours to water associated with five seeps on Whitewood Creek, Butte County, SD. The five seeps used in the test were selected after a reconnaissance visit to determine which seeps were flowing. For four seeps, the site identification numbers designated by personnel of the U.S. Fish and Wildlife Service (USFWS) and South Dakota Game, Fish and Parks (SDGFP) for Whitewood Creek during a previous reconnaissance were WC023R, WC031L, WC032L, and WC033L (Figure 1). These seep locations were designated 23R, 31L, 32L, and 33L, respectively, for the present study. One seep not previously identified by the USFWS or SDGFP, but used in the study was designated WC023L. The universal transverse mercator (UTM) for these sites were as follows: 23R, UTM (N)16212691.76, UTM (E)2036145.5; 31L, UTM (N)16213845.56, UTM (E)2030244.2; 32L, UTM (N)16214243.34, UTM (E)2038342.58; 33L, UTM (N)16214524.3, UTM (E)2038357.78. No UTM identifier was available for seep 23L, which was located about 40 m west of seep 23R in a ravine draining into Whitewood Creek about 5 m below seep 23 R and on the opposite side of the creek.

These five seeps were selected from a total of 52 seeps examined in a screening study previously conducted by the USFWS and SDGFP. The selection of the seeps was based on a combination of measurable flow of the seep, visual precipitate, seep draining tailings, and the professional judgement of personnel of the USFWS, SDGFP, and U.S. Geological Survey/Biological Resources Division (USGS/BRD) that the seep could potentially pose a risk to fish and wildlife resources. Access to sites was by foot from a highway bridge or public land access points, and along the creek below the high water mark.

Figure 1. Map showing locations of the five seeps along Whitewood Creek, South Dakota, used in a toxicity test with young fathead minnow.


Belle
Fourche
River
N
(Drawing Not to Scale)
Whitewood
Creek
WC033L
WC032L
WC031L
WC023L
WC023R
Hwy 79
Vale

Test procedure

The acute toxicity tests were conducted in the laboratory at the SDGFP Regional Office, Rapid City, SD. Each static acute toxicity test was conducted with a separate seep water, and closely followed standard methods (USEPA 1993) by exposing the fish for 96 hours to creek water from locations collected above the seep, seep water, and water at the mixing zone of the seep with Whitewood Creek. A total of 15 waters (5 sites × 3 locations/site) were tested plus a reference water (described below). Waters from the seeps and mixing zones were collected using polyethylene beakers, and composited in a 4-L polyethylene bottle. This composite water sample was subsampled for water quality analyses, renewal of the fish toxicity test, and analysis of inorganic element concentrations (described below). Water upstream of seeps was collected by grab sampling in the middle of the channel.

A reference water was used as the control treatment with the tests of creek, seep, and mixing zone waters, and was a blended water that simulated Whitewood Creek water (hardness, 668 mg/L as $CaCO_3$). The reference water was blended by diluting well water from Gavins Point National Fish Hatchery, Yankton, SD, with deionized water, which is an approved method to formulate water to a hardness higher than standard test waters given in USEPA (1993). The reference water was blended at the Yankton ERS, used in fish culture, and transported to the Rapid City test facility in 20-L polyethylene carboys.

Three replicate exposure beakers, termed a, b, and c, were used with each test water, and ten fish were exposed in each test beaker. Fathead minnows were exposed in a 1-L beaker containing 600 ml of test water. The beakers were calibrated and marked at the 300 ml level. Fish were randomly stocked into test vessels within 60 minutes after the initial addition of test

water to the exposure beakers. Test waters were full strength, i.e., no dilutions, and were introduced to exposure beakers using a polyethylene graduated cylinder. The test water was renewed with fresh test water after 24, 48, and 72 hours of exposure. Half the test water (300 ml) in each exposure beaker was removed by siphoning prior to addition of fresh water. Renewal of test water was by slowly pouring test water from a graduated cylinder along the side of the exposure beaker, which minimized disturbance of fish during the water renewal process. Exposure beakers were not cleaned during the tests.

Fish were not fed for 1 day before the test, but were fed after 48 hours of exposure as described in USEPA (1993). For the feeding at 48 hours of exposure, 2 hours before water renewals, 50 ml of nauplii of brine shrimp were removed from their culture jar and sieved through a 80 μm mesh plankton cloth, then reconstituted in 50 ml of reference water. Fish in each exposure beaker were fed 0.2 ml of brine shrimp solution.

Fish were tested at room temperature, which was maintained as close to 20°C as possible, with test vessels held on a laboratory table. Test vessels were randomly assigned to their position on the table using a random numbers table. Temperature in the reference test vessel was monitored daily with a precision-grade mercury thermometer.

Survival of fish in each test vessel was recorded at the end of each 24-hour exposure period. The effect criterion for fish was death, which was defined as the lack of gill movement. Abnormal behavior of fish was recorded at the end of each 24-hour exposure period. After the 96-hour exposure, three groups of fish (group 1 had 9 fish, group 2 had 10 fish, and group 3 had 10 fish) in the reference treatment were measured for total length, and two composite samples of fish (due to their small size; composite 1 had 8 fish and composite 2 had 9 fish) were measured for weight.

General water sample collection

Water samples were collected by personnel wearing waders and polyethylene gloves, entering the stream downstream of the sampling location and proceeding upstream to the sampling point, and rinsing the gloves in ambient water before collecting the sample. The composite water samples were collected in 4-L polyethylene bottes that were acid-cleaned prior to transport to the sampling site, and conditioned with sample water before the composite sample was collected. Water samples collected for total mercury analysis were composited directly in the specially-prepared 500-ml teflon bottles supplied by the analytical facility at USGS, Water Resources Division, Wisconsin District Mercury Laboratory (WDML), Middleton, WI.

Decontamination procedures

During laboratory and field sampling and measurement activities, disposable equipment was used when possible. However, some equipment was used repeatedly. Reusable equipment was decontaminated to minimize cross-contamination errors. This equipment was conditioned before sampling at each sample site. Reusable equipment in the laboratory was decontaminated before each use. In the field, decontamination involved rinsing equipment three times with deionized water, then conditioning in ambient water. In the laboratory, decontamination involved rinsing with 10% HCl followed by three rinses with deionized water.

Water quality characteristics

Test waters were analyzed in-situ for general water quality characteristics using standard methods (APHA et al. 1995) for the following characteristics: conductivity using a Yellow

Springs Instrument (YSI) model 33 meter, and pH and temperature using an Orion model 290A meter. Upon arrival of the test water at the test facility, the water was allowed to approach ambient room temperature, and then the following water quality characteristics were measured using standard methods (APHA et al. 1995): alkalinity, hardness, calcium, and pH. Magnesium concentrations were calculated by subtracting the calcium concentration from the hardness concentration.

Subsamples of the composite water were collected in 250-ml polyethylene bottles for transport to the Yankton ERS for analysis of sulfate and chloride (200-ml sample stored at 4°C, no preservative used) by standard methods (APHA et al. 1995). A subsample of the composite water was collected in 125-ml polyethylene bottles for transport to the Yankton ERS for analysis of ammonia (125-ml sample preserved with 0.5 ml concentrated H_2SO_4 to reduce pH below 2) by ion-selective electrode and following the procedure for low concentration measurements of the manufacturer (Orion 1990). Water samples for sulfate and chloride analysis were transported in a cooler with wet ice, and for ammonia at ambient temperature.

Dissolved oxygen concentrations, pH, and temperature were measured with meters (described above) directly in one replicate of the reference water and one replicate of each test water before and after water renewal.

Test chemical analysis of test water and fish diet

Water samples were collected daily for analysis by inductively-coupled argon plasma (ICP) spectrophotometry for concentrations of beryllium, cadmium, chromium, copper, nickel, lead, and zinc. A 125-ml sample of each test water was collected in an acid-cleaned polyethylene

bottle from the 4-L composited samples. Samples of reference water were collected on days 0 and 3, and a reagent blank was collected on days 1 and 3 for analysis of the same inorganic elements. The reagent blank consisted of deionized water from the mobile laboratory combined with the acid preservative. Replicate water samples of two test waters were collected on days 1 and 2. All water samples were acidified with ultrapure HNO_3 and stored at room temperature until analyzed. Analyses were conducted by the Environmental Trace Substances Laboratory (ETSL), University of Missouri, Rolla, MO, and incorporated appropriate quality assurance/quality control (QA/QC) procedures such as standardizing equipment with certified reference material, determination of limit of detection, analysis of reagent blanks, spiked samples, duplicate field samples, duplicate analysis samples, and certified reference materials. Analysis of inorganic elements by ICP was based on USEPA method 6010 (USEPA 1983).

Water samples were collected daily for analysis of arsenic and selenium concentrations by atomic absorption spectrophotometry. For each analysis, one 100-ml sample of each test water was collected in an acid-cleaned polyethylene bottle from the 4-L composited sample. Samples of reference water were collected on days 0 and 3, and a reagent blank (described above) was collected on days 1 and 3 for analysis of arsenic and selenium concentrations. Replicate water samples of two test waters were collected on days 1 and 2. All water samples were acidified with ultrapure HCl and stored at room temperature until analyzed. Analysis were conducted by the ETSL, and incorporated appropriate QA/QC procedures as described above. Analysis of arsenic concentrations was based on USEPA method 7061, and analysis of selenium concentrations was based on USEPA method 7000 (USEPA 1983).

