Determination Of Point And Nonpoint Source Toxicity In The Clark Fork River Basin Using The Daphnid, *Ceriodaphnia Dubia*

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Abstract— *Ceriodaphnia dubia, a small planktonic daphnid was used to biomonitor point sources of toxicity in wastewater and nonpoint source toxicity in stream samples obtained from the Clark Fork River Basin, MT. Brief descriptions, results, and discussions are presented for studies of wastewater from a kraft mill near Frenchtown, MT and potential toxicity of water samples from 19 sites along the Clark Fork River in 1985. In 1987, dilutions of Missoula, MT municipal wastewater fortified with ammonia were tested, as was the wastewater before and after chlorination. Potential toxicity of water samples from eight sites along the upper Clark Fork River were also tested. All studies were cooperative efforts with the Montana Departments of Health and Environmental Sciences; and Fish, Wildlife and Parks.*

Ceriodaphnia appear to be indicators of toxicity in a variety of test conditions such as ammonia in wastewater and metals from past mining activities. The daphnids indicated toxicity from other substances in the wastewater or perhaps the influence of characteristics of the wastewater that increased ammonia toxicity. An example of nonpoint source effects was toxicity in samples from Silver Bow Creek MT, where impaired conditions to aquatic life resulting from the presence of metals have been reported for years. During some of the tests with wastewater, toxicological endpoints were observed using the actual number of daphnids that reproduced in a test, not the average number of young. There was circumstantial evidence in 1985 that copper alone was responsible for the toxicity in Silver Bow Creek. However, the later studies performed under different hydrological conditions found toxicity was probably due to a combination of metals, some of which had not been measurable earlier. For well-defined "control" of "standard" conditions during testing, there are indications that waters to be used as reference media for Ceriodaphnia *need further research. Nevertheless, the use of daphnids to test the ambient conditions described in this paper should encourage environmental managers to consider approaches with this or similar species in the future.*

INTRODUCTION

In 1985 and 1987, a planktonic daphnid, *Ceriodaphnia dubia*, was used by the Environmental Protection Agency (EPA) Region VIII Denver, CO, to determine toxic conditions in various Montana waters. All of the testing was conducted in the Clark Fork River Basin, which has documented water quality problems resulting from historic mining activities and pollution by municipal wastewaters and industries such as pulp processing (Anonymous, 1986). The approach used the daphnid as an environmental "sensor" or biomonitor in much the same way as a pH meter "senses" the hydrogen ion concentration, except that the organisms integrate the toxicity of all constituents and their interactions within the matrix of the chemistry of the sample.

Results from some of these studies have been considered in management decisions regarding permit limits, treatment technologies etc., but all results were used for the development of, or the validation of testing methods using *Ceriodaphnia* in field situations. The demonstration of the procedures to various state and federal agency personnel, training of agency personnel, technical assistance to state and local officials as well as obtaining experience in waters of differing characteristics were considerations for conducting the tests.

The purposes of this paper are (1) to demonstrate how daphnids were used in biomonitoring, (2) to note some important observations and discoveries made during the testing, and (3) to include various (baseline) toxicological and chemical parameters measured during the testing so comparison may be made in successive studies in the Clark Fork River Basin.

MATERIALS AND METHODS

Locations and Test Conditions

Mobile laboratories were located at Frenchtown, MT on property owned by a kraft liner board mill (currently Stone Container), May through June, 1985 and at the Missoula Wastewater Treatment Plant, May through June, 1987. Locations of ambient sampling stations along the Clark Fork River and mobile laboratory sites are shown in Figure 1.

Two studies were conducted in 1985 (Table 1):

1. a chronic 7-day static renewal test of kraft mill wastewater, Frenchtown, MT; and
2. a chronic 7-day static renewal test of 19 ambient samples obtained (daily) along the Clark Fork River from Butte to Noxon Dam, MT.

Two studies were conducted in 1987 (Table 1):

1. 48-hour acute and chronic 7-day static renewal tests of Missoula, MT, wastewater fortified with ammonia singly, and chlorine singly; the latter as part of standard treatment measures at the wastewater treatment facility; and
2. a chronic 7-day static renewal test of eight ambient samples obtained on four separate days from the upper Clark Fork River, including Silver Bow Creek (Butte to Missoula, MT).

Sample Collection, Preservation and Analysis

For the studies of wastewater from both the kraft mill at Frenchtown (1985) and the city of Missoula (1987) grab samples were diluted with Clark Fork River water. It also served as a reference water. The kraft mill samples were unaltered except for preparation in a dilution series of 4.0, 2.0 1.5, 1.12, 0.64, 0.36, 0.2 and 0% wastewater (0% being 100% river water).

Figure 1. Location of ambient sampling stations along the Clark Fork River and mobile laboratory sites.

