COMPARISON OF ON-SITE AND LABORATORY TOXICITY TESTS: DERIVATION OF SITE SPECIFIC CRITERIA FOR UN-IONIZED AMMONIA IN WASTEWATER

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ABSTRACT

Acute tests with fathead minnows (Pimephales promalas, Johnny darters (Etheostoma nigrum), and white suckers (Catostomus commersoni), and acute and chronic tests with Ceriodaphnia dubia were conducted to evaluate whether St. Vrain River water would ameliorate or enhance toxicity of un-ionized ammonia as compared to laboratory (well) water and LC50 values found in the literature. Concurrently, tests were conducted on dilutions of Longmont, CO wastewater to evaluate its toxicity at several ammonia concentrations. Tests were conducted at two temperatures (6 and 20 C.) to simulate seasonal differences.

LC50s for fishes in river water were similar to LC50s in the literature and laboratory water indicating there was no site water effect. Either constituents in or else characteristics of the wastewater appeared to enhance ammonia toxicity. Literature values for resident aquatic organisms and the new LC50 data for Johnny darter on site were used to derive site-specific criteria for un-ionized ammonia. Greater sensitivity of tested species to ammonia at cold versus warm temperatures suggests that colder low flow conditions may be a critical period for warm-water aquatic communities with regard to ammonia toxicity.

Keywords Ammonia toxicity Site-specific Johnny darter Wastewater

INTRODUCTION

In the early 1980s the population of Longmont, Colorado began to exceed design capacity of the wastewater treatment plant. As a result, the city became eligible for funding by the Environmental Protection Agency (EPA) for construction of additional facilities. The National

Environmental Policy Act (NEPA) of 1969 required that either an Environmental Impact Statement (EIS) or a Finding of No Significant Impact (FONSI) be issued prior to EPA's obligating funds for a construction grant. Of particular concern in the wastewater was the concentration of ammonia that Longmont discharged into Segment Three of the St. Vrain River, classified as Class I Warm Water Aquatic Life. The Colorado Discharge Permit contained no effluent limitation for ammonia, although 0.06 mg/L un-ionized ammonia was the ambient water quality standard generally applied to Class I warmwater streams in Colorado. The NEPA required that state or federal wildlife agencies address whether threatened or endangered species would be affected by construction of additional facilities. Prior to interest in this project, the Colorado Division of Wildlife (DOW) indicated that a State-listed threatened species, the Johnny darter (Etheostoma nigrum) existed in Segment Three of the St. Vrain River, and might be affected by the discharge of un-ionized ammonia or other toxicants from the Longmont wastewater treatment plant. But, a broader implication of this study was that the discharge of Longmont's wastewater into the St. Vrain River was an example of many front range streams dominated by an effluent during much of the year. Typically, it is believed that this situation occurs during late summer and fall during seasonal low flows and the concern has been that ammonia and other toxicants may pose a hazard to fishes and other aquatic life.

Recognizing that local environmental conditions may affect the toxicity of a substance, EPA provided flexibility for states to modify published National Water Quality Criteria. The process is usually referred to as development of site-specific criteria (1). These modifications (often the basis for state standards) are appropriate when there is a question

whether national criteria are under or overprotective for aquatic life use classifications due to local conditions. To aid in development of site-specific criteria, EPA provided procedures and protocols to assist states by taking into account local conditions such as species composition, habitat characteristics, stream flows, water chemistry, etc. In addition, the Colorado Water Quality Control Commission began to address site-specific issues by encouraging the use of data from toxicity tests performed on site. Test guidance was coordinated through an advisory bioassay committee in Colorado (2).

The primary objective of this study was to obtain data in support of a site-specific criterion for ammonia in the St. Vrain River in the vicinity of Longmont, Colorado. To achieve this objective the following testing was performed: (1) Recalculation Procedure in which an additional resident species (Johnny darter) is tested in laboratory water and the data added to the National data base prior to the final calculation; (2) Indicator Species Procedure which determines whether physical or chemical characteristics of water at a site may influence biological availability or toxicity of a substance (water effect ratio); and (3) additional testing at cold water conditions (i.e., 6 to 7 C.) to compare the toxicity of ammonia to warm-water conditions because of the current warm-water classification of the stream.

MATERIALS AND METHODS

Site-specific testing was conducted within Segment Three of the St. Vrain River at Longmont, Colorado, and laboratory testing was performed at the Division of Wildlife, Fort Collins, Colorado. Segment Three is

located between Hygiene Canal and the River's confluence with the South Platte River east of Longmont and north-northeast of Denver (Figure 1).