Water samples were collected daily for analysis of total mercury concentrations by cold

vapor/atomic absorption spectrophotometry using low concentration techniques (Olson and DeWild 1999). One 500-ml sample was collected by grab sampling in a telfon bottle (supplied by WDML) from creek water above the seep, seep water, and water at the mixing zone of the seep with the creek. Samples of reference water were collected on days 0 and 3, and a reagent blank (deionized water supplied by WDML preserved with 50% HCl supplied by WDML) was collected on days 1 and 3 for analysis of total mercury. Replicate water samples of two test waters were collected on days 1 and 2. All water samples were acidified with 50% HCl supplied by WDML and stored at room temperature until analyzed. Analysis were conducted by the WDML, and incorporated appropriate QA/QC procedures such as analysis of reagent blanks, duplicate field samples, duplicate analysis samples, and certified reference standards. All samples were analyzed in duplicate, and if the difference between the samples was greater than 10%, the samples were reanalyzed.

A 30-g sample of nauplii of brine shrimp was collected by compositing samples from several culture jars of brine shrimp, stored frozen in Whirl-Pak bags, and analyzed by ICP for concentrations of aluminum, arsenic, boron, barium, beryllium, cadmium, chromium, copper, iron, magnesium, manganese, molybdenum, nickel, lead, selenium, strontium, vanadium, and zinc. Moisture content in the brine shrimp sample was also determined. Analyses were conducted by the ETSL, and incorporated appropriate QA/QC procedures such as standardizing equipment with certified reference material, analysis of spiked samples, duplicate analysis samples, and certified reference materials. Analysis of inorganic elements in brine shrimp was based on USEPA method 6010 (USEPA 1983).

Proximate analysis of brine shrimp

One sample of nauplii of brine shrimp (31 g wet weight) was collected for proximate analysis to determine protein, fat, carbohydrate, crude fiber, and moisture content. A second sample (43 g wet weight) was collected to determine gross energy content measured by bomb calorimetry. Samples were stored frozen and shipped packed with dry ice to the CN Laboratories, Courtland, MN, for analyses. Proximate analysis of brine shrimp was based on standard methods (AOAC 1990, 1995) and gross energy by instrument manuals (Parr Instruments, Moline, IL).

Statistics

Data were analyzed using computer programs from Statistical Analysis System Institute, Inc. (SAS 1990). Analysis of variance testing was used for comparisons of water quality characteristics and inorganic element concentrations among various test waters. When significant differences were observed, means were compared by the Bonferroni (Dunn) multiple mean comparison test (Snedecor and Cochran 1967). Correlation analysis of the means with standard deviation and variance were conducted to determine if transformations were needed to meet the assumptions of normality and homogeneity of variance (M. Ellersieck, University of Missouri, Columbia, personal communication). The data for inorganic element concentrations in water were log10 transformed prior to analysis of variance.

RESULTS

Analytical quality control

For ICP analyses of water, the estimated limit of detection (LOD) for inorganic elements was as follows: beryllium 0.2 μg/L, cadmium 0.2 μg/L, chromium 0.8 μg/L, copper 0.9 μg/L, nickel 3 μg/L, lead 0.2 μg/L, and zinc 2 μg/L. The procedure blanks (n=4) and field blanks (n=2) had background concentrations less than or close to the LOD, which indicated no contamination from reagents or sample handling. Spiked samples analyzed for matrix suppression or enhancement had recoveries ranging from 97 to 102% (mean 99, standard error [SE] 1.2%, n=4), which indicated no interference from other water components. The percent relative standard deviation for duplicate sample preparation and analysis ranged from 0 to 5.6% (mean 2.6, SE 1.3%, n=4), which indicated consistent sample handling during preparation, digestion, and analysis. The percent relative standard deviation of duplicate field samples ranged from 0 to 28.1% (mean 11.9, SE 5.9%, n=4), which indicated consistent sample collection in the field. Recoveries of inorganic elements in Environmental Resources Associates reference water ERA9969TM (n=4) was within the recommended range, which indicated the digestion and analysis procedure accurately measured inorganic element concentrations.

For arsenic analyses of water, the LOD was <0.4 μg/L. The procedure blanks (n=4) and field blanks (n=2) had background concentrations less than the LOD. Spiked samples analyzed for matrix suppression or enhancement had arsenic recoveries ranging from 100 to 115% (mean 110, SE 3.6%, n=4). The percent relative standard deviation for duplicate sample preparation and analysis ranged from 1.3 to 5.9% (mean 3.3, SE 1.2%, n=4). The percent relative standard deviation of duplicate field samples ranged from 2.5 to 17.3% (mean 7.4, SE 3.4%, n=4).

Recoveries of arsenic in Environmental Resources Associates reference water ERA9969TM (n=4) was within the recommended range.

For selenium analyses of water, the LOD was 1 μg/L. The procedure blanks (n=4) and field blanks (n=2) had background concentrations less than the LOD. Spiked samples analyzed for matrix suppression or enhancement had selenium recoveries ranging from 88 to 97% (mean 92, SE 2%, n=4). The percent relative standard deviation for duplicate sample preparation and analysis ranged from 0 to 67% (mean 50, SE 17%, n=4); however, the four samples used in the duplicate analysis contained near LOD concentrations and therefore the percent relative standard deviation were unrealistically high. The percent relative standard deviation of duplicate field samples ranged from 0 to 67% (mean 17, SE 17%, n=4); however, the four samples used in the duplicate analysis contained near LOD concentrations and therefore the percent relative standard deviation were unrealistically high. Recoveries of selenium in Environmental Resources Associates reference water ERA9969TM (n=4) was within the recommended range.

For total mercury analyses of water, the LOD was 0.07 ng/L. The field blanks (n=2) had background concentrations less than the field water samples. Two samples of deionized water supplied by WDML and used as procedure blanks contained a mean of 0.33 ng/L (SE 0.06 ng/L). Spiked samples analyzed for matrix suppression or enhancement had mercury recoveries ranging from 89 to 108% (mean 99, SE 3%, n=7). The average difference between analysis of duplicate analysis of all samples was 4.09%. The percent relative standard deviation of duplicate field samples ranged from 0 to 57.4% (mean 23.2, SE 12.5%, n=4). Recoveries of total mercury in National Institute of Standards and Technology certified standard 3133 (water, n=14) was within the recommended range.

For ICP analysis of brine shrimp, the estimated LOD for inorganic elements was as follows: aluminum 5 μg/g, arsenic 5 μg/g, boron 0.4 μg/g, barium 0.08 μg/g, beryllium 0.2 μg/g, cadmium 0.2 μg/g, chromium 0.8 μg/g, copper 0.6 μg/g, iron 1 μg/g, magnesium 0.5 μg/g, manganese 0.2 μg/g, molybdenum 0.4 μg/g, nickel 0.9 μg/g, lead 2 μg/g, selenium 4 μg/g, strontium 0.05 μg/g, vanadium 0.4 μg/g, and zinc 0.8 μg/g. The procedure blank (n=1) had a background concentration less than the LOD. A spiked sample analyzed for matrix suppression or enhancement had an average recovery of 102% (the recoveries for the individual 18 elements ranged from 96 to 138%). The percent relative standard deviation for duplicate sample preparation and analysis was 1.5% (the percent relative standard deviations for the individual 18 elements ranged from 0 to 12.9%). Recoveries of 10 certified trace elements in dogfish muscle (reference material DORM2, n=1) from the National Research Council of Canada was within the recommended range for each element except for cadmium, which was below the LOD. The percent moisture in brine shrimp was 86.0% (percent relative standard deviation was 0.1%, n=2).

Water quality

Water quality characteristics measured in Whitewood Creek above the five seeps were not significantly different among the five seeps (Table 1, Appendix 1). For seep water at the five seep sites, pH was not significantly different among seeps, but dissolved oxygen was significantly higher at seep 32L compared to the other four seeps (Table 1). Conductivity in seep waters was significantly different among the seeps, and was highest at the most upstream seep (23R) and lowest at the most downstream seep (33L) (Table 1).

For waters collected in Whitewood Creek above the five seeps and measured in the

Table 1. Mean (standard error in parentheses; n=4) water quality characteristics measured on site in Whitewood Creek, South Dakota. For each measure and within a seep, locations with the same lower case letter are not significantly different (P=0.05). For each measure and the "Seep" location, means with the same upper case letter are not significantly different (P=0.05).