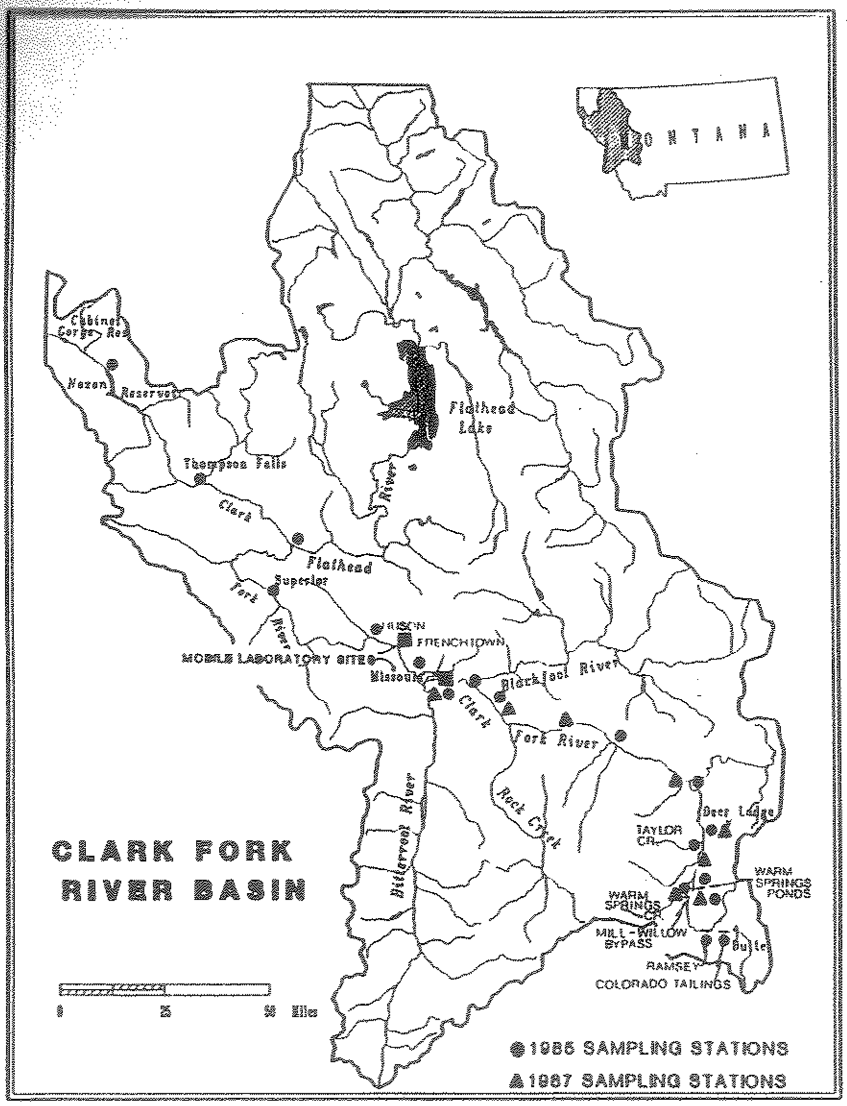

Table 1. Dates, test waters, test conditions, and locations of 1985 and 1987 studies using *Ceriodaphnia*.

Sample Tested	Test Conditions	Locations	Dates
Kraft Mill Treated Wastewater	Static-Renewal	Stone Container Frenchtown, MT	May-June 1985
Clark Fork River	Static-Renewal Ambient Samples plus Silver Bow Dilutions	19 stations Butte to Noxon Dam, MT	May-June 1985
Missoula Wastewater Treatment Plant	Static-Renewal	Missoula, MT	May-June 1987
Clark Fork River	Static-Renewal Ambient Samples plus Silver Bow Dilutions	8 stations, Silver Bow to Missoula, MT	May-June 1987

For the Missoula wastewater, two series of test dilutions were prepared, both fortified with ammonia using the methods and procedures of Nimmo et al., (1989). One series of dilutions was wastewater plus ammonia diluted with river water for a range of 100, 75, 56, 32, 18, 10 and 0% (the latter being river water without ammonia). The other dilutions were river water and river water plus ammonia in a similar series; 100, 75, 56, 32, 18, 10 and 0% (the latter also river water without ammonia). The reason for this design was to conduct the tests within the matrices of wastewater dilutions versus Clark Fork River water to provide information on the potential effects of other constituents in wastewater compared to the effects of ammonia. A comparison of results from the two series of dilutions showed them to be a fair simulation of conditions in the mixing zone of the Clark Fork River below the wastewater treatment plant. Because of the volatility of ammonia, the dilutions prepared were immediately dispensed into test containers and results of the testing are reported as milligrams-per-liter (mg/l;ppm) un-ionized (NH_3). For the pre- and post-chlorinated tests, grab samples of wastewater were obtained from the treatment plant immediately before chlorination and at the discharge point. Both were diluted with Clark Fork River water in a series as above then dispensed into the test containers.

For ambient tests, grab samples of Clark Fork River water were collected for seven consecutive days, May 10-16, 1985 and in 1987, collected four times; May 25, 27, 29, and June 1. In 1985, samples were collected from 19 sites; in 1987, eight sites. Upon arrival at the mobile laboratories each sample was mixed with dilution water and split into two samples, with one used without alteration for the daphnid test, the other used for chemical analysis and analysis of water characteristics.

In 1985, a portion of the unaltered samples were composited, with 1125 mls of test water added daily to an autoclavable polypropylene shipping bottle. The bottles were maintained at ambient river temperature (10-12°C) by floating in a covered tank in which river water was pumped continuously. On the last (7th) day of sampling, the bottles were shipped via ground transportation to the U.S. EPA Laboratory, Corvallis, OR. Elemental chemical analysis was performed on filtered samples using an inductively coupled plasma atomic emission spectrophotometer (ICAP) using the methods of U.S. EPA (1979, 1982). In 1987, uncomposited and unaltered samples destined for analysis of metals were maintained on ice at 4°C and transported to Missoula, then repacked and shipped on ice to the U.S. EPA Laboratory, Corvallis, OR, where they were filtered and analyzed as above. Because Silver Bow Creek water was acutely toxic to the daphnids, samples were diluted with local reference water believed to be free from the influence of metals, and tested separately using a series of dilutions as described above with the wastewater tests. The various reference waters used in these tests are discussed in the results section.