Figure 1

Toxicity tests were conducted from December 11, 1983 through February 28, 1984 using the following procedures (3, 4) and draft guidance documents (5, 6). On-site at Longmont, flow-through tests were conducted in a mobile laboratory using two vacuum siphon diluter systems. Chemical analyses on test waters and static-renewal tests using Ceriodaphnia dubia (7), were conducted in a separate mobile support laboratory adjacent to the unit described above. In Fort Collins, at the Division of Wildlife laboratory, testing with fishes was accomplished with proportional diluters (8). A diagram showing the experimental design and sequence of all testing is shown in Figure 2. Species of fish tested were: Johnny darters, Etheostoma nigrum; white suckers, Catostomus commersoni; and fathead minnows, Pimephales promelas, both juveniles and larvae. Various tanks and pumps were used to store and deliver test water to the diluters. Inside the workplace at Longmont were the diluters and two 568-L stainless steel holding tanks used to prepare and test solutions. Outside the mobile laboratory, two 1300-L polyethylene tanks were used to store the dilution water obtained from the St. Vrain River above the City of Longmont. Each day, unchlorinated wastewater was pumped directly from a prechlorinated basin into one of the stainless steel holding tanks; the other was filled with St. Vrain River water. The wastewater was adjusted to pH 8.1 to 8.2 with NaOH, then both wastewater and St. Vrain River water were fortified with NH_4Cl. Fluid metering pumps transported both test solutions and appropriate volumes of dilution water to the diluters via stainless steel tubing.

Figure 2

Organisms for the tests were obtained from commercial suppliers and out-of-state government agencies. Upon arrival the animals were transported to the Longmont study site and divided into two groups: one was maintained at Longmont; the other, transported to the Division of Wildlife Laboratory, Fort Collins, Colorado. Both groups were acclimated to laboratory conditions and held until tested (3). Ceriodaphnia were obtained from the Environmental Research Laboratory, Duluth, Minnesota, and maintained in continuous culture until used. At the Longmont site, fish were acclimated to test temperature in a 563-L fiberglass tank equipped with a heating/chilling unit filled with St. Vrain water obtained upstream of the wastewater discharge. About 50 percent of the dilution water was replaced each day to prevent build-up of metabolic wastes. At the Fort Collins laboratory, the fish were held in 265-L fiberglass tanks supplied with flowing well water at 15 C. Because larval fathead minnows have little reserves of energy, they were fed 48-hr-old Artemia hatched daily.

Five experiments with elevated concentrations of ammonia in laboratory well water were conducted at the Fort Collins laboratory using Johnny darters and fathead minnows (larval and juvenile stages). A proportional diluter (8) delivered five test concentrations of ammonia and a control. Flow conditions were adjusted to provide 99 percent replacement every three hours. This was done to match flows and volume conditions of tests conducted at Longmont which were delivered every three minutes to maintain nominal concentrations. The pH was not adjusted because it was constant throughout in all test dilutions (Fig. 2).

Two temperature regimes, 20 C and 6 C, were used to compare the

toxicity of ammonia to fathead minnows and Johnny darters. These temperatures were typical of Colorado's summer and winter stream conditions along the segment of the St. Vrain River.

Procedures for testing Ceriodaphnia

Test solutions were obtained daily from each diluter during the 96-hour testing of fish, and subsequently used to test Ceriodaphnia. However, additional dilutions of 40% and 26% (V/V) were prepared to provide a series of 100, 75, 56, 40, 32, 26, 18, 10 percent and control. Immediately upon receipt of the stock culture, additional cultures were begun and maintained throughout the testing at the Longmont site. Because of the need to transfer test organisms into test media with a pH different by greater than 0.2 units, a period of acclimation was provided for several cultures. Two days prior to testing, mature reproducing females were isolated, about 20 per beaker at pH 8.0, and fed with yeast suspension until brood sizes of between 8 and 15 neonates per female were obtained. Tests were initiated with 24-hour old neonates.

Tests were conducted in 30-ml disposable plastic beakers (6) containing 15 ml of test solution. Eight test dilutions plus control were used with ten replicates per dilution, each containing one neonate. The animals were transferred to clean beakers and new solution daily, an account of events being recorded during the transfer. Mortality was recorded for the first three days of the test, as were other observations such as locomotion, number of molts, and growth. On the 4th through the 7th days, living or dead adults and the number of neonates produced were recorded. All animals were fed daily a suspension of dry yeast dissolved

in distilled water at a rate of 0.05 ml per beaker per day. Fresh yeast suspensions were prepared every two days. During the warmwater tests, the test solutions were maintained at 25 ± 1 C in a BOD incubator, whereas in the coldwater tests, the daphnids were maintained at 7 + 1 C.

Chemical analyses of water

Samples of the unmodified wastewater at St. Vrain River water upstream of Longmont were taken daily before renewing the 100% test solutions. The samples were analyzed immediately for pH, temperature, and ammonia. Likewise, samples from the previous day's 100% test solution in their respective holding tanks were analyzed for pH, temperature, and ammonia before renewal. Analyses of priority pollutants were performed on Longmont wastewater samples and on St. Vrain water samples after seven days.