Seep site	Location	Water temperature (°C)	pH	Corrected conductivity (μmhos/cm)	Dissolved oxygen (mg/L)
23R	Above seep	3.5 (1.2)	8.3a (0.4)	1,170a (118)	13.4c (0.4)
	Seep	5.4 (0.3)	6.9aX (0.4)	4,310cZ (94)	4.4aX (0.6)
	Mixing zone	3.6 (0.6)	7.0a (0.4)	3,160b (247)	7.6b (0.8)
23L	Above seep	3.5 (1.1)	8.4a (0.5)	1,180a (128)	13.3b (0.4)
	Seep	4.8 (0.6)	7.1aX (0.4)	3,740bY (106)	6.0aX (1.3)
	Mixing zone	3.9 (1.0)	8.1a (0.4)	1,510a (101)	12.7b (0.5)
31L	Above seep	3.0 (0.9)	8.3a (0.5)	1,200a (124)	13.4b (0.2)
	Seep	7.6 (0.3)	6.8aX (0.4)	3,020cX (44)	4.0aX (0.5)
	Mixing zone	3.7 (1.0)	7.3a (0.3)	1,620b (24)	11.6b (0.6)
32L	Above seep	2.3 (0.7)	8.7a (0.3)	1,210a (131)	13.6b (0.2)
	Seep	2.8 (0.7)	8.1aX (0.3)	2,740bWX (85)	12.4aY (0.2)
	Mixing zone	2.2 (0.7)	8.8a (0.3)	1,210a (119)	13.6b (0.2)

Table 1. Continued.

Seep site	Location	Water temperature (°C)	pH	Corrected conductivity (μmhos/cm)	Dissolved oxygen (mg/L)
33L	Above seep	1.8 (0.7)	9.0a (0.4)	1,240a (126)	13.7b (0.2)
	Seep	3.6 (0.6)	7.7aX (0.4)	2,590bW (100)	5.2aX (0.5)
	Mixing zone	2.9 (0.8)	8.8a (0.3)	1,370a (53)	13.3b (0.2)

laboratory, hardness was not significantly different among the five seep sites and the reference water (Table 2, Appendix 2). For other water quality characteristics, waters above the seeps were generally similar and not significantly different among the collection points, but they were significantly different from the reference water. Water quality characteristics of the seep waters such as hardness, calcium, magnesium, and sulfate was generally highest at the most upstream seep (23R) and lowest at the most downstream seep (33L). Ammonia was elevated at seep 23L, which was adjacent to an active sheep pasture, and at seep 31L, which was adjacent to an active cattle pasture. Although not significantly different among "above seep" locations, there was a trend for the lowest hardness and calcium at the most upstream site (23R) and the highest at the most downstream (33L), which was probably a result of very hard water entering the stream from the seeps used in the present study and most likely from the other 48 seeps examined in a screening study previously conducted by the USFWS and SDGFP, but not used in the present study.

Inorganic elements

There was no difference in the inorganic element concentrations among waters collected from the creek above the seeps at five seep sites including the reference water (Table 3, Appendix 3). However, there were significant differences among seep waters in concentrations of arsenic and nickel (Table 3). The highest arsenic concentration was at seep 33L and lowest at seep 23R, whereas the highest nickel concentration was at seep 31L and the lowest at seep 33L.

The five seeps each had one or more inorganic elements at higher concentrations than in the creek (Table 3). Arsenic concentrations in water from seeps 23R, 23L, 31L, and 33L were

Table 2. Mean (standard error in parentheses, n=4 except where noted in brackets) water quality characteristics of water collected from various sites in Whitewood Creek, South Dakota, and measured in the laboratory. For each measure and within a Seep site, means with the same lower case letter are not significantly different (P=0.05). For each measure and within the "Above seep" or "Seep" locations, means with the same upper case letter are not significantly different (P=0.05).

Seep site	Location	Hardness (mg/L as $CaCO_3$)	Ca (mg/L)	Mg (mg/L)	pH	Ammonia (mg/L as N)	Un-ionized ammonia (mg/L as N)	Alkalinity (mg/L as $CaCO_3$)	Cl (mg/L)	SO_4 (mg/L)
23R	Above seep	635aX (6)	146aX (2)	66aY (1)	8.0cXY (0)	0.01 (0)[2]	0	185aY (1)	22bY (0)	514aX (6)
	Seep	2,970cP (15)	560cP (1)	380cN (3)	6.9aM N (0)	<0.1[1]	-[2]	467cP (1)	19aM (0)	2,831cP (29)
	Mixing zone	2,170b (112)	417b (20)	274b (15)	7.0b (0)	<0.1	-	359b (14)	18a (0)	2,059b (129)
23L	Above seep	635aX (6)	146aX (2)	66aY (1)	8.0cXY (0)	<0.1	-	184aY (1)	22aY (0)	511aX (13)
	Seep	2,565bO (84)	398cN (6)	382bN (24)	7.0aNO (0)	0.22bX (0.04)	0.002aX (0.002)	336bM (11)	56bP (1)	2,462bO (98)
	Mixing zone	1,020a (144)	196b (19)	129a (24)	7.7b (0.1)	0.08aN (0.03)	0.004a (0.004)	210a (10)	26a (2)	883a (132)
31L	Above seep	640aX (7)	148aX (2)	66aY (1)	8.0cX (0)	0.02aX (0)[2]	0.002X (0.002)[2]	185aY (1)	22bY (0)	512aX (12)
	Seep	2,055cN (5)	432cO (1)	237cM (1)	6.8aM (0)	0.11bM (0.02)	0	410cO (2)	18aM (0)	1,856cN (37)
	Mixing zone	875b (50)	196b (10)	93b (6)	7.2b (0.1)	0.02c (0.01)	0	218b (7)	21b (1)	724b (51)

Table 2. Continued.

Seep site	Location	Hardness (mg/L as $CaCO_3$)	Ca (mg/L)	Mg (mg/L)	pH	Ammonia (mg/L as N)	Un-ionized ammonia (mg/L as N)	Alkalinity (mg/L as $CaCO_3$)	Cl (mg/L)	SO_4 (mg/L)
32L	Above seep	645aX (6)	150aX (1)	66aY (1)	8.0aX (0)	0.01 -[1]	-	188aY (1)	22aY (0)	513aX (14)
	Seep	1,785bM (5)	391bN (2)	196bM (0)	7.6bP (0.1)	<0.01	-	366bN (1)	23bN (0)	1,548bM (21)
	Mixing zone	672a (11)	156a (2)	69a (2)	8.0a (0)	0.01 -[1]	0 -[1]	192a (2)	22ab (0)	548a (13)
33L	Above seep	649aX (5)	150aX (1)	67aY (0)	8.0aX (0)	0.01aX (0)[2]	0.002bX (0)[2]	188aY (1)	21aY (1)	491aX (16)
	Seep	1,620bM (0)	334bM (1)	191bM (1)	7.1bO (0)	0.04aM (0.01)	0	378bN (3)	26bO (0)	1,410bM (14)
	Mixing zone	732a (66)	165a (12)	78a (9)	7.9a (0.1)	0.02a (0)[2]	0.001a (0)[2]	205a (11)	22a (0)	581a (58)
Reference		630X (2)	185Y (1)	40X (0)	8.2Y (0)	0.04X (0.01)	0.002X (0)	150X (2)	14X (0)	547X (5)

[1]<: less than detection limit of method.
[2]-: not calculated.

Table 3. Mean (standard error, number of analyses greater than the limit of detection in brackets) concentrations of inorganic elements (μg/L) in water collected from various sites in Whitewood Creek, South Dakota. For each element and within a seep site, means with the same lower case letter are not significantly different (P=0.05). For each element and the "Above seep" or "Seep" location, means with the same upper case letter are not significantly different (P=0.05).

Seep site	Location	Inorganic element									
		As	Se	Hg	Be	Cd	Cr	Cu	Pb	Ni	Zn
23R	Above seep	32a (1) [4]	2 (0) [4]	0.0196b (0.0103) [4]	0.8 (-) [1]	<0.2[1]	2 (0) [3]	1.7 (0.4) [3]	0.4 (-) [1]	6 (-) [1]	4 (-) [1]
	Seep	89cN (2) [4]	1 (-) [1]	0.0011a (0.0006) [4]	0.3 (0.1) [3]	0.4 (-) [1]	2 (0) [4]	1.3 (0.3) [3]	0.6 (-) [1]	18OP (3) [4]	17 (3) [3]
	Mixing zone	43b (2) [4]	<1	0.0023a (0.0003) [4]	0.8 (-) [1]	0.8 (-) [1]	2 (0) [3]	1.5 (0.5) [2]	0.4 (0.2) [2]	12 (2) [4]	9 (1) [3]
23L	Above seep	33a (0) [4]	1 (0) [2]	0.0107b (0.0004) [4]	0.3 (-) [1]	<0.2	2 (-) [1]	2.1b (0.1) [4]	0.3 (0.2) [2]	4a (0) [2]	3 (-) [1]
	Seep	290bO (67) [4]	<1	0.0011a (0.0004) [4]	0.4 (-) [1]	0.4 (0) [2]	1 (-) [1]	1.0a (0) [2]	0.2 (-) [1]	12bN (1) [4]	12 (2) [4]
	Mixing zone	70a (15) [4]	1 (-) [1]	0.0145b (0.0045) [4]	<0.2	0.3 (-) [1]	4 (-) [1]	1.9c (0.1) [4]	0.4 (0) [2]	5a (0) [4]	8 (2) [2]

Table 3. Continued.