Methods for acute tests (48-hour) were those of U.S. EPA (1985b) and ASTM (1980) in which the daphnids were exposed to toxicants for a portion of their life cycle. The endpoint considered was lethality which is defined as an LC50, or the lethal concentration to 50% of the test organisms. In some cases, an EC50 (an effect observed other than lethality) was considered as an endpoint. To begin the acute test, one neonate (young daphnid) less than 24 hours old was placed in each of ten 30-ml beakers filled with test sample and incubated at $25^{\circ}\pm1^{\circ}$ C; for a total of 10 test organisms per dilution. The sample was renewed the next day, for a total of 48 hours exposure time. Daphnids were not fed during the test.

For 7-day chronic tests, methods of Mount and Norberg (1984) and U.S. EPA (1985a) were used and involved exposure of the daphnids through a life cycle. Endpoints considered were lethality, number of young produced per female, and in the case of wastewater from Missoula where some unusual findings were discovered, the number of adults that produced young—regardless of how many young were produced. Under controlled conditions where no toxicity is involved, the parthenogenetic females begin producing young on the fourth day and within three more days two additional broods are produced, for a total of 20 to 40 neonates per female.

Chronic tests were begun by placing one neonate, less than 24 hours old, in each of ten 30-ml beakers filled with test sample, for a total of 10 test organisms per dilution. Every day for seven consecutive days, organisms were transferred into renewed test solutions. A diet of commercially-prepared dried cereal leaves, trout chow and bakers yeast supplemented with algae (*Selenastrum capricornutum*) was fed daily to each neonate. Test temperature was $25^{o}\pm1^{o}C$ and conditions of the animals (living or dead), number of young per female or any unusual behavior were recorded daily.

Lethal concentrations (LC50s), or effective concentrations (EC50s) and their corresponding 95% confidence intervals for acute tests were computed by the trimmed Spearman Karber method (Hamilton et al., 1977). For the ambient tests of stream water, significance of survival was analyzed by Student's "t" test. Data from reproduction (number of young per female) were first tested for homogeneity of variance, then analyzed by the Analysis of Variance (ANOVA) procedure. In the case of unequal numbers of replicates (survivors in the seven-day tests), a "t" test with Bonferroni's Adjustment was used to identify differences in reproduction of test animals among sampling sites compared to those from a control site. When there was an equal number of replicates, Dunnett's procedure was performed. If the data were not normally distributed, a nonparametric test such as the Wilcoxon's Rank Sum Test with Bonferonni Adjustment was used. (Florence Kessler, U.S. EPA, personal communication).

RESULTS AND DISCUSSION

Kraft Mill Wastewater Study, Frenchtown, MT, 1985

Due to public concern about water quality in the middle and lower Clark Fork River, in particular the release of treated wastewater from the kraft mill, a chronic toxicity test was conducted. Results of this effort were considered along with other relevant data for modification of a discharge permit originally issued by the state of Montana, April 1984. *Ceriodaphnia* were tested for seven days on dilutions of wastewater ranging from 4% wastewater to control (Clark Fork River water) as previously discussed. To provide a range of dilutions bracketing discharge volumes, the 4% dilution was chosen as a doubling of the 2% dilution, the latter being a permit limit for the facility based on low flow conditions in the receiving stream.

Daphnids produced significantly fewer young in the 4% dilution (4% wastewater, 96% river water) but there was no significant effect of wastewater on survival of daphnids (Table 2) at a dilution of 2%. Analysis of priority pollutants were conducted on two different occasions on both the wastewater and river water, but they did not reveal any chemical constituents that appeared responsible for chronic toxicity at 4% (Nimmo et al., 1985).

Table 2. Results of 1985 seven-day chronic test with *Ceriodaphnia* showing survival and reproduction in Frenchtown, MT kraft mil wastewater diluted with Clark Fork River water.

Percent Wastewater	Percent Survival	Average Number of Young per Female	95% Confidence Interval
0.0	80	10.6	6.5-14.7
0.2	90	9.9	7.2-12.6
0.36	90	10.9	5.8-16.0
0.64	90	6.6	4.2-9.0
1.12	90	12.2	7.2-17.2
1.5	100	10.1	6.6-13.6
2.0	100	9.9	4.9-14.9
4.0	90	3.4[1]	1.0-5.8

[1] Significantly different from control (0.0) at $P \leq 0.05$.

Missoula, MT Wastewater Study, 1987

For a number of years there were concerns about operational problems at the Missoula Wastewater Treatment Plant, particularly about dairy wastes, and the potential for providing treatment of sewage from outlying areas. Data collected by the Montana Water Quality Bureau in 1984 and 1985 indicated the possibility of ammonia toxicity in the mixing zone of the Clark Fork River, especially during low flows. There was also concern about chlorine, which was seasonally added as a disinfectant beginning in June and ending in September of each year. The limitations on chlorine and ammonia were part of a permit renewal in September of 1987, after substantial expansion of the plant had been completed May, 1987.

