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1992
Journal Article WWRC-92-07

In

Environmental Toxicology and Chemistry

Volume 11

Submitted by

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Environmental Toxicology and Chemistry, Vol. 11, pp. 1187–1195, 1992
Printed in the USA. Pergamon Press Ltd.

0730-7268/92 $5.00 + .00

ENVIRONMENTAL EFFECTS OF SALINE OIL-FIELD DISCHARGES ON SURFACE WATERS

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(*Received* 9 *April* 1991; *Accepted* 25 *October* 1991)

Abstract—Ambient water samples from streams receiving discharges of coproduced brines from the Salt Creek oil field in Wyoming were collected and tested for toxicity from 1988 to 1990. Exposure to waters downstream from the oil field significantly ($P < 0.05$) reduced survival and reproduction of *Ceriodaphnia dubia* compared to the upstream control, but fathead minnows (*Pimephales promelas*) did not appear to be sensitive to the test conditions. Toxicity to *Ceriodaphnia dubia* increased as stream flow decreased. Toxicity could not be attributed to H_2S, trace metals, or nonpolar organic compounds based on toxicity identification evaluation (TIE) methods. Major inorganic ions (Na^+, K^+, Cl^-, HCO_3^-, and CO_3^{2-}) appeared to account for the observed toxicity.

Keywords—Oil-field brines Ambient toxicity *Ceriodaphnia dubia* Fathead minnows

INTRODUCTION

Concerns about the environmental effects of discharging saline, coproduced waters from oil fields have been reported from as early as 1924 [1,2]. Recent implementation of toxicity-testing requirements for effluent discharges in the United States has led to a reevaluation of saline discharges from oil and gas wells [3]. During oil and gas production, a mix of petroleum and water that is trapped in the petroleum-bearing rocks is pumped to the surface. Coproduced waters are then separated from the oil and either injected into a well or discharged to surface waters. Typically high in total dissolved solids, coproduced waters can also contain petroleum contaminants, sulfide (from the bacterial reduction of sulfate), and trace metals, including Al, Ba, B, Cd, Cu, Fe, Hg, Li, Mn, Pb, Se, Sr, and Zn [4]. Some of these elements are found naturally in subsurface waters, and some are added during production and separation of oil and water.

Many of these components, including high total dissolved solids, can be toxic to freshwater biota in receiving waters. To assess this potential problem, this study evaluated the ambient toxicity of saline oil-field discharge waters in Salt Creek and downstream in the Powder River, both in northeastern Wyoming. Subsurface waters in the Salt Creek oil field typically contain high concentrations of Na^+, Cl^-, and HCO_3^- and sometimes SO_4^{2-}, K^+, Ca^{2+}, Mg^{2+}, and CO_3^{2-} [5]. Petroleum production operations in the drainage release large quantities of produced water to Salt Creek, which derives almost all of its continuous flow from these discharges [6] and contributes a significant proportion of the flow in the Powder River.

Our objectives were to (a) estimate the ambient toxicity of Salt Creek waters and the persistence of this toxicity downstream and (b) determine the contribution of salts, organic compounds, and other materials to the toxicity of Salt Creek and Powder River waters.

METHODS

Sample locations

Salt Creek originates approximately 40 km north of Casper, Wyoming, and flows north through the Salt Creek oil field for approximately 56 km before joining the Powder River (Fig. 1). Three study sites were selected on Salt Creek. The first (USC) was located upstream from any oil treater facility (SE1/4 S26 T39N R78W); the second (LIN) was located 26 km downstream from USC (SE1/4 S8 T42N R79W) in the Linch, Wyoming area; and the third (CSC) was about 9.1 m above the confluence of Salt Creek and the Powder River (SE1/4 S15 T43N R79W) (Fig. 1). USC served as the upstream control. All the oil companies releasing discharges that reach Salt Creek are located between USC and LIN.

Three sample sites were chosen on the Powder River. The first (CPR) was approximately 0.6 km

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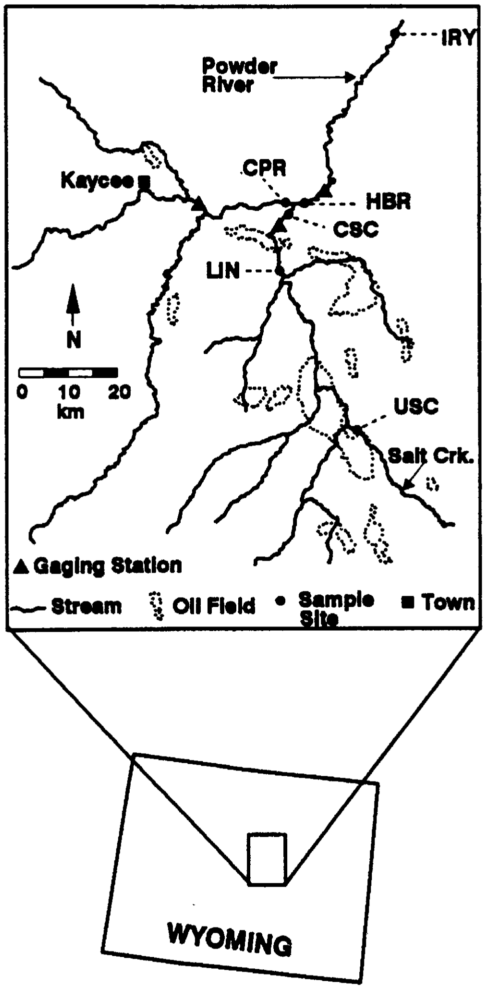