All test concentrations at the Longmont study site, including replicates (100, 75, 56, 32, 18, 10, and control), were analyzed daily for dissolved oxygen, pH, and temperature. Dissolved oxygen and temperature were measured with a YSI meter (Yellow Springs Instrument Model 57) standardized for dissolved oxygen daily by comparison with results of a Winkler test (9). The pH was measured with an Orion Model 201 meter, calibrated daily. Samples for ammonia, nitrite, total dissolved solids and bicarbonate were taken (prior to renewing the 100% test solutions) from each test concentration and analyzed daily by EPA's Region VIII Laboratory in Denver. Dissolved solids (Method No. 100.1), nitrite (Method No. 354.1) and ammonia (Method 350.2 with Nessler color development) were analyzed according to the methods of U.S. EPA (10). Bicarbonates were analyzed according to method 403 of Standard Methods (11).

Duplicate samples from the same test concentration were taken at 4, 12, 24, 48, 72, and 96 hours and analyzed immediately for ammonia (Method 350.3) with an Orion Model 407A meter and Orion Model 95-10 probe according to U.S. EPA (10). Following test solution renewal, samples were analyzed for ammonia and pH to ensure that resultant levels of ammonia were consistent with nominal test concentrations. Because analyses of ammonia in wastewater are usually conducted with a 95-10 probe, the results of this method were used to calculate LC50s. Analyses of the parameters above were performed the first five days in the Ceriodaphnia tests.

Analysis of metals and priority pollutants was conducted on seven samples of Longmont wastewater and St. Vrain River water, each collected on separate sampling days.

In Fort Collins, events were monitored periodically with mortality of test organisms, total ammonia, temperature, and pH recorded daily. Hardness, alkalinity, conductivity, and dissolved oxygen were measured at the beginning and end of each test (11). Total ammonia was determined with a HNU Systems Inc. ammonia gas sensing probe (Model ISE 10-10-00) and pH was measured with a Beckman Model 3560 millivolt pH meter.

In all tests, un-ionized ammonia (NH_3-N) was estimated from measured concentrations of total ammonia and from calculations of pH and temperature (12, 13).

Statistical methods

Median lethal concentrations (LC50s) or median effective concentrations (EC50s) and corresponding 95% confidence intervals for acute tests

were computed by the trimmed Spearman-Karber method (14). In some instances with Ceriodaphnia, the binomial method (15) was used.

Ceriodaphnia dubia chronic tests were analyzed with a procedure based on the Poisson distribution (16) or the "bootstrap", and "all data estimator" (M.A. Hamilton, personal communication). The criteria used for determining sublethal effects of ammonia were survival of Ceriodaphnia and decrease in average number of young (neonates) produced per female.

RESULTS AND DISCUSSION

Testing fishes under warm conditions

Toxicity studies with fishes under warm conditions using Longmont wastewater, St. Vrain River water, and well water in Fort Collins gave data similar to those reported in the literature (Table 1). For example, the LC50s reported for the orangethroat darter (Etheostoma spectabile), a relative to the Johnny darter, were 0.90 and 1.07 mg/L (17) whereas the LC50 for the Johnny darter in St. Vrain water was 1.15 mg/L and in laboratory water, was 1.12 mg/L. Results with fathead minnows in this study were similar to those reported by Thurston et al. (18) who reported LC50s from 29 tests that ranged from 0.75 to 3.4 mg/L (sizes of fish ranged from 0.1 to 2.3 g). In St. Vrain water, the LC50 was 0.94 mg/L for larval fathead minnows and 1.40 mg/L for juveniles. For larval fathead minnows tested in laboratory (well) water, the LC50 was 1.12 mg/L. However, ammonia LC50s were lower (more toxic) for fathead minnows in Longmont wastewater, i.e., 0.56 mg/L for larval fathead minnows and 0.79 mg/L for juvenile fathead minnows. The data base for white suckers (Catostomus commersoni) is not as extensive as the one for fathead

Table 1

minnows. Reinbold and Pescitelli (19) reported LC50s of 1.35 and 1.40 mg/L (temperatures of about 15 C; pH 8.0 to 8.28). Swigert and Spacie (20) reported an LC50 of 0.79 mg/l, and West (personal communication) reported LC50s ranging from 0.76 to 2.22 mg/L (temperature range, 3.6 to 15.3; pH range, 7.8 to 8.2). In the study at Longmont, an LC50 for white suckers was not obtained in St. Vrain water (LC50 > 0.94 mg/L but in Longmont wastewater, the LC50 was 0.57 mg/L (20.2 C; pH, 7.83 to 7.95). It is possible that the suckers, due to their size relative to the volumes and flows of St. Vrain water in the aquaria water, artificially lowered the pH due to their own metabolic activity. Lowered pH may have reduced the concentration of un-ionized ammonia and thus its toxicity.