Results of the 48-hour *Ceriodaphnia* test in ammonia-fortified dilutions of wastewater mixed with Clark Fork River water and ammonia-fortified Clark Fork River water are shown in Table 3. Significantly more daphnids died in dilutions of wastewater than in river water (LC50 1.93 mg/l ammonia in wastewater vs. an LC50 >1.95 mg/l in river water). The latter value refers to insufficient lethality at a measured 1.95 mg/l in river water to calculate a lethal concentration; and, that increased toxicity, other than can be attributed to ammonia, is occurring in the wastewater. Another possibility is that the characteristics of wastewater mixed with the riverwater, compared to riverwater alone, enhance the toxicity of ammonia.

Table 3. Results and LC50's from 1987 48-hour acute test with *Ceriodaphnia* in dilutions of Clark Fork River water and Missoula, MT wastewater fortified with unionized ammonia.

Clark Fork River Water			Missoula Wastewater		
Percent Dilution	Un-ionized Ammonia[1] (mg/l)	Percent Survival	Percent Dilution	Un-ionized Ammonia[2] (mg/l)	Percent Survival
Control	0.04	100	Control	0.03	100
10	0.23	90	10	0.21	100
18	0.39	100	18	0.39	100
32	0.73	100	32	0.68	100
56	1.21	100	56	1.24	100
75	1.55	90	75	1.74	80
100	1.95[3]	100	100	2.50[3]	0

[1] Forty-eight hour LC50 was > 1.95 mg/l un-ionized ammonia.

[2] Forty-eight hour LC50 was 1.93 mg/l un-ionized ammonia (95 percent confidence interval, 1.77-2.10).

[3] Twenty-four hour average measured concentration.

Compared to the acute tests above, results of chronic tests in Missoula's ammonia-fortified wastewater and ammonia-fortified Clark Fork River water were not significantly different, with a seven-day LC50 in wastewater of 1.68 mg/l ammonia and in river water, 2.36 mg/l (Table 4). However, survival and reproduction (average number of daphnids produced per female) of daphnids in wastewater was significantly different at 1.33 mg/l ammonia compared to 1.74 mg/l in river water. It was also noted that the number of daphnids that did not reproduce in seven days in the river water was directly correlated with the concentration of ammonia; therefore, using this endpoint, the effective concentration or EC50 was 0.44 mg/l. A similar endpoint was not observed in the tests in wastewater.

Results of the pre- and post-chlorination study where ammonia was not added, showed the LC50 for unchlorinated wastewater was approximately 68% wastewater; whereas, the EC50 based on reproductive success was 79% wastewater (Table 5). Results of the post-chlorinated test showed the LC50 and EC50 to be about the same, both greater than 75% wastewater (Table 6). In these tests the number of young produced per female daphnid was between 32 and 35 in reference waters, or significantly greater than in reference waters from previous studies at the kraft mill (Table 2) and in Missoula wastewater (Table 4).

Table 4. Results, LC50's and EC50 from 1987 seven-day chronic test with *Ceriodaphnia* in dilutions of Clark Fork River water and Missoula, MT wastewater fortified with unionized ammonia.

Clark Fork River Water					Missoula Wastewater				
Percent Dilution	Un-ionized Ammonia[1] (mg/l)	Percent Survival	Number that Reproduced (per total at 7 days)[2]	Avg. # Per Female	Percent Dilution	Un-ionized Ammonia[3] (mg/l)	Percent Survival	Number that Reproduced (per total at 7 days)	Avg. # Per Female
Control	0.04	100	9 (9)	9.7	Control	0.04	100	10 (10)	9.8
10	0.26	90	7 (8)	12.4	10	0.23	100	6 (6)	18.6
18	0.42	100	5 (10)	8.6	18	0.40	100	10 (10)	14.8
32	0.74	100	2 (9)	5.0	32	0.74	100	9 (10)	10.4
56	1.36	80	1 (8)	5.0	56	1.33	80	8 (10)	11.1
75	1.90	80	3 (10)	3.3	75	1.74	60	5 (7)	6.8
100	2.40	50	2 (5)	5.5	100	2.45	0	---	---

[1] The LC50 of un-ionized ammonia in riverwater was 2.36 mg/l (95 percent confidence interval not reliable).

[2] The EC50 based on number of daphnids that did not reproduce was 0.44 mg/l (95 percent confidence interval, 0.31 - 1.62).

[3] The LC50 of un-ionized ammonia in wastewater was 1.68 mg/l (95 percent confidence interval, 1.45 - 1.94).

Table 5. Results, LC50 and EC50 from 1987 seven-day chronic test with *Ceriodaphnia* in dilutions of prechlorinated Missoula, MT wastewater and Clark Fork River water.

Percent Dilution	Chlorine mg/l[1]	Percent Survival[2]	Average Number young Per Female	95 Percent Confidence Interval	Number Reproducing Females (per total at 7 days[3])
Control	ND	100	34.8	30.2-39.2	9 (9)
10	ND	100	32.8	26.6-39.0	10 (10)
18	ND	100	30.7	22.7-38.6	9 (9)
32	ND	100	31.2	24.3-38.1	9 (9)
56	ND	90	30.4	19.3-41.5	8 (9)
75	ND	30	23.5	13.7-33.3	8 (9)
100	ND	0	---	-------	1 (9)

[1] Chlorine as total residual chlorine, not detected

[2] LC50, 67.8 percent wastewater (95% confidence interval, 60.3-76.1).