Fig. 1. Location of Salt Creek and Power River study area. Sample sites: USC = upstream Salt Creek; LIN = Linch; CSC = Salt Creek just upstream from confluence; CPR = Powder River just upstream from confluence; HBR = Powder River 6 km downstream from confluence at Highway 192 bridge; IRY = Powder River 68 km downstream from confluence at Irigary Bridge.

above the confluence of Salt Creek and the Powder River (SE1/4 S15 T43N R79W); the second (HBR) was located near Highway 192 bridge, 6 km downstream from the confluence (NW1/4 S13 T43N R79W); and the third (IRY) was 68 km downstream from the confluence at the Irigary Bridge (SW1/4 S19 T46N R77W) (Fig. 1).

Sample collection

Samples were collected from all study sites three times in year 1 (1988), during high (May), medium (October), and low (August) flow regimes. Stream flow data from 1986 [7] (gaging stations shown in Fig. 1) were used to estimate these flow regimes. During year 2 we collected samples from the LIN and HBR sites in September 1989 (USC was dry at this time) and from the USC and LIN sites in February 1990.

Water quality measurements in the field included temperature (degrees Celsius), dissolved oxygen (milligrams per liter) using a YSI model 64A dissolved oxygen meter (Yellow Springs Instrument Co., Yellow Springs, OH), and stream flow (cubic feet per second; 1 cubic foot per second = 0.028 cubic meters per second) using a Teledyne Gurley Pygmy (No. 625) current meter (Teledyne Gurley, Troy, NY). At each sample location, a 19-L grab sample was collected in a polyethylene jug and stored on ice for transport to the Red Buttes Environmental Biology Laboratory at the University of Wyoming, Laramie. There, samples were refrigerated at 4°C for subsequent tests. A 500-ml aliquot was drawn from each sample and filtered with a 0.45-μm cellulose acetate filter, acidified with redistilled HNO_3 (1 ml/L), and stored at 4°C for later chemical analyses.

Sediments

Sediment samples were collected in February 1990 at the USC and LIN sites for pore water and sediment extract analyses. Sediments were scraped from approximately the top 5 cm of stream bottom and stored in 1-L polyethylene bottles for transport to Laramie. There, excess water was decanted and the contents of each bottle thoroughly homogenized by hand.

For pore water analyses, four 50-ml centrifuge tubes were each filled with 40 g of homogenized sediment from one sample location. Samples were centrifuged for 10 min at 7,000 rpm in a model B-20A centrifuge (International Equipment Co., Needham Heights, MD). Supernatants from all four tubes were combined and filtered through a plankton net before toxicity testing to remove visible invertebrates.

Sediment extracts were prepared by combining homogenized sediment samples with hard reconstituted water in a 1:4 (v/v) ratio of sediment to water [8]. Each mixture was placed in a 2-L polyethylene container with a lid and shaken vigorously on a shaker table at 150 rpm for 30 min. After settling for 1 hour, the supernatant from each container was decanted and centrifuged for 15 min at 10,000 rpm. The supernatant was again decanted for toxicity tests.

Laboratory chemical analyses

Standard methods [9] were used to analyze routine chemical parameters of temperature, pH (Corning model 10 pH meter, Corning Scientific Instruments, Medfield, MA), conductivity (Extech model 440 conductivity meter, Extech, Boston, MA), alkalinity and hardness, and total ammonia (Orion Analyzer model 407A with a selective-ion ammonia probe, Orion Research Inc., Boston, MA). Major cations, Na^+, K^+, Ca^{2+}, and Mg^{2+}, and SO_4^{2-} were analyzed by using a Perkin-Elmer model 5500 inductively coupled argon plasma spectrophotometer (ICP) (Perkin-Elmer Corp., Norwalk, CT). Ten trace elements (Al, As, Fe, Hg, Li, Mn, Ni, Pb, Se, and Sr) were analyzed by ICP during year 1. In year 2, samples were analyzed for these elements plus Cd, Cr, Cu, and Zn. Cl^- was measured with a Buchler-Cotlove model 4-2000 chloridometer (Buchler Instruments, Fort Lee, NJ) [10]. Concentrations of HCO_3^- and CO_3^{2-} were calculated from total alkalinity and phenolphthalein alkalinity titration values.

Toxicity identification evaluation (TIE)

We characterized the toxicity of the sample collected in September 1989 at the LIN site by using phase I toxicity identification evaluation (TIE) procedures developed by the U.S. Environmental Protection Agency (EPA) [11]. Manipulations included pH adjustment, filtration, aeration, C_{18} Sep-pak (J.T. Baker Inc., Phillipsburg, NJ), oxidant reduction, EDTA chelation, and graduated pH tests. The LIN sample collected in February 1990 was not toxic to the test organisms; therefore, TIE procedures could not be performed on this sample. Instead, a second TIE characterization of the LIN water that was still toxic after being refrigerated since September 1989 was performed. Also tested was a reconstituted salt solution containing major cations and anions in concentrations approximating those of toxic stream waters.