Seep site	Location	Inorganic element									
		As	Se	Hg	Be	Cd	Cr	Cu	Pb	Ni	Zn
31L	Above seep	31a (0) [4]	1a (0) [3]	0.0168b (0.0024) [4]	0.3 (-) [1]	0.2 (-) [1]	1 (-) [1]	3.0 (1.3) [4]	0.4 (0.2) [2]	4a (0) [3]	7 (-) [1]
	Seep	399cOP (9) [4]	<1	0.0008a (0.0004) [4]	0.4 (-) [1]	<0.2	1 (-) [1]	0.9 (0.1) [2]	0.4 (-) [1]	24cP (1) [4]	10 (0) [4]
	Mixing zone	100b (24) [4]	2b (-) [1]	0.0123b (0.0026) [4]	<0.2	<0.2	2 (-) [1]	2.0 (0) [4]	0.4 (0.1) [3]	8b (1) [4]	7 (2) [2]
32L	Above seep	31 (1) [4]	1 (0) [3]	0.0154 (0.0012) [4]	<0.2	<0.2	2 (0) [2]	2.5 (0.5) [3]	0.5 (0.2) [3]	4a (1) [4]	27 (-) [1]
	Seep	27M (5) [4]	1 (0) [2]	0.0097 (0.0051) [4]	0.5 (-) [1]	<0.2	2 (0) [2]	1.4 (0.6) [2]	0.6 (0.2) [2]	14bON (1) [4]	17 (4) [3]
	Mixing zone	30 (1) [4]	1 (0) [3]	0.0185 (0.0017) [4]	0.6 (-) [1]	0.4 (-) [1]	2 (1) [3]	2.0 (0) [3]	0.4 (0.1) [4]	5a (1) [4]	8 (2) [2]

Table 3. Continued.

Seep site	Location	Inorganic element									
		As	Se	Hg	Be	Cd	Cr	Cu	Pb	Ni	Zn
33L	Above seep	33a (2) [4]	1 (0) [3]	0.0149b (0.0020) [4]	0.4 (0) [4]	0.3 (-) [1]	3 (-) [1]	1.9 (0.1) [3]	0.5 (0) [2]	4 (0) [4]	6 (2) [2]
	Seep	787bP (62) [4]	<1	0.0005a (0.0004) [4]	0.6 (0) [2]	0.3 (0) [2]	1 (0) [3]	1.4 (0.6) [2]	0.3 (-) [1]	5M (0) [4]	11 (1) [3]
	Mixing zone	55a (22) [4]	1 (0) [4]	0.0216b (0.0062) [4]	0.5 (0.2) [2]	0.2 (0) [2]	4 (3) [2]	2.6 (0.4) [4]	1.2 (0.6) [4]	6 (1) [4]	21 (14) [2]
Reference		0 (0) [2]	1 (0) [2]	0.0106 (0.0008) [2]	0.7 (-) [1]	<0.2	2 (0) [2]	1.0 (-) [1]	0.3 (-) [1]	<3	10 (-) [1]

[1]<: Less than limit of detection.

significantly higher than in creek water collected from above the respective seep. Nickel concentrations in water from seeps 23L, 31L, and 32L were significantly higher than in creek water collected above the respective seep. On the contrary, mercury concentrations in water from seeps 23R, 23L, 31L, and 33L, and copper concentrations in water from seep 23L, were significantly lower than creek water collected above the respective seep. There were no differences in concentrations of selenium, beryllium, cadmium, chromium, lead, and zinc among waters collected in the creek above the seep, seep water, or the mixing zone of the seep with the creek at the five seeps.

Brine shrimp

Proximate analysis of brine shrimp reported the following results: 0.9% fiber, 1.0% ash, 6.7% protein, 1.5% fat, 89.8% moisture, and 544 calories/g energy (wet weight; 5.32 kcal/g dry weight based on 89.78% moisture). The inorganic element concentrations in brine shrimp were as follows: aluminum 6 μg/g, arsenic 10 μg/g, boron 8.3 μg/g, barium 0.62 μg/g, beryllium <0.2 μg/g, cadmium <0.2 μg/g, chromium <0.8 μg/g, copper 8.6 μg/g, iron 206 μg/g, magnesium 3,140 μg/g, manganese 17 μg/g, molybdenum <0.4 μg/g, nickel <0.9 μg/g, lead <2 μg/g, selenium <4 μg/g, strontium 24 μg/g, vanadium <0.4 μg/g, and zinc 100 μg/g.

Test procedure and larvae survival

The stock of fathead minnows used in this study were routinely tested at Aquatic Bio Systems to determine if good quality fish were produced by the facility. The facility used standard USEPA methods (USEPA 1994) and KCl as a reference toxicant. Their report showed that the 48-hour and 96-hour toxicity data for the offspring produced in February 1999 were

similar to offspring produced during the period March 1998 through February 1999.

Daily renewal of water during the test caused little change in dissolved oxygen concentrations, pH, and water temperature in the exposure beakers (Appendix 4). The mean water quality characteristics were as follows: dissolved oxygen concentration before renewal was 7.1 mg/L (SE 0 mg/L, n=64) and after was 7.6 mg/L (SE 0.1 mg/L, n=48), pH before was 8.0 (SE 0.1, n=64) and after was 7.8 (SE 0.2, n=48), and water temperature before was 20.4 °C (SE 0.4 °C, n=64) and after was 20.3 °C (SE 0.3 °C, n=48).

There was no significant reduction in survival of fathead minnow exposed to seep water (≥87%) compared to fish survival in the reference water (97%) or in the respective creek water collected above the seep (≥97%) or the mixing zone of the seep with the creek (≥87%; Table 4, Appendix 5). The highest mortality of fish (40%) occurred in replicate c of seep water from seep 23R. Some fish exposed to seep water or mixing zone water had a orangish precipitate on their body surface (Appendix 5). For fish in the reference water treatment, mean total length was 5.6 mm and weight was 0.0006 g.

DISCUSSION

Current national water quality criteria for most inorganic pollutants consists of two numbers, the Criterion Continuous Concentration and the Criterion Maximum Concentration (Stephan et al. 1985). The Criterion Continuous Concentration is the 4-day average concentration that should not be exceeded more than once every 3 years, and was derived primarily from chronic toxicity data. The Criterion Maximum Concentration is the 1-hour average concentrations that should not be exceeded more than once every 3 years, and was derived from acute toxicity data, i.e., 96-hour LC50s. These two values are generally derived

Table 4. Mean (standard error in parentheses, n=3) survival of fathead minnow exposed to waters collected from various sites in Whitewood Creek, South Dakota.

Seep site	Location	Exposure period (hour)			
		24	48	72	96
23R	Above seep	100 (0)	100 (0)	100 (0)	100 (0)
	Seep	93 (7)	90 (10)	90 (10)	87 (13)
	Mixing zone	97 (3)	97 (3)	97 (3)	87 (3)
23L	Above seep	100 (0)	100 (0)	100 (0)	97 (3)
	Seep	97 (3)	97 (3)	97 (3)	97 (3)
	Mixing zone	100 (0)	97 (3)	97 (3)	97 (3)
31L	Above seep	100 (0)	100 (0)	100 (0)	100 (0)
	Seep	97 (3)	97 (3)	97 (3)	90 (6)
	Mixing zone	97 (3)	97 (3)	97 (3)	97 (3)
32L	Above seep	100 (0)	100 (0)	100 (0)	100 (0)
	Seep	100 (0)	100 (0)	100 (0)	100 (0)
	Mixing zone	100 (0)	100 (0)	100 (0)	100 (0)
33L	Above seep	100 (0)	100 (0)	100 (0)	100 (0)
	Seep	93 (3)	93 (3)	93 (3)	93 (3)
	Mixing zone	97 (3)	97 (3)	97 (3)	93 (3)
Reference		97 (3)	97 (3)	97 (3)	97 (3)

from tests with a variety of aquatic organisms including aquatic invertebrates and several species of fish, and are designed to protect 95% of the species. The acute-derived Criterion Maximum Concentration for the inorganic elements measured in seep water were as follows: arsenic 340 μg/L, beryllium 130 μg/L[1], cadmium 19 μg/L (based on hardness of 400 mg/L as $CaCO_3$, equation $e^{(1.128[\ln \text{hardness}]-3.6867)}$, and freshwater conversion factor of 1.138672-(ln hardness) (0.041838)), and chromium (IV) 16 μg/L (based on freshwater conversion factor of 0.982), copper 50 μg/L (based on hardness of 400 mg/L as $CaCO_3$, equation $e^{(0.9422[\ln \text{hardness}]-1.700)}$, and freshwater converstion factor of 0.960), lead 280 μg/L (based on hardness of 400 mg/L as $CaCO_3$, equation $e^{(1.273[\ln \text{hardness}]-1.460)}$, and freshwater conversion factor of 1.46203-(ln hardness) (0.145712)), nickel 1,510 μg/L (based on hardness of 400 mg/L as $CaCO_3$, equation $e^{(0.8460[\ln \text{hardness}]+2.255)}$, and freshwater conversion factor of 0.998), mercury 1.4 μg/L (based on a freshwater conversion factor of 0.85), selenium 20μg/L, and zinc 380 μg/L (based on hardness of 400 mg/L as $CaCO_3$, equation $e^{(0.8473[\ln \text{hardness}]+0.884)}$, and freshwater conversion factor of 0.978) (USEPA 1986, 1987, 1995, 1998, 1999). The hardness value of 400 mg/L was used in the above calculations because it is generally the upper limit of the water qualities used in the acute toxicity database used in establishing national water quality criteria. The concentrations of inorganic elements in seep water were 8-fold less for chromium and 10-fold less for beryllium, cadmium, copper, mercury, nickel, lead, selenium, and zinc than the Criterion Maximum Concentration for those elements. However, arsenic concentrations in seep water were about 2-fold higher than the

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[1]Footnote: Insufficient data to develop criterion. Value presented is the lowest observed effect level (USEPA 1986).