[3] EC50, based on number of reproducing females per total females that began the test, 79.0 percent wastewater (95% confidence interval, 71.8-89.1)

Table 6.--Results, LC50 and EC50 from 1987 seven-day chronic test with <u>Ceriodaphnia</u> in dilutions of post-chlorinated Missoula, MT wastewater and Clark Fork River water.

Percent Dilution	Chlorine[1] mg/l	Percent Survival[2]	Average Number Young Per Female	95 Percent Confidence Interval	Number Reproducing Females (per total at 7 days[3])
Control	ND	100	31.9	22.5-41.3	9 (9)
5	0.01	100	33.4	26.9-39.9	10 (10)
10	0.02	100	33.9	22.7-45.1	9 (9)
18	0.04	100	35.7	33.0-38.3	10 (10)
32	0.05	100	33.4	20.4-46.4	10 (10)
56	0.08	90	31.4	25.6-37.2	8 (8)
75	0.12	70	29.3	24.1-34.5	9 (9)
100	--	--	--	--	--

[1] Chlorine was measured as total residual chlorine and the 0.12 mg/l is an estimate based on a rate of chlorine loss over a 12-hour period.

[2] LC50, >75% wastewater.

[3] EC50, based on number of reproducing females per total females that began the test, >75% wastewater.

Ambient Testing of the Clark Fork River, 1985

The purpose of this study was to determine and document nonpoint sources of toxicity from metals or other constituents in the Clark Fork River. Results of toxicity testing and chemical constituents are shown in Table 7 and suggest the following:

1. Using survival of daphnids as a criterion, all stations on Silver Bow Creek were acutely toxic.
2. Analysis of Variance (ANOVA) of the reproduction data (average number of young produced per female in water from each site) showed all stations were significantly less than the Taylor Creek control except the station at Huson. However, based on the 95% confidence interval around the mean, daphnids reproduced as well in water from the confluence of the Little Blackfoot and Clark Fork Rivers, as in Taylor Creek water used as a reference.
3. When Silver Bow Creek samples were diluted with Taylor Creek water and tested for seven days, the toxicity of Silver Bow Creek water decreased downstream (Table 8). For instance, the effect/no effect dilution at the Colorado Tailings was between 18% and 10% ambient water; at Ramsay, it was 32% and 18%; finally, just above Warm Springs Ponds, 75% and 56% Silver Bow Creek water (Fig. 1).
4. An association appears between toxicity of the dilutions discussed in (3) above and copper concentrations. At the effect/no effect dilutions, estimated concentrations of copper measured in 100% Silver Bow water ranged from geometric means of 7.2 to 11.2 µg/l, or a fairly restricted range. By contrast, the geometric mean of zinc ranged from 65.3 µg/l at Colorado tailings to 48.9 µg/l at Ramsay; but was only 10.2 µg/l just above Warm Springs Ponds (Fig. 1). It is possible that the interaction of the copper and zinc or other constituents of the water influenced toxicity.

Ambient Testing of the Clark Fork River, 1987

Some observations from the 1985 ambient testing of the upper Clark Fork River prompted a repeat study in 1987. In 1985, only Silver Bow Creek samples were acutely toxic to the daphnids and their reproduction in samples from the remaining stations was less than in the Taylor Creek control water (Table 4) except at Huson and perhaps in water from the Little Blackfoot River. Surprisingly, lethality was not found in water from some upper stations of the Clark Fork River such as the confluence below Warm Springs Creek, Deer Lodge and stations below the Little Blackfoot River confluence (Fig. 1). It was hypothesized that due to unusually dry conditions of the season, metals in the watershed were not mobilized. No precipitation occurred immediately prior to- or during the period of study.

Table 7. Results of 1985 seven-day chronic test with *Ceriodaphnia* showing survival, reproduction and analysis of ambient samples from the Clark Fork River. (units expressed as mg/l;ppm)

Stn. #	Description	N	Percent Survival	Young/ Female (X)	95% C.I.	$CaCO_3$ Hardness (mg/l)	Conduct.	Dissolved Oxygen (mg/l)	pH
1	Taylor Creek above CFR	20	100	41	37.7-44.4	--	--	--	--
2	Silver Bow Colo. tailings	10	0	0	--	142	426	8.4	6.9
3	Silver Bow at Ramsay	10	0	0	--	144	458	8.8	7.2
4	Silver Bow above WSP	10	0	0	--	114	320	9.0	7.2
5	Warm Springs Pond #2 disch.	9	100	32	28.2-35.8	155	443	8.9	7.8
6	CFR below Warm Springs Creek	10	100	31.7	29.7-33.7	135	377	9.0	7.4
7	CFR at Deer Lodge	10	100	35.2	32.7-37.7	182	448	9.0	7.5
8	CFR below Little Bl. Ft	10	90	35.6	32.6-38.6	151	413	8.9	7.6
9	CFR at Drummond	10	100	35.5	33.4-37.6	157	390	9.0	7.6
10	CFR at Turah	10	90	29.8	28.6-31.0	90	228	9.1	7.4
11	CFR below Milltown Dam	10	100	30.6	26.2-35.0	88	191	9.3	7.5
12	CFR above Missoula WWTP	10	100	30.0	28.4-31.6	76	166	9.3	7.5
13	CFR at Harper Bridge	9	90	30.6	27.8-33.4	66	147	9.1	7.3
14	CFR at Huson below Stone Cont.	10	100	39.9	35.9-43.9	62	149	9.2	7.3
15	CFR at Superior	10	90	32.1	30.3-33.9	61	140	9.2	7.4
16	CFR above Fl. Head River	10	90	33.8	28.9-38.7	60	135	9.1	7.3
17	CFR at Thomp. Falls	10	90	30.2	27.5-32.9	66	158	9.2	7.5
18	CFR below Thomp. Falls	10	100	30.6	28.1-33.1	71	150	9.3	7.5
19	CFR below Noxon Dam	10	100	31.3	28.7-33.9	69	151	9.2	7.4