Toxicity tests

To test instream toxicity during year 1, we conducted 7-d survival and growth tests with fathead minnows (*Pimephales promelas*) and 7-d survival and reproduction tests with *Ceriodaphnia dubia*, using standard protocols developed by the EPA [12]. Stock cultures of fathead minnows and *C. dubia* were originally obtained from the EPA's Environmental Research Laboratory in Duluth, Minnesota, and have been cultured at the Red Buttes Environmental Biology Laboratory for many years. Larvae and neonates from these stock cultures were used for all toxicity tests conducted in this study.

Undiluted stream water (ambient water sample) was tested for each sample location. The USC sample served as the ambient control, and a hard reconstituted water (pH 8.5, conductivity 600 μS/cm, alkalinity 114 mg/L as $CaCO_3$, and hardness 168 mg/L as $CaCO_3$) was used as the laboratory control. For statistical comparisons, the USC sample was used as the control.

Temperature for all tests was maintained at 25 ± 2°C by using recirculating water baths, and tests were run under a 16:8-h light:dark photoperiod. Fathead minnow tests were conducted in 1-L glass beakers with 250 ml of test water, with four replicate beakers for each test water and the laboratory control. Each beaker contained 10 larval fathead minnows, for a total of 40 minnows per test water. At the start of each test, the minnows were between 3 and 7 d old and were hatched within 48 h of each other. They were fed 0.1 ml newly hatched brine shrimp (*Artemia salina*) two times per day during the test. Test waters were renewed daily, and after 7 d surviving minnows in each replicate beaker were dried and weighed as a group and average dry weight per fish was calculated.

Ceriodaphnia dubia tests were started with neonates <24 h old and released within 4 h of each other. One neonate was placed in each of 10 replicate beakers for each test water and the laboratory control. Test beakers were 30-ml plastic cups, each containing 15 ml of test water. Animals were transferred to fresh test water daily and fed 100 μl per beaker with a mixture of algae (*Selenastrum capricornutum*) and No. 1 trout starter food (U.S. Fish and Wildlife Service Specification Diet SD9-30, obtained from A.J. Balshi Inc., Catawissa, PA) slurry. The mean number of young per female was calculated at the end of the test by dividing the total number of offspring produced in each test water by the number of females that started the test in that water. If a female died before reproducing, her offspring total was recorded as zero.

During year 2, toxicity tests were conducted on instream, ambient waters with *C. dubia* only, because it was the more sensitive species. The test for the water samples collected in September 1989 was started as a chronic test (10 replicates with one organism per replicate), but because all *C. dubia* in stream water samples died in 24 h, the test was discontinued after 48 h and considered an acute test. Thereafter, for the instream water samples, 48-h static acute tests were conducted using four replicate beakers of five *C. dubia* (<24 h old) for each test water. Each beaker of *C. dubia* was fed 100 μl algae (*S. capricornutum*) and 100 μl of a yeast–Cerophyl® (Agri-Tech, Kansas City, MO)–trout starter food mixture (YCT) at the start of the test. For the TIE samples, sediment pore waters, sediment extracts, and salt solution, we conducted 48-h static acute tests with four replicate beakers of 15 ml test water, five *C. dubia* (<24 h old), and 125 μl of a food mix of algae and YCT.

Statistical analyses

Data for all 7-d toxicity tests were analyzed by using the computer program TOXSTAT Version 2.1 [13], which applies all the statistical procedures recommended in the EPA's protocol [12]. For the 48-h acute tests, we calculated LC50 values with the program described in the EPA's manual for acute tests [14], which uses the probit, binomial, and moving-average methods.

For year 1 survival tests, we used stepwise logistic regression for fathead minnow and *C. dubia* survival vs. chemical and physical variables. We also used multiple linear regression for fathead minnow weights and *C. dubia* number of young vs. chemical and physical variables. For these regressions, we used the BMDP statistical program [15]. Pearson product-moment correlation coefficients were computed using SPSS-X [16] to evaluate the covariance among physical and chemical variables, including Na^+, Ca^{2+}, Mg^{2+}, K^+, Cl^-, SO_4^{2-}, alkalinity, pH, conductivity, and stream flow. BMDP and SPSS-X statistical programs were run on the VAX mainframe computer at the University of Wyoming.

RESULTS

Flow

In year 1, flows in the Powder River ranged from 3.5 cubic feet per second (cfs) at the CPR site

Table 1. Water chemistry parameters and flow in Salt Creek and the Powder River during 1988, 1989, and 1990[a]