Criterion Maximum Concentration. This comparison suggested that there might have been some acute toxicity from arsenic in seep water in the test, but probably not from the other elements.

The lack of acute toxicity to fathead minnow exposed to undiluted seep water in the present study was probably due to the low inorganic element concentrations in seep water compared to their individual toxicity values to fish. Inorganic element concentrations in seep water in the present study were substantially below those in the published literature for the individual elements tested specifically with fathead minnow (Table 5). The toxic unit (TU) concept can be used to assess the toxic components in a mixture (Sprague 1970). The theoretical toxic contribution of each component to the mixture is expressed as a TU. A TU is defined as the concentration of a chemical in a mixture divided by its individual toxic concentration for the endpoint measured. Mixtures with summed TU values close to one are considered to be additive in toxicity. The summed TU was 0.07 for seep 33L, which had the highest TU value of the seeps tested. This low value suggested little, if any, toxicity would be expected from this seep water or from the other seep waters. The TUs derived in Table 5 should be considered as only approximations, because the toxicity values were not directly comparable due to differences in test conditions (e.g., age, size, and quality of test organisms, water quality, test chambers) used in the present study and those cited.

The lack of mortality in the present study was supported by the results of Buhl (1998). He tested young fathead minnow with a mixture of 11 elements in the proposed Criterion Maximum Concentrations that was suggested as a site-specific water quality criteria for selected streams in the northern Black Hills of South Dakota. For 9 of the 11 elements, the proposed site-specific criterion concentrations exceeded current national water quality criteria derived by

Table 5. Comparisons of inorganic concentrations in water from seep 33L tested with fathead minnow to published individual acute toxicity values for fathead minnow.

Inorganic element	Concentration in seep (μg/L)	Individual acute toxicity value and test conditions				Toxic unit[2]
		96-hour LC50 (μg/L)	Age, size, or life stage	Hardness (mg/L as CaCO3)	Reference[1]	
Arsenic (assume As^{+3})	787	12,600	30-day old (0.15 g)	44	1	0.06
Beryllium	0.6	3,250	3-month old (0.14 g)	140	2	<0.01
Cadmium	0.3	90	14-30-day old	200	3	<0.01
Chromium (assume Cr^{+6})	1	43,300	30-day old (0.15 g)	44	1	<0.01
Copper	1.4	490	6-week old (22 mm)	202	4	<0.01
Lead	0.3	125,500[3]	Not reported	160	5	<0.01
Mercury	0.005	168	Juvenile (3-month old)	46	6	<0.01
Nickel	5	27,900[4]	Immatures	207	7	<0.01
Selenium (assume Se^{+4})	<1	1,700	30-day old	51	8	<0.01

Table 5. Continued.

Inorganic element	Concentration in seep (μg/L)	Individual acute toxicity value and test conditions				Toxic unit[2]
		96-hour LC50 (μg/L)	Age, size, or life stage	Hardness (mg/L as CaCO3)	Reference[1]	
Zinc	11	870	1-day old	174-198	9	0.01

[1]Reference: 1, Spehar and Fiandt (1986); 2, Cardwell et al. (1976); 3, Hall et al. (1986); 4, Pickering et al. (1977); 5, USEPA (1984); 6, Snarski and Olson (1982); 7, Pickering 1974; 8, USEPA (1987); 9, Pickering and Vigor (1965).
[2]Inorganic concentration in seep water ÷ its individual 96-hour LC50) value. Summed units = 0.07.
[3]96-hour LC50 value estimated from least squares regression of the logarithms of 96-hour LC50 values on natural logarithms of hardness values for tests with fathead minnow given in reference.
[4]Geometric mean of four values.

the USEPA (USEPA 1986, 1987). He reported no mortality of fish exposed to the mixture of the proposed Criterion Maximum Concentration in a 96-hour period (mixture contained 360 μg/L arsenic, 6.7 μg/L cadmium, 28 μg/L chromium, 59 μg/L copper, 59 μg/L cyanide, 484 μg/L lead, 2.4 μg/L mercury, 2,110 μg/L nickel, 41 μg/L selenium, 37 μg/L silver, and 254 μg/L zinc). These inorganic concentrations were substantially above the concentrations found in the five seeps tested in the present study. Buhl (1998) reported that testing the mixture at two times the proposed Criterion concentrations resulted in 85-90% mortality, which suggested a relatively narrow margin between the proposed Criterion concentrations and concentrations causing adverse effects in young fathead minnow.

Fathead minnow may not have been the most sensitive species to test for effects of contaminants in seep water. For example, in the water quality criteria document for arsenic (USEPA 1995), the element in the present study that seemed to be present at toxic concentrations, fathead minnow were ranking sixth in sensitivity behind an amphipod (ranked most sensitive), cladocerans (ranked 2nd to 4th), and rainbow trout (ranked 5th). Buhl (1998) also reported that a cladoceran was more sensitive than fathead minnows in the toxicity tests with a mixture of elements suggested as a site-specific water quality criteria for selected streams in the Black Hills. Because the area of Whitewood Creek where seeps were tested was more of a warm water habitat than a cold water habitat, it was more appropriate to test fathead minnow rather than rainbow trout. Likewise, the paucity of aquatic invertebrates in Whitewood Creek precluded the selection of an appropriate aquatic invertebrate for testing.

Although acute toxicity of seep waters to young fathead minnow was not demonstrated in the present study, effects from chronic, long-term waterborne, dietary, or sediment exposure on

aquatic invertebrates and fish could be occurring in Whitewood Creek, Belle Fourche River, Cheyenne River, and the Cheyenne Arm of Lake Oahe. Several investigators have reported elevated inorganic element concentrations in water, sediment, fish, and fish-eating birds collected from these areas (Walter et al. 1973, Hesse et al. 1975, Sowards et al. 1991, Ruelle et al. 1993). Preliminary information from other recent investigations of total mercury and methyl mercury in water and sediments of the Whitewood Creek-Belle Fourche-Cheyenne River system indicate that methyl mercury was present at some locations in elevated concentrations (S. Sando, USGS, written communication) that could potentially adversely affect aquatic organisms.

Others have reported that environmental stresses may adversely affect fish in the Whitewood Creek-Belle Fourche River-Cheyenne River system. For example, Eide (1999) used a necropsy-based health and condition technique to assess the health of channel catfish (*Ictalurus punctatus*) collected from the Belle Fourche River. He reported that the general population of fish seemed to be in good condition, but the most notable abnormalities were small whitish bumps on the skin of fish collected at sites in close proximity to the Whitewood Creek confluence. Although undiagnosed, these abnormalities suggested that fish might be under some stress. Cordes (1996) reported small raised ulcers on the skin of channel catfish collected from the Cheyenne River near Red Shirt, SD, were probably due to bacterial infection of attachment sites of leeches. However, he did not believe the lesions were a result of contaminants entering the river. However, Byrne (1997) concluded that an environmental stress that was probably related to contaminants caused lesions characteristic of bacterial infections on flathead chub (*Platygobio gracilis*) and channel catfish, but not on longnose dace (*Rhinichthys cataractae*), collected from the Cheyenne River.

CONCLUSIONS

Water from five seeps in Whitewood Creek was not acutely toxic to young fathead minnows. However, Whitewood Creek was being loaded with arsenic from four seeps and with nickel from three seeps. Whitewood Creek water contained higher total mercury concentrations than were found in waters coming from seeps. Additional research is needed to determine if long-term exposure to inorganic elements in water, diet, or sediments is adversely affecting aquatic organisms in Whitewood Creek, the Belle Fourche River, and the Cheyenne River.

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Appendix 1. Water quality characteristics measured in-situ in Whitewood Creek, South Dakota.

Seep site	Location	Date	Day of exposure	Water temperature (°C)	pH	Corrected conductivity (μmhos/cm)	Dissolved oxygen (mg/L)
23R	Above seep	03/02/99	0	3.4	8.03	851	13.4
		03/03/99	1	4.0	9.46	1,170	13.0
		03/04/99	2	6.2	8.23	1,250	12.6
		03/05/99	3	0.4	7.44	1,420	14.4
	Seep	03/02/99	0	5.0	6.58	4,210	4.3
		03/03/99	1	6.0	7.88	4,240	4.0
		03/04/99	2	5.7	6.78	4,590	3.4
		03/05/99	3	5.0	6.27	4,210	6.1
	Mixing zone	03/02/99	0	3.6	6.82	2,450	9.4
		03/03/99	1	4.3	8.06	3,560	6.0
		03/04/99	2	4.7	6.96	3,430	6.8
		03/05/99	3	1.9	6.30	3,220	8.3
23L	Above seep	03/02/99	0	4.9	7.86	812	13.3
		03/03/99	1	2.9	9.83	1,250	13.0
		03/04/99	2	5.5	8.31	1,280	12.4
		03/05/99	3	0.7	7.69	1,400	14.4
	Seep	03/02/99	0	4.7	6.60	3,760	5.7
		03/03/99	1	5.7	8.23	4,040	3.9
		03/04/99	2	5.6	7.01	3,570	4.6
		03/05/99	3	3.1	6.54	3,610	9.6
	Mixing zone	03/02/99	0	4.6	7.58	1,310	12.7
		03/03/99	1	4.8	9.20	1,790	11.8
		03/04/99	2	5.3	8.10	1,440	12.2
		03/05/99	3	0.8	7.59	1,490	14.2

Appendix 1. Continued.