Table 7 (continued)

Stn. #	Description	Ca	Mg	Cu	Cr	Zn	Ni	Cd	As	Pb	Al	Mn	S
1	Taylor Creek above CFR	--	--	--	--	--	--	--	--	--	--	--	--
2	Silver Bow Colo. tailings	40.3	9.9	0.05	<0.004	0.49	<0.006	<0.002	<0.015	<0.025	<0.025	0.66	24.9
3	Silver Bow at Ramsay	41.4	9.8	0.05	<0.004	0.20	<0.006	<0.002	<0.015	<0.025	<0.025	0.74	28.6
4	Silver Bow above WSP	33.1	2.6	0.02	<0.004	0.02	<0.006	<0.002	<0.015	<0.025	<0.025	0.83	17.5
5	Warm Springs Pond #2 disch.	42.5	11.8	<0.007	<0.004	<0.002	<0.006	<0.002	<0.015	<0.025	<0.025	<0.001	40.2
6	CFR below Warm Springs Creek	38.4	9.4	<0.007	<0.004	<0.002	<0.006	<0.002	<0.015	<0.025	<0.025	0.04	27.9
7	CFR at Deer Lodge	52.4	12.3	<0.007	<0.004	<0.002	<0.006	<0.002	<0.015	<0.025	<0.025	<0.001	33.3
8	CFR below Little Bl. Ft	41.3	11.5	<0.007	<0.004	<0.002	<0.006	<0.002	<0.015	<0.025	<0.025	<0.001	25.9
9	CFR at Drummond	44.0	11.3	<0.007	<0.004	<0.002	<0.006	<0.002	<0.015	<0.025	<0.025	<0.001	22.9
10	CFR at Turah	24.8	7.0	<0.007	<0.004	<0.002	<0.006	<0.002	<0.015	<0.025	<0.025	<0.001	10.8
11	CFR below Milltown Dam	24.5	7.0	<0.007	<0.004	<0.002	<0.006	<0.002	<0.015	<0.025	<0.025	<0.001	10.7
12	CFR above Missoula WWTP	23.3	7.2	<0.007	<0.004	<0.002	<0.006	<0.002	<0.015	<0.025	<0.025	<0.001	4.6
13	CFR at Harper Bridge	19.8	6.4	<0.007	<0.004	<0.002	<0.006	<0.002	<0.015	<0.025	<0.025	<0.001	4.1
14	CFR at Huson below Stone Cont.	17.8	5.1	<0.007	<0.004	<0.002	<0.006	<0.002	<0.015	<0.025	<0.025	<0.001	3.3
15	CFR at Superior	17.1	4.7	<0.007	<0.004	<0.002	<0.006	<0.002	<0.015	<0.025	<0.025	<0.001	3.7
16	CFR above Fl. Head River	16.6	4.7	<0.007	<0.004	<0.002	<0.006	<0.002	<0.015	<0.025	<0.025	<0.001	3.2
17	CFR at Thomp. Falls	16.5	4.5	<0.007	<0.004	<0.002	<0.006	<0.002	<0.015	<0.025	<0.025	<0.001	2.9
18	CFR below Thomp. Falls	19.6	5.3	<0.007	<0.004	<0.002	<0.006	<0.002	<0.015	<0.025	<0.025	<0.001	2.0
19	CFR below Noxon Dam	19.1	5.1	<0.007	<0.004	<0.002	<0.006	<0.002	<0.015	<0.025	<0.025	<0.001	2.0

Table 8. Results of 1985 seven-day chronic test with *Ceriodaphnia* showing survival, reproduction and metal concentrations in dilutions of water from three Silver Bow Creek, MT sampling stations showing toxicity.

Station and Dilution[1]	Percent Survival	Average Number Young/Female	95% Confidence Interval	Estimated[2] Concentrations of Filtered Metals, µg/l Zn	Cu	Cr	Al
Taylor Creek (control)	90	20.6	19.4-21.8	--	--	--	--
10% SCB, Colo. tailings	90	18.8	17.1-20.5	49.4	5.4	<0.004	<0.005
18% SBC, Colo. tailings	0[4]	22.0[5]	--	88.9	9.7	<0.004	<0.005
32% SBC, Colo. tailings	0	--	--	158.0	17.3	<0.004	<0.005
56% SBC, Colo. tailings	0	--	--	--	--	--	--
75% SBC, Colo. tailings	0	--	--	--	--	--	--
100% SBC, Colo. tailings	0	--	--	--	--	--	--
10% SBC, Ramsay	70	19.5	17.2-23.3	20.4	4.9	<0.004	<0.005
18% SBC, Ramsay	100	19.8	16.3-23.3	36.7	8.2	<0.004	<0.005
32% SBC, Ramsay	20[4]	16.0	--	65.3	15.7	<0.004	<0.005
56% SBC, Ramsay	0	--	--	--	--	--	--
75% SBC, Ramsay	0	--	--	--	--	--	--
100% SBC, Ramsay	0	--	--	--	--	--	--
10% SBC, WSP[3]	90	22.8	20.6-25.0	1.6	1.7	<0.004	<0.005
18% SBC, WSP	100	20.4	18.8-22.0	2.9	3.1	<0.004	<0.005
32% SBC, WSP	100	19.9	17.0-22.8	5.1	5.4	<0.004	<0.005
56% SBC, WSP	80	18.8	15.8-22.8	9.0	9.5	<0.004	<0.005
75% SBC, WSP	30[4]	20.7	--	12.0	12.8	<0.004	<0.005
100% SBC, WSP	0	--	--	--	--	--	--