Site[b]	pH units	Conductivity (μS/cm)	Alkalinity (mg/L CaCo$_3$)	Hardness (mg/L CaCO$_3$)	Ammonia (mg/L total N)	Dissolved oxygen (mg/L)	Temp. (°C)	Flow (cfs)
May 1988, high flow								
USC	8.30	4,270	265	1040	<0.10	6.90	23.5	0.70
LIN	8.50	6,090	594	410	0.18	7.10	19.0	48.00
CSC	8.50	6,740	732	424	0.13	7.10	24.0	48.00
HBR	8.40	1,815	222	312	<0.10	7.00	20.0	357.00
IRY	8.35	1,688	203	308	<0.10	7.00	16.0	389.00
CPR	8.25	828	129	274	<0.10	6.90	20.0	258.00
Oct. 1988, medium flow								
USC	8.20	4,170	281	992	<0.10	6.85	13.8	1.50
LIN	8.52	6,000	784	220	<0.10	7.80	14.5	43.00
CSC	8.72	6,190	730	190	0.11	6.90	8.0	53.00
HBR	8.38	5,930	498	345	<0.10	7.10	11.8	83.00
IRY	8.50	3,590	342	365	<0.10	6.50	10.5	104.00
CPR	8.30	2,500	250	590	<0.10	6.60	6.8	26.00
Aug. 1988, low flow								
USC	8.30	4,840	252	1070	<0.10	6.65	30.0	0.04
LIN	8.82	6,400	813	152	<0.10	6.20	19.5	31.00
CSC	8.93	6,420	774	142	<0.10	6.50	27.0	30.00
HBR	8.72	4,420	706	206	<0.10	6.80	30.1	53.00
IRY	8.86	5,820	519	314	<0.10	6.60	15.5	18.90
CPR	8.19	2,240	173	870	<0.10	6.70	29.0	3.50
Sept. 1989								
LIN	8.70	6,330	784	156	–[c]	7.30	21.0	24.80
HBR	8.60	5,830	712	236	–	7.00	20.5	–
Feb. 1990								
USC	7.90	4,220	286	1070	<0.10	8.50	5.0	0.03
LIN	8.15	4,750	550	667	0.20	8.50	0.0	60.00

[a] Dissolved oxygen, temperature, and flow were measured at sample sites in the field; pH, conductivity, alkalinity, hardness, and ammonia were measured in the laboratory.

[b] See Figure 1 for sample site locations and codes.

[c] Not measured.

in August to 389 cfs at IRY, the site furthest downstream, in May (Table 1). Flows in Salt Creek were 0.04 cfs at the USC site in August and 53 cfs at the CSC site in October. Salt Creek contributed 16% of the water in the Powder River during high flow, 67% during medium flow, and 89% during low flow.

Flows at the LIN site in year 2 were 24.8 cfs in September 1989 and 60.0 cfs in February 1990 (Table 1). In September 1989, the streambed at the USC site was essentially dry; the flow was low (approximately 0.03 cfs) in February 1990, but there was enough water to collect a sample.

Chemical analyses

Due to the nature of the surrounding soils and bedrock, waters at the USC site have naturally high hardness and conductivity [6] (Table 1). Conductivity, alkalinity, and pH increased within the area of oil-field discharges (LIN site) and then decreased after Salt Creek entered the Powder River (HBR and IRY). In contrast, hardness decreased with the addition of coproduced waters. Dissolved oxygen measurements for all sampling locations were similar to the upstream control and remained consistent throughout sampling periods (Table 1).

Concentrations of major inorganic ions (Table 2) followed a trend that would be expected from conductivity, alkalinity, and hardness measurements. Concentrations of Na^+, K^+, Cl^-, HCO_3^-, and CO_3^{2-} increased with the addition of oil-field discharges at the LIN site and then generally decreased downstream, whereas SO_4^{2-}, Ca^{2+}, and Mg^{2+} decreased at the LIN site and downstream.

Concentrations of Fe, Mn, Ni, and Sr were relatively low, with few exceeding the upstream Salt Creek values, except for the 3.39-mg/L value for Fe in the February 1990 LIN sample (Table 3). Values for Li increased at the LIN site and then decreased downstream. Concentrations of As, Hg,

Table 2. Concentrations of major inorganic ions in Salt Creek and the Powder River during 1988, 1989, and 1990[a]

	Cations				Anions			
Site[b]	Na^+	Ca^{2+}	Mg^{2+}	K^+	Cl^-	SO_4^{2-}	HCO_3^-	CO_3^{2-}
May 1988, high flow								
USC	845	219	109	9	8	809	323	0.0
LIN	1,255	55	52	18	1,042	383	712	6.0
CSC	1,410	47	62	17	1,066	466	878	7.2
HBR	268	71	32	4	171	152	268	1.8
IRY	239	70	31	4	139	149	242	2.4
CPR	73	70	24	2	8	85	157	0.0
Oct. 1988, medium flow								
USC	805	229	112	9	8	804	343	0.0
LIN	1,390	30	30	16	1,294	242	934	10.8
CSC	1,395	25	33	16	1,299	260	876	7.2
HBR	845	51	42	9	777	282	604	1.8
IRY	685	74	53	11	552	273	414	1.8
CPR	200	161	64	5	144	320	305	0.0
Aug. 1988, low flow								
USC	895	235	132	11	8	914	307	0.0
LIN	1,420	24	24	14	1,123	204	927	31.8
CSC	1,430	20	26	15	1,238	215	859	42.0
HBR	1,290	24	33	14	1,118	249	831	15.0
IRY	1,205	37	53	15	1,006	322	599	16.8
CPR	254	208	93	8	82	419	211	0.0
Sept. 1989								
LIN	1,015	16	21	39	1,301	543	–[c]	–
HBR	–	–	–	–	–	–	–	–
Feb. 1990								
USC	510	254	127	17	24	–	349	0.0
LIN	643	170	52	42	730	–	671	0.0

[a] Values expressed as milligrams per liter.

[b] See Figure 1 for sample site locations and codes.

[c] Not measured.