Seep site	Location	Date	Day of exposure	Water temperature (°C)	pH	Corrected conductivity (μmhos/cm)	Dissolved oxygen (mg/L)
31L	Above seep	03/02/99	0	4.2	7.79	846	13.4
		03/03/99	1	3.8	9.64	1,260	13.2
		03/04/99	2	3.7	8.14	1,260	13.0
		03/05/99	3	0.2	7.66	1,420	14.1
	Seep	03/02/99	0	7.2	6.32	2,890	4.0
		03/03/99	1	7.1	7.78	3,040	3.8
		03/04/99	2	8.4	6.64	3,080	3.1
		03/05/99	3	7.5	6.27	3,080	5.3
	Mixing zone	03/02/99	0	5.2	6.72	1,580	10.4
		03/03/99	1	4.5	8.21	1,680	11.1
		03/04/99	2	4.5	7.19	1,640	11.7
		03/05/99	3	0.7	7.10	1,590	13.4
32L	Above seep	03/02/99	0	3.0	8.88	828	14.1
		03/03/99	1	2.3	8.68	1,320	13.6
		03/04/99	2	3.6	9.17	1,270	13.0
		03/05/99	3	0.3	7.88	1,420	13.9
	Seep	03/02/99	0	3.7	8.19	2,530	12.3
		03/03/99	1	3.0	8.04	2,670	12.4
		03/04/99	2	3.8	8.61	2,860	11.9
		03/05/99	3	0.7	7.39	2,890	13.1
	Mixing zone	03/02/99	0	3.0	8.89	862	14.0
		03/03/99	1	2.3	9.39	1,380	13.5
		03/04/99	2	3.4	9.09	1,280	13.0
		03/05/99	3	0.1	7.88	1,340	14.0

Appendix 1. Continued.

Seep site	Location	Date	Day of exposure	Water temperature (°C)	pH	Corrected conductivity (μmhos/cm)	Dissolved oxygen (mg/L)
33L	Above seep	03/02/99	0	2.7	8.91	871	14.2
		03/03/99	1	1.1	9.43	1,380	13.5
		03/04/99	2	3.1	9.66	1,290	13.1
		03/05/99	3	0.3	7.89	1,420	14.0
	Seep	03/02/99	0	3.4	7.56	2,300	6.6
		03/03/99	1	3.6	8.39	2,710	4.4
		03/04/99	2	5.3	8.11	2,730	4.3
		03/05/99	3	2.3	6.80	2,650	5.3
	Mixing zone	03/02/99	0	4.3	8.78	1,240	12.9
		03/03/99	1	2.9	9.40	1,380	13.3
		03/04/99	2	3.8	9.21	1,340	13.1
		03/05/99	3	0.6	7.84	1,500	13.9

Appendix 2. Water quality characteristics of water collected from various sites in Whitewood Creek, South Dakota, and measured in the laboratory.

Seep site	Location	Date	Day of exposure	Hardness (mg/L as $CaCO_3$)	Ca (mg/L)	Mg (mg/L)	pH	Ammonia (mg/L as N)	Un-ionized ammonia (mg/L as N)	Alkalinity (mg/L as $CaCO_3$)	Cl (mg/L)	SO_4 (mg/L)
23R	Above seep	03/02/99	0	620	140	66	8.06	<0.01[1]	-[2]	182	21	501
		03/03/99	1	640	148	66	7.98	<0.01	-	186	22	518
		03/04/99	2	650	150	67	8.09	0.01	0	186	22	529
		03/05/99	3	630	146	64	8.01	0.01	0	185	22	510
	Seep	03/02/99	0	2,930	561	372	6.88	<0.01	-	468	18	2,830
		03/03/99	1	2,960	561	379	6.88	<0.01	-	465	19	2,751
		03/04/99	2	3,000	565	386	6.90	<0.01	-	466	19	2,881
		03/05/99	3	2,980	561	384	6.82	<0.01	-	469	19	2,863
	Mixing zone	03/02/99	0	2,400	457	306	7.04	<0.01	-	387	18	2,324
		03/03/99	1	1,880	365	235	6.92	<0.01	-	323	19	1,726
		03/04/99	2	2,280	441	286	6.98	<0.01	-	370	18	2,183
		03/05/99	3	2,120	405	269	6.95	<0.01	-	355	18	2,005
23L	Above seep	03/02/99	0	620	142	64	8.04	<0.01	-	182	22	481
		03/03/99	1	640	148	66	8.00	<0.01	-	187	22	515
		03/04/99	2	650	150	67	8.10	<0.01	-	181	22	544
		03/05/99	3	630	144	66	7.98	<0.01	-	185	22	505

Appendix 2. Continued.

Seep site	Location	Date	Day of exposure	Hardness (mg/L as $CaCO_3$)	Ca (mg/L)	Mg (mg/L)	pH	Ammonia (mg/L as N)	Un-ionized ammonia (mg/L as N)	Alkalinity (mg/L as $CaCO_3$)	Cl (mg/L)	SO_4 (mg/L)
23L	Seep	03/02/99	0	2,700	393	418	6.92	0.30	0	350	58	2,503
		03/03/99	1	2,720	385	427	6.88	0.27	0.006	358	59	2,718
		03/04/99	2	2,420	413	337	7.08	0.15	0	317	54	2,266
		03/05/99	3	2,420	401	345	7.01	0.18	0	319	55	2,360
	Mixing zone	03/02/99	0	1,420	248	195	7.42	0.15	0	238	31	1,244
		03/03/99	1	1,040	200	131	7.60	0.09	0.015	213	26	917
		03/04/99	2	830	172	97	7.87	0.03	0	194	25	699
		03/05/99	3	790	164	92	7.82	0.03	0	194	24	673
31L	Above seep	03/02/99	0	620	144	63	8.03	0.02	0	181	20	485
		03/03/99	1	650	150	67	7.95	0.01	0.003	187	22	538
		03/04/99	2	650	150	67	8.09	<0.01	-	185	22	528
		03/05/99	3	640	146	67	8.00	<0.01	-	186	22	499
	Seep	03/02/99	0	2,060	433	238	6.74	0.15	0	411	18	1,788
		03/03/99	1	2,060	433	238	6.78	0.15	0.001	414	18	1,956
		03/04/99	2	2,060	433	238	6.78	0.08	0	405	19	1,862
		03/05/99	3	2,040	429	235	6.85	0.07	0	411	18	1,819

Appendix 2. Continued.

Seep site	Location	Date	Day of exposure	Hardness (mg/L as $CaCO_3$)	Ca (mg/L)	Mg (mg/L)	pH	Ammonia (mg/L as N)	Un-ionized ammonia (mg/L as N)	Alkalinity (mg/L as $CaCO_3$)	Cl (mg/L)	SO_4 (mg/L)
31L	Mixing zone	03/02/99	0	1,010	224	109	6.99	0.04	0	237	19	875
		03/03/99	1	870	196	92	7.24	0.02	0	218	21	688
		03/04/99	2	850	192	90	7.27	0.02	0	213	22	683
		03/05/99	3	770	174	82	7.46	0.01	0	206	22	651
32L	Above seep	03/02/99	0	630	148	63	8.00	0.01	-	184	20	474
		03/03/99	1	655	152	67	7.97	<0.01	-	191	22	539
		03/04/99	2	655	152	67	8.09	<0.01	-	189	22	530
		03/05/99	3	640	148	66	7.99	<0.01	-	188	22	510
	Seep	03/02/99	0	1,780	389	196	7.46	<0.01	-	365	23	1,541
		03/03/99	1	1,780	389	196	7.53	<0.01	-	365	23	1,607
		03/04/99	2	1,800	397	196	7.72	<0.01	-	369	23	1,507
		03/05/99	3	1,780	389	196	7.61	<0.01	-	367	23	1,538
	Mixing zone	03/02/99	0	650	152	66	7.97	<0.01	-	189	22	516
		03/03/99	1	700	160	73	7.90	<0.01	-	197	22	578
		03/04/99	2	660	154	67	8.07	<0.01	-	191	22	542
		03/05/99	3	680	156	71	7.98	0.01	0	190	23	556

Appendix 2. Continued.