Derivation of site-specific criteria

Data selected for the site-specific calculations follow. The water effect ratio (WER) was equal to one, which means there was no significant difference in the LC50 of fathead minnows exposed to un-ionized ammonia in St. Vrain (site water) versus the LC50 of minnows exposed in the laboratory (well) water, i.e., . . .

Site Water LC50 and 95% confidence limit, 0.94 (0.87 to 1.02)

Laboratory Water LC50 and 95% confidence limit, 1.12 (0.96 to 1.30).

Similarly, tested under the same conditions, the WER for the Johnny darter was one, i.e., 1.15 (1.01 to 1.31) and 1.12 (0.96 to 1.30) for the site water and laboratory water respectively. Therefore, the data base from the literature (21) was modified by removing the data for walleye (Stizostedon vitreum) which does not occur in the St. Vrain River and substituted the data from the Johnny darter tested in laboratory water. By using the

guidelines for deriving numerical water quality criteria, the site-specific maximum (0.56 mg/L) and chronic value (criterion) of 0.05 mg/L for un-ionized ammonia were derived for Longmont (Table 2). This value, although not identical, supports the 0.06 mg/L standard usually applied to Class I warm-water streams in Colorado.

Table 2

Testing fishes under cold conditions

Ammonia was more toxic to fishes at cold, rather than warmwater, temperatures (Table 1). Juvenile fathead minnows in Longmont wastewater were about twice as sensitive at 6.0 C as at 20.0 C and in St. Vrain water, more than three times as sensitive. Johnny darters were almost three times more sensitive in wastewater at 6.0 C than at 20.0 C, i.e., 0.27 versus 0.73 mg/L respectively and five times as sensitive in St. Vrain water under cold rather than warm conditions (0.23 versus 1.15 mg). The results were virtually identical in laboratory (well) water (Table 1). Larval fathead minnows were almost six times as sensitive at 6.0 C as at 20.0 C; and Johnny darters were more than 6.2 times more sensitive in cold laboratory water than in warm water.

Results of tests with Ceriodaphnia

EC50s of Ceriodaphnia exposed to ammonia under warm versus cold conditions are shown in Table 3 and values were similar to those reported in the literature for the same genus. Mount (20) reported a 48-hr. LC50 of 0.77 mg/L (24 C; pH, 7.06) for two-hour-old Ceriodaphnia acanthina. Russo et al. (20) reported Daphnia magna LC50s of 0.53 to 2.77 (19.6 to 22.0 C; pH, 7.4 to 8.15). In tests with St. Vrain water, the EC50 for

Table 3

C. dubia was 1.43 mg/L, whereas in Longmont wastewater the EC50 was 1.06 mg/L.

In seven-day chronic tests with Ceriodaphnia, sublethal effects were evident with increasing concentrations of ammonia in dilutions of wastewater in St. Vrain River water (Table 4). Ceriodaphnia failed to produce as many neonates at 0.70 mg/L in Longmont wastewater; in the St. Vrain water, reduced number of neonates per female occurred at 0.88 mg/L. The chronic value, i.e., calculated maximum acceptable toxicant concentration (MATC) was between 0.62 and 0.70 mg/L in wastewater with a geometric mean of 0.66. In St. Vrain water, the MATC was between 0.68 and 0.88 with a geometric mean of 0.79 mg/L. Acute/chronic ratios were 1.61 in Longmont wastewater and 1.81 in St. Vrain river water. Interestingly, we found excellent agreement between the range of acute toxicities of fishes and Ceriodaphnia "chronic" limits under both cold and warm test conditions (Table 5). Further research may show that this daphnid may be a useful surrogate to test a variety of wastes or substances instead of the more-involved and expensive tests with fishes.

Table 4

Table 5

Factors affecting ammonia toxicity

An unequivocal consensus about the effects of temperature on ammonia toxicity is not available; however, three reports show increasing toxicity of ammonia with decreasing temperature. Thurston et al. (18) reported that the toxicity of ammonia increased with a decrease in temperature with fathead minnows. In another study (22), bluegills and fathead minnows were tested between 4.0 to 4.6 C and 23.9 to 25.2 C, and rainbow trout were tested at 3.0 versus 15 C. Both species were more sensitive to

ammonia at the lower temperatures, toxicity being 1.5 to 5 times greater in the colder water. A third report showed LC50s for bluegills, channel catfish, and largemouth bass at 28 to 30 C were approximately twice that of 22 C (22). In the tests at Longmont using St. Vrain water or those in laboratory well water at Fort Collins, the LC50s ranged from about two to six times lower at cold than at warm temperatures (Table 1). The greatest difference in toxicity between warm and coldwater tests were with Johnny darters tested in well water where LC50s were 0.18 mg/L at 7.2 C and 1.12 mg/L at 20.6 C, or a factor of 6.2 times lower. Likewise, toxicity of ammonia to Ceriodaphnia increased at colder temperatures, e.g., 3.2 times more toxic at 7 C than at 25 C in St. Vrain river water (Table 3). We note that temperature extremes in the St. Vrain and other streams along the eastern plains streams of Colorado often are well below the 6 to 7 C temperature regime of this study. It is possible that temperature could be a more important environmental factor relative to the toxicity of un-ionized ammonia in warmwater streams than has been considered--especially during winter conditions at low flows.

In warmwater tests, ammonia was more toxic in dilutions of Longmont wastewater than in St. Vrain water (Table 1). LC50s in wastewater were lower than in St. Vrain river water by about 40 percent for white suckers, Johnny darters, and larval or juvenile fathead minnows. For Ceriodaphnia, the EC50 was about 30 percent lower. At cold temperatures fish LC50s were the same in waste and river water but the EC50 for Ceriodaphnia was about 40 percent lower in wastewater. Many factors are known to affect the toxicity of ammonia to aquatic organisms and most are discussed in the criteria document for ammonia (21). These factors include concentration

of dissolved oxygen, temperature, pH, previous acclimation of test organisms to ammonia, fluctuating or intermittent exposures during the testing, concentration of carbon dioxide, salinity, and concentrations of other toxicants.

The possibility exists that in some of the tests conducted in dilutions of wastewater that low dissolved oxygen alone may have resulted in toxicity, despite continuous introduction of compressed air into each aquarium and at least six complete turnovers of test solution per 24 hours. In two tests, dissolved oxygen concentrations were lower than 2.0 mg/L in 100 percent wastewater averaging only 60 percent of that in the 100 percent St. Vrain water. Spoor (24) reported loss of largemouth bass larvae after only 3-hr. exposure at a dissolved oxygen concentration of 2.5 mg/L; however, adults and juveniles of non-salmonid species survived for at least a few hours at concentrations of oxygen as low as 3 mg/L. Another possibility is that a decrease in dissolved oxygen can increase the toxicity of ammonia to aquatic species--and it is possible that, during some tests, this occurred. Selesi and Vamos (25) and Thurston et al. (26) reported ammonia to be more lethal than expected due to lower dissolved oxygen concentrations.

Additional toxicants in wastewater

At least three metals in the wastewater could have contributed to the toxicity of ammonia and the first to be discussed is aluminum. Concentrations of aluminum were averaging about 2.5 mg/L, presumably due to the use of alum as a coagulant at the wastewater treatment plant. The following data on the toxic effects of aluminum to aquatic species are a 96-hr. EC50

for Daphnia magna of 9 mg/L (27); 96-hr. LC50 for brook trout (Salvelinus fontinalis) of 3.6 mg/L (28); and a 96-hr. LC50 for fathead minnows of 18.9 mg/L (29). Toxicity of aluminum may be related to pH and thus its solubility in water. Toxicity of aluminum to rainbow trout was directly related to the concentration of the soluble aluminum that passed through a 0.45nm membrane filter (30). Toxicity of various concentrations of aluminum to trout were highly pH dependent, greatest toxicity (LC50 = 0.52 mg/L) occurring at pH 7.0 (31). However, among trout exposed to concentrations of dissolved or suspended aluminum well below 0.5 mg/L, growth and behavior were normal and pathological anomalies were absent (32, 33).

The second metal that could have been toxic was copper. Copper is unique in that its salts are widely used to eliminate algae in water supplies and in antifouling paints. Therefore, its toxic properties to aquatic species are well known. Using the formula in the Ambient Water Quality Criteria for Copper (33), a two part criterion based on hardness can be calculated as (1) a four-day average concentration in ug/L, expressed as total recoverable copper, not to be exceeded by more than once every three years and (2) a one-hour average concentration in ug/L, expressed as total recoverable, which does not exceed the numerical value more than once every three years. Therefore, for the St. Vrain River with a hardness of 174 mg/L as Ca CO_3 the criteria are as follows:

A four day criterion =

$$= e^{0.8545\,[\ln(\text{hardness, } 174)] - 1.465}$$

$$= 18.9 \text{ ug/L}$$

and a one-hour criterion =

$$= e^{0.9422\,[\ln(\text{hardness}, 174)] - 1.464}$$

$$= 29.8 \text{ ug/L}$$

Copper in the wastewater was at or above both criteria (mean, 29.7 ug/L) through most of the testing at Longmont.

Lastly, nickel was above the limits of detection in three samples of Longmont wastewater and these averaged 61.6 ug/L. This value is a fraction of the four-day average concentration of nickel (once every three years) based on hardness of 174 mg/L (35). The calculation is as follows:

Four-day criteria =

$$= e^{0.8460\,[\ln(\text{hardness}, 174)] + 1.1645}$$

$$= 210.5 \text{ ug/L}$$

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Table 1. Comparison of 96-hr. LC50s (95% confidence limits) of fishes exposed in various test waters containing un-ionized ammonia.

Species	Laboratory (well) (Fort Collins)	St. Vrain River Water	Longmont Wastewater
	Warm Test Conditions 20 C		
Johnny Darters	1.12 (0.99 - 1.26)	1.15 (1.01 - 1.31)	0.73 (0.68 - 0.78)
Larval Fathead Minnows	1.12 (0.96 - 1.30)	0.94 (0.87 - 1.02)	0.56 (0.52 - 0.61)
Juvenile Fathead Minnows	---	1.40 (1.33 - 1.46)	0.77 (-)
White Suckers	---	>0.94 (-)	0.57 (0.45 - 0.74)
	Cold Test Conditions 6 C		
Johnny Darters	0.18 (-)	0.23 (0.21 - 0.26)	0.27 (0.25 - 0.29)
Larval Fathead Minnows	0.19 (-)	---	---
Juvenile Fathead Minnows	0.30 (0.27 - 0.33)	0.40 (0.35 - 0.45)	0.43 (0.40 - 0.46)

Table 2. Fish and macroinvertebrate species list in the derivation of site-specific criteria for un-ionized ammonia in the St. Vrain River, Colorado.

Species	Species Mean Acute Value ($mg/L\ NH_3$-N)
Johnny Darter[1]	1.26
White Sucker	1.29
Fathead Minnow	1.50
Green Sunfish	1.59
Carp	1.65
Mayfly (Callibaetis sp.)	1.94
Red Shiner	2.56
Mayfly (Ephemerella sp.)	5.11
Riffle Beetle	6.82
---	---
Final Acute Value (0.05)[2]	1.13
Maximum Un-ionized Ammonia[2] (mg/L, NH_3-N)	0.56
Final Chronic Value[2]	0.05

[1]The species mean acute values included the LC50 for Johnny Darter derived in the laboratory (well) water.

[2]These values were derived using the guidelines for deriving numerical national water quality criteria for the protection of aquatic life and their uses 1985. (NTIS Number PB85-227049), Federal Register 50 No. 145: 30784-30796.

Table 3. Comparison of 48-hr. EC50s (mg/L un-ionized ammonia) for Ceriodaphnia at warm (25 C) versus cold (7 C) conditions.

Test Water	Warm	Cold
St. Vrain	1.43	0.46 (0.40 - 0.54)
Wastewater	1.06 (0.99 - 1.15)	0.25 (-)

Table 4. Results of chronic testing with un-ionized ammonia using Ceriodaphnia with St. Vrain River and Longmont wastewater.

Test Solution as Percentage	Un-ionized Ammonia (mg/L) as NH_3-N	Average Number Neonates/Female	95-Percent Confidence Interval
Longmont Wastewater			
Control	ND[1]	12.3	9.1 - 15.3
10	0.16	14.8	11.4 - 18.2
18	0.25	15.8	14.4 - 17.2
26	0.40	17.2	14.8 - 19.6
32	0.43	16.3	13.9 - 18.7
40	0.62	13.0	10.3 - 15.7
56	0.70	6.2[2]	4.8 - 7.9
75	0.90	1.3[2]	0.5 - 2.1
100	1.40	-	-
St. Vrain River Water			
Control	ND[1]	13.3	9.5 - 17.1
10	0.19	11.3	7.9 - 14.7
18	0.31	13.8	10.6 - 16.8
26	0.44	10.1	6.3 - 13.4
32	0.53	9.2	6.6 - 11.9
40	0.68	9.4	6.6 - 12.2
56	0.88	6.0[2]	4.8 - 6.8
75	1.16	4.7[2]	2.1 - 7.3
100	1.43	1.3[2]	0.2 - 2.5

[1]ND, Not Detectable

[2]Significantly different from controls, using the "all data" method of analysis

Table 5. Comparison of "acute" 96-hr. LC50s of fishes and Ceriodaphnia "chronic limits" (mg/L un-ionized ammonia).

Test Waters	Fishes	Ceriodaphnia[1]
	"Acute" Range of LC50s	Range of "Chronic Limits"
	----------Warm Conditions----------	
St. Vrain	0.94 - 1.40	0.68 - 0.88
Wastewater	0.52 - 0.77	0.62 - 0.72
	----------Cold Conditions----------	
St. Vrain	0.23 - 0.40	0.40 - 0.54
Wastewater	0.27 - 0.43	0.25[2]

[1] Acute/Chronic ratios were wastewater, 1.61; St. Vrain, 1.81.

[2] Test was discontinued after 96 hr.

Figure 1. St. Vrain River and Watershed.

Hygiene Canal is the beginning of Segment 3; the end is the confluence with the South Platte River. Dilution water was obtained at Point A and transported to the test site, Point B, location of the mobile laboratory.

Figure 2.

Testing ammonia in a well characterized laboratory water compared to site water was the basis of the Indicator Species Procedure--or the possibility that physical or chemical characteristics of water at a site may influence biological availability and toxicity of a substance. Testing within the matrices of wastewater diluted with St. Vrain River versus River water alone provided information on potential effects of other constituents in the wastewater as well as ammonia.

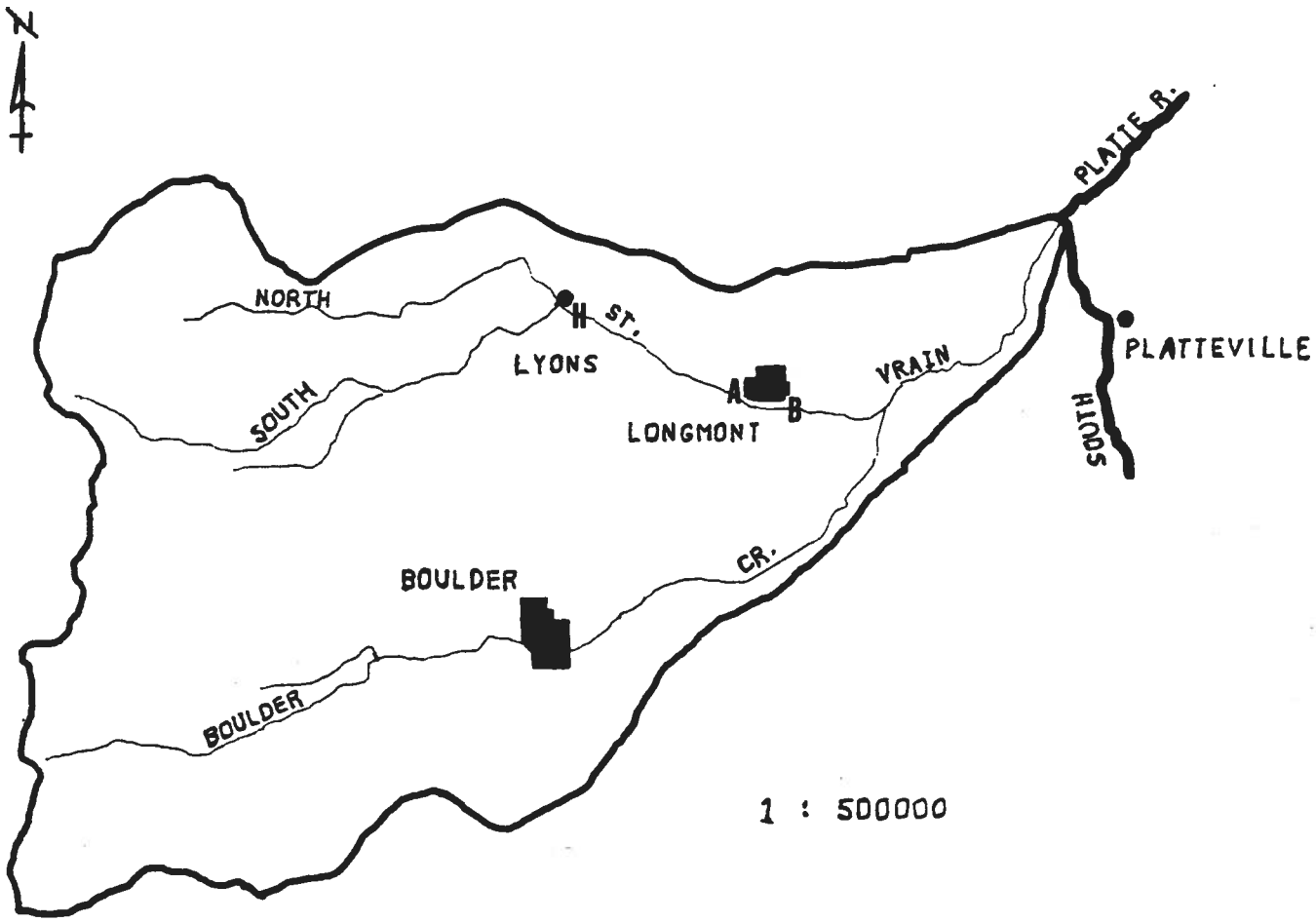
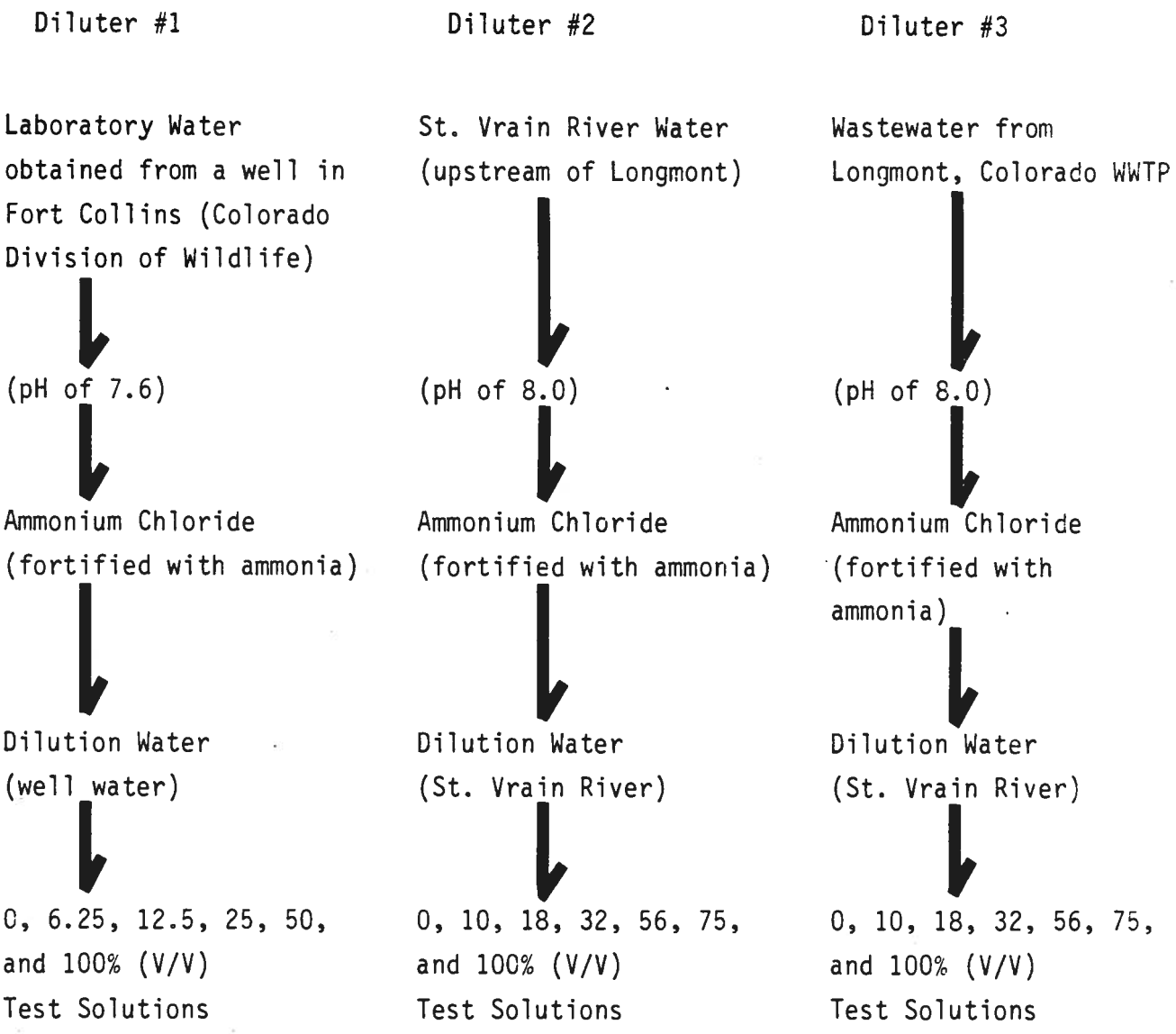

N
PLATTE R.
NORTH
ST.
LYONS
VRAIN
SOUTH
A
B
LONGMONT
PLATTEVILLE
SOUTH
CR.
BOULDER
BOULDER
1 : 500000

Figure 2


Diluter #1
Diluter #2
Diluter #3
Laboratory Water obtained from a well in Fort Collins (Colorado Division of Wildlife)
St. Vrain River Water (upstream of Longmont)
Wastewater from Longmont, Colorado WWTP
(pH of 7.6)
(pH of 8.0)
(pH of 8.0)
Ammonium Chloride (fortified with ammonia)
Ammonium Chloride (fortified with ammonia)
Ammonium Chloride (fortified with ammonia)
Dilution Water (well water)
Dilution Water (St. Vrain River)
Dilution Water (St. Vrain River)
0, 6.25, 12.5, 25, 50, and 100% (V/V) Test Solutions
0, 10, 18, 32, 56, 75, and 100% (V/V) Test Solutions
0, 10, 18, 32, 56, 75, and 100% (V/V) Test Solutions