Table 3. Concentrations of selected trace elements in Salt Creek and the Powder River during the study[a]

Site[c]	Element[b]					
	Al	Fe	Li	Mn	Ni	Sr
May 1988, high flow						
USC	1.20	0.09	0.02	0.17	0.04	3.91
LIN	0.40	0.09	0.26	0.01	0.02	2.33
CSC	0.50	0.07	0.24	0.01	0.04	2.11
HBR	0.70	<0.01	0.02	<0.01	0.03	0.89
IRY	0.70	<0.01	0.02	0.01	0.01	0.95
CPR	0.70	0.03	<0.01	0.01	0.04	0.65
Oct. 1988, medium flow						
USC	0.80	0.04	0.01	<0.01	0.03	4.05
LIN	1.90	<0.01	0.26	0.01	0.02	1.87
CSC	0.80	0.01	0.26	<0.01	0.03	1.97
HBR	1.50	<0.01	0.13	<0.01	<0.01	1.73
IRY	1.10	<0.01	0.13	<0.01	<0.01	1.69
CPR	0.80	<0.01	0.05	<0.01	0.03	1.97
Aug. 1988, low flow						
USC	1.60	0.09	0.02	0.01	0.05	4.97
LIN	0.80	0.05	0.26	<0.01	<0.01	1.68
CSC	0.60	0.05	0.27	<0.01	0.02	1.30
HBR	1.70	0.03	0.23	<0.01	0.03	1.24
IRY	0.70	0.02	0.21	<0.01	0.04	1.63
CPR	0.60	0.04	0.08	0.06	0.04	2.88
Sept. 1989						
LIN	0.80	0.31	0.26	<0.01	<0.01	1.57
Feb. 1990						
USC	1.20	0.26	0.02	0.06	<0.01	3.82
LIN	1.60	3.39	0.26	0.02	<0.01	3.05

[a] Additional analyzed elements were below detection limits: As, Hg, Pb, Se (<0.20 mg/L) and Cd, Cu, Cr, Zn (<0.01 mg/L).

[b] Values expressed as milligrams per liter.

[c] See Figure 1 for sample site locations and codes.

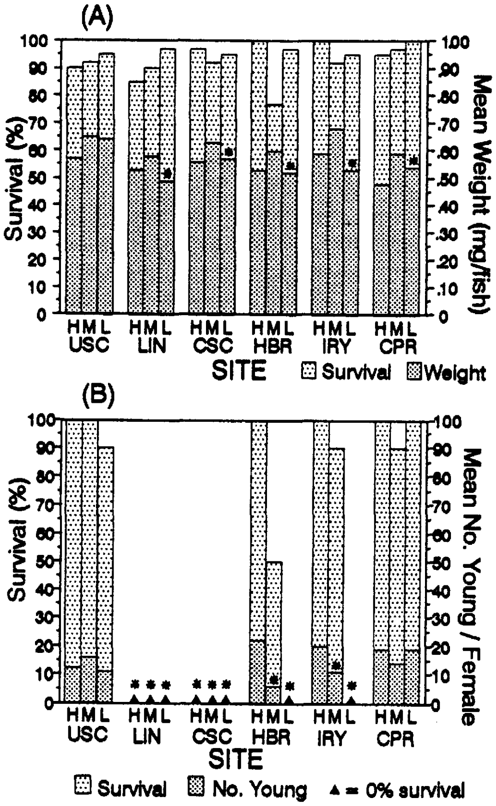

Fig. 2. (A) Fathead minnow (*Pimephales promelas*) survival and weight and (B) *Ceriodaphnia dubia* survival and reproduction in toxicity tests conducted in waters collected during high (H), medium (M), and low (L) flow periods in 1988. USC = upstream Salt Creek; LIN = Linch; CSC = Salt Creek just upstream from confluence; HBR = Powder River 6 km downstream from confluence at Highway 192 bridge; IRY = Powder River 68 km downstream from confluence at Irigary Bridge; CPR = Powder River just upstream from confluence. * = Survival, weight, or number of young significantly ($P < 0.05$) lower than upstream control during that flow period.

Pb, and Se were below detection limits (<0.20 mg/L), as were values of Cd, Cr, Cu, and Zn measured in year 2 samples (<0.01 mg/L).

Hydrogen sulfide had been a suspected toxicant, but we did not detect the characteristic odor of H_2S in any of the stream samples. In year 2, two samples from the LIN site were analyzed for sulfide and found to contain <1 mg/L.

Toxicity tests

There were no significant effects on fathead minnow survival in any of the water samples collected from Salt Creek and the Powder River during this study. Fathead minnow weights at all sites were significantly lower than weights at the USC site during the low flow period in 1988 (Fig. 2A). No *C. dubia* survived in ambient water samples collected during all three flow regimes in year 1 from the LIN site or from the CSC site (Fig. 2B). In Powder River waters, survival was 90 to 100% in water collected at the CPR site but significantly reduced at the HBR site during medium flow. No *C. dubia* survived at this site and the IRY site during low flow (Fig. 2B).

Sites where all organisms died (LIN and CSC during all flow regimes and HBR and IRY during low flow) were not included in statistical comparisons of *C. dubia* mean number of young. No significant effects on the number of young were seen in the Powder River sites during high flow (Fig. 2B). But during the medium flow period, the mean number of young produced was significantly reduced at the HBR site and the IRY site.