[1] Silver Bow Creek water diluted with Taylor Creek (control).

[2] Concentrations of metals were estimated by analysis of a composited 7-day sample and calculating the percent dilution of that sample.

[3] Warm Springs Ponds.

[4] Significantly different from control at $P \leq 0.05$ based on survival.

[5] One daphnid produced 22 young then died on the seventh day of the study.

The purpose of the 1987 study was to repeat the 1985 study during conditions of precipitation, which fortunately occurred during the sampling. Precipitation recorded at Butte, MT was 0.19 inches on 5/25; 0.62 on 5/26; 0.84 on 5/27; 0.06 on 5/28; trace on 5/29; none on 5/30; 0.26 on 5/31; and trace on 6/1. Results again indicated that Silver Bow Creek was acutely toxic to daphnids (Table 9), and that toxicity was associated with zinc, copper and other metals. However, there was no significant toxicity at any of the other stations sampled in the upper Clark Fork River, despite increased concentrations of metals. For example, all the daphnids survived, and produced an average of 41 young per female in water from the Mill-Willow Bypass above the Warm Springs Creek confluence that had 31 µg/l zinc and 29 µg/l copper (Table 9). By comparison, in the 1985 study none of the daphnids survived in Silver Bow water that had only 20 µg/l zinc and 20 µg/l copper (Table 7). Though an apparent mobilization of metals occurred due to runoff during the 1987 study compared to 1985, based on nickel, chromium, and arsenic found in upper Clark Fork River samples, there was no indication of acute or chronic toxicity except in water from Silver Bow Creek (Table 9). It was noted that the sample from Silver Bow Creek collected on 5/27/87, after approximately 1.5 inches of rain the two previous days, showed a peak concentration of 502 µg/l zinc and 117 µg/l copper. The only samples consistently having chromium were those of Silver Bow Creek and the only site where arsenic was measured on each of the sampling dates was the Mill-Willow Bypass above Warm Springs Creek.

Samples from Silver Bow Creek and Mill-Willow Bypass, taken above the confluence with Warm Springs Creek, were used to determine the effect/no effect dilutions with *Ceriodaphnia* (Table 10). This threshold was found to be between 50% and 25% with metal concentrations measured as 102 µg/l zinc, 18 µg/l copper and 19 µg/l nickel (Table 10). Nickel, perhaps mobilized by the rain showers in 1987, was not measured at any of the three Silver Bow Creek stations in 1985 (Table 7). It is possible that nickel could be more toxic to *Ceriodaphnia* than previously considered, especially in combination with other metals,. Again, differences in the reproductive success of the daphnids were noted in reference water from different sources: an average of 41 in Taylor Creek (Table 7); an average of 21 in another study using Taylor Creek water (Table 8); and an average of 41 in water from Mill-Willow Bypass (Table 9). In artificially-produced "control" water in the 1987 study, an average of 31 young per female were produced (Table 9).

DISCUSSION AND CONCLUSIONS

Ceriodaphnia are believed to be a sensitive indicator of certain kinds of toxicity judging from the tests with wastewater. Daphnids were significantly affected (based on number of young per female) in a dilution of 4% kraft mill wastewater but not at a 2% dilution (Table 2) and were also more affected by exposure to the Missoula, MT wastewater (LC50, 1.93 mg/l ammonia) than to Clark Fork River water, with an LC50 greater than 1.95 mg/l ammonia (Table 3). These findings are similar to those reported for ammonia as nitrogen (expressed as NH_3-N) in a study in Colorado (Nimmo, et al., 1989) where the 48-hour LC50 of *Ceriodaphnia* in ammonia-fortified wastewater from the city of Longmont was 1.06 mg/l; whereas, the LC50 in ammonia-fortified St. Vrain River water was greater than 1.43 mg/l. Results from both studies suggest that approximately equal concentrations of ammonia in each of the wastewater dilutions versus the ammonia in the respective river water dilutions do not necessarily produce the same toxic responses. Therefore, other constituents in the wastewater apparently contributed to its toxicity.

This difference was also observed in 7-day chronic testing in which survival and reproduction of daphnids were reduced in dilutions of ammonia-fortified wastewater compared to ammonia-fortified Clark Fork River reference water.

Table 9. Results of 1987 seven-day chronic test with *Ceriodaphnia* showing survival, reproduction and metal concentrations in samples from the Clark Fork River.