Seep site	Location	Date	Day of exposure	Hardness (mg/L as $CaCO_3$)	Ca (mg/L)	Mg (mg/L)	pH	Ammonia (mg/L as N)	Un-ionized ammonia (mg/L as N)	Alkalinity (mg/L as $CaCO_3$)	Cl (mg/L)	SO_4 (mg/L)
33L	Above seep	03/02/99	0	640	148	66	7.97	<0.01	-	186	21	501
		03/03/99	1	660	152	68	7.98	0.01	0.002	188	18	443
		03/04/99	2	655	152	67	8.10	0.01	0.003	190	22	507
		03/05/99	3	640	148	66	8.00	<0.01	-	189	22	512
	Seep	03/02/99	0	1,620	337	189	7.06	0.04	0	385	27	1,420
		03/03/99	1	1,620	333	192	7.09	0.05	0	373	26	1,422
		03/04/99	2	1,620	333	192	7.06	0.02	0	378	26	1,369
		03/05/99	3	1,620	333	192	7.11	0.03	0	376	26	1,431
	Mixing zone	03/02/99	0	930	200	105	7.67	0.02	0.001	239	22	754
		03/03/99	1	670	154	69	7.92	<0.01	-	196	21	504
		03/04/99	2	660	154	67	8.05	0.01	0.001	192	23	526
		03/05/99	3	670	152	71	7.98	<0.01	-	192	23	542
Reference		03/02/99	0	635	186	41	8.16	0.05	0.003	154	14	536
		03/03/99	1	630	186	40	8.14	0.04	0.002	153	14	541
		03/04/99	2	625	184	40	8.24	0.02	0.002	147	14	556
		03/05/99	3	630	184	41	8.09	0.06	0.003	147	14	555

[1]<: less than detection limit of method.
[2]-: not calculated.

Appendix 3. Concentrations of inorganic elements (μg/L) in water collected from various sites in Whitewood Creek, South Dakota. Concentrations in replicate samples are given in parentheses.

Seep site	Location	Date	Day of exposure	Inorganic element									
				As	Se	Hg	Be	Cd	Cr	Cu	Pb	Ni	Zn
23R	Above seep	03/02/99	0	31	2	0.00888	<0.2[1]	<0.2	2	2.0	<0.2	<3	<9
		03/03/99	1	34	2	0.01022	<0.2	<0.2	2	<0.9	<0.2	<3	<9
		03/04/99	2	30	1	0.00872	<0.3 (0.8)	<0.2 (<0.2)	<2 (<2)	2.2 (2.0)	0.4 (0.5)	6 (6)	4 (3)
		03/05/99	3	32	2	0.05044	<0.2	<0.2	1	1.0	<0.2	<3	<9
	Seep	03/02/99	0	91	1	0.00277	0.2	<0.2	2	1.0	<0.2	13	<9
		03/03/99	1	90 (85)	<1	0.00051	<0.2	<0.2	1	<0.9	<0.2	18	20
		03/04/99	2	95	<1	0.00092 (0.00166)	0.4	<0.3	2	2.0	0.6	26	10
		03/05/99	3	84	<1	0.00040	0.4	0.4	2	1.0	<0.2	17	20
	Mixing zone	03/02/99	0	46	<1	0.00246	<0.2	<0.2	2	<0.9	0.3	16	10
		03/03/99	1	39	<1	0.00296	<0.2	<0.2	2	<0.9	<0.2	8	<9
		03/04/99	2	48	<1	0.00245	<0.3	<0.2	<2	1.0	<0.3	15	6
		03/05/99	3	42	<1	0.00138	0.8	0.8	2	2.0	0.6	11	10

Appendix 3. Continued.

Seep site	Location	Date	Day of exposure	Inorganic element									
				As	Se	Hg	Be	Cd	Cr	Cu	Pb	Ni	Zn
23L	Above seep	03/02/99	0	34	1	0.01184	<0.2	<0.2	2	2.0	0.2	<3	<9
		03/03/99	1	34	<1	0.00993	<0.3	<0.2	<2	2.3	0.5	5	3
		03/04/99	2	29 (34)	<1	0.01067	<0.2	<0.2	<1	2.0	<0.2	4	<9
		03/05/99	3	33	1	0.01054	0.3	<0.2	<1	2.0	<0.2	<3	<9
	Seep	03/02/99	0	410	<1	0.00015	<0.2	<0.2	1	<0.9	0.2	11	10
		03/03/99	1	400	<1 (<1)	0.00101	0.4 (<0.3)	<0.2 (<0.2)	<2 (<2)	1.0 (0.9)	<0.3 (<0.3)	17 (13)	10 (9)
		03/04/99	2	166	<1	0.00104	<0.2	0.3	<1	<0.9	<0.5	10	10
		03/05/99	3	184	<1	0.00220	<0.2	0.4	<1	1.0	<0.2	10	20
	Mixing zone	03/02/99	0	110	<1	0.02529	<0.2	<0.2	4	2.0	0.4	5	10
		03/03/99	1	78	<1	0.00658	<0.3	<0.2	<2	1.7	0.4	6	6
		03/04/99	2	49	<1	0.00751	<0.2	<0.2	<1	2.0	<0.2	4	<9
		03/05/99	3	43	1	0.01869	<0.2	0.3	<1	2.0	<0.2	4	<9

Appendix 3. Continued.

Seep site	Location	Date	Day of exposure	Inorganic element									
				As	Se	Hg	Be	Cd	Cr	Cu	Pb	Ni	Zn
31L	Above seep	03/02/99	0	31	1	0.01943	0.3	<0.2	1	1.0	<0.2	<3	<9
		03/03/99	1	32	<1	0.02221	<0.3	<0.2	<2	6.8	0.6	5	7
		03/04/99	2	30	<1 (1)	0.01366	<0.2 (<0.2)	<0.2 (0.2)	<1 (<1)	2.0 (2.0)	0.3 (<0.2)	4 (4)	<9 (<9)
		03/05/99	3	32	1	0.01182	<0.2	<0.2	<1	2.0	<0.2	4	<9
	Seep	03/02/99	0	420	<1	0.00147	<0.2	<0.2	<1	<0.9	<0.2	22	10
		03/03/99	1	400 (410)	<1	0 (0)	<0.3	<0.2	<2	0.8	<0.3	26	12
		03/04/99	2	390	<1	0.00020	<0.2	<0.2	<1	<0.9	<0.2	23	10
		03/05/99	3	380	<1	0.00148	0.4	<0.2	1	1.0	0.4	24	10
	Mixing zone	03/02/99	0	170	<1	0.00627	<0.2	<0.2	2	2.0	0.6	8	9
		03/03/99	1	88	<1	0.01631	<0.3	<0.2	<2	2.1	<0.3	10	5
		03/04/99	2	73	<1	0.00954	<0.2	<0.2	<1	2.0	0.3	6	<9
		03/05/99	3	70	2	0.01711	<0.2	<0.2	<1	2.0	0.3	6	<9

Appendix 3. Continued.

Seep site	Location	Date	Day of exposure	Inorganic element									
				As	Se	Hg	Be	Cd	Cr	Cu	Pb	Ni	Zn
32L	Above seep	03/02/99	0	28	2	0.01581	<0.2	<0.2	2	<0.9	<0.2	4	<9
		03/03/99	1	32	1	0.01861	<0.3	<0.2	<2	3.6	0.9	6	27
		03/04/99	2	33 (31)	1	0.01398	<0.2	<0.2	<1	2.0	0.5	3	<9
		03/05/99	3	32	<1	0.01339	<0.2	<0.2	1	2.0	0.2	4	<9
	Seep	03/02/99	0	40	<1	0.01128	<0.2	<0.2	2	<0.9	<0.2	12	<9
		03/03/99	1	31	<1 (<1)	0.00197	<0.3	<0.2	<2	0.9	0.4	16	21
		03/04/99	2	15	1	0.00166 (0.00244)	<0.2	<0.2	<1	<0.9	<0.2	13	10
		03/05/99	3	22	1	0.02345	0.5	<0.2	3	2.0	0.9	14	20
	Mixing zone	03/02/99	0	29	1	0.02197	<0.2	<0.2	3	<0.9	0.6	5	10
		03/03/99	1	31	<1	0.01959	0.6	<0.2	<2	2.1	0.4	7	7
		03/04/99	2	28	1	0.01814	<0.2	0.4	1	2.0	0.4	5	<9
		03/05/99	3	31	2	0.01410	<0.2	<0.2	1	2.0	0.3	4	<9

Appendix 3. Continued.

Seep site	Location	Date	Day of exposure	Inorganic element									
				As	Se	Hg	Be	Cd	Cr	Cu	Pb	Ni	Zn
33L	Above seep	03/02/99	0	32	1	0.02088	0.3	<0.2	3	<0.9	0.5	4	9
		03/03/99	1	35	2	0.01187	0.4	<0.2	<2	1.8	0.5	5	4
		03/04/99	2	28	1 (1)	0.01364	0.4	0.3	<1	2.0	<0.2	4	<9
		03/05/99	3	36	<1	0.01325	0.5	<0.2	<1	2.0	<0.2	3	<9
	Seep	03/02/99	0	850	<1	0	<0.2	<0.2	2	<0.9	<0.2	5	10
		03/03/99	1	700	<1	0.00028 (0.00031)	1.0 (0.4)	0.3 (<0.2)	<2 (<2)	0.7 (0.9)	<0.3 (0.3)	7 (7)	14 (11)
		03/04/99	2	929	<1	0.00175	<0.2	0.3	1	<0.9	<0.2	5	10
		03/05/99	3	670	<1	0	0.6	<0.2	1	2.0	<0.2	5	<9
	Mixing zone	03/02/99	0	120	1	0.03934	<0.2	<0.2	7	2.0	2.9	9	35
		03/03/99	1	37	2	0.02087	<0.3	0.3	<2	3.1	0.5	6	7
		03/04/99	2	30	1	0.01223	0.3	0.2	1	2.0	0.6	4	<9
		03/05/99	3	33	1	0.01409	0.7	<0.2	<1	3.4	0.6	4	<9
Reference		03/02/99	0	0	1	0.01135	<0.2	<0.2	3	<0.9	0.3	<3	10
		03/03/99	1	-	-	-	-	-	-	-	-	-	-
		03/04/99	2	-	-	-	-	-	-	-	-	-	-
		03/05/99	3	0	1	0.00981	0.7	<0.2	2	1.0	<0.2	<3	<9

[1]<: Less than limit of detection.

Appendix 4. Dissolved oxygen, pH, and temperature of water in exposure beakers before and after water renewal. Waters were collected from various sites in Whitewood Creek, South Dakota.

Seep site	Location	Replicate	Date	Exposure duration (hour)	Dissolved oxygen (mg/L)		pH		Temperature (°C)	
					Before	After	Before	After	Before	After
23R	Above seep	a	03/03/99	24	7.4	8.1	8.06	8.02	20.4	20.4
		b	03/04/99	48	7.1	7.9	8.04	8.04	20.3	20.3
		c	03/05/99	72	7.1	8.2	8.06	8.06	19.9	20.0
		a	03/06/99	96	7.3	-[1]	8.00	-	21.0	-
	Seep	a	03/03/99	24	7.0	7.2	7.82	7.86	20.2	20.6
		b	03/04/99	48	7.1	6.5	7.91	7.56	20.3	20.6
		c	03/05/99	72	6.9	6.3	7.85	7.18	19.9	20.2
		a	03/06/99	96	6.9	-	7.68	-	20.9	-
	Mixing zone	a	03/03/99	24	7.1	6.5	7.76	7.26	20.1	20.4
		b	03/04/99	48	6.9	6.1	7.86	7.23	20.4	20.4
		c	03/05/99	72	6.9	6.7	7.84	7.34	19.9	20.0
		a	03/06/99	96	7.0	-	7.78	-	20.8	-
23L	Above seep	a	03/03/99	24	7.3	8.0	8.06	8.01	20.4	20.5
		b	03/04/99	48	7.2	8.0	8.04	8.05	20.5	20.4
		c	03/05/99	72	7.1	8.2	8.06	8.06	19.9	20.1
		a	03/06/99	96	7.3	-	8.01	-	21.1	-

Appendix 4. Continued.

Seep site	Location	Replicate	Date	Exposure duration (hour)	Dissolved oxygen (mg/L)		pH		Temperature (°C)	
					Before	After	Before	After	Before	After
23L	Seep	a	03/03/99	24	7.1	7.1	7.95	7.82	20.4	20.7
		b	03/04/99	48	7.1	7.1	8.06	7.90	20.4	20.7
		c	03/05/99	72	7.0	6.7	8.05	7.47	20.2	20.3
		a	03/06/99	96	6.9	-	7.91	-	21.0	-
	Mixing zone	a	03/03/99	24	7.4	8.0	8.08	7.91	20.2	20.4
		b	03/04/99	48	7.3	8.0	8.12	8.04	20.4	20.3
		c	03/05/99	72	7.4	8.2	8.13	8.05	20.1	20.2
		a	03/06/99	96	7.3	-	8.06	-	21.0	-
31L	Above seep	a	03/03/99	24	7.5	8.3	8.05	7.99	20.3	20.2
		b	03/04/99	48	7.1	7.9	8.01	8.02	20.7	20.5
		c	03/05/99	72	7.1	8.0	8.04	8.05	20.1	20.1
		a	03/06/99	96	7.3	-	7.99	-	20.8	-
	Seep	a	03/03/99	24	7.0	6.0	7.79	7.27	20.1	20.7
		b	03/04/99	48	6.9	6.6	7.81	7.57	20.3	20.7
		c	03/05/99	72	6.9	6.6	7.91	7.16	20.0	19.9
		a	03/06/99	96	7.0	-	7.70	-	20.9	-

Appendix 4. Continued.

Seep site	Location	Replicate	Date	Exposure duration (hour)	Dissolved oxygen (mg/L)		pH		Temperature (°C)	
					Before	After	Before	After	Before	After
31L	Mixing zone	a	03/03/99	24	7.3	7.7	7.91	7.64	20.2	20.3
		b	03/04/99	48	7.1	7.7	8.00	7.70	20.7	20.6
		c	03/05/99	72	7.1	8.0	7.99	7.81	20.0	20.0
		a	03/06/99	96	7.3	-	7.97	-	20.9	-
32L	Above seep	a	03/03/99	24	7.3	8.2	8.03	7.98	20.4	20.4
		b	03/04/99	48	7.2	7.9	8.01	8.02	20.4	20.3
		c	03/05/99	72	7.1	8.1	8.03	8.04	20.1	20.1
		a	03/06/99	96	7.3	-	7.99	-	21.1	-
	Seep	a	03/03/99	24	7.3	7.9	7.98	7.81	20.4	20.4
		b	03/04/99	48	7.2	7.9	7.89	7.79	20.4	20.3
		c	03/05/99	72	7.1	7.6	7.89	7.80	19.9	19.9
		a	03/06/99	96	7.1	-	7.82	-	21.2	-
	Mixing zone	a	03/03/99	24	7.4	8.2	8.06	8.01	20.4	20.3
		b	03/04/99	48	7.0	8.0	8.01	8.00	20.7	20.5
		c	03/05/99	72	7.2	8.1	8.03	8.04	20.0	19.9
		a	03/06/99	96	7.2	-	7.98	-	21.0	-

Appendix 4. Continued.

Seep site	Location	Replicate	Date	Exposure duration (hour)	Dissolved oxygen (mg/L)		pH		Temperature (°C)	
					Before	After	Before	After	Before	After
33L	Above seep	a	03/03/99	24	7.4	8.3	8.01	7.99	20.2	20.2
		b	03/04/99	48	7.1	8.1	8.01	8.00	20.4	20.2
		c	03/05/99	72	7.1	8.1	8.02	8.04	20.0	19.9
		a	03/06/99	96	7.2	-	7.98	-	20.9	-
	Seep	a	03/03/99	24	7.1	7.2	7.91	7.95	20.1	20.6
		b	03/04/99	48	7.1	6.5	7.97	7.55	20.4	20.7
		c	03/05/99	72	6.9	7.2	7.92	7.47	19.9	19.9
		a	03/06/99	96	7.0	-	7.84	-	21.0	-
	Mixing zone	a	03/03/99	24	7.4	8.3	8.01	8.00	20.1	20.0
		b	03/04/99	48	7.1	7.8	7.98	7.99	20.5	20.5
		c	03/05/99	72	7.0	8.5	8.01	8.03	19.9	19.5
		a	03/06/99	96	7.3	-	7.98	-	20.8	-
Reference		a	03/03/99	24	7.0	7.1	8.09	8.11	20.7	20.7
		b	03/04/99	48	6.9	7.1	8.10	8.12	20.7	20.7
		c	03/05/99	72	7.0	7.3	8.12	8.12	19.9	19.8
		a	03/06/99	96	7.1	-	8.04	-	21.3	-

[1]-: No water renewal at end of exposure period.

Appendix 5. Percent survival of fathead minnow exposed to waters collected from various sites in Whitewood Creek, South Dakota.

		Exposure duration, date, and replicate number											
		24 hour (03/03/99)			48 hour (03/04/99)			72 hour (03/05/99)			96 hour (03/06/99)		
Seep site	Location	a	b	c	a	b	c	a	b	c	a	b	c
23R	Above seep	100	100	100	100	100	100	100	100	100	100	100	100
	Seep	100[1]	100[1]	80[1]	100[1]	100[1]	70[1]	100[1]	100[1]	70[1]	100[1]	100[1]	60[1]
	Mixing zone	100[1]	90[1]	100[1]	100[1]	90[1]	100[1]	100[1]	90[1]	100[1]	90[1]	80[1]	90[1]
23L	Above seep	100	100	100	100	100	100	100	100	100	90	100	100[2]
	Seep	100	100	90	100	100	90	100	100	90	100	100[3]	90
	Mixing zone	100	100	100	90	100	100	90	100	100	90	100	100
31L	Above seep	100	100	100	100	100	100	100	100	100	100	100	100
	Seep	100[1]	100[1]	90[1]	100[1]	100[1]	90[1]	100[1]	100[1]	90[1]	100[1]	80[1]	90[1,4]
	Mixing zone	100	90	100	100	90	100	100	90	100	100	90	100
32L	Above seep	100	100	100	100	100	100	100	100	100	100	100	100
	Seep	100	100	100	100	100	100	100	100	100	100[5]	100[6]	100[5]
	Mixing	100	100	100	100	100	100	100	100	100	100	100	100
33L	Above seep	100	100	100	100	100	100	100	100	100	100	100	100
	Seep	90	90	100	90	90	100	90	90	100	90	90[3]	100
	Mixing zone	90	100	100	90	100	100	90	100	100	90	90	100
Reference		100	100	90	100	100	90	100	100	90	100	100	90

[1]Most fish with a light coating of precipitate on body.
[2]One fish with curved spine.
[3]One fish with a heavy coat of precipitate.
[4]One fish immobilized and one lethargic.
[5]One fish lethargic.
[6]One fish surfacing.