Table 4. Year 2 *Ceriodaphnia* survival and LC50s[a] in ambient stream samples and in sediment pore waters, sediment extracts, and a salt solution reconstituted to match major ion concentrations at the LIN site

	% Survival in 100% water		LC50 (%)	
Date/sample	24 h	48 h	24 h	48 h
Sept. 1989/LIN	0	0	86 (75–100)[b]	66 (50–75)
Sept. 1989/HBR	0	0	–[c]	–
Feb. 1990/LIN	100	100	–	–
Feb. 1990/USC	100	100	–	–
Feb. 1990/USC sediment pore water	100	100	–	–
Feb. 1990/USC sediment extract	100	100	–	–
Feb. 1990/LIN sediment pore water	100	75	–	–
Feb. 1990/LIN sediment extract	100	100	–	–
Reconstituted salt solution	0	0	74 (65.7–78.1)	58 (50–75)

[a] LC50 = median lethal concentration.

[b] 95% C.I.s shown in parentheses.

[c] No dilutions were tested, so LC50 could not be determined.

In year 2, samples collected at the LIN site and the HBR site in September 1989 were toxic to *C. dubia* (Table 4). No test organisms survived in undiluted water samples, and we calculated a 48-h LC50 of 66% for the LIN sample (Table 4). All *C. dubia* survived in the USC and LIN samples collected in February 1990 (Table 4). Sediment pore waters and extracts from the USC sediments were not toxic to *C. dubia* nor were extracts from sediments at the LIN site (Table 4). However, 48-h survival in the sediment pore water from the LIN site was reduced to 75% (Table 4). In all tests, survival of laboratory control organisms was 90 to 100%, and in 7-d tests *C. dubia* young production averaged 19 young per female and fathead minnow weights averaged 0.51 mg per fish.

Statistical regressions that separately regressed cations, anions, alkalinity, pH, conductivity, and flow (cubic feet per second) against year 1 toxicity test results revealed that none of these parameters were significant in accounting for variance in fathead minnow weights. Several parameters did significantly account for the variance in *C. dubia* survival in year 1 toxicity tests (Table 5). Alkalinity and pH, for example, accounted for 94 and 81% of the variance, respectively. The inorganic ions Cl^-, Na^+, and K^+ accounted for 94, 90, and 82% of the variance, respectively. Two of these ions, Na^+ and K^+, significantly contributed to the variance in the mean number of *C. dubia* young (55 and 45%, respectively), but not as much as alkalinity, which accounted for 70% (Table 5). Chemical variables that significantly accounted for toxicity (Na^+, K^+, Cl^-, alkalinity, and pH) were highly correlated with each other and with other chemical and physical parameters (Table 6).

None of the standard TIE manipulations reduced the toxicity of the LIN sample collected in September 1989, suggesting that nonpolar organics, cationic metals, or sulfide was not the cause of toxicity. To test whether major ions accounted for the toxicity, we used average values of major cations and anions from the toxic LIN samples (Na^+, 1,272 mg/L; Ca^{2+}, 31 mg/L; Mg^{2+}, 32 mg/L; K^+, 22 mg/L; Cl^-, 1,195 mg/L; SO_4^{2-}, 424 mg/L; and alkalinity, 746 mg/L as $CaCO_3$) to prepare a reconstituted salt solution. Analyzed concentrations in the reconstituted solution were Na^+, 1,270 mg/L; Ca^{2+}, 15 mg/L; Mg^{2+}, 24 mg/L; Cl^-, 1,740 mg/L; SO_4^{2-}, 427 mg/L; and alkalinity, 678

Table 5. Significant ($P < 0.05$) regression analyses for *Ceriodaphnia dubia* survival and reproduction during 7-d toxicity tests in Salt Creek and Powder River waters conducted in year 1

Toxicity end point	Parameter	Coefficient	Intercept	R^2
% survival	Alkalinity	−0.0200	8.81	0.94
	Cl^-	−0.0083	5.63	0.94
	Na^+	−0.0100	10.41	0.90
	K^+	−0.9056	10.44	0.82
	pH	−14.2850	120.70	0.81
	Ca^{2+}	+0.0811	−4.89	0.63
	Conductivity	−0.0009	4.05	0.34
	Mg^{2+}	+0.0358	−1.84	0.25
	cfs	+0.0083	−5.69	0.12
	SO_4^{2-}	+0.0026	−0.86	0.06
Mean no. young/female	Alkalinity	−0.0416	25.95	0.70
	Na^+	−0.0114	20.90	0.55
	K^+	−1.0310	22.38	0.45
	Conductivity	−0.0025	20.70	0.30

Table 6. Correlations among chemical and physical variables measured in Salt Creek and Powder River waters during 1988

	Bivariate correlation coefficient (r) between variable 1 and variable 2[a]				
	Variable 2				
Variable 1	Na^+	K^+	Cl^-	Alkalinity	pH
Na^+	1.00	0.94[b]	0.86[b]	0.92[b]	0.68[b]
Ca^{2+}	−0.46	−0.41	−0.80[b]	−0.69[b]	−0.70[b]
Mg^{2+}	−0.15	−0.07	−0.58[b]	−0.46	−0.54[b]
K^+	0.94[b]	1.00	0.84[b]	0.85[b]	0.62[b]
Cl^-	0.86[b]	0.84[b]	1.00	0.96[b]	0.77[b]
SO_4^{2-}	0.09	0.11	−0.40	−0.25	−0.40
Alkalinity	0.92[b]	0.85[b]	0.96[b]	1.00	0.73[b]
Conductivity	0.69[b]	0.70[b]	0.57[b]	0.70[b]	0.30
pH	0.68[b]	0.62[b]	0.77[b]	0.73[b]	1.00
Flow (cfs)	−0.57[b]	−0.61[b]	−0.31	−0.40	−0.15

[a] $n = 18$.

[b] Significant correlation ($P < 0.05$).

mg/L as $CaCO_3$. The 48-h LC50 for the reconstituted solution (58%) was essentially the same as the 48-h LC50 for the September 1989 LIN sample (66%) (Table 4).

DISCUSSION

Several researchers have noted detrimental effects on biota from oil-field brines [17–19]. Birge et al. [20] reported a strong negative relationship between stream chloride concentrations and fish and macroinvertebrate community structure downstream from a brine outfall.

The Salt Creek oil field has been a suspected source of pollution to surface waters since at least 1968 [21]. Researchers reported free and emulsified oil concentrations up to 11 mg/L in Salt Creek and Powder River waters but concluded these levels did not detrimentally affect fish populations [21,22].

Taraldsen et al. [3] tested five effluents from Salt Creek oil treater facilities and ambient water samples from Salt Creek above and below one of the dischargers for toxicity to fathead minnows and *C. dubia*. They also conducted TIEs on the effluents. One effluent was not acutely toxic, H_2S was the suspected toxicant in three effluents, and nonpolar organic compounds accounted for the toxicity of the fifth effluent. Ambient Salt Creek water collected above one of the dischargers was not toxic, but water immediately below the discharge was toxic to both *C. dubia* and fathead minnows [3].

Woodward and Riley [23] reported petroleum hydrocarbons and Cd, Cr, Cu, Pb, and Zn in water and sediments downstream from an oil-field discharge to the Little Popo Agie River in Wyoming. They noted a reduction in species diversity of macrobenthos in downstream sampling stations.

In our study, toxicity tests showed adverse effects on *C. dubia* from exposure to surface waters from the Linch site and from sites far downstream in the Powder River. In August 1988, when Salt Creek comprised about 89% of the Powder River flow, toxicity was observed at the IRY site, 68 km downstream from the confluence of Salt Creek and the Powder River. Survival and reproduction were significantly reduced at the Linch site and at one or more downstream sites at all sampling times except February 1990, when stream flow was the highest recorded at that site during our study. Toxicity increased as stream flow decreased. At low flow, no *C. dubia* survived in ambient stream waters from the Linch site and all downstream sites. Exposure to sediment pore water from the Linch site reduced survival of *C. dubia*, but the limited volume of this sample precluded further analysis.

None of the trace elements analyzed in this study appeared to contribute to the toxicity of the stream waters. Toxicity due to Hg, Pb, or Se can occur at concentrations much lower than the detection limits used in this study, but evidence from the EDTA chelation tests performed in the TIE and from the reconstituted salt solution tests indicated that these elements did not appear to be present at toxic concentrations. And although Taraldsen et al. [3] found that H_2S and nonpolar organics were suspected toxicants in the effluents, these components had apparently dissipated, decayed, or were diluted to nontoxic concentrations at sites further downstream where we collected our samples.

This study illustrates the importance of using more than one test organism whenever possible, as *C. dubia* was more sensitive than fathead minnows to the test waters. Similarly, a study of produced waters from a degasification field in Alabama revealed that *Ceriodaphnia* spp. were more sensitive to the waters than fathead minnows and that chloride accounted for most of the toxicity [24]. At the produced water 48-h LC50 for *Ceriodaphnia* (71%), O'Neil et al. [24] reported a chloride concentration of 994 mg/L, similar to the chloride concentration of 859 mg/L we found at the LC50 for the September 1989 Linch sample (66%). Birge et al. [20] reported LC50 values for chloride (as NaCl) of 6,570 mg/L for fathead minnows (96 h) and 1,470 mg/L for *Daphnia pulex* (48 h), again demonstrating the greater sensitivity of invertebrates to chlorides. Our study found significant correlations between toxicity and individual parameters, especially Cl^-, Na^+, K^+, and alkalinity. However, singling out any one parameter would be misleading due to covariance among the variables. The toxicity of the reconstituted solution was very similar to toxic stream waters from the Linch site. Also, none of the standard TIE manipulations, which do not address toxicity due to salinity, reduced toxicity. Therefore, it appears that major ions accounted for the toxicity of these stream waters.

Acknowledgement—We thank Fred Carr, Amoco Oil Co., and the U.S. Naval Petroleum Reserve for providing access to study sites. Sampling locations were suggested by John Wagner and Kurt King of the Wyoming Department of Environmental Quality. Joe Fernandez, Dave Gulley, Connie Boese, Mike Szumski, and Sheryl Hill assisted with sample collection, toxicity tests, and statistical and chemical analyses at the University of Wyoming. Funding was provided by the U.S. Geological Survey through the Wyoming Water Research Center at the University of Wyoming, Laramie.

REFERENCES

1. **Wiebe, A.H., J.G. Burr** and **H.E. Faubion.** 1924. The problem of stream pollution in Texas with special reference to salt water from the oil fields. *Trans. Am. Fish. Soc.* **64**:81–85.
2. **Clemens, H.P.** and **W.H. Jones.** 1954. Toxicity of brine water from oil wells. *Trans. Am. Fish. Soc.* **84**:97–109.
3. **Taraldsen, J.E., J.R. Amato** and **D.I. Mount.** 1988. Toxicity testing and characterization of toxicants from effluents of the Powder River Basin, Wyoming. NETAC Technical Report B-88. U.S. Environmental Protection Agency, Duluth, MN.
4. **Collins, A.G.** 1975. *Geochemistry of Oilfield Waters.* Elsevier, Amsterdam, The Netherlands.
5. **Crawford, J.G.** 1964. Rocky Mountain oil field waters. Section 5. Chemical and Geological Laboratories, Casper, WY.
6. **Wyoming Department of Environmental Quality.** 1986. Wyoming 1986 water quality assessment. Water Quality Division, Cheyenne, WY.
7. **U.S. Department of the Interior.** 1986. Water resource data for Wyoming. USGS Water-Data Report WY-86-1. U.S. Geological Survey, Cheyenne, WY.
8. **Nebeker, A.V., M.A. Cairns, J.H. Gakstatter, K.W. Malueg, G.S. Schuytema** and **D.F. Krawczyk.** 1984. Biological methods for determining toxicity of contaminated freshwater sediments to invertebrates. *Environ. Toxicol. Chem.* **3**:617–630.
9. **American Public Health Association, American Water Works Association** and **Water Pollution Control Federation.** 1980. *Standard Methods for Examination of Water and Wastewater*, 15th ed. American Public Health Association, Washington, DC.
10. **Cotlove, E., H.V. Trantham** and **R.L. Bowman.** 1958. An instrument for and method for automatic, rapid, accurate, and sensitive titration of chloride in biological samples. *J. Lab. Clin. Med.* **50**:358–371.
11. **U.S. Environmental Protection Agency.** 1988. Methods for aquatic toxicity identification evaluations. Phase I toxicity characterization procedures. EPA 600/3-88-034. Environmental Research Laboratory, Duluth, MN.
12. **U.S. Environmental Protection Agency.** 1989. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. EPA 600/4-89-001. Environmental Monitoring and Support Laboratory, Cincinnati, OH.
13. **Gulley, D.D., A.M. Boelter** and **H.L. Bergman.** 1988. TOXSTAT Version 2.1. University of Wyoming, Laramie, WY.
14. **U.S. Environmental Protection Agency.** 1985. Methods for measuring the acute toxicity of effluents to freshwater and marine organisms. EPA 600/4-85-013. Environmental Monitoring Systems Laboratory, Cincinnati, OH.
15. **Dixon, W.J., M.B. Brown, L. Engleman, J.W. Frane, M.A. Hill, R.I. Jennrich** and **J.D. Toporek.** 1985. *BMDP Statistical Software.* University of California Press, Berkeley, CA.
16. **SPSS.** 1988. *SPSS-X User's Guide*, 3rd ed. Chicago, IL.
17. **Charles, J.R.** 1967. Effects of oilfield brines. *Proceedings*, 18th Annual Conference Southeast Association Game Fish Commission, Clearwater, FL, Oct. 18–21, 1964, pp. 371–403.
18. **Harrel, R.C.** and **T.C. Dorris.** 1968. Stream order, morphometry, physico-chemical conditions, and community structure of benthic macroinvertebrates in an intermittent stream system. *Am. Midl. Nat.* **80**:220–251.
19. **Van Hassel, J.H., D.S. Cherry, J.C. Hendricks** and **W.L. Specht.** 1988. Discrimination of factors influencing biota of a stream receiving multiple-source perturbations. *Environ. Pollut.* **55**:271–287.
20. **Birge, W.J., J.A. Black, A.G. Westerman, T.M. Short, S.B. Taylor, D.M. Bruser** and **E.D. Wallingford.** 1985. Recommendations on numerical values for regulating iron and chloride concentrations for the purpose of protecting warmwater species of aquatic life in the Commonwealth of Kentucky. Memorandum of Agreement 5429. Kentucky Natural Resources and Environmental Protection Cabinet, Lexington, KY.
21. **Mueller, J.W.** 1968. Oil pollution investigations on Powder River, middle and south forks of Powder River and Salt Creek, 1967. Administrative Report, Project 0367-07-6503. Wyoming Game and Fish Department, Cheyenne, WY.
22. **Millis, R.** 1972. Salt Creek oil investigations. Administrative Report, Project 0371-07-7101. Wyoming Game and Fish Department, Cheyenne, WY.
23. **Woodward, D.F.** and **R.G. Riley.** 1983. Petroleum hydrocarbon concentrations in a salmonid stream contaminated by oil field discharge water and effects on macrobenthos. *Arch. Environ. Contam. Toxicol.* **12**:327–334.
24. **O'Neil, P.E., S.C. Harris, M.F. Mettee, K.R. Drottar, D.R. Mount** and **J.P. Fillo.** 1989. Biomonitoring of a produced water discharge from the Cedar Grove Degasification Field, Alabama. GRI-89/0073. Final Report. Gas Research Institute, Chicago, IL.