Stations	Percent Survival	Average Young/Female (C.I.)[1]	Average Dissolved Oxygen, (ppm)	Average pH	Concentrations of Metals, µg/l (range)				
					Zinc	Copper	Nickel	Chromium	Arsenic
Recon water[2]	100	30.9 (12.5-49.3)	7.2	7.4	--	--	--	--	--
Silver Bow Creek	0	--	7.0	7.5	426 (366-502)	96 (73-117)	28 (ND-74)	30 (20-43)	6 (ND-25)
Mill-Willow Bypass	100	41.4 (21.8-61.0)	7.2	8.1	31 (17-44)	29 (19-36)	ND	ND	39 (ND-63)
CFR below Racetrack Creek	90	32.7 (17.8-47.6)	7.2	8.0	14 (8-19)	2 (ND-7)	ND	ND	3 (ND-10)
CFR at Deer Lodge	100	37.0 (16.2-57.8)	7.2	8.1	29 (19-38)	29 (23-37)	11 (ND-45)	10 (ND-27)	29 (25-34)
CFR at Gold Creek	100	32.1 (15.5-48.7)	7.3	8.2	22 (18-26)	19 (10-33)	ND	10 (ND-27)	13 (ND-28)
CFR at Bearmouth	100	38.2 (24.5-51.9)	7.2	8.2	26 (16-43)	17 (13-22)	10 (ND-38)	7 (ND-14)	7 (ND-29)
CFR at Clinton	100	42.2 (28.7-55.7)	7.2	8.2	34 (23-52)	15 (10-20)	24 (ND-72)	16 (ND-50)	ND
CFR above Missoula WWTP	100	39.2 (12.9-65.5)	7.3	8.2	28 (14-54)	17 (6-26)	ND	10 (ND-38)	ND

[1] 95 percent confidence interval.

[2] Reconstituted water, (1985b).

For example, survival and reproduction were less in equivalent concentrations of ammonia in wastewater (50% survival in 2.4 mg/l ammonia) than in Clark Fork River water (0% survival in 2.45 mg/l ammonia) (Table 4), and were similar to results from the Longmont, CO study. (Nimmo et al., 1989)

One intriguing finding during the chronic testing of Missoula wastewater was an apparent inverse relationship between the absolute number of daphnids that reproduced and concentrations of ammonia. This was especially noted in the dilutions of river water (Table 4). An inverse association was expected between average number of neonates produced per female and the concentrations of ammonia in both wastewater and river water. It is believed that the yeast - trout chow - cerephyl diet prepared for the organisms at Missoula was nutritionally inadequate; but, this situation was apparently corrected in successive tests because reproduction based on young produced per female increased in successive tests (Tables 5 and 6).

Ambient tests of water from Silver Bow Creek using *Ceriodaphnia* confirm the obvious—that the creek is toxic under dry or wet conditions (Tables 7 and 9); and apparently during dry conditions it is less toxic as the water travels downstream. This finding is based on the decreasing percent of dilution necessary for daphnids to survive and reproduce and the corresponding amount of copper in those dilutions (Table 8). For instance, in 1985, during a period of dry conditions, daphnids survived and reproduced in dilutions of Silver Bow water with zinc concentrations between 9 and 65.3 µg/l but a narrow range of only 5.4 to 9.5 µg/l of copper, suggesting that the latter is the metal responsible for the toxicity. However, under conditions of increased runoff in 1987, where concentrations of both metals were greater, toxicity was not observed; suggesting that additional characteristics (i.e. alkalinity, hardness, concentrations of organic acids) of the waters may have influenced toxicity (Tables 9 and 10). Daphnids survived and reproduced in waters with copper concentrations of 29 µg/l (Table 9) and 18 µg/l (Table 10). During the later study however, nickel, chromium and arsenic were also measured (Table 9), which confounds the issue of the specific metal attributable to toxicity in Silver Bow Creek.

A question arising from these studies is still being addressed in laboratory studies and field testing with *Ceriodaphnia*. What should be the selection of - or nature of - acceptable reference or "control" waters? Was Taylor Creek the appropriate reference in the 1985 ambient study (Table 7)? Was an artificially-prepared water an adequate reference water in 1987 (Table 9) or should it have been from the Mill-Willow Bypass above the confluence with Warm Springs Creek or Clark Fork River below the confluence with the Little Blackfoot River? While these questions do not invalidate the results of the studies in which waters within a basin are compared, they suggest that further research on this important question should be undertaken.

Table 10. Results of 1987 seven-day chronic test with Ceriodaphnia showing survival, reproduction and estimated metal concentrations in dilutions of Silver Bow and Mill-Willow Bypass water obtained above confluence with Warm Springs Creek.

Dilution Percent	Percent Survival	# Young Per Female (95% C.I.)	Estimated Concentrations of Metals, µg/l[1] Zn	Cu	Ni	Cr	As
Silver Bow Creek	0	0[2]	409	73	74	20	25
50	30[2]	15.6 ± (6.6[2])	286	51	52	14	18
25	100	36.7 ± (5.4)	102	18	19	5	6
15	100	33.3 ± (5.6)	61	11	11	3	4
10	90	40.0 ± (7.3)	41	7	7	2	3
5	100	34.4 ± (11.3)	20	4	4	1	1
Clark Fork above Warm Spngs. (Control)	100	32.5 ± (8.9)	28	19	0	20	25

[1] Concentrations based on dilutions of the sample collected from 5/25/87.

[2] Significantly different from Mill-Willow Bypass (reference) water ($P = < 0.05$ level